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INSTRUCTIONS TO AUTHORS

GENERAL INFORMATION: Nigerian Journal of Plant Protection, is a scientific and periodic Journal that covers various aspects of plant protection, in the areas of agricultural biology, environmental biology and crop protection. It also falls into the multidiscipline of mycology, bacteriology, virology, nematology, weed science, etc, as it affects protection of agricultural crops. Review articles, original research reports and short communication are welcome from authors for publication in the Journal.

PREPARATION OF MANUSCRIPTS: Manuscripts should be prepared in standard English Language and typed in double space one side A4 size paper leaving 0.5 cm margins on all sides of the paper. The manuscripts should not be more than 15 pages, tables, figures and plates inclusive. However, more pages attract additional charges. Coloured prints at request will attract additional charges as decided by the Editor-in-Chief.

TITLE: The title should be concise and typed in bold capital letters. Scientific names should be used as appropriate and should be italicized. The title should generally reflect the content of the article.

RUNNING TITLE: A running title of not more than eight words, which is a paraphrase that gives an idea about the study should be indicated in parenthesis below the main title.

AUTHOR(S) NAMES: The name(s) of the author(s) should be indicated below the title of the article in full surname(s) and initials, followed by their full addresses. The chief or the corresponding author should be so indicated. The phone number(s) and e-mail address(es) should be written after the postal address.

SUMMARY: The summary should be written in concise form without losing the focus and clarity of the study. The summary should be able to answer the question on the objectives of the study, how it was carried out, the results obtained, the inference drawn from the results and the conclusion. The summary should not exceed 250 words. Uninformative sentences such as ‘the significance of the results is discussed’ are not acceptable.

KEYWORDS: Key words of not more than seven that conform with the content of the article should be provided as footnote of the abstract for indexing and other purposes.
INTRODUCTION: This should contain brief introduction of the background information of the study, its purpose and significance. Besides, a short review of the pertinent literature should be provided.

MATERIALS AND METHODS: These aspects should contain detailed information of the methodology utilized in the study. It should contain repeatable methods which will remove any doubt in the confidence in the work of the author. In case of universal methodology, proper reporting and citations of relevant literature should be put in place for further referencing, and any deviation should be indicated with proper justification. Also, appropriate experimental design, mode of data collection and statistical analyses should be clearly indicated.

RESULTS: The results should be concise and clear with relevant supporting data, transformation/aids such as, tables, graphs and pictures (plates). Appropriate numbering of the data transformation (tables, graphs and plates) in the relevant sections of the text where the supporting aids belong should be put in place. The results should only be reported in the section without discussing them. Duplication between this section and the discussion must be avoided.

DISCUSSION: The discussion should be the inference drawn from the results as indicated in the previous section. Furthermore, implications and consequences of the results reported here should be backed up with previous relevant and recent literature from past workers on the subject matter. Comparative analyses should be drawn from the inference in relation to previous works.

CONCLUSION: The contribution to scientific knowledge and suggestions for further research in line with the study should be indicated here.

REFERENCES: In the reference list, name and initials of all the authors must be given with the year of publication (not in parenthesis), article title, journal title in full, volume, number, first and last page numbers. If there are three or more authors, the first author’s name should be followed by et al., ensuring the different references with the same name and date are differentiated by a, b, etc. For references to books (if different from authors of chapters), volume number, edition, page numbers of the chapter, publishers and town of publication should be indicated. Ensure that all references cited in the text are listed in the reference list and vice versa, and be consistent in the spelling of authors’ names, date, etc. The list of references cited should be numbered and the number should be used in citing the reference within the text instead of their names and years. This information is very important as the manuscript will be rejected if not strictly
adhered to. Note that the manuscript may be rejected, or publication delayed if it departs in any way from the required style.

**SUBMISSION OF MANUSCRIPTS AND CORRESPONDENCE**

Manuscripts should be sent via e-mail (as an attachment in Microsoft Word) to the Editor-in-Chief (nsppjournal@gmail.com). Submission of manuscript will be held to imply that it reports original unpublished research, that it is not under consideration for publication elsewhere and that if accepted, it will not be published again in any form either in English or in any other language without the Editor’s prior consent. A scanned copy of evidence of payment of the handling charge of three thousand naira (N3,000.00) should be sent along with the manuscript. Upon acceptance, a publication fee of twenty thousand naira (N20,000.00) only will be charged per manuscript. All payments are to be made into the Nigerian Society for Plant Protection account (0036642563) domiciled with Union Bank Plc. Advertisement rates can be obtained from the Editor-in-Chief.
PRESIDENTIAL ADDRESS BY PROFESSOR (MRS.) OLUFUNMILOLA ALABI

I give glory to God the Almighty for the many blessings to us and the Nigerian Society for Plant Protection (NSPP). I sincerely appreciate the golden opportunity to be alive to contribute to the science of plant protection for the good of society and humanity. The NSPP was founded on May 16, 1970. Since then, the Society has grown in leaps and bounds, creating impacts on agriculture and the economy of Nigeria. There has been tremendous increase in the knowledge and practice of plant protection over the years as members of the society immensely contribute sacrificially in their spheres of influence.

Members of the Nigerian Society for Plant Protection are up to the task of fulfilling their mission and making their contributions to the growth of the nation. One way the NSPP has demonstrated this is the regular publishing of results on professionally researched topical issues of significance to agricultural growth, environmental protection and national economy, especially as in relation to plant pests and diseases. A veritable tool that the NSPP has deployed in achieving this is the Nigerian Journal of Plant Protection (NJPP) published by NSPP. The current volume is the 31st in series since the inception of NSPP. I appreciate the tenacious efforts of our teeming authors and reviewers who continue to patronize this platform to publish the results of their researches for societal good, and to ensure that the quality of papers published is of high value and profitable for scientific and professional advancement. It is my sincere hope that individuals, groups and corporate entities will take advantage of the quality of information available in NJPP for improved plant protection practices. I must thank the Editorial Board members who have tirelessly anchored the production of NJPP for NSPP. It is my utmost desire and wish that these efforts will continue to yield the expected results. I plead for your unalloyed support and patronage.

Thank you most sincerely.

Prof Olufunmilola Alabi FNSPP, FASI
President
(National President)
BRIEF FROM THE EDITOR-IN-CHIEF

The current volume (31) of Nigerian Journal of Plant Protection (NJPP) is a product of hard work, commitment, untiring and sacrificial efforts from a team of dedicated and professional authors and reviewers, as well as, the support of the Board of Trustees (BOT) and Executive Committee (EXCO) of the Nigerian Society for Plant Protection (NSPP). I must salute the crop of professional plant protectionists who turned in the results of their meticulously researched and documentedendeavours for publication. It must be stated that the quality of the papers submitted, and professional comments of the reviewers were the veritable guides for the selection of papers published in Volume 31 of NJPP.

While, I admit that the quality of papers received was generally good, I will appeal that authors, especially those that are still learning the ropes of research and publication, have their manuscripts peer reviewed by senior colleagues in their disciplines before submission to fast track the review process and guarantee the high chance of acceptance. I must also advise on the need for strict adherence to the requirements of the journal aptly captured in the Instructions to Authors to avoid outright rejection. You will observe the lack of good representation of some disciplines of plant protection in the number of papers published in the current volume of NJPP. I can only encourage that colleagues in the under-represented disciplines should endeavour to send in their papers for publication in the journal.

I owe the numerous reviewers a debt of gratitude for finding time out of “no time” to respond to my numerous requests and reminders for review of manuscripts without commensurate rewards. This could have only been possible because of your deep interest towards the progress of NSPP’ and NJPP. In fact, some reviewed at no cost to NSPP. I can only say a huge thank you and pray that God will continue to bless you and make your lines fall in pleasant places.

Finally, let me emphasize that the sustenance of NJPP will dependlargely, on the quality of papers received from members of NSPP. So, forward those quality results and NJPP will publish and publicize them on your behalf. I mustthank the members BOT, EXCO and Plant Protection family for giving me the opportunity to lead the NJPP Editorial Board, and my predecessor, Prof. O. A. Enikuomehin for laying a good foundation for my smooth take off. I again, acknowledge the authors for having faith in NJPP and the reviewers for their selfless and professional contributions to NJPP.

Professor B. D. Kashina
Editor-in-Chief
Influence of Ambient Ecology and Cypermethrin Insecticide on Aphid Population Density, Virus Disease Incidence and Yield of Two Tomato (*Solanum lycopersicon* L.) Cultivars

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Summary

RomaVF and Beske tomato seedlings were grown in sterilized topsoil in the nursery inscreened cage for four weeks with cypermethrin application at 0.002 a.i/2 litres of water before transplanting in May 2013 and 2014. The experimental design was split plot fitted into Randomized Complete Block with three replicates. The treatments were RomaVF tomato without cypermethrin application (unsprayed), RomaVF tomato with cypermethrin application at 0.004 a.i/plot (sprayed), Beske sprayed and Beske unsprayed. Data on disease incidence, disease severity and aphid population were taken weekly from 2 to 6 weeks after transplanting (WAT), while leaf samples for virus indexing using Enzyme-linked Immunosorbent Assay (ELISA) were collected at 4 WAT. Tomato fruit yields were determined at physiological maturity. Data collected were analyzed using Analysis of Variance (ANOVA). In 2013, at low rainfall (between 4 and 10.5 mm) the aphid population increased and gradually declined as the rainfall increased (between 10.5 and 27 mm) whereby, the lowest aphid population was at 2 WAT with 116 counts when rainfall was high while aphid population peaked at 4 WAT with 287 counts when rainfall was lowest. In 2014, the trend was the same, but reverse of the 2013. Cypermethrin application reduced virus disease incidence in both years. In 2013, the virus disease incidence for Beske was 93.2% for unsprayed and 63.5% in sprayed plots, while for Roma VF it was 91.1% unsprayed and 70.9 % for sprayed plots. In 2014 the trend was the same. There was significantly (p<0.05) higher fruit yield in sprayed tomato plots than unsprayed plots. Correlation analysis showed a significant but negative relationship between the aphid populations trapped and yield of the tomato cultivars in both years. The
ELISA tests indicated positive reactions for *Pepper veinal mottle virus* and *Potato virus Y*.

**Keywords:** Tomato, ambient ecology, aphid vectors, Roma, Beske, disease incidence

**TOMATO** (*Solanum lycopersicum* L.), a member of the plant family Solanaceae is an annual herbaceous fruiting plant. According to FAO report (9), tomato is still the most important vegetable grown among other vegetables in the tropics. It is widely cultivated in the northern parts of Nigeria and all other parts of the country mostly by subsistence farmers (10).

Despite its importance, tomato farmers face several adverse conditions in growing the crop throughout the tropics. Low tomato yields are due to environmental factors, insect pests and diseases (23, 19, 17). Aphid species are one of the most common polyphagous insect pests (4). The pest affects almost all the aerial parts of the tomato plant from the early growth stages till the fruit maturation stage (7, 6) thereby resulting in stunting, curling or yellowing of plant foliage (4).

Globally, tomato is susceptible to more than 200 diseases, out of which 40 are caused by viruses (20, 18). Virus diseases, besides other biological agents such as insect pests like African bollworm (*Helicoverpa armigera*), thrips (*Thrips tabaci*), whitefly (*Bemisia tabaci*) and aphids (*Myzus persicae*) are reported to infect tomato (8, 22). Bacteria, fungi and nematodes have been long associated with yield losses ranging from 10 to 100% in field grown tomato crops, depending on the virus-host vector relationships, as well as, prevailing epidemiological factors. Virus-infected plants are typically stunted, crinkled, spotted and have small fruits, or no fruits at all. In some cases, infected plants show die-back symptoms before flowering (11).

Similarly, 80 - 100% crop losses are caused by *Cucumber mosaic virus* in tomatoes (14) while other viruses that infect tomato in southwest Nigeria are *Pepper veinal mottle virus* and *Potato virus Y* (2). Tomato varieties bred for resistance to viral diseases are not yet available in this agro-ecological zone and perhaps, in the country at large.

Over the years, virus-like symptoms have been observed in field-grown tomatoes in southwestern Nigeria and investigations on disease incidence have reported the occurrence of viruses. In Nigeria, especially in Southwest where viruses and their
vectors are prevalent, there is the need to enhance tomato production and improve on its yield for increased food security and wealth generation.

Various cost-effective measures have been reported for controlling tomato virus diseases. They include cultural practices, vector manipulation, inoculum source elimination, etc. Hence, the objectives of this study were, to determine the influence of ambient ecology and cypermethrin application on aphid population density, virus disease incidence and yield of two tomato cultivars grown in Abeokuta, southwest Nigeria.

**MATERIALS AND METHODS**

**Study location**

Field experiments were conducted in the early rainy season between May and September of 2013 and 2014 each year at the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta (FUNAAB) located between latitude 7° 15’N and longitude 3° 25’E, 32 metres above sea level. The experimental area had over the years been cultivated with cowpea, yam and or cassava. The area has a bimodal rainfall pattern with the peak in July and September and a short dry spell in August.

**Sources of Seeds**

Two commonly grown tomato cultivars by farmers in northern Nigeria RomaVF (improved tomato cultivar) and Beske grown in southwest Nigeria were both sourced from FUNAAB tomato seed Centre (FUNAAB/KNSUT/DFID-DELPHE/5/ACRN).

**Nursery and field operations**

Nursery trays each of 48 cm diameter and 50 cm depth filled with sterilized top soil and moistened with water were sown with tomato seeds by broadcasting and covering lightly with soil in insect-free and insect-proof cages for a period of four weeks. Cypermethrin insecticide was sprayed in and around the cages 0.002 a.i/2 litres of water after one week for four weeks to ensure that the tomato seedlings were free of virus vectors.

The tomato seedlings were transplanted in split plot arrangement fitted into Randomized Complete Block Design (RCBD) in three replicates according to the treatments namely; RomaVF tomato without cypermethrin application (unsprayed), RomaVF tomato with cypermethrin application applied at 0.004g a.i/plot (sprayed), Beske with cypermethrin and Beske without cypermethrin application. Each plot size was 2 m x 2 m and separated apart by 1 m border in order to reduce inter-plot effects.
The plant spacing for the tomato was 1 m x 0.5 m at one seedling of tomato plant per hole.

Four-week old tomato seedlings from the nursery were transplanted on May 30th of both years to a prepared field. Weeding was done at three and six weeks after transplanting. Cypermethrin insecticide was applied on the specified plots weekly.

**Disease Incidence (DI)**

Disease incidence was determined from two to six weeks after transplanting (WAT) by counting the number of plants showing virus-like symptoms and expressing them as percentage of the total number of plants within the plot.

**Disease Severity**

The degree of virus symptoms expression was assessed weekly from 2 to 6 WAT using a virus disease severity scale developed by Ayo-John et al. (2) where: 1 = No visible symptoms, 2 = Mild symptoms (less than 10% of the total leaves) such as mosaic, mottling, yellowing or necrosis, 3 = Moderate symptoms greater than 10% but less than 50%, 4 = Severe symptoms greater than 50% but less than 75% accompanied with reduced leaf lamina or distortion with stunting, 5 = Severe symptoms greater than 75% plus leaf distortion and general stunting of the plant.

**Vector count**

Virus vector (aphids) population in the field was monitored by placing a yellow plastic bowl containing 98% water, 1.5% liquid detergent mixed with 0.5% formalin preservative solution within the plots to trap the aphids (1). The solution was changed weekly. The aphids were trapped weekly from 2 to 6 WAT and visually counted with the aid of a light microscope (Olympus, China).

**Collection of leaf samples showing virus-like symptoms**

Leaf samples expressing virus-like symptoms such as mosaic, mottling, yellowing, chlorosis, leaf distortions were collected from each treatment plot of tomato cultivar at 4 WAT during 2013 and 2014 planting seasons. Four leaf samples were collected from each treatment per replicate making a total of sixteen leaf samples from each for RomaVF and Beske cultivars. A total of 96 leaf samples were collected in 2013, while 50 leaf samples were collected in 2014, due to loss of plants to wilting. After collection, leaf samples were immediately transported to the laboratory of the Department of Crop Protection, FUNAAB for preservation.
Detection of viruses in tomato leaf samples using Double antibody Sandwich (DAS) ELISA

Approximately 0.1g of leaf samples were taken from four leaves collected per cultivar and bulked together before testing. The leaf samples were ground in Phosphate buffered saline with 0.05% v/v Tween-20 (PBS-T) at pH of 7.4 plus 2% Polyvinyl pyrrollidone (PVP) using a sample extraction bag. The extracted sap was then transferred into a 1.5 ml microcentrifuge tube.

The sap extracted from tomato leaf tissue was used in Double Antibody Sandwich ELISA (DAS-ELISA) with Pepper veinal mottle virus (PVMV), Cucumber mosaic virus (CMV), Potato virus Y (PVY) and Tobacco etch virus (TEV) specific polyclonal antibodies sourced from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig, Germany as described by Clark and Adams (5) for the detection of the respective viruses.

The absorbance of the contents of the ELISA wells was read after incubation at room temperature at 1 hour using microplate reader (Mindray MR 96, China) at 405 nm. Samples were regarded as positive to PVMV, CMV, PVY and TEV viruses if the absorbance reading were double that of healthy control samples.

Weather data collected

Agro-metrological data on rainfall, relative humidity and temperature, were obtained from the Agro-metrological Information Centre of the Federal University of Agriculture Abeokuta, Ogun State Nigeria for the period, between June and August 2013 and 2014 rainy seasons, respectively to determine the effects of weather on the incidence of virus diseases and aphid population.

Data collected on tomato fruit yield

At physiological maturity, tomato fruits were harvested, and their weights were determined using a Mettler electronic balance (Model: MT-2000, China). These were later divided by the total number of plants from which the fruits were harvested to determine the actual number of plants and to estimate the actual yield per plot (ton/ha).

Data analyses

Data on disease incidence, disease severity, yield parameters, vector count and weather parameters were analyzed using GenStat and the significant difference between treatment means were separated using least significant difference (LSD) at 5% level of probability. The trapped aphid numbers were logarithm transformed before analysis. Also, correlation between aphid population
and weather data were determined using Pearson’s correlation procedure to determine the relationships.

RESULTS

Influence of ambient ecology on aphid population density in tomato plots

The aphid population varied over time. It was observed in 2013 growing season that the lowest aphid vector population was at 2 WAT with 116 counts while, the peak was observed at 4 WAT and 287 aphids were collected. However, in 2014, the trend was the reverse of what was observed in 2013. The lowest aphid vector population was at 4 WAT and it was 104 while, the peak aphid population was at 2 WAT which was 152 (Figure 1). In 2013, rainfall pattern was relatively low from 2 to 4 WAT where it was below 10 mm. A 5 WAT it was 10 mm and increased to over 25 mm when the aphid population dropped. The relative humidity was between 75 and 84%. The temperature was 27.5°C when the aphid population was at its peak and dropped to 26.5 °C from the 5th week (Figures 2, 3 and 4). In 2014, the rainfall pattern was opposite that of 2013. The rainfall between 2 to 3 WAT was 15 mm which dropped below 5 mm at 4 WAT when the aphid population peaked. The temperature was at 26.3 °C while the relative humidity was at 80%.

![Figure 1](image_url)

**Figure 1:** Populations of aphid vector trapped weeks after transplanting of tomato during the 2013 and 2014 early seasons in Abeokuta
Figure 2: Mean weekly rainfall pattern during the 2013 and 2014 early growing seasons.

Figure 3: Mean weekly temperature during the 2013 and 2014 early growing seasons.
Rainfall significantly influenced the population of aphids in both years. At consistently low rainfall pattern (between 4 and 10.5 mm) the aphid population increased and gradually declined as the rainfall increased (between 10 and 27 mm) in 2013. In 2014, aphid population peaked at 2 WAT when the temperature was at 26.6 °C and relative humidity was at 80%, and gradually declined at 3 and 4 WAT when rainfall rose to between 11 mm and 18 mm). However, temperature and relative humidity did not significantly correlate with the aphid population (Figures 2, 3 and 4).

**Effects of cypermethrin application on virus disease incidence and yield of tomato cultivars**

Generally, cypermethrin application significantly reduced virus disease incidence in 2013 and 2014 at 3-6 weeks after transplanting and application of cypermethrin. There was no significant (p>0.05) difference between Roma VF and Beske tomato varieties on virus disease incidence in both years (Table 1).

In 2013, the virus disease incidence for Beske was 93.2% for no cypermethrin applied plot and 63.5% in plots with cypermethrin application while, for Roma VF it was 91.1% for...
no cypermethrin and 70.9 % with cypermethrin. In 2014 the trend was the same. However, significant differences were shown in the response of Roma VF and Beske at 4 and 5 WAT to virus symptoms (Table 1).

Cypermethrin application influenced the yield of the two tomato cultivars. There were significantly higher fruit yields per plant, average fruit weight per plant and total fruit yield in cypermethrin applied tomato plots than tomato plots without cypermethrin. Roma VF also had significantly higher fruit weight per plant than Beske, but there was no significant difference between both tomato varieties in the number of fruits per plot, fruit yield per plant and total fruit yield per plot (Table 2).

**Table 1:** Virus disease Incidences on two tomato cultivars in 2013 and 2014 early rainy seasons.

<table>
<thead>
<tr>
<th>WAT</th>
<th>Tomato Cultivars</th>
<th>Virus symptoms incidence (%) on treatments</th>
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<tr>
<td></td>
<td>No cypermethrin</td>
<td>Cypermethrin applied</td>
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<tr>
<td></td>
<td>applied</td>
<td>applied</td>
</tr>
<tr>
<td>2</td>
<td>Roma VF</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>Beske</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>L.S.D(0.05)</td>
<td>V = 10.2 (ns)</td>
</tr>
<tr>
<td>3</td>
<td>Roma VF</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>Beske</td>
<td>33.8</td>
</tr>
<tr>
<td></td>
<td>L.S.D(0.05)</td>
<td>v = 10.5 (ns)</td>
</tr>
<tr>
<td>4</td>
<td>Roma VF</td>
<td>48.9</td>
</tr>
<tr>
<td></td>
<td>Beske</td>
<td>47.8</td>
</tr>
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WAT = weeks after transplanting; Least Significant Differences (L.S.D) are the following comparisons at 5% probability; v = varieties; t= Treatment; ns= not significant at p ≤ 0.05; s = significant at p ≤ 0.05.

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<tr>
<th>L.S.D (0.05)</th>
<th>v = 10.2 (ns)</th>
<th>t = 5.9 (s)</th>
<th>v = 4.7 (s)</th>
<th>t = 5.0 (s)</th>
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<tr>
<td>5 Roma-VF</td>
<td>75.6</td>
<td>46.5</td>
<td>70.1</td>
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<td>Beske</td>
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<td>t = 6.3 (s)</td>
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Table 2: Effect of cypermethrin application on the yield of Roma VF and Beske tomato cultivars grown at FUNAAB in 2013 and 2014.

<table>
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<td>L.S.D (0.05)</td>
<td>V= ns</td>
<td>t = ns</td>
<td>V= 8.5 (s)</td>
</tr>
<tr>
<td>Average fruit weight per plant (g)</td>
<td>Roma-VF</td>
<td>30.0</td>
<td>49.5</td>
</tr>
<tr>
<td></td>
<td>Beske</td>
<td>19.2</td>
<td>34.0</td>
</tr>
<tr>
<td>L.S.D (0.05)</td>
<td>V= 8.9 (s)</td>
<td>t = 7.7 (s)</td>
<td>v = 5.0 (s)</td>
</tr>
<tr>
<td>Fruits yield per plant (g)</td>
<td>Roma-VF</td>
<td>179.9</td>
<td>224.0</td>
</tr>
<tr>
<td></td>
<td>Beske</td>
<td>101.0</td>
<td>133.0</td>
</tr>
<tr>
<td>L.S.D (0.05)</td>
<td>v = (ns)</td>
<td>t = 52.6 (s)</td>
<td>v = (ns)</td>
</tr>
<tr>
<td>Total fruits yield per plot (t/ha)</td>
<td>Roma-VF</td>
<td>16.1</td>
<td>20.1</td>
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<tr>
<td></td>
<td>Beske</td>
<td>9.0</td>
<td>11.8</td>
</tr>
<tr>
<td>L.S.D (0.05)</td>
<td>v= ns</td>
<td>t = 4.7 (s)</td>
<td>v = (ns)</td>
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</tbody>
</table>

Significant Differences (L.S.D) are the following comparisons at 5% probability. v = varieties; t= Treatment; v × t = interaction of varieties and treatment; ns= not significant at p ≤ 0.05; s = significant at p ≤ 0.05.
Correlation of aphid population density and yield of tomato cultivars

There were significant but negative relationships between the aphid population trapped and the yield of RomaVF and Beske tomato cultivars grown in both years at \( p \leq 0.05 \). In 2013, the correlation coefficient \( (r) \) was -0.342 and -0.924 for RomaVF and Beske tomato cultivars while in 2014 it was -0.687 and -0.792 (Table 3).

Table 3: Correlation coefficient analysis between aphid vector populations trapped and yield of two tomato cultivars grown in 2013 and 2014

<table>
<thead>
<tr>
<th>Vector trapped</th>
<th>Year</th>
<th>Variety grown</th>
<th>( r ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aphids</td>
<td>2013</td>
<td>Roma-VF</td>
<td>-0.342*</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>Beske</td>
<td>-0.924*</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>Roma-VF</td>
<td>-0.687*</td>
</tr>
<tr>
<td></td>
<td>2014</td>
<td>Beske</td>
<td>-0.792*</td>
</tr>
</tbody>
</table>

*Significant at \( P \leq 0.05 \)

DISCUSSION

Ambient ecology cannot be ignored in epidemiology, as such, the ambient ecology of the farm area, as well as the population of aphids trapped were noted in the study period. Rainfall significantly influenced the aphid population density. The rainfall pattern therefore, had an influence on the peak population of aphids in the field. Low rainfall coincided with peak aphid population density. This agrees with the findings of Kaushik (15) who reported that heavy shower within a short time had a significant negative impact on pest population structure in the field. The temperature observed at peak aphid population density was between 26.3 and 26.6 °C while the relative humidity was 80%. In West Africa, rainfall, temperature and wind were identified as key virus weather variables affecting diseases in cereal, vegetable and tuber crop
Some researchers have reported lesser or higher temperature depending on the prevalent ambient ecology and aphid species. Muhmmad et al. (21) reported higher maximum temperature of 30 °C, minimum temperature of about 13 °C, and relative humidity ranging from 60–70% were the most favorable environmental conditions for building up the aphid population beyond the economic threshold level. Also, Kumar et al. (16) reported that average temperature of 18.06 °C (Maximum 22.81 and minimum 13.31 °C) under the influence of high relative humidity with the range from 80.71 to 86.5% provided conductive conditions for aphid incidence.

The ELISA result confirmed the presence of PVMV and PVY in some samples tested. Some leaf samples that showed virus-like symptoms did not react positively with any of the antibodies used. The thought of other viral infections cannot be over ruled. Other aphid-transmitted virus infections have been reported on tomato (2). PVMV and PVY are important Potyviruses infecting tomato and pepper in southwest Nigeria (2, 3). Both viruses are aphid-transmitted and have been reported to cause yield losses in susceptible crops. Control methods that include strategies for aphid control should be incorporated into integrated disease management for tomato. Also, there was a significant but negative correlation between aphid population and yield of tomato, thus high aphid population will lead to low yield in tomato. Therefore, tomato yield can be improved with the control of aphid vectors.

Cypermethrin insecticide significantly reduced the virus disease incidence and consequently, improved the yield of tomato in both cultivars used. Insecticide application for the control of virus vectors is effective. However, it may have negative impact on farmers, consumers and the environment (13). As such, pesticides should be used with caution and should be handled by trained personnel. To avoid aphid-transmitted viral diseases, the vulnerable growth stage of tomato that is, when the plants are young should not coincide with peak aphid population.

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Morphological and Molecular Characterization of *Magnaporthe Oryzae* Isolates Causing Blast Disease of Rice (*Oryza sativa*) In Nigeria

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Summary

Rice blast disease, caused by the fungus *Magnaporthe oryzae*, is one of the most devastating diseases of rice in Nigeria. Diversity studies of *M. oryzae* were conducted in the laboratories at the University of Ibadan and the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The *M. oryzae* isolates were separated from diseased rice plants from six locations in Nigeria (Epe, Edozhigi, Abeokuta, Badeggi, Ikenne and Ibadan) and used for this study. Morphological attributes and molecular sequence analysis were used in the characterization of the pathogen. *Magnaporthe oryzae* colonies for all the isolates were greyish-black in colour with regular margins on rice powder agar plates. Conidia shapes were pyriform, and all had hyaline colour. Molecular characterization of ITS region of ribosomal DNA and the actin gene revealed at least 95.7% and 99.1% similarities with *M. oryzae* sequences in GenBank, respectively. Phylogenetic analyses revealed similar clustering patterns of *M. oryzae* among Nigerian isolates. This is the first report of molecular characterization of rice blast fungi in Nigeria.

**Keywords:** *Magnaporthe oryzae*, Actin gene, ITS gene, Identification, Conidia

**RICE** is the most important cereal crop in terms of production and contribution to diet in the developing world (10). It is consumed by more than 4.8 billion people in 176 countries and is the most important food crop for over 2.89 billion people in Asia, over 40 million people in Africa and over 150.3 million people in America (3). Rice straw is a useful source of fodder for livestock and it provides 40 to 70% of total animal food calories (1). Rice
is a source of income for millions of farmers and provides food for about 40% of the world’s human population (9). It is also used in making wine, beer, spirit and vinegar (8). Rice blast disease is one of the important factors limiting rice production in Nigeria. It is considered the principal of all rice diseases due to its wide distribution and high incidence under favourable conditions (6). Rice blast disease was first recorded in Port Harcourt (River state) in 1956 (2) and since then, the fungus has been observed anywhere rice is grown in the country. In any rice-growing area of the world, a blast outbreak could cause losses of about 35–50% of rice yield, and up to 100% of yield could be lost in a serious outbreak of the disease (12). Although, planting of resistant varieties has been proven to be the most effective and economical way of controlling blast disease in rice fields, it has also been discovered that the fungus is able to break plant resistance within a few years of cultivation (6). The breakdown of resistance may be due to high variability of the pathogen (14) and prevailing environmental conditions (11). This implies that a rice variety that shows resistance in one locality may be found susceptible in another location. Thus, this research was carried out to investigate rice blast pathogen diversity in Nigeria.

**MATERIALS AND METHODS**

The research was conducted at the Department of Crop Protection and Environmental Biology, University of Ibadan, Oyo State, Nigeria. Molecular analysis was carried out at the Bio-Science Laboratory, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Six *Magnaporthe oryzae* isolates from Epe, Edozhigi, Abeokuta, Badeggi, Ikenne and Ibadan were obtained from the Plant Pathology unit of the AfricaRice Centre, Cotonou, Republic of Benin. Culture media were prepared by mixing (with a spatula) 10 g of milled rice, 2 g of yeast extract powder and 15 g of agar powder in 1 litre of distilled water and then sterilized in an Autoclave at 15 psi, 121 °C for 15 minutes. Three drops of lactic acid were added into the prepared sterile rice powder agar using a dropper to prevent bacterial growth. The media was poured out into 9 cm diameter petri dishes, covered and left in the laminar flow to gel. Each isolate was cultured on the prepared media and incubated at 37°C for four weeks. At two weeks, the conidia were photographed for comparison.

Extraction of fungal nucleic acid was carried out using the method of Nabi et al. (7). About 100 mg of mycelia were filtered from each pure culture of the six *M. oryzae* isolates grown on Potato Dextrose broth. Mycelia were macerated in 1.5 ml microtubes
containing 1 ml of DNA extraction buffer (100 mM Tris-HCl; 8.5 mM EDTA; 500 mM NaCl) with proteinase K (0.05 mg/ml). Fifty microliters of 20% Sodium Dodecyl Sulphate was added to each microtube and incubated at 65 °C for 30 minutes. After the addition of 7.5 M Potassium acetate (100 µl), microtubes were mixed briefly and centrifuged at 13,000 rpm for 10 minutes. Supernatants were transferred into new microtubes and 2/3 volumes of cold isopropanol was added to each tube, inverted gently and incubated at –20°C for 1 hour. Microtubes were again centrifuged at 13,000 rpm for 10 minutes and supernatants decanted. Pellets of DNA were washed in 300 µl of 70% ethanol, air-dried at room temperature, suspended in 30 µl of sterile distilled water and stored at 4 °C.

The primer pairs ITS 1 (5’-TCCGTAGGGTAAACCTGCGG-3’); ITS 4 (5’TCCCTCGCTTTATTGATATGC-3’) (13) and ACT-512F (5’-ATGTGCAAGGCCGGTTGC-3’); ACT-783R (5’-TACGAGTCTTCTGGCCCAT-3’) (4) directed towards the internal transcribed spacer (ITS) region and actin gene of fungal ribosomal DNA were used for molecular characterization, respectively. Nucleic acid extract (2µl) from each M. oryzae isolate was used as template in total reaction volumes of 12.5 µl containing 5X Green GoTaq® Flexi buffer (Promega, Madison, USA), 0.2 mM dNTPs, 1.5 mM MgCl2, 10 µM of each primer pair and 0.3 U of Taq DNA polymerase (Promega, Madison, USA). Reactions were placed in a Veriti 96 well thermocycler (Thermo Fisher Scientific, MA, USA) with amplification set at 94 °C for 5 minutes followed by 35 cycles at 94 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 90 seconds followed by a final extension at 72 °C for 7 minutes. Reaction products were run through a 1.5 % agarose-TAE gel stained with ethidium bromide at 100 V for 1 hour. Ethanol-purified fragments obtained from each M. oryzae isolate were sequenced in both directions, manually edited using BioEdit v7.2.5 software (15) and verified by the nucleotide option of the basic local alignment online search tool (http://www.ncbi.nlm.nih.gov) (16). Isolates were submitted to GenBank and were assigned accession numbers as shown in Table 1. The sequenced isolates were compared with ITS and Actin sequences of M. oryzae from other parts of the world available at GenBank. Construction of phylogenetic trees was carried out using Molecular Evolutionary Genetics Analysis Program (MEGA v6.06) (17).
Table 1: GeneBank Accession characteristics of six *Magnaporthe oryzae* isolates from Nigeria

<table>
<thead>
<tr>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibadan</td>
<td>NG-IBD</td>
<td>KX146524</td>
<td>KX496542</td>
</tr>
<tr>
<td>Abeokuta</td>
<td>NG-ABK</td>
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<td>KX496543</td>
</tr>
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<td>NG-LAG</td>
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<td>KX496544</td>
</tr>
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<td>KX496545</td>
</tr>
<tr>
<td>Badeggi</td>
<td>NG-BAD</td>
<td>KX146528</td>
<td>KX496546</td>
</tr>
<tr>
<td>Ikenne</td>
<td>NG-IK</td>
<td>KX146529</td>
<td>KX496547</td>
</tr>
</tbody>
</table>

I= Location, II= Isolate, III= ITS gene sequence accession numbers (sequences submitted on 26 April and confirmed on 5 June 2016), IV= Actin gene sequence accession numbers (sequences submitted on 29 June and confirmed on 5 July 2016).

**RESULTS AND DISCUSSION**

Morphological studies indicated that all isolates were as identical. Mycelia colour of all isolates were greyish black in colour with regular margin when cultured on rice powder agar (Plate 1). Conidia shapes were pyriform with round base and a narrow apex. The colours were hyaline, and they were three septate (Plate 2).
Plate 1: Cultures of *Magnaporthe oryzae* isolates from six locations in Nigeria at four weeks after inoculation. (a) Edozhigi (KX496545) (b) Ibadan (KX496542) (c) Epe (KX496544) (d) Badeggi (KX496546) (e) Ikenne (KX496547) and (f) Abeokuta (KX496543).

Plate 2: Conidia photomicrograph of six *Magnaporthe oryzae* isolates from Nigeria
(a) KX496545 (b) KX496542 (c) KX496544 (d) KX496546 (e) KX496547 and (f) KX496543
Expected amplicon sizes of 660 bp and 406 bp were obtained from each *M. oryzae* for the ITS and actin gene, respectively (Plate 3). Nucleic acid sequence comparison among the six *M. oryzae* isolate revealed varying levels of polymorphisms. For the ITS gene sequences, four polymorphisms were identified among the isolates. At positions 102, only isolates NG-LAG, NG-EDZ and NG-IKE had similar nucleotides while an extra 'T' was added for NG-LAG at position 103 (Figure 1). For NG-BAD, nucleotide polymorphism occurred at position 160 while at position 193, polymorphisms occurred for isolates NG-LAG and NG-BAD (Figure 1). For the actin gene sequences, a total of seven polymorphic sites were observed among the six isolates, corresponding to positions 32, 121, 195, 234, 237, 278 and 279. At the amino acid level, only one polymorphic site was observed, at position 11(Figure 1).

**Plate 3:** Partial amplicons of *Magnaporthe oryzae* ITS (a) and Actin (b) genes from six isolates in Nigeria.

M= Marker, Lane 1= KX496542, Lane 2= KX496543, Lane 3= KX496544, Lane 4= KX496545, Lane 5= KX496546, and Lane 6= KX496547
Figure 1: Nucleotide and Amino acid sequence alignments and positions of polymorphism for *Magnaporthe oryzae* ITS and Actin genes from six isolates in Nigeria.

When *M. oryzae* sequences from Nigeria were compared with other retrieved sequences from GenBank through the BLASTn tool, isolates had at least 95.7% and 99.1% similarities with other *M. oryzae* sequences for ITS and actin gene sequences obtained from rice and other graminaceous hosts occurring in different countries, respectively. Phylogenetic analyses revealed that the ITS sequences of the six *M. oryzae* isolates clustered up together in one clade, suggesting similar origin and common ancestor (Figure 2). Similar clustering patterns were observed within the actin gene sequences with all six isolates forming a distinct sub-clade (tree) as shown in figure 3.
ITS sequence from Ikenne (KX146529) shows a closer relationship with a sequence from Egypt (KM484893) (Figure 2) and thus, suggests similar evolution. Since both occur in Africa, analyses also suggest a common origin. All the sequences (for ITS and ACTIN gene) showed relationship with the sequences of Chinese and Japanese isolates as compared to Indian isolates (areas where rice is believed

Figure 2: Phylogenetic tree showing relationship among partial ITS genes of Magnaporthe oryzae isolates from Nigeria with other parts of the world.
Figure 3: Phylogenetic tree showing relationship among partial actin genes of *Magnaporthe oryzae* isolates from Nigeria with other parts of the world.

This could be supported by Couch *et al.* (5) who proposed that rice blast pathogen (*M. oryzae*) has a single origin and could have moved to other parts of the world in association with rice cultivation. Moreover, most of the *M. oryzae* sequences under study were grouped according to the host species (rice). To the best of our knowledge, these are the first *M. oryzae* isolates to be sequenced and submitted from Nigeria.

**CONCLUSION**

The results from this study demonstrate low level of genetic diversity among isolates of *M. oryzae* from various locations in Nigeria. The isolates were morphologically and genetically similar with variations in nucleotide sequence of both ITS and actin gene segments.
It is, therefore, recommended that varying characterization methods should be considered in rice breeding programmes against *M. oryzae* in Nigeria. Additionally, other genomic or full segments of *M. oryzae* should be determined to understand the properties of this important fungus.

ACKNOWLEDGEMENT

The authors are grateful to Dr. Drissa Silue of AfricaRice Centre, Cotonou, Benin Republic for kind provision of the *M. oryzae* isolates used in this study.

REFERENCES


Seasonal Variations in Incidence and Severity of Bacterial Spot and Bacterial Speck of Tomato (*Solanum lycopersicum* L.) Under Rain-Fed and Irrigated Conditions In Samaru Zaria, Nigeria

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**Summary**

Tomato production is hampered by many constraints, including insect pests and diseases. Among the important diseases are bacterial spot and bacterial speck, caused by *Xanthomonas vesicatoria* and *Pseudomonas syringae* pv. *tomato*, respectively. These diseases have been reported to cause remarkable yield loss in all tomato producing areas. This study was therefore undertaken to evaluate the reaction of some tomato varieties to bacterial spot and bacterial speck diseases under screenhouse and field conditions and determine the seasonal variation in the incidence and severity of the disease on the field under natural infection. Screenhouse experiments were carried out in a completely randomized design while field experiments were done using randomized complete block design. Four tomato varieties (Roma VF, UC - 82, Tima and Rio Grande) were evaluated, and Koch’s postulate was confirmed to ascertain infection by the pathogens. The results indicated that all seed samples obtained from farmer saved stocks were infected with bacterial spot while those from the open markets/certified seed vendors were not infected with the pathogen. Among the varieties evaluated for resistance, none was resistant to the pathogens with Rio Grande being the most tolerant. On the field, incidence (35.0%) and severity (1.6±0.17) of bacterial spot was more severe in the wet season than in the dry season in
2010/2011 also incidence (33.0%) and severity (1.7±0.23) of bacterial spot was more severe in the wet season than in the dry season of 2011/2012 while bacterial speck was also not detected in both seasons.

**Keywords**: Bacterial spot, Bacterial speck, Incidence, severity, Tomato diseases

**TOMATO** (*Solanum lycopersicum* Mill) is a crop of great importance in Nigeria and the world over, because of its popularity and versatility in fresh and processed forms, as well as its adaptability as a horticultural crop (15). Its importance is largely due to its nutritional significance as being rich in lycopene and as a source of vitamins A and C. Lycopene is an important antioxidant present in ripe red tomato fruit which has some anticancer property (7). The plant is also highly adaptive to diverse environmental conditions, making it possible for its cultivation all year round in most parts of the world (13). Tomato is cultivated mostly in the northern states of Nigeria (8). Despite the increase in tomato production in Nigeria, average yields were just 57.8 tons/ha in 2012, which was much lower than the average global yield of 336.8 tons/ha (5). These losses are partly due to activities of insect pests and diseases (4). Diseases caused by fungi, bacteria, nematodes and viruses count for a significant portion of the yield losses on tomato (16). Bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) and bacterial speck (*Pseudomonas syringae* pv. *tomato*) are among the major bacterial diseases affecting tomato in all tomato-growing regions of the world. The efficiency of pathogen infection, symptom development and yield responses of host plants are greatly influenced by environmental conditions such as temperature and light variations, seasons of the year, nutrition and water supply. For phytobacterial pathogens, environmental conditions have a significant impact on their survival and proliferation, since they are non-spore-forming (6). Almost all varieties of tomato are highly susceptible to diseases, especially during the wet season. This a daunting challenge to increasing production especially in the tropics where the diseases are extremely difficult to control under the moist and warm conditions prevalent in tropical and subtropical tomato growing regions. In general, seasonal climatic change is one of the key factors that influence disease infection, because if the climatic conditions are not
favourable, infection may not be established (3).

The objectives of this study were to determine the prevalence and seasonal variation in the occurrence of bacterial spot and bacterial speck on tomato, and to screen some locally available varieties for resistance to bacterial spot and bacterial speck diseases.

MATERIALS AND METHODS

Study Location

Zaria lies between Latitude 11°03’ N and 11°15’ N; Longitude 7°30’ E and 7°45’ E and altitude of 550-700 meters in Kaduna State. The town experiences distinct wet and dry seasons. The wet season (May-October) is characterized by convectional rainfall with a mean annual rainfall is about 1000 mm. The dry season (November - April) is characterized by a period of low temperatures (21ºC) (December-February) and the hot, dry season (March - April) where temperatures are as high as 32ºC. Relative humidity is high during the rainy season (75%) but decreases during the dry season (21%), (12).

The screenhouse experiment was conducted in the Department of Crop Protection while the field experiments were carried out on the Institute for Agricultural Research (IAR) Research Farms in Samaru, Zaria. Four tomato varieties were used in the experiment namely Roma VF, UC-82, TIMA and Rio Grande. Sterile soil was used in the screenhouse experiments to prevent contamination by other pathogens.

Screenhouse Experiment

Preparation of bacterial Inoculum and inoculation of tomato seedlings

Pure cultures of Xanthomonas campestris pv. vesicatoria (Xcv) and Pseudomonas syringae pv. tomato (Pst) were obtained from the Bacteriology laboratory at the Crop Protection Department of the Faculty of Agriculture, Institute for Agricultural Research, Ahmadu Bello University Zaria. Identification of the bacterial pathogens was done using physiological tests, (Gram reaction and hypersensitive reaction), and growth characteristics on selective media (Kings Media B (KB) for Pst and Yeast Dextrose Carbonate agar (YDC) for Xcv respectively). The petri dishes were then incubated at 25 – 28 ºC and examined daily. When colonies had formed on the petri-dishes, they were harvested to prepare individual bacterial suspensions that were adjusted to a concentration of 10^8 cfu ml⁻¹ for inoculation of the tomato plants.
Screening varieties of tomato for resistance to bacterial spot

Four varieties, namely; Roma VF, UC-82, Tima, Rio Grande, were evaluated under screenhouse conditions for resistance to the bacterial spot. Seedlings of each variety were raised in 14.75× 8.25×1 inches trays containing sterile sandy-loam soil and transplanted at 21 days after sowing (DAS) into plastic pots (15cm diameter ×14cm depth). The pots were arranged on a bench in a completely randomized design (CRD) with three replications. At 35 DAS, seedlings were inoculated with Xanthomonas campestris pv. vesicatoria suspension using the aerosol method in which the abaxial and adaxial leaf surfaces were sprayed to runoff. Plants treated with sterile distilled water (SDW) served as control.

Screening varieties of tomato for resistance to bacterial speck diseases

Four varieties, namely; Roma VF, UC-82, Tima, Rio Grande, were evaluated under screenhouse conditions for resistance to bacterial speck. The same procedure as described above was repeated. At 35 DAS, seedlings were inoculated with the bacterial suspension (Pseudomonas syringae pv. tomato) using aerosol method. Plants serving as control were treated with sterile distilled water (SDW).

Plants were thereafter observed for the expression of a hypersensitive reaction or typical bacterial speck and spot symptoms between 3 and 4 days after inoculation (DAI). At 14 days, plants were scored for disease severity using the scale described by (14).

Data collected were analyzed using the analysis of variance (ANOVA) on the SAS package. Treatment means were separated using Least Significant Difference (LSD) at 5% level of significance. All the experiments were repeated twice. The experiments were carried out both in the wet and dry season. A combined analysis of the two seasons on each parameter was done.

Field Survey

Determination of the occurrence of bacterial diseases of tomato.

Experiments were conducted at Institute for Agricultural Research Samaru in August and November of 2010 and 2011 representing the rainy and dry seasons respectively. The following varieties namely; Roma VF, UC-82, Tima and Rio Grande, were planted on the field with recommended spacing (1.5 m wide × 3 m long with intra row spacing of 45cm while inter row spacing was 75cm). Disease incidence was determined by
taking the percentage of the ratio of the total number of diseased plants to the total number of plants examined.

\[
\text{Disease incidence} = \frac{\text{Total number of diseased plants}}{\text{Total number of plants examined}} \times 100
\]

\[
\text{Disease severity index} = \frac{\text{sum of individual plant rating}}{\text{Total number of plants assessed} \times \text{maximum score}} \times 100
\]

Disease severity of bacterial spot was scored using a scoring scale described by (14) where:

1 = no disease, 2 = 1-3 % infection, 3 = 5-12 % infection, 4 = 12-25 % infection, 5 = 25-50 % infection and 6 = above 50 % infection.

Disease severity of bacterial speck was scored using scale described by (14) where;

1 = no lesions, 2 = 1-10 lesions on leaves, 3 = 11-20 lesions on leaves, 4 = 21-40 lesions on leaves, 5 = more than 40 lesions on leaves.

Diseases were assessed in August (rainy season) and November (dry season) of 2010 and 2011 to compare variations in the levels of disease incidence and severity.

**RESULTS**

Results from greenhouse experiments showed that all the tomato varieties were susceptible to bacterial spot and bacterial speck diseases. Their susceptibilities were however not severe (Tables 1 and 2). In screening for resistance to bacterial spot, there was no significant difference between the varieties (Table 1). A similar trend was observed when screening for resistance to bacterial speck, with no significant difference between the means (Table 2).

In determining the occurrence of bacterial diseases of tomato, in the first year, Roma VF had the highest incidence of bacterial spot on both leaves and fruits while Rio Grande had the lowest incidence in the wet season (Table 3). The difference between the varieties with highest incidence and lowest incidence was significant. With severity of disease on leaves, there were no significant differences in the wet season but the difference among the varieties in the dry season was significant. In the dry season however, Roma VF and UC-82 had highest incidence on leaves with Tima and Rio Grande having no incidence of infection on the leaves (Table 3). In the dry season, there was no disease incidence on the fruits.
In the second year, the incidence and severity of the diseases on leaves and fruits followed a similar trend, but there were no significant differences among the varieties in both the wet and dry seasons (Table 4). There was no incidence of bacterial speck in both seasons on any of the varieties grown (Tables 3 and 4).

**Table 1:** Reaction of tomato varieties to bacterial spot under inoculation at Samaru.

<table>
<thead>
<tr>
<th>Tomato variety</th>
<th>Disease severity at 14 DAI</th>
<th>Combined Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2010</td>
<td>2010</td>
</tr>
<tr>
<td>Roma VF</td>
<td>3.00a</td>
<td>2.30a</td>
</tr>
<tr>
<td>UC-82</td>
<td>3.30ab</td>
<td>2.30a</td>
</tr>
<tr>
<td>Tima</td>
<td>3.00a</td>
<td>2.00a</td>
</tr>
<tr>
<td>Rio Grande</td>
<td>3.00a</td>
<td>1.70ab</td>
</tr>
<tr>
<td>Control</td>
<td>1.00 b</td>
<td>1.00b</td>
</tr>
<tr>
<td>SE</td>
<td>0.20</td>
<td>0.22</td>
</tr>
</tbody>
</table>

All means with the same letter show no significant difference at 5% level of probability.

SE = Standard error

DAI = Days after inoculation

**Table 2:** Reaction of tomato varieties to bacterial speck under inoculation at Samaru.

<table>
<thead>
<tr>
<th>Tomato variety</th>
<th>Disease severity at 14 DAI</th>
<th>Combined Mean</th>
</tr>
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<tr>
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</tr>
<tr>
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<td>1.70a</td>
</tr>
<tr>
<td>Rio Grande</td>
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<tr>
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</table>

All means with the same letter show no significant difference at 5% level of probability.

SE = Standard error
DAI = Days after inoculation

**Table 3:** Incidence and severity of bacterial spot on tomato varieties, 2010/2011 wet and dry seasons

<table>
<thead>
<tr>
<th>Tomato variety</th>
<th>Wet Season</th>
<th>Dry Season</th>
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<tr>
<td></td>
<td>Leaves</td>
<td>Fruits</td>
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<tr>
<td></td>
<td>Incidence (%)</td>
<td>Severity</td>
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All means with the same letter show no significant difference at 5% level of probability

SE = Standard error

**Table 4:** Incidence and severity of bacterial spot on tomato varieties, 2011/2012 wet and dry seasons.

<table>
<thead>
<tr>
<th>Tomato variety</th>
<th>Wet Season</th>
<th>Dry Season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaves</td>
<td>Fruits</td>
</tr>
<tr>
<td></td>
<td>Incidence (%)</td>
<td>Severity</td>
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<tr>
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</tbody>
</table>

All means with the same letter show no significant difference at 5% level of probability
SE = Standard error

**DISCUSSION**

This study was aimed at evaluating the reactions of some tomato varieties to bacterial spot and speck diseases which are among the major diseases affecting tomato worldwide and determining the seasonal variations if any of the disease.

The results from the screenhouse experiments showed that all the varieties screened were susceptible to bacterial spot and bacterial speck diseases. This agrees with (3) who observed that almost all varieties of tomato are highly susceptible to diseases, especially during the wet season. The incidence and severity of the disease in the field studies showed that the bacterial spot was prevalent in the wet season and less so in the dry season, implying that the pathogen was more prevalent in the wet season as a result of favorable weather conditions suitable for disease development. This agrees with (6) who observed that favorable environmental conditions facilitate disease expression with a significant impact on their survival and proliferation. However, in the dry season, the spot symptoms were less severe on the leaves and were not expressed on the fruits suggesting that the environmental conditions play a critical role in the disease expression and pathogen proliferation.

In Nigeria, (9) observed about 5 percent loss of marketable fruits due to bacterial diseases. (11) further reported crop yield losses of 50%. To estimate the yield losses, (10) conducted several field trials and found that marketable fruit yield was reduced by 30 percent in control plots as compared to treated plots as a result of the disease infection. In Tanzania, (2) reported that a survey of tomato fields (from 1997 – 1998) showed that bacterial spot of tomato was widespread in all the vegetable-growing regions of the country. The findings of this study showed that bacterial spot is widespread while bacterial speck was not detected in tomato fields at the time the experiments were carried out. Although the results do not corroborate the findings of earlier studies which reported widespread occurrence of bacterial speck and bacterial spot diseases on farmers’ fields throughout the tomato-producing areas in Tanzania by (14), as an emerging disease in Nigeria, bacterial speck is not endemic to the study area yet, but a more comprehensive study in a diverse range of agro-ecologies will provide a clear overview of the distribution of the disease in Nigeria.
CONCLUSION

This work has shown that the incidence and severity of bacterial spot diseases in the dry season is less than that of the wet season while bacterial speck was not detected in the study area. It is therefore advisable that farmers in Nigeria should adhere to dry season production of tomatoes. However, since wet season production is inevitable, farmers should use the least susceptible variety (Rio Grande). This study also revealed that among the varieties screened for resistance, none of them showed any resistance to the pathogens. As much work is yet to be carried out on yield loss assessment on bacterial speck disease of tomato in Nigeria, more research should be carried out on epidemiology and yield loss assessment of both diseases. More indigenous varieties should also be subjected to research to develop disease resistant genotypes that are suitable for use by farmers in Nigeria.

Systemic efforts are warranted to undertake studies on aspects of epidemiology and assessment of losses caused by these diseases. There is also an urgent need to combine integrated disease management techniques combining the use of various cultural practices, chemicals, bio-agents and host resistance which would be cost effective and provide disease free tomato.

REFERENCES


Evaluation of Neem Seed Extract for the Management of Early Blight (*Alternaria solani*) Disease of Tomato (*Solanum lycopersicum* L.)

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**Summary**

Early blight disease of tomato caused by the fungus, *Alternaria solani*, accounts for significant yield losses in tomato production annually. This research evaluated the use of aqueous neem seed extract for the management of early blight disease of tomato (*Solanum lycopersicum* L.) in a screenhouse experiment. Infected samples of tomato leaves and fruits were collected from an experimental plot at the University of Ibadan for isolation of associated fungi. Five kilogrammes of sterilized soil were filled into 20cm diameter experimental pots laid out in a completely randomized design with six treatments and three replications. A susceptible variety of tomato (‘Cobra’) was used as the test crop. Three concentrations, 50 g, 100 g, and 200 g per litre of neem seed extract were applied a week before and after inoculation. Synthetic mancozeb fungicide was used as positive control. The inoculum was quantified using the serial dilution method, and a concentration of $10^6$ conidia/ml was applied on the plants. Pathogenicity, incidence and severity of isolates were determined following standard procedures. Data were collected on number of leaves, plant height (cm), stem diameter (cm), cumulative fruit weight (g) and total dry matter yield (g) per plant. Plants inoculated and sprayed with aqueous neem seed extract had the lowest disease incidence (40%) and severity score (1) of the early blight disease relative to other treatments. Plants inoculated and sprayed with aqueous neem seed extract also showed significant increase in yield and growth parameters and compared favourably with mancozeb synthetic fungicide. Neem concentrations used in this study reduced the incidence and severity of early blight disease to 33.3-45.1% and 20.7-29.4%, respectively relative to plants treated with mancozeb fungicide (12.8% and 10.6%, respectively). Although aqueous neem seed extract showed
potential in the management of early blight disease of tomato, further studies and field investigations should be carried out to explore the possible application of neem seed extract in the integrated management of the disease.

**Keywords:** Neem seed extract, Pathogenicity, *Alternaria solani*, Early blight, Conidia

**TOMATO** (*Solanum lycopersicum* L.) is a vegetable crop which belongs to the nightshade family, Solanaceae, and it is widely cultivated for its fruits throughout the humid and subtropics. Its production is severely affected by several diseases caused by fungi, bacteria, viruses and nematodes at all growing stages from seedling to maturity, resulting in considerable reduction in yield (36). Of these diseases, early blight caused by the necrotrophic fungus *Alternaria solani* (Ellis and Martin) Jones and Grout, is one of the most devastating foliar diseases occurring over a wide range of climatic conditions (15). The disease can lead to complete defoliation in areas with high relative humidity greater than 75% and temperature of between 24 and 29°C, and in semiarid climates where frequent and prolonged night dews occur (8, 28). Tomato plants are most susceptible at 8-10 weeks of growth.

Symptoms associated with the disease include collar rot or basal stem lesions at the seedling stage, extensive necrosis surrounded by yellow halo on leaves, stem lesions and fruit rot in the adult plant stage (11, 33). The leaf blight phase, commonly referred to as early blight, is the most important phase of the disease and can result in complete loss of the crop when incidence is severe (17). Collar rot due to early blight damage can cause seedling losses in the field from 20 to 40% and yield losses up to 79% (34). The disease occurs in major tomato-producing areas and symptoms develop more rapidly during periods when favourable environmental conditions alternate between humidity and drought (21, 35).

The main methods of controlling *Alternaria* leaf blight include preventing long periods of wetness on the leaf surface, sanitation, cultivation of resistant varieties and application of fungicides (19, 27). It has been reported that cultivation of resistant varieties is the ultimate control of this disease, but some tomato cultivars have been reported to show low level of genetic resistance to *Alternaria* leaf blight (13). Besides, farmers in pursuance of high yield are inclined to cultivate some varieties which may be less resistant to disease. Chemical control using synthetic fungicides is not a viable option either, due to
environmental concerns, pathogen resurgence and human safety. Hence, it has become imperative to explore alternative measures of disease control.

Organic pesticides are increasingly becoming popular in the management of plant diseases because their products have been found to be biodegradable, eco-friendly and safe for human health (5, 12, and 20). Due to its efficacy and minimum side effects, azadirachtin, a tetra terpenoid obtained from neem seeds, has emerged as a natural biopesticide and a viable alternative to synthetic pesticides (23, 29). Neem (*Azadirachta indica* A. Juss.) is an evergreen tree belonging to the family Meliaceae. It thrives well in tropical and sub-tropical regions, having unique attributes of fast growth and resistance to drought conditions. All parts of the tree including seeds, leaves, roots, bark, constitute a rich source of medicinal drugs (6, 31). The tree has been reported to possess insecticidal, antiseptic, antifungal, antibacterial and anti-malaria properties among several other uses (10, 25, 30). Neem contains a vast array of biologically active compounds that are chemically diverse and structurally variable with more than 140 compounds isolated from different parts of the tree (39). Quercetin and β-sitosterol, were the first polyphenolic flavonoids purified from neem tree and were known to have antibacterial and antifungal properties (22). Also, neem is readily available in Nigeria and cheap unlike the synthetic pesticides. This study, therefore, evaluated the potential of neem seed extract in the management of early blight disease of tomato.

**MATERIALS AND METHODS**

**Field sampling for early blight disease and isolation of associated fungi**

Infected tomato leaf samples showing characteristic symptoms of early blight disease such as circular irregular black or brown spots on the older leaves of the plants including series of distinct dark concentric rings in the center of the spots were collected from an experimental plot at the roof top garden of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan, southwest Nigeria using random sampling technique. Fifty diseased specimens were collected for isolation of infecting fungi; while seeds of a susceptible tomato variety, ‘Cobra’ were used for the experiment. Infected leaves were washed with sterile distilled water (SDW), surface-sterilized in 10% sodium hypochlorite for 1 minute, rinsed with three changes of SDW to remove surface contaminants and air-dried on
sterilized tissue paper. Plating of samples was done on potato dextrose agar (PDA) medium dispensed in 9 cm diameter Petri dishes and incubated at 28±2°C under alternating conditions of 12 - hour darkness and light. Alternaria solani was further sub-cultured on Czapeck agar to enhance sporulation, while other fungi sporulated on PDA. A litre of the medium consisted of Sucrose 30 g, Sodium nitrate 2g, Dipotassium phosphate 1g, Magnesium sulphate 0.500 g, Potassium chloride 0.500g, Ferrous sulphate 0.010, and agar-agar 15g.

Identification of isolated fungi

The various fungal isolates from each of the samples were sub-cultured on PDA to obtain pure cultures for identification. The cultural features of each fungal isolate were carefully observed and recorded. Wet mounts of each isolate were prepared on a microscope slide and stained with lactophenol cotton blue. Themounts were then observed under a microscope and detailed structural features of the isolates were recorded. The features of the organisms were compared with those described in a standard manual of fungi following standard procedures (4, 9, 37). Further confirmation of isolates was made at the Mycological Herbarium in the Germplasm Health Unit of the International Institute of Tropical Agriculture (IITA), Ibadan.

Pathogenicity of isolates

The experiment for pathogenicity was laid out in a completely randomized design with three replications. The treatments consisted of three fungi isolated from the infected tomato samples: Fusarium oxysporum, Colletotrichum sp. and Alternaria solani. Seeds of a susceptible tomato variety, ‘Cobra’ were sown in sterilized soil and transplanted into experimental pots filled with 5 kg sterilized soil at three weeks after planting (WAP). At three weeks after transplanting, the seedlings were inoculated with a 10⁶ conidial/ml of the test isolates; inoculum concentration was quantified using serial dilution and sprayed on the leaves using a hand sprayer. Control treatment was set up in a similar manner, except that sterile distilled water was used instead of inoculum. Symptom development was evaluated at 14 days after inoculation. Re-isolation and reinoculation was again done to establish Koch’s postulates for pathogenicity.

Nursery bed preparation and experimental layout

Loamy soil for seedbed nursery and planting was obtained from the Crop Garden of the Department of Crop Protection and Environmental
Biology, University of Ibadan. The soil was sterilized at 170°C for 4 hours in an electrical sterilizer (Model SS-5-20, Philip Avent, USA) to eliminate microbial contamination. Sterilized soil was poured into a sterilized wooden tray measuring 90 cm × 40 cm which served as the nursery bed, and sufficiently watered before seeds of a susceptible tomato variety (‘Cobra’) were sown by drilling. Germinated tomato seedlings were transplanted after 3 weeks to experimental pots measuring to 20 cm in diameter and filled with 5kg of soil at one seedling per pot in a screenhouse. The experiment was laid out in a completely randomized design with six treatments and three replicates. The treatments were as follows:

Treatment 1: Plants were sprayed with sterile distilled water and served as control.

Treatment 2: Plants were inoculated with the pathogen, but no fungicide or crude neem seed extract application

Treatment 3: Plants were inoculated with the pathogen and sprayed with 50g/L of crude neem seed extract.

Treatment 4: Plants were inoculated with the pathogen and sprayed with 100g/L of crude neem seed extract.

Treatment 5: Plants were inoculated with the pathogen and sprayed with 200g/L of crude neem seed extract.

Treatment 6: Plants were inoculated with the pathogen and sprayed with 0.5g/L of mancozeb fungicide.

Preparation of crude neem seed extract and inoculation of seedlings

Freshly harvested healthy neem seeds were air-dried at room temperature for two weeks and then, ground with a high-speed rotary blender (WPB80 Model 1122217, Warring, USA) into fine powder. The powder was dissolved in weights of 50, 100 and 200g per litre of sterile distilled water while 0.5g Mancozeb fungicide was dissolved per litre. The crude extract and fungicide were applied 2 times on the plant before and after inoculation using a hand sprayer. A 10-fold serial dilution method was used for inoculum preparation and a concentration of $10^6$ conidia/mL was used for inoculation using a hand sprayer. Control treatment was sprayed with sterile distilled water. Data on number of leaves, stem
diameter, plant height, disease incidence and severity were collected weekly, while total fruit weight and biomass were collected at 12WAT. Number of leaves was determined by visual counting, plant height and stem diameter were measured using meter rule and vernier caliper (Model 830-104, Tresna, USA) respectively.

**Determination of dry matter**

The tomato plants were harvested from the middle of each plot by uprooting. The roots of the freshly harvested plants were washed with running tap water and plants from each plot were separately weighed to determine the total fresh weight. This was followed by partitioning of the component parts of each plant in order to determine shoot fresh weight and root fresh weight. The shoot was also partitioned into stem and leaves to determine fresh stem weight and fresh leaf weight. These were separately packed into paper bags. The samples were labelled and placed in an oven at 70°C for 72 hours until constant weight was achieved. The samples were weighed using an electric Mettler balance Model PI210, USA to express the dry weight in grammes (14).

**Determination of disease incidence and severity**

Assessment of disease incidence and severity started at two weeks after transplanting (WAT). Disease incidence was determined as percentage of infected plants. This was calculated thus:

\[
\text{Percentage disease incidence} = \frac{C}{D} \times 100
\]

Where \( C \) = number of plants infected in a treatment

\( D \) = total number of plants in the treatment

Disease severity was rated on a scale of 1-5 following standard procedure (3).

1 = No symptom (0% leaf tissue infected)
2 = Mild symptom (<5% leaf tissue infected)
3 = Moderate symptom (>5%\( \leq \) 30% leaf tissue infected)
4 = Severe symptom (> 30% \( \leq \) 50% leaf tissue infected)
5 = Very severe symptom (> 50% leaf tissue infected)

Thus, disease severity was calculated as:

\[
\text{Disease severity index (%)} = \frac{\text{Sum of disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100
\]
Data analysis

All data were subjected to analysis of variance (ANOVA) and means were separated using Duncan Multiple Range Test (DMRT). Least Significant Difference (LSD) was determined at 5% level of probability using SAS version 9.1 (38).

RESULTS

Pathogenicity, disease incidence and severity

Three fungal isolates: *Colletotrichum* sp., *Fusarium oxysporum* and *Alternaria solani* were associated with early blight disease symptoms, with only *A. solani* being pathogenic on re-inoculation following Kock’s postulates. *Colletotrichum* sp., *Fusarium oxysporum* tested negative in the pathogenicity test and were therefore discarded. Disease assessment showed that the plants inoculated with *Alternaria solani*, but unsprayed with either neem seed extract or mancozeb fungicide had the significantly (p<0.05) highest incidence of 17.7% relative to other treatments and control with 0% incidence (Table 1). This trend continued progressively until 8WAT with all inoculated plants in that treatment, which showed early blight symptoms and recorded 85.4% incidence. Similarly, the same treatment exhibited the highest severity of disease symptoms of 78.7%. Tomato plants that were inoculated with the test pathogen and sprayed with mancozeb (T6) showed the least incidence and disease severity of 12.8% and 10.6%, respectively at 8WAT compared to all the other treatments. Mean incidence and severity were lowest at 4WAT but increased progressively reaching a maximum of 36.4 % and 26.7%, respectively.

Table 1: Effect of neem seed extract on percent incidence and severity of early blight disease symptoms

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<th>3WAT I</th>
<th>3WAT S</th>
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<th>4WAT S</th>
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**Incidence; **Severity; WAT= Weeks after transplanting; T1=Uninoculated control, T2 =Inoculated but unsprayed, T3=Inoculated and sprayed (50g/L extract), T4=Inoculated and sprayed (100g/L), T5=Inoculated and sprayed (200g/L), T6=Inoculated and sprayed Mancozeb fungicide (0.5g/L).

Means followed by same letter along a column are not significantly different at p = 0.05, using Duncan’s Multiple Range Test (DMRT).

**Effect of aqueous neem seed extract on number of leaves per tomato plant inoculated with A. solani**

Tomato plants that were inoculated and sprayed with synthetic mancozeb fungicide produced the significantly (p<0.05) highest mean number of leaves (58.5) and 97.0 at 2 and 4WAT, respectively. This was closely followed by treatment sprayed with 50g/L of aqueous neem seed extract (53.1) but there was no significant difference between the two treatments (p>0.05) (Fig. 1). The application of 50g/L of the extract apparently encouraged production of significantly (p<0.05) highest number of leaves (115) at 6WAT relative to other treatments and control with no significant difference between both treatments. At 8 WAT, the plants inoculated and sprayed with 100g/L of aqueous neem seed extract (T4) produced the highest mean number of leaves (90.5) at 8WAT. This was followed by the plants inoculated and sprayed with 50g/L and 200g/L of aqueous neem seed extract with mean number of leaves of 90 and 89, respectively relative to the control. There were no significant differences (p>0.05) amongst the treatments. However, the plants that were inoculated and sprayed with aqueous neem seed extract and those that were inoculated with the pathogen but unsprayed differed significantly (p<0.05).
Figure 1: Effect of aqueous neem seed extract on number of tomato leaves inoculated with *A. solani*

T1 = Control, T2 = Inoculated (unsprayed), T3 = Inoculated and sprayed 50 g/L, T4 = Inoculated and sprayed 100 g/L, T5 = Inoculated and sprayed 200 g/L, T6 = Mancozeb 0.5 g/L

**Effect of aqueous neem seed extract on height of tomato plants inoculated with *A. solani***

The plants inoculated and sprayed with fungicide (mancozeb) were the tallest (27.6 cm) at 2 WAT. This was followed by those inoculated and unsprayed with inoculum and those that were inoculated and sprayed with 50 g/L of the extract with plant heights of 26.9 cm and 26.2 cm, respectively (Fig. 2). However, there were no significant differences (p>0.05) among the treatments at 2 WAT. Similarly, tomato plants that were sprayed with mancozeb recorded the highest plant height at 4WAT. This was again followed by the plants inoculated and unsprayed (T2) with a mean height of 42.5 cm. There was no significant difference among tomato plants that were inoculated but unsprayed with either neem seed extract or mancozeb fungicide at 7 and 8WAT.

**Effect of aqueous neem seed extract on stem diameter of tomato plants inoculated with *A. solani***

Tomato plants that were inoculated and sprayed with mancozeb fungicide had significantly (p < 0.05) highest
mean stem diameter of 0.39 cm followed by plants inoculated and unsprayed (0.38 cm) at 2 WAT (Fig. 3). The lowest mean stem diameter of 0.28 cm was obtained from the plants that were inoculated with the pathogen and sprayed with 200 g/L of the extract. There were however, no significant (p > 0.05) differences among the five treatments. The plants inoculated and sprayed with mancozeb had the highest mean stem diameter of 0.62 cm and was significantly different from all other treatments at 4WAT. On the contrary, plants that were inoculated and sprayed with 200g/L of aqueous neem seed extract had the lowest mean stem diameter of 0.28 cm and were significantly different from other treatments at the same period. Similarly, mean stem diameter at 6 and 8 WAT was 0.61 and 0.60 cm, respectively among plants that were inoculated with the test pathogen and sprayed with mancozeb, which differed significantly from the other treatments.

**Figure 2:** Effect of aqueous neem seed extract on height of tomato plants inoculated with *A. solani*

T1= Control, T2= Inoculated (unsprayed), T3= Inoculated and sprayed 50 g/L, T4= Inoculated and sprayed 100 g/L, T5= Inoculated and sprayed 200 g/L, T6= Mancozeb 0.5 g/L
T1= Control, T2= Inoculated (unsprayed), T3= Inoculated and sprayed 50 g/L, T4= Inoculated and sprayed 100 g/L, T5= Inoculated and sprayed 200 g/L, T6= Mancozeb 0.5 g/L

**Effect of aqueous neem seed extract on cumulative fruit yield of tomato inoculated with A. solani**

Tomato plants that were inoculated and sprayed with mancozeb (T6) produced significantly (p<0.05) highest cumulative fruit yield (119.9 g) (Fig. 4). The lowest cumulative fruit yield of 34.5 g was recorded in treatment 2 which consisted of plants that were inoculated with *Alternaria solani* but unsprayed with either neem seed extract or mancozeb fungicide and was significantly different (P<0.05) from other treatments.
Figure 4: Effect of aqueous neem seed extract on cumulative fruit yield of tomato plants inoculated with *A. solani*

T1= Control, T2= Inoculated (unsprayed), T3= Inoculated and sprayed 50 g/L, T4= Inoculated and sprayed 100 g/L, T5= Inoculated and sprayed 200 g/L, T6= Mancozeb 0.5 g/L

**Effect of aqueous neem seed extract on total dry matter of tomato plants inoculated with *A. solani***

The tomato plants inoculated and sprayed with 50g/L of aqueous neem seed extract had the highest total dry matter yield of 11.1 g (Fig. 5) followed by plants that were inoculated and sprayed with mancozeb (10.1 g). Control plants that were sprayed with sterile distilled water produced the lowest dry matter yield of 6.96 g and differed significantly from other treatments.
Figure 5: Effect of aqueous neem seed extract on total dry matter of tomato plants inoculated with *A. solani*

T1= Control, T2= Inoculated (unsprayed), T3= Inoculated & sprayed 50 g/L, T4= Inoculated and sprayed 100 g/L, T5= Inoculated and sprayed 200 g/L, T6= Mancozeb 0.5 g/L

DISCUSSION

Early leaf blight disease of tomato caused by the fungus, *Alternaria solani*, is a devastating disease of tomato which causes significant reduction in yield, quality and market value of the crop. It is an important disease which is prevalent in the tropical and sub-tropical regions of the world. The early blight pathogen can cause disease on all parts including the leaves, stems and fruits of the plant which results in severe damage during all stages of plant development (1). However, blight symptoms were more predominant on the tomato leaves because they were the site of application of inoculums. Although three fungal isolates, *Fusarium oxysporum*, *Colletotricum* sp. and *Alternaria solani* were associated with early blight disease symptoms in this study, only *A. solani* tested positive in the pathogenicity study. Several authors also give credence to *A. solani* as the causal organism of early blight disease of tomato (13, 15, 21). The highest incidence and severity of early blight disease symptoms were
observed on the leaves of treatment inoculated with *A. solani* but unsprayed with neither neem seed extract nor synthetic mancozeb fungicide. The non-application of pesticides to the treatment was probably responsible for the high rate of infection since the activity of the blight-inducing pathogen was not inhibited. On the contrary, lower incidence and severity rates were observed in plants inoculated but sprayed with neem seed extract and mancozeb, which implied that the inhibitory action of the extracts reduced disease incidence and severity in the test plants. Previous authors had reported high disease incidence and severity of early blight among susceptible tomato varieties inoculated with *A. solani* (24, 27, 30). Plants sprayed with 50-200 g/L of neem seed extract had significantly lower disease incidence relative to the treatments that were inoculated but unsprayed with either the botanical or synthetic fungicide. This indicated that the extract competed favourably and even surpassed the efficacy of the synthetic fungicide. The potential of neem extract in the management of several pathogens and diseases had been articulated by previous authors. Hassanein *et al.* (16) conducted a research on the efficacy of neem leaves against blight and wilt inducing pathogens of tomato and found the botanical to have considerably reduced the incidence and severity of both diseases. Similarly, Bokhari *et al.* (7) reported a significant reduction in sclerotia formation and disease incidence in the evaluation of the efficacy of neem extract for the control of *Rhizoctonia solani* in infected soils. In another study, Adepoju *et al.* (2) found inhibitory effect of neem oil extract against four pathogens, *Fusarium* sp. *Rhizopus* sp. *Curvularia* sp. and *Aspergillus* sp. The significant reduction of disease incidence and severity among inoculated tomato plants, gives credence to the postulation that natural plant products are important sources of new agrochemicals for the control of plant diseases and the use of these environmentally safe methods in sustainable agriculture calls for reduction in the use of synthetic chemical fungicides (18, 32).

The application of aqueous neem seed extract significantly increased vegetative growth of the test plants, especially in the number of leaves per plant relative to plants that were sprayed with mancozeb. This implies that the neem seed extract in this study apparently enhanced vegetative growth better than fungicidal treatment with mancozeb. This result is consistent with previous findings of Nahak and Sahu (27) that evaluated the effect of neem leaf extract on
growth performance of tomato and reported a positive correlation between the application of the extract and the performance of the crop under field conditions. Similarly, Rajput et al. (31) reported the efficacy of neem products in the control of damping-off disease of shisham seedlings caused by *Fusarium solani*, and the enhancement of the overall growth of the test plants. Also, the plants that were inoculated with *A. solani* and sprayed with 50 g/L aqueous neem seed extract had significantly higher yields relative to those plants that were inoculated with the pathogen but unsprayed as well as other treatments. This showed that the application of neem seed extract did not only reduce the incidence of early blight disease, but also increased ultimate yield of tomato fruits. Conversely, the poor yield of treatments that were inoculated and unsprayed could be attributed to the unimpeded activity of the blight pathogen which was neither sprayed with the test extract nor the synthetic fungicide. Sale et al. (37) also conducted a research on the effect of neem extract preparations and nutrient sources on field performance of okra plants and reported impressive growth performance and yield from plants that were treated with the botanical. Moyin-Jesu (26) similarly established significant effect of modified neem leaf and wood extracts on disease reduction and soil improvement for the enhancement of growth and yield of water melon.

Although the synthetic mancozeb fungicide used in this study considerably reduced the incidence and severity of early blight disease, it could not completely eradicate the disease. Another deficiency is that the use of this synthetic fungicide in the management of the early blight disease of tomato is fraught with problems of environmental degradation, pathogen resurgence, elimination of natural enemies and other beneficial microbes in the ecosystem that make this option untenable. Neem seed extract compared favourably with mancozeb and significantly influenced vegetative growth and yield, with a concomitant reduction of disease incidence and severity on tomato plants evaluated. It is, therefore, strongly recommended in the management of early blight disease especially by virtue of being readily available, cheap, drought tolerant and eco-friendly with minimal residual effect. More importantly, neem seed extract at lower concentration of 50 g/L enhanced better yields of the test plants than those obtained at higher concentrations of application, which makes its usage largely economical.
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rice by leaf extract of *Datura metel* against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. *oryzae*. *Physiology and Molecular Plant pathology* 65: 91-100.


watermelon (*Citrullus luna*us) (sole and intercrop) 3 (1): 90-97.


Occurrence of Fungi on *Cola Nitida* (L) Nuts Obtained in Southwestern States of Nigeria

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Summary

Fungi are ubiquitous, especially in healthy and diseased agricultural product. This study was conducted to identify pathogenic fungi associated with kolanut from Ondo and Osun States, Nigeria in 2014. The kolanut collected were plated on potato dextrose agar modified with streptomycin to isolate the constituent fungal species. Cultural and morphological characteristics of the fungal isolate were recorded. A total of twelve fungi were isolated from both healthy and infected nut. Five organisms namely: Aspergillus niger, A. flavus, Fusarium oxysporum, Lasiodiplodia theobromea and Rhizopus stolonifer were isolated from the two States, while Botrytis cinerea, Collectotrichum gloeosporioides, Penicillium atramentosum and Colletotrichum lindemuthianum from Ondo State and Fusarium solani, Aspergillus fumigatus and Penicillium expansum from those from Osun State. The pathogenicity test confirmed eight of the fungal isolates as pathogenic.

Key word: Cultural characters, Fungi, kolanut, pathogenicity.

**KOLA** is a plant of the genus *Cola*, family *sterculiaceae*, tribe sterculaeae and order Malvales (6, 24).The genus *Cola* contains 50 species and are widely distributed across tropical Africa. The *Cola nitida* known as gbanja or goro, *C. acuminata* (Obi gidi or abata), *C. verticillata* (Obi Olooyo or slimy kola) and *C. millenii* (Obi edun) are the common fruit bearing species in Nigeria. *C. nitida* and *C. acuminata* are of high economic value, but *C. nitida* is more popular as kola of commerce (22, 26).

The kola plant has a wide range of uses both traditionally and industrially. The nuts contain high amount of caffeine, theobromine, kolatin and theophylline. They are
eaten to induce strength, alertness and concentration as well as prevent depression in human due to their active properties. (7, 12, 18, 29, 30). The nuts are also used by food and pharmaceutical industries for the production of food, drugs, soft drinks, wines, candies and beverages (14, 15, 21). Methanolic and aqueous extracts from the nut has been found to control microbial activities of Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Candida albicans (9, 27, 28). In human medicine, the extracts from the nuts are used to treat malaria, fever, ringworm, scabies, gonorrhea and dysentery (20). Nut of kola contain tannic acid which precipitate toxins in the gut thereby preventing their absorption (17).

Attempts to improve the nut yield and quality of the nuts will help to achieve food and nutrition security in Nigeria. Constraints to the strategies adopted in kolanuts improvement is disease infestation. Disease is responsible for most of the spoilage of the nuts and this affects the nutritional and biochemical quality of the nuts (16). Fungi everywhere are excellent degraders of organic matter including nuts of which they cover with grayish mycelia while the internal tissues are decayed or rotten (25). Botrytis spp, Botryodiplodia theobromae, Paecilomyces variotii, Mucor spp and Fusarium spp had been reported as the main fungi causing mould and rots of the nuts (1, 4, 5, 23).

Preventing mould and rot of the nuts during long storage has been a challenge to farmers and marketers. It is important to know the features of fungi associated with deterioration of kolanut for effective control. In view of this, the study was carried out to identify and describe the cultural and morphological attributes of mycoflora associated with deterioration in kolanuts.

MATERIALS AND METHODS

Experimental material

Healthy and infected nuts of C. nitida were sourced from farmers’ fields and markets at Atakummosa East (7°30’00N 4°04’00E), Atakummosa West (7°05’00N 4°37’00N) and Odo Otin (8°01’N4°42’E) Local Government Area (LGA) in Osun States while Ile-Oluji (7°13’N4°52’0E) and Ifedore (7°23’N 5°4’E) LGA were visited in Ondo States, Southwest Nigeria. Kolanut samples collected from the different LGAs within each States were bulked together and small quantity of the nut was selected randomly to evaluate the mycofloral occurrence.

Morphological characters of isolated mycoflora

This experiment was carried out at the Pathology Laboratory of Cocoa
Research Institute of Nigeria (CRIN), Ibadan in 2014. Healthy and infected samples from each location were cut into small pieces (3 mm) with sterilize scalpel and surface sterilized with 2% sodium hypochlorite for 2 min and then rinsed in five changes of sterile distilled water. Each sterilized sample was blotted dry on sterile filter paper and inoculated into freshly prepared solidified potato dextrose agar (PDA) in four replicates. Colony growth after 4 days of inoculation was subsequently subculture on freshly prepared PDA until pure culture was obtained. The cultures were incubated at 28 ± 2°C in the incubator. Characters of the emerged colony and reproductive structures were observed at 6 days after inoculation and compared with fungi descriptors (8, 13, 31). The occurrence of each fungus was calculated using the formula

\[ \text{Fungi occurrence} = \frac{\text{Frequency of occurrence of isolate}}{\text{Total occurrence of all the isolates}} \times 100 \]

**Pathogenicity test**

Pathogenicity test was carried out to determine virulence of each fungal isolate. Kolanut samples were surface sterilized and 5 mm sterilized cork borer was used to make holes in the nuts. Five millimetre diameter of 7 days-old culture of each isolates was inserted into the holes made on the nuts and covered vaseline. The inoculated samples were transferred to a micro-humidity chamber and incubated at 28 ± 2°C for 51 days in the incubator. The nuts were observed for symptom development at 24 hrs interval. Samples which showed disease symptoms after the period of inoculation were surface sterilized and re-inoculated into freshly prepared PDA to confirm Koch’s postulate. Means and percentages were used to analyse data collected.

**RESULTS**

A total of 12 mycoflora were isolated from both infected and healthy kolanuts nuts (Table 1). Their cultural and morphological characteristics of the mycoflora were presented (Plate I to III). Morphological characters of the isolates were matched with fungi identification guides. The 12 fungi were associated with at least one of the two States, A. niger, A. flavus, F. oxysporum, L. theobromea and R. stolonifer were associated with nuts from the two States (Table 2) while Botrytis cinerea, C. gloeosporioides and C. lindemuthianum are specific to Ondo State while Fusarium solani and Penicillium expansum to Osun State.

The percent occurrence of each fungal isolates was observed on both the healthy and infected nuts (Table 3). Greater than 10% occurrence was indicated for C. lindemuthianum (24.82%), F. oxysporum (18.18%), P. expansum (13.64%) and A. flavus
(13.09%) on the healthy nuts, while *Fusarium oxysporum* (25.18%), *L. theobromae* (16.09%) and *C. lindemuthianum* (15.02%) and *F. solani* (11.49%) had high occurrence on the infected nuts. The test showed that *A. flavus, A. niger, B. cinerea, C. lindemuthianum, F. oxysporum, L. theobromae, P. expansum* and *R. stolonifer* causes deterioration on inoculated nuts but *C. gloeosporioides, A. fumigatus, F. solani* and *P. atramentosum* induced no visible symptoms (Table 3).

**Table 1:** Cultural characteristics of fungi isolated on nuts of *Cola nitida* in 2014

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Texture</th>
<th>Surface colour</th>
<th>Reverse colour</th>
<th>Zonation</th>
<th>Conidia shape</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>Powdery</td>
<td>Greenish yellow</td>
<td>Cream</td>
<td>Slightly furrowed</td>
<td>Globose</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td>Powdery</td>
<td>Whitish with typical black spores</td>
<td>Light yellow</td>
<td>Slightly furrowed</td>
<td>Globose</td>
</tr>
<tr>
<td><em>A. fumigatus</em></td>
<td>Powdery</td>
<td>Brown colony</td>
<td>Cream coloured</td>
<td>Slightly furrowed</td>
<td>Globose</td>
</tr>
<tr>
<td><em>Botrytis cinerea</em></td>
<td>Cottony</td>
<td>Dirty white to grey</td>
<td>Dark grey to black</td>
<td>No zonation</td>
<td>Globose</td>
</tr>
<tr>
<td><em>Colletotrichum gloeosporioides</em></td>
<td>Woolly</td>
<td>Light orange</td>
<td>Orange</td>
<td>Slight zonation</td>
<td>Globose to ellipsoidal</td>
</tr>
<tr>
<td><em>C. lindemuthianum</em></td>
<td>Slightly Powdery</td>
<td>Light pink</td>
<td>Light pink</td>
<td>Slightly radially furrowed</td>
<td>Cylindrical</td>
</tr>
<tr>
<td><em>Fusarium solani</em></td>
<td>Fluffy</td>
<td>White to creamy</td>
<td>Creamy</td>
<td>No zonation</td>
<td>Boat shaped to oval</td>
</tr>
<tr>
<td><em>F. oxysporum</em></td>
<td>Fluffy</td>
<td>Whitish with traces of pink or violet</td>
<td>Light pink or violet</td>
<td>No zonation</td>
<td>Straight and fusiform</td>
</tr>
<tr>
<td><em>Lasiodiplodia theobromae</em></td>
<td>Fluffy</td>
<td>Whitish changes to gray and then black with age</td>
<td>Grey to black</td>
<td>No zonation</td>
<td>ellipsoidal</td>
</tr>
<tr>
<td><em>Penicillium expansum</em></td>
<td>velvety</td>
<td>Bluish green with white borders</td>
<td>Orange</td>
<td>No zonation</td>
<td>Oval shape</td>
</tr>
<tr>
<td><em>P. atramentosum</em></td>
<td>Thick velvety</td>
<td>Olive green colony surrounded by cream border</td>
<td>Cream coloured and heavily</td>
<td>Slightly furrowed</td>
<td>Globose to subglobose</td>
</tr>
</tbody>
</table>
**Plate 1:** Colony growth, colour and pattern for *Aspergillus fumigatus* (a), *A. niger* (b), *Penicillium expansum* (c), *Fusarium solani* (d), *Lasiodiplodia theobromae* (e), *P. atramentosum* (f). g-l show the pattern and colour of the reverse for each fungus, respectively.

**Plate II:** Colony growth, colour and pattern for *Colletotrichum gloeosporoides* (a), *C. lindemuthianum* (b), *Botrytis cinerea* (c), *Fusarium oxysporum* (d), *Aspergillus flavus* (e), *Rhizopus stolonifer* (f). g-l show the pattern and colour of the reverse for each fungus, respectively.
Plate III: Morphological structure of each fungal isolate showing the reproductive structures of *Aspergillus* spp. (a), *Penicillium* spp. (b), *Fusarium solani* (c), *Lasiodiplodia theobromae* (d), *Colletotrichum gloeosporioides* (e), *C. lindemuthianum* (f), *Botrytis cinerea* (g), *Rhizopus stolonifer* (h), *F. oxysporum* (i).

Table 2: Distribution of fungi on infected kolanuts obtained from Osun and Ondo States in Nigeria in 2014.

<table>
<thead>
<tr>
<th>Organism</th>
<th>State</th>
<th>Osun</th>
<th>Ondo</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus flavus</em></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>A. niger</em></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>A. fumigates</em></td>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Botrytis cinerea</em></td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Colletotrichum gloeosporioides</em></td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>C. lindemuthianum</em></td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Fusarium oxysporum</em></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>F. solani</em></td>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Lasiodiplodia theobromae</em></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fungi</td>
<td>Occurrence (%)</td>
<td>Pathogenicity</td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------------</td>
<td>---------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Healthy nuts</td>
<td>Infected nuts</td>
<td></td>
</tr>
<tr>
<td><strong>Aspergillus flavus</strong></td>
<td>13.09</td>
<td>10.1</td>
<td>+</td>
</tr>
<tr>
<td>A. niger</td>
<td>6.38</td>
<td>1.15</td>
<td>+</td>
</tr>
<tr>
<td>A. fumigates</td>
<td>a</td>
<td>1.25</td>
<td>-</td>
</tr>
<tr>
<td>Botrytis cinerea</td>
<td>a</td>
<td>8.09</td>
<td>+</td>
</tr>
<tr>
<td>Colletotrichum</td>
<td>4.55</td>
<td>a</td>
<td>-</td>
</tr>
<tr>
<td><strong>Colletotrichum glœosporoides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. lindemuthianum</td>
<td>24.82</td>
<td>15.20</td>
<td>+</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>18.18</td>
<td>25.41</td>
<td>+</td>
</tr>
<tr>
<td>F. solani</td>
<td>9.09</td>
<td>11.49</td>
<td>-</td>
</tr>
<tr>
<td>Lasiodiplodia theobromea</td>
<td>9.00</td>
<td>16.39</td>
<td>+</td>
</tr>
<tr>
<td>Penicillium expansum</td>
<td>13.64</td>
<td>4.00</td>
<td>+</td>
</tr>
<tr>
<td>P. atramentosum</td>
<td>a</td>
<td>7.02</td>
<td>-</td>
</tr>
<tr>
<td><strong>Rhizopus stolonifer</strong></td>
<td>a</td>
<td>1.15</td>
<td>+</td>
</tr>
</tbody>
</table>

Susceptible (+); Not susceptible (-); a- not present

**DISCUSSION**

The different fungal species isolated were identified with respect to their colony character and morphological features namely *Aspergillus niger*, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Colletotrichum lindemuthianum*, *Fusarium oxysporum*, *Fusarium solani*, *Lasiodiplodia theobromea*, *Penicillium expansum* and *Rhizopus stolonifer*. This finding corroborates the report of several researchers. Adeniyi et al. (2) isolated *L. theobromea*, *Aspergillus* spp., *Penicillium* spp. and *Rhizopus stolonifer* among others from retailed kolanut obtained from north central. Agbeniyi (3) and Chuku et al. (11) on mycoflora on kolanut, isolated *F. oxysporum*, *A. niger*, *R. stolonifer*, *A. fumigatus* and *A. flavus* with several...
other fungi from kolanut obtained from the eastern part of Nigeria.

The number of fungi isolated from healthy nuts shows the vulnerability of the nut to fungal pathogens. The pathogenicity test reveals the ability of most of the fungal isolates to induce rot in agricultural produce. The findings of Sharma and Pandey (28) revealed the occurrence of A. niger, Penicillium sp, and Fusarium sp on decayed vegetables, also Norhayati et al. (19), isolated L. theobromae from kenaf seed as the black rot pathogen of kenaf seeds, similary Chuku and Emelike (10) isolated F. oxysporum, A. niger, R. stolonifer among others from cucumber and water melon.

The pathogenicity test also reveals that most of the fungi which causes deterioration in storage are carried from the field to storage where they become active. Botrytis cinerea, Collectroticum gloeosporioides, Penicillium atramentosum and Colletotrichum lindemuthianum were prevalent in kolanuts from Ondo State while Fusarium solani, Aspergillus fumigatu and Penicillium expansum were isolated from those from Osun State. Fungi exist on seemingly healthy agricultural produce. These fungi can induce rot and thereby reducing the quality of the produce. Most fungi which causes deterioration in storage usually infect the seed on the field or during the processing stage. Hence proper handling and preservation methods will eliminate these associated fungi and reduce their contamination of food in storage.

It is therefore important that fundamental knowledge of diseases be well understood for proper control measures in various environments.

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Comparative Study of Three Insecticidal Plant Powders Admixed With Cattle Urine for the Management of Wood Termites (*Macrotermes bellicosus*) in Calabar, Nigeria

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Summary

Termites come into conflict with man when they damage farm and household infrastructures, and plants of economic importance to him. Eco-friendly control strategy as alternative to the prevalently used hazardous synthetic insecticides is needed. This study evaluated the potential for controlling the wood termite, *Macrotermes bellicosus*, with cow urine, seed powders of *Jatropha curcas* L. (Euphorbiaceae), *Dennettia tripetala* Bak. f. (Annonaceae) and *Cedrela odorata* L. (Meliaceae) each applied at the rate of 0.5 g, 1.0 g and 2.0 g / 20 g of *Alstonia scholaris* (L.) dust contained in 3 kg capacity jars. Other treatments were the seed powders mixed with 10 ml of cow urine and an untreated control. Each treatment was replicated four times in completely randomized design in the laboratory of the Department of Forestry and Wildlife Resources Management, University of Calabar. Each jar was infested with 20 termites and mortality was quantified in percentage from 12 h to 60 h post-infestation. The 2.0 g rate of *C. odorata*, *D. tripetala*, and *J. curcas* mixed with cow urine caused the highest mortality respectively 83.3 %, 98.3 %, and 99.7 % at 60 h post-infestation compared with 60.0 % for cow urine alone. Mortality generally increased with rate of application and duration of exposure. It is concluded that seed powders of *C. odorata*, *D. tripetala*, and *J. curcas* have potential to mitigate damage by the wood termite.

Key words: Pesticidal plants, wood termite, cow urine, eco-friendly, botanicals, seed powders
Termites exert negative impact on human welfare by causing damage to plants of economic importance to man in the natural ecosystem as well as in agro-ecosystem, and by causing damage to farm and household infrastructural facilities (11, 37, 28, 32). The cost of controlling termites, added on to the cost of replacing or repairing damaged infrastructure, can be exorbitant. The use of synthetic organic insecticides for termite control is prevalent. Sole reliance upon this method has resulted in pesticide resistance, polluted environment, upset equilibrium of non-target populations (32, 35). Aneco-friendlier method of mitigating termite damage is desirable giving their role in recycling nutrient and improving soil fertility (20, 18, 21, 7). In Sub-Saharan Africa the use of plant-derived insecticide is an ancient practice (2). Many plants, for example neem (Azadirachta indica) leaves and seeds, garlic (Allium sativum) bulbs, physic nut (Jatropha spp.) seeds, Cedrela odorata leaves have demonstrated bioactivity against termites in agricultural lands (9, 2, 29, 19). Bioactivity is evidenced by toxicity 16, 35), feeding inhibition (38, 35), growth retardation (6), oviposition deterrence (41), reduction of fecundity and fertility and inhibition of tunnelling (24). Chawla et al. (8) documented the efficacy of cattle urine alone or in combination with plant extracts against termites.

In this study, we evaluated toxicity of seed powders of J. curcas, D. tripetala and C. odorata, alone or in combination with cattle urine, to the wood termite, Macrotermes bellicosus. Jatropha curcas L. has been touted as a wonder plant. It is drought resistant, making it suitable in areas with low amount of rainfall (15, 40). The seeds contain an average of 33 % oil that can be processed to produce good-quality biodiesel and soap (15, 1, 23, 34). Unfortunately, the remaining high-protein seed particles cannot be used as food or feed because it contains toxic phorbol esters (39). Studies have shown that the nutritional value of detoxified J. curcas seed cake is comparable to soya bean, and better than sunflower and cotton seed meal, making it an ideal stock feed (23).

Pepper fruit, Dennettia tripetala (G.) (Baker f.) G.E. Schatz (Annonaceae), is a woody forest and spicy plant, cultivated in Southern states of Nigeria, where the leaves and fruits are used in combination with other herbs for the treatment of cough, infantile convulsion, and worm infestation (13). D. tripetala extracts have also been reported to exhibit insecticidal (12), and antifungal properties (27). Cedrela odorata L. (Red cedar) is a species that is
primarily located in humid ecosystems, specifically in the evergreen tropical forest, sub-deciduous tropical forest, tropical deciduous forest and in the mesophytic mountain forest (10). Cedrela odorata is characteristic of rainforests of economic and ecological importance, but the lack of quantitative studies to understand the characteristics of the seeds of this species have been some of the causes that limit its cultivation and propagation (3). Leopoldo et al. (19) reported that 3 to 4 g of C. odorata leaf powder has the capacity of killing termites within 20 to 25 seconds.

It is pertinent to produce control method, which is environmentally friendly and sustainable. The objective of the study therefore, was to assess the efficacy the three plant powders (Jatropha curcas, Dennettia tripetala and Cedrela odorata) admixed with cattle urine on the management of wood termites.

MATERIALS AND METHODS

Study area

The experiment was conducted in the laboratory of the Department of Forestry and Wildlife Resources Management, University of Calabar, Nigeria (Latitude 4°55′50″ to 4°57′37″ and Longitude 8°20′25″ to 8°21′30″; see Fig. 1.).
Figure 1: Map of Nigeria showing the location of University of Calabar and the coordinates

Materials used

Seeds of *D. tripetala*, *J. curcas* and *C. odorata* were collected from the Departmental Arboretum while cattle urine was obtained from the Department of Animal Science Research Farm. The seeds were cleaned, shelled and the kernels and hulls were separated manually. The kernels were dried, ground to fine powder, and stored in glass vials until used.

Wood termite collection and culture

*Macrotermes bellicosus* adults were collected from the Departmental Arboretum and cultured for one month in a medium containing *Alstonia scholaris* (L.) dust. The progenies that
developed and emerged from the cultures were collected and used for the experiments.

**Laboratory bioassays**

Seed powders of *J. curcas*, *D. tripetala* and *C. odorata* each at rate of 0.5, 1 and 2 g were thoroughly mixed with 10 ml of cattle urine and incorporated into 10 g of *A. scholaris* dust contained in a 3 kg capacity jar. The same rate of seed powders without urine was also admixed with the dust. These, and a urine only as well as an untreated control treatment were replicated four times in completely randomized design. Each jar was infested with 20 adult termites and kept at 25±1°C and 65–70 % relative humidity.

**Data collection and analysis**

The number of dead termites was counted and expressed as a percentage of the number infested. A termite was considered dead if it showed no movement sequel to pricking an upward-facing abdominal segment. Data were subjected to analysis of variance using StatView statistical software (Version 1992 – 98 SAS). Significantly different means were separated using Duncan Multiple Range Test at 5 % level of probability.

**RESULTS AND DISCUSSION**

In all treatments, mortality tended to increase with increase in the rate of applied seed powder as well as with duration of exposure (Table 1). In the untreated control, mortality ranged from 9.7 % - 18.3 %. Treatment with cattle urine alone, plant powder alone or with plant powder mixed with cattle urine caused significantly higher mortality. The highest level of mortality caused by cattle urine was 52.0%, mortalities were 21.7 %, 31.7 %, and 38.0 % higher in 1.0 g *C. odorata*, 2.0 g *D. tripetala*, and 2.0 g *J. curcas*, respectively, at 60 h post-infestation. *J. curcas*, with or without urine, was the quickest acting causing >70.0 % termite mortality within 12 h of exposure. Mixing the seed powders with urine increased mortality significantly only with 0.5 g and 1.0 g rate of *C. odorata* at 36 h post-treatment and 1.0 g and 2.0 g seed powder of *D. tripetala* at 24 h and 36 h post-treatment, respectively (Table 1).

Each of the applied rates of plant powder caused significantly higher mortality than that in the untreated control. Mixing the powders in 10 ml of cattle urine had no significant effect (Table 1). Mortality increased as the rate of application increased; the 2.0 g consistently caused significantly higher mortality than the 0.5 g from 12 h to 60 h post-infestation. In contrast, the differences in mortality between 1.0 g and 2.0 g were statistically significant only from 12 h to 24 h post-infestation. *J. curcas* tended to be the most toxic to the wood termite causing an average of 56.2 % mortality over the five observation periods (12 h – 60 h post-infestation) compared with 50.8 % and 45.4 % for *D. tripetala* and *C. odorata*, respectively. The latter two were statistically comparable except at the last observation (60 h post-
infestation) when *D. tripetala* caused higher mortality (Tables 2a-e).

**Table 1:** Effects of pesticidal plant powders on mortality of wood termites at different times of exposure

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Rates</th>
<th>12 h</th>
<th>24 h</th>
<th>36 h</th>
<th>48 h</th>
<th>60 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. odorata</em></td>
<td>0 g</td>
<td>9.67±0.33e</td>
<td>10.33±0.88e</td>
<td>10.67±0.67e</td>
<td>13.33±3.33e</td>
<td>16.67±3.33d</td>
</tr>
<tr>
<td></td>
<td>0.5 g</td>
<td>26.67±1.67d</td>
<td>28.67±1.67d</td>
<td>31.67±4.41d</td>
<td>51.67±7.27d</td>
<td>55.67±1.67c</td>
</tr>
<tr>
<td></td>
<td>1.0 g</td>
<td>35.00±2.89e</td>
<td>40.00±0.00cd</td>
<td>45.00±2.89e</td>
<td>76.67±4.41bc81.67±1.67ab</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0 g</td>
<td>43.33±3.33bc</td>
<td>42.00±2.89ed</td>
<td>55.00±5.00bc</td>
<td>76.67±8.33b</td>
<td>80.00±5.77ab</td>
</tr>
<tr>
<td></td>
<td>0.5 g*</td>
<td>26.67±1.67d</td>
<td>33.33±3.33d</td>
<td>41.67±4.41e</td>
<td>53.33±6.01d</td>
<td>61.67±1.67c</td>
</tr>
<tr>
<td></td>
<td>1.0 g*</td>
<td>38.33±1.67c</td>
<td>45.00±2.89e</td>
<td>65.00±2.89b</td>
<td>81.67±6.01b</td>
<td>81.67±4.41b</td>
</tr>
<tr>
<td></td>
<td>2.0 g*</td>
<td>50.00±2.89b</td>
<td>48.33±1.67c</td>
<td>70.00±5.77b</td>
<td>80.00±5.00b</td>
<td>83.33±3.33ab</td>
</tr>
<tr>
<td></td>
<td>10 ml of urine</td>
<td>25.00±2.89d</td>
<td>33.33±3.33d</td>
<td>55.00±5.00bc</td>
<td>53.33±8.82d</td>
<td>60.00±6.67c</td>
</tr>
<tr>
<td><em>D. tripetala</em></td>
<td>0 g</td>
<td>9.67±0.33e</td>
<td>10.00±0.57e</td>
<td>11.33±0.67e</td>
<td>14.33±2.96e</td>
<td>18.33±1.67d</td>
</tr>
<tr>
<td></td>
<td>0.5 g</td>
<td>30.67±2.33c</td>
<td>33.33±1.67d</td>
<td>40.00±2.89e</td>
<td>65.00±2.89c</td>
<td>70.00±2.89b</td>
</tr>
<tr>
<td></td>
<td>1.0 g</td>
<td>33.33±1.67c</td>
<td>36.67±1.67d</td>
<td>43.33±1.67c</td>
<td>75.00±2.89bc</td>
<td>83.33±1.67ab</td>
</tr>
<tr>
<td></td>
<td>2.0 g</td>
<td>50.00±2.89b</td>
<td>63.33±1.67b</td>
<td>65.00±5.00b</td>
<td>88.33±1.67a</td>
<td>91.67±1.67a</td>
</tr>
<tr>
<td></td>
<td>0.5 g*</td>
<td>35.00±2.89c</td>
<td>41.67±1.67cd</td>
<td>55.00±5.00bc</td>
<td>68.33±4.41c</td>
<td>73.33±3.33b</td>
</tr>
<tr>
<td></td>
<td>1.0 g*</td>
<td>36.67±3.33c</td>
<td>46.67±3.33c</td>
<td>56.67±3.37bc</td>
<td>83.33±3.33b</td>
<td>93.33±3.33a</td>
</tr>
<tr>
<td></td>
<td>2.0 g*</td>
<td>53.00±3.33b</td>
<td>65.00±2.89b</td>
<td>80.00±5.77a</td>
<td>91.67±4.41a</td>
<td>98.33±1.67a</td>
</tr>
<tr>
<td></td>
<td>10 ml of urine</td>
<td>25.00±2.89d</td>
<td>33.33±3.33d</td>
<td>55.00±5.00bc</td>
<td>53.33±8.82d</td>
<td>60.00±5.78c</td>
</tr>
<tr>
<td><em>J. curcas</em></td>
<td>0 g</td>
<td>9.67±0.33e</td>
<td>10.00±0.57e</td>
<td>11.33±0.67e</td>
<td>14.33±2.96e</td>
<td>18.33±1.67d</td>
</tr>
<tr>
<td></td>
<td>0.5 g</td>
<td>36.67±2.89c</td>
<td>49.33±5.21c</td>
<td>56.67±4.41bc</td>
<td>65.00±5.77c</td>
<td>70.00±2.89b</td>
</tr>
<tr>
<td></td>
<td>1.0 g</td>
<td>52.67±2.67b</td>
<td>55.00±2.89bc</td>
<td>83.33±3.33a</td>
<td>88.33±4.41a</td>
<td>96.67±1.67a</td>
</tr>
<tr>
<td></td>
<td>2.0 g</td>
<td>73.33±3.33a</td>
<td>86.67±4.41a</td>
<td>81.67±10.93a</td>
<td>93.33±3.33a</td>
<td>98.00±1.53a</td>
</tr>
<tr>
<td></td>
<td>0.5 g*</td>
<td>38.33±1.67c</td>
<td>50.00±5.77c</td>
<td>58.33±4.41bc</td>
<td>66.67±4.41c</td>
<td>71.67±4.41b</td>
</tr>
<tr>
<td></td>
<td>1.0 g*</td>
<td>53.33±3.33b</td>
<td>56.67±3.33bc</td>
<td>85.00±2.89a</td>
<td>90.00±5.77a</td>
<td>98.33±1.67a</td>
</tr>
<tr>
<td></td>
<td>2.0 g*</td>
<td>73.33±3.33a</td>
<td>86.67±3.33a</td>
<td>95.00±2.89a</td>
<td>95.00±2.89a</td>
<td>99.67±0.33a</td>
</tr>
<tr>
<td></td>
<td>10 ml of urine</td>
<td>25.00±2.89d</td>
<td>33.33±3.33d</td>
<td>55.00±5.00bc</td>
<td>53.33±8.82d</td>
<td>60.00±5.67c</td>
</tr>
</tbody>
</table>

Means in the same column with different letter(s) differ significantly (p<0.05).
**Table 2a:** ANOVA table showing effects of plant powders on mortality, 12 hours post infestation.

<table>
<thead>
<tr>
<th>SV</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F-Value</th>
<th>P-Value</th>
<th>Lambda</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treat</td>
<td>2</td>
<td>2374.083</td>
<td>1187.042</td>
<td>59.311</td>
<td>&lt;0001</td>
<td>118.622</td>
<td>1.00</td>
</tr>
<tr>
<td>Rate</td>
<td>7</td>
<td>16174.611</td>
<td>2310.659</td>
<td>115.453</td>
<td>&lt;0001</td>
<td>808.169</td>
<td>1.00</td>
</tr>
<tr>
<td>Treats x Rate</td>
<td>14</td>
<td>1522.139</td>
<td>108.724</td>
<td>5.432</td>
<td>&lt;0001</td>
<td>76.054</td>
<td>1.00</td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td>960.667</td>
<td>20.014</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SV = source of variability; DF = degree of freedom; SS = sum of squares; MS = mean squares

**Table 2b:** ANOVA table showing effects of plant powders on mortality, 24 hours post application.

<table>
<thead>
<tr>
<th>SV</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F-Value</th>
<th>P-Value</th>
<th>Lambda</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treat</td>
<td>2</td>
<td>2942.583</td>
<td>1471.292</td>
<td>54.020</td>
<td>&lt;0001</td>
<td>108.040</td>
<td>1.00</td>
</tr>
<tr>
<td>Rate</td>
<td>7</td>
<td>22182.611</td>
<td>3168.944</td>
<td>116.351</td>
<td>&lt;0001</td>
<td>814.456</td>
<td>1.00</td>
</tr>
<tr>
<td>Treats x Rate</td>
<td>14</td>
<td>2304.972</td>
<td>164.641</td>
<td>6.045</td>
<td>&lt;0001</td>
<td>84.629</td>
<td>1.00</td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td>1307.333</td>
<td>27.236</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SV = source of variability; DF = degree of freedom; SS = sum of squares; MS = mean squares

**Table 2c:** ANOVA table showing effects of plant powders on mortality, 36 hours post application.

<table>
<thead>
<tr>
<th>SV</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F-Value</th>
<th>P-Value</th>
<th>Lambda</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treat</td>
<td>2</td>
<td>1978.861</td>
<td>989.431</td>
<td>17.17</td>
<td>&lt;0001</td>
<td>34.233</td>
<td>1.00</td>
</tr>
<tr>
<td>Rate</td>
<td>7</td>
<td>32372.611</td>
<td>4624.659</td>
<td>80.004</td>
<td>&lt;0001</td>
<td>560.026</td>
<td>1.00</td>
</tr>
<tr>
<td>Treats x Rate</td>
<td>14</td>
<td>1833.139</td>
<td>130.938</td>
<td>2.265</td>
<td>0.018</td>
<td>31.712</td>
<td>1.00</td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td>2774.667</td>
<td>57.806</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SV = source of variability; DF = degree of freedom; SS = sum of squares; MS = mean squares
Table 2d: ANOVA table showing effects of plant powders on mortality, 48 hours post application.

<table>
<thead>
<tr>
<th>SV</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F-Value</th>
<th>P-Value</th>
<th>Lambda</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treat</td>
<td>2</td>
<td>881.694</td>
<td>440.847</td>
<td>5.238</td>
<td>.0081</td>
<td>10.657</td>
<td>.824</td>
</tr>
<tr>
<td>Rate</td>
<td>7</td>
<td>41024.000</td>
<td>5860.571</td>
<td>70.835</td>
<td>&lt;0001</td>
<td>495.842</td>
<td>1.000</td>
</tr>
<tr>
<td>Treats x Rate</td>
<td>14</td>
<td>827.417</td>
<td>59.101</td>
<td>.714</td>
<td>.749</td>
<td>10.001</td>
<td>.372</td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td>3971.333</td>
<td>82.735</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SV = source of variability; DF = degree of freedom; SS = sum of squares; MS = mean squares

Table 2e: ANOVA table showing effects of plant powders on mortality, 60 hours post application.

<table>
<thead>
<tr>
<th>SV</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F-Value</th>
<th>P-Value</th>
<th>Lambda</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treat</td>
<td>2</td>
<td>885.083</td>
<td>442.542</td>
<td>11.723</td>
<td>&lt;0001</td>
<td>23.446</td>
<td>.996</td>
</tr>
<tr>
<td>Rate</td>
<td>7</td>
<td>43383.208</td>
<td>6197.601</td>
<td>164.175</td>
<td>&lt;0001</td>
<td>1149.224</td>
<td>1.000</td>
</tr>
<tr>
<td>Treats x Rate</td>
<td>14</td>
<td>497.583</td>
<td>35.542</td>
<td>.942</td>
<td>.5236</td>
<td>13.181</td>
<td>.497</td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td>1812.000</td>
<td>37.750</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SV = source of variability; DF = degree of freedom; SS = sum of squares; MS = mean squares

Termiticidal properties of *J. curcas*, *D. tripetala* and *C. odorata* powders admixed with cattle urine showed significant effects on mortality of wood termite. The treatment of wood with natural extracts against wood termite attack provides an alternative to synthesized chemicals. Blaske *et al.* (5) and Jembere *et al.* (17) concluded that above-ground and below-ground application of plant extracts has potential for controlling termites. Several authors have alluded to the possibility of using cattle urine alone or in combination with plant-derived insecticides to control insect pests (4, 8, 29). Gupta (14) reported effective control of mustard aphids with combination of neem product and cow urine. In this study, cattle urine was only moderately toxic to the wood termite and when mixed with test seed powders it did not consistently enhance toxicity significantly. The numerically higher mortality obtained might be because the urine served as
an extractive of the active ingredients of the test plants (30). Of the seed powders tested, *J. curcas* was the most toxic. In an earlier study by Singh and Sushilkumar (33), 20% *Jatropha* oil maximally protected wood against attack by *M. beesoni*. The observed toxic effect might be due to plant’s high content of phorbol esters, anti-nutritional diterpenes (35, 25). Bioactivity of *D. tripetala* against post-harvest coleopteran pests (12) and termites (17) has been reported. Termicidal property of the plant, because of silybin, silidianin, silychristin constituents (17), was confirmed in this study. Similarly, our results agree with the findings of Leopoldo *et al.* (19) on potency of *C. odorata* leaf extract to termites. *Jatropha curcas* also produced the highest mortality compared with the other two powders with *Cedrela odorata* as the least efficient. These results confirm previous reports mentioning elsewhere that the efficiency of termite management depends upon species and kind of plant parts used (31). Preventing damage may be exploited in several situations in agricultural ecosystem where seed or plant parts may be prevented from access of termites (35).

**CONCLUSION**

Based on the results obtained from the study, it may be concluded that *Jatropha curcas* and *Dennettia tripetala* powders have termicidal properties and could be recommended for the management/control of wood termites. We do hope that findings of this research serve farmers in Sub-Saharan Africa and many other developing countries.

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Termites (*Cryptotermes cavifrons* BANKS (Insecta: Isoptera: Kalotermitidae) infestation. *Greener Journal of Agricultural Sciences.* 5(6): 210-216,


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Fungitoxicity of Aqueous And Methanolic Extracts of Marigold Leaf and Root Against *Fusarium oxysporum* f.sp *lycopersici in Vitro*

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**Summary**
Fusarium wilt caused by *Fusarium oxysporum* f. sp *lycopersici* is an economically important disease known to cause major losses in tomato crop. Efficacies of aqueous and methanolic extracts of marigold leaf and root were evaluated against *F. oxysporum* f. sp *lycopersici* in vitro. The experiment was laid up in completely randomized design with four replications. Infected tomato leaves were collected at the National Horticultural Research Institute field for pathogen isolation. The result showed that both aqueous and methanolic extracts of marigold leaf or root caused significant (P ≤ 0.05) reduction in mycelial growth of *F. oxysporum* f.sp *lycopersici*. Radial growth inhibition induced by methanolic leaf extract at 10 mg/ml was the highest (61.49 %), followed by aqueous leaf extracts at 10 mg/ml and methanolic extract at 8 mg/ml with mycelial growth inhibitions of 56.89 and 56.51 %, respectively. Of all the concentrations of marigold root, the highest (67.8 %) inhibitory effect was induced by the application of 10 mg/ml aqueous extract. There were no significant P ≤ 0.05 differences in mycelial inhibition induced by the aqueous extract of marigold root at 8 and 6 mg/ ml and methanolic root extract at 6 and 4 mg/ ml. However, least percentage inhibition was recorded against marigold root aqueous extract at 4 and 2 mg/ ml with percentage inhibition of 12.64 and 16.28 %, respectively. It was observed that marigold root aqueous extract and marigold leaf methanolic extract at 10 mg/ml had the highest (67.81 and 61.49 %) inhibitory effect on *F. oxysporum* f. sp *lycopersici in vitro*.  

**Keywords:** Fusarium wilt, control, growth inhibition, extracts, marigold
Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) is one of the most damaging diseases limiting tomato production throughout the world (11). It becomes one of the most prevalent diseases wherever tomatoes are grown intensively because the pathogen can persist indefinitely in infected soils (1). The fungus is characterized by wilted plants, yellowed leaves and decrease crop yield. Yield losses of about 30 to 40% have attributed to this disease and may go up to 80% under favourable weather conditions (12).

The most cost-effective and environmentally safe method of control is the use of resistant cultivars where they are available (18). Moreover, breakdown of resistance in the face of high pathogenic variability in the pathogen population restricted the usefulness of many resistant cultivars to only few years (13). Pre-soil fumigants such as methyl bromide have been proven to be effective, but they are costly and environmentally damaging (8). Methyl bromide has been assigned an ozone depletion of 0.4 by the World Meteorological Organization in 1998 which categorizes it as a “Class I” ozone-depleting substance (3). Consequently, according to the 1997 Montreal Protocol, methyl bromide production was to be phased out in developed nations by the year 2005 and in developing nations by year 2015 (3).

Over the years, many investigations have been conducted on the antimicrobial effects of various plant species and derivatives on plant pathogens (15). Plants contain physiologically active substances which may serve as novel alternative sources of pesticides (17). Over 2000 species have been reported to contain secondary metabolites that possess disease control properties (10). Effectiveness of natural product when compared with some commercial fungicides has been investigated (5). Due to an increasing demand for environmentally safe agrochemicals, there is need to develop alternative methods for controlling *Fusarium oxysporum* f. sp. *lycopersici* to promote sustainable and increase food production while maintaining high levels of disease control. The objective of this study was to evaluate the effect of aqueous and methanolic extracts of leaves and root of marigold against *F. oxysporum* f. sp. *lycorpesici*.

**MATERIALS AND METHODS**

**Isolation of pathogen from diseased plant**

At three weeks after transplanting, seedlings of Ibadan local tomato variety exhibiting symptoms of wilt disease were collected from experimental plot of vegetable
research programme of the National Horticultural Research Institute (NIHORT), Ibadan. Samples were taken to laboratory for isolation of pathogen. Isolation was done within 24 h by placing the whole seedlings under running water to remove soil particles and surface sterilized for 3 min in 0.5 % NaOCl and rinsed severally. The root and the stem bases were excised aseptically and cut into pieces using sterile scalpel and placed on Potato dextrose agar supplemented with streptomycin. The plates were incubated for 5 days at room temperature 28±2 °C and examined daily for fungal growth. Sub culturing was done to obtain a pure culture of the isolate.

**Plant collection**

Leaves and roots of marigold (*Tagetes erecta*) were collected from an established plot at NIHORT experimental field, Ibadan between 6 and 7 a. m throughout the study period. The leaves and roots were air dried at 28±2 °C in the laboratory for 21 days, blended into powder with an electric blender Marlex Excella (India) and kept at room temperature (28±2 °C) in clean bottles until needed.

**Preparation of aqueous extracts**

Aqueous extract of leaves and roots of each plant was prepared by adding 100 ml of sterile distilled water to 10 g of ground tissues in 250 ml beaker. The mixture was stirred vigorously, allowed to settle for 4 hr and then filtered through sterile filter paper to obtain the stock. The residue was oven dried at 80 °C for 2 hrs. The weight of plant material that dissolved in cold water was calculated using the formula:

\[
W_S = W_1 - (W_2 + W_3)
\]

Where: \(W_S\) = weight of dissolved samples  
\(W_1\) = initial weight of sample before oven dried drying  
\(W_2\) = weight of the oven dried sample  
\(W_3\) = weight of filter paper (7)

**Methanolic extraction**

Methanolic extract was obtained with 90 % methanol in a soxhlet extractor. The extract was concentrated by steam distillation, stored in clean bottles and kept in refrigerator at -4°C until needed. A stock (10mg/ml) was prepared with 20 % propylene glycol and lower concentrations (8, 6, 4 and 2 mg/ml) were prepared from the stock using serial dilution method.

**Preliminary phytochemical screening of extracts**

Phytochemical analysis for some major phyto-constituents of the plant extracts were carried out according to Ozcan and Boyraz
Plant extracts were screened for the presence of biologically active compounds such as Tannins, Cardiac glycosides, Anthraquinones, Saponins and Alkaloids.

**Antifungal screening of plant extracts**

Fungitoxic effect of extracts on fungal mycelial growth was evaluated using the method of Al-Abed *et al.* (2). Four equal sections were created on the plate by drawing two perpendicular lines at the reverse side of each plate. One millilitre of aqueous and methanolic extracts of the botanical at varying concentrations was dispensed into 9 cm diameter Petri dishes with sterile pipette while control were plates without plant extracts but with sterile distilled water. Fifteen millilitres of PDA containing (5µg/ml streptomycin) was dispensed into petri dishes, gently swirled and allowed to solidify. Three millimetres of mycelial disc of the fungal species obtained from the edge of a 7-day old culture was placed upside down at the point of intersection which indicated the centre of the plate. Plates were incubated at room temperature (28±2 °C) for 7 days. Radial growth was measured daily as the mean growth along the two pre-drawn perpendicular lines on the reverse side of the plate.

Fungitoxicity was expressed as percentage inhibition of mycelia growth using the formula:

\[ M_P = \frac{M_I - M_2}{M_I} \times 100 \]

Where:

- \( M_P \) = Percentage inhibition of mycelia growth
- \( M_I \) = Mycelial growth in control plate
- \( M_2 \) = Mycelial growth in plate containing plant extracts (4)

Data collected were subjected to Analysis of variance and the means were separated using Duncan’s Multiple Range Test.

**RESULTS**

The phytochemical screening of either leaf or root extracts of marigold revealed the presence of tannins, cardiac glycoside and saponins. However, anthraquinine and alkaloids were absent both in leaf and root extracts (Table 1). Aqueous and methanolic extracts of marigold leaf significantly reduced the mycelial growth of *F. oxysporum f.sp lycopersici* *in vitro* (Plate 1). Of all the concentrations of marigold leaf extract, 10 mg/ml aqueous extract resulted in least radial growth (37.5 mm) with percentage mycelial inhibition of 56.9 % while the lowest mycelial inhibition (20.7 %) was
exhibited at 2mg/ml (Table 2). Other concentrations of marigold leaf aqueous extract caused a range of between 31.0 – 51.2 % mycelial growth inhibitions. Mycelial growth inhibited by methanolic extracts of marigold leaf at 10 and 8 mg/ml were not significantly different from each other (Table 2). However, lower concentrations (4 – 8 mg/ml) of methanolic extract induced range of between 37.2 – 56.5 % mycelial growth inhibitions on *F. oxysporum* f.sp *lycopersici*. Aqueous extract of marigold root at 10 mg/ml induced the least mycelial growth of 28.00 mm and highest mycelial inhibition of 67.8 % (Table 2 and Plate 2). Mycelial growth inhibition induced at 8 and 6 mg/ml concentrations of marigold root aqueous extract were not significantly different (P ≤ 0.05). Similar trend was observed at 4 and 2 mg/ml concentrations. (Table 1). Among other concentrations of marigold root methanolic extract, 2 mg/ml had the least growth inhibition of 27.2 %.

**DISCUSSION**

Plants are rich in a wide variety of naturally occurring, secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, quinone, essential oils to mention but a few. These secondary metabolites possess wide range of biological activities. This offers pool of structurally diverse antifungal agents that can provide an alternative to synthetic fungicides (19). The plant extracts studied were found to contain one or more of the following phytochemical components namely; tannins, cardiac glycosides and saponins. Both aqueous and

<table>
<thead>
<tr>
<th>Table 1: Occurrence of some phytochemicals in extract of test plants</th>
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<tbody>
<tr>
<td>Extract</td>
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<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Marigold leaf</td>
</tr>
<tr>
<td>Marigold root</td>
</tr>
</tbody>
</table>

+ = present, - = absent
**Table 2:** Effect of aqueous and methanolic extracts of marigold leaf or root on radial growth of *Fusarium oxysporum* f.sp lycopersici in vitro.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentration (mg/ml)</th>
<th>Radial length (mm)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Marigold leaf</td>
<td>Marigold root</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aqueous</td>
<td>10</td>
<td>37.50^g (56.89)</td>
<td>28.00^a (67.81)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>42.50^f (51.15)</td>
<td>53.33^d (38.70)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>49.50^e (43.10)</td>
<td>55.33^d (36.40)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>60.00^b (31.03)</td>
<td>76.00^b (12.64)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>69.00^c (20.69)</td>
<td>72.83^b (16.28)</td>
<td></td>
</tr>
<tr>
<td>Methanolic</td>
<td>10</td>
<td>33.50^g (61.49)</td>
<td>40.00^d (54.02)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>37.83^g (56.51)</td>
<td>44.83^e (48.47)</td>
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</tr>
<tr>
<td></td>
<td>6</td>
<td>45.33^d (47.89)</td>
<td>52.50^d (39.65)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>54.66^d (37.17)</td>
<td>53.33^d (38.70)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>56.00^c (35.63)</td>
<td>63.33^c (27.20)</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>87.00^a (0.00)</td>
<td>87.00^a (0.00)</td>
<td></td>
</tr>
</tbody>
</table>

Values in parenthesis are percentage reductions in radial length. Values are obtained from mean of 4 replicate plates of fungal pathogen per treatment. Means with the same alphabet in the same column are not significantly different (P ≤ 0.05) Duncan’s Multiple Range Test.
Plate 1: Growth inhibition of *Fusarium oxysporum* f.sp *lycopersici* by different concentrations of Marigold leaf; (a) aqueous and (b) Methanolic extracts after 7 days incubation at 28 ± 2 °C. A: 10 mg/ml; B: 8 mg/ml; C: 6 mg/ml; D: 4 mg/ml; E: 2 mg/ml; F: control (No extract)

Plate 2: Growth inhibition of *F. oxysporum* f.sp *lycopersici* by different concentrations of Marigold root; (a) aqueous and (b) Methanolic extracts after 7 days incubation at 28 ± 2 °C. A: 10 mg/ml; B: 8 mg/ml; C: 6 mg/ml; D: 4 mg/ml; E: 2 mg/ml; F: control (No extract)

methanolic extracts of marigold leaf and root were found to be effective in suppressing the radial mycelial growth of *F. oxysporum* f.sp *lycopersici*. All concentrations of marigold aqueous extracts induced significant reduction in mycelial growth of the pathogen *in vitro*. The effectiveness of aqueous
extracts in this study may be attributed to their relative solubility in water as earlier reported by (2, 17). Significantly higher mycelial inhibition exhibited by both leaf and root aqueous extracts at 10 mg/ml concentration implied that increase in concentrations of these extracts corresponded to an increase in the active ingredients of the solutions which acted on the fungus thereby affecting its physiological processes and consequently lowering the growth of the fungus. Tanins have been reported to possess antibacterial properties which act by different mechanisms, including enzyme inhibition, reduction in oxidative phosphorylation and iron deprivation amongst others (16). Saponins have also been found to exhibit antimicrobial properties particularly against fungi bacterial and protozoa (6). The effectiveness of marigold root extracts in this study could be traceable to the presence of thiopene in its tissues as earlier reported (9, 14). Efficacy of methanolic extracts of marigold is also in accordance with the work of (20) who reported that ethanol extraction of betel leaf at 500 ppm caused 40 % growth inhibition of A. flavus.

REFERENCES


In Vitro Assessments Potential of *Trichoderma harzianum* for Biological Control of *Botryodiplodia theobromae* on White Yam (*Dioscorea rotundata* Poir) Tuber

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**Summary**

The potential of *Trichoderma harzianum* as biocontrol agent of *Botryodiplodia theobromae* of white yam (*Dioscorea rotundata*) tuber rot was carried out at Advanced Plant Pathology Laboratory, Federal University of Agriculture, Makurdi, Nigeria. The antagonist was introduced at three different times (same time with the pathogen, 2 days before the inoculation of the pathogen and 2 days after the inoculation of the pathogen) on potato dextrose agar. The dual culture and control plates were incubated at ambient room temperature for 192 hours. Measurements of mycelial radial growths were done at 24 hours interval beginning from the 72 hour. In the dual culture tests, the mycelial growths of the pathogen were inhibited by the antagonist, while the control grew uninhibited. The mean percentage growth inhibition of mycelial of *B. theobromae* when inoculated same time with *T. harzianum* was 48.38% compared with 70.19% inhibition when the antagonist was introduced 2 days after inoculation of the pathogen. The highest mean percentage growth inhibition of 70.24% was recorded when the antagonist was introduced 2 days before inoculation of the pathogen. The results revealed that *T. harzianum* was able to significantly \((P \leq 0.05)\) inhibit the growth of *B. theobromae* in culture at three different times of introduction of the antagonist. Minimum inhibition concentration (MIC) for the three treatments showed that *T. harzianum* was more effective in controlling *B. theobromae* when it was introduced 2 days before inoculation of the pathogen. It is therefore, concluded that
in vitro control using *T. harzianum* is a good antagonist for biological control of *B. theobromae* causing dry rot in yam tubers.

**Keywords:** Antagonist, *Botryodiplodia theobromae*, radial growth, yam, inhibition, rots

**YAMS** (*Dioscorea* spp.) are mostly cultivated and ranked second after cassava in the study of root and tuber crops in West Africa (1, 2). Nigeria is the largest producer of the crop, producing about 38.92 million metric tonnes annually (3, 4). It is reported that nearly one billion people in the World are challenged by severe hunger of which 10% actually die from hunger-related complications. A substantial part of this hunger problem is traced from inadequate agricultural storage and produce preservation associated with microbes-induced spoilages (5, 6). Postharvest losses of crop produce caused by plant diseases are the most devastating (7). Research conducted on different varieties of yam have shown that between 50% and 56% of yam tubers harvested are lost to rot organisms after 6 months of storage in the yam barn (8). This is a major threat to food security in developing countries where there are no adequate facilities for storage of yam tubers. Rot of yam tubers in storage is caused by a variety of fungi organism which include, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus niger*, *Botryodiplodia theobromae*, *Collectotrichum* spp., *Fusarium oxysporum*, *Fusarium solani*, *Fusarium moniliforme*, *Geotrichum candidum*, *Penicillium chrysogenum*, *Penicillium digitatum*, *Rhizoctonia* spp. (9, 10, 11, 12, 13)

To reduce the incidence of these pathogenic organisms causing rots in yam tubers, various control measures have been researched and adopted. Chemicals such as sodium orthophenylphenate, borax, captan, thiabendazole, mancozeb and Benomyl have proved to be effective in reducing storage losses of yam (14, 15, 16). The use of micro-organisms such as *Trichoderma harzianum* and *Bacillus subtilis* in the control of fungi pathogens have also been reported (17, 18, 19, 12, 13) but have not been fully adopted by resource poor farmers in developing countries. Plant extracts have been used to control yam tuber rot diseases (20, 21, 22, 23, 16). Fungicides may have a role in the management of yam tuber rot but their cost of application, tolerance of target pathogens and environmental and health concerns may limit application. Thus alternative methods to control post harvest diseases, particularly those that are environmentally safe are urgently needed (24, 25). The research was therefore aimed at evaluating the
in-vitro antagonist capabilities of *T. harzianum* on mycelial growth of *B. theobromae* which is one of the major pathogens of yam tuber dry rot diseases in storage.

**MATERIALS AND METHODS**

**The study area**

The study area lies between longitudes 9° 25' and 9° 28'E, and on latitude 7° 32' and 7° 35'N in Zaki-Biam, Ukum local government area of Benue State, Nigeria.

**Source of the Antagonist**

The antagonist used in this study was *T. harzianum* which was obtained from yam Pathology Unit of University of Ibadan, Oyo State, Nigeria. Stock cultures of the isolate were maintained on slants of acidified potato dextrose agar (PDA) in McCartney bottles for subsequent studies.

**Collection of diseased yam tubers**

Rotted yam tubers of *Ogoja* white yam varieties (*Dioscorea rotundata*) showing various diseased symptoms of rots were obtained from yam farmers from various storage barns in the study area. The rotten yam tubers were packaged in sterile polyethylene bags and were protected using wire mesh to prevent rodent attack. Potato Dextrose Agar (PDA) was the medium used. Test fungus for this study was *B. theobromae*.

**Isolation and identification of *B. theobromae***

*B. theobromae* was isolated from rotted white yam tubers. Tissue segments of about 2 mm diameter were cut from the periphery of infected portions of the tuber using sterilized scalpel. The pieces were surface sterilized by dipping completely in a concentration of 5% sodium hypochlorite solution for 2 minutes; the sterilized sections were then removed and rinsed in four successive changes of sterile distilled water (SDW) as reported by (26).

**Inoculation**

Rotted yam pieces were aseptically transferred onto solidified sterile potato dextrose agar (PDA) medium in Petri dishes. Four pieces of the yam sections were placed on three PDA plates each. The plates were incubated at ambient room temperature (30 ± 5°C) for 192 hours. Plates were examined daily for the development of fungal growth.

**Characterization and identification**

Fungi that grew from infected tissues were sub-cultured on separate sterile acidified PDA plates and incubated to obtain pure cultures of pathogens. Macroscopic and microscopic examination and morphological
characteristics and identification were done to ascertain its identity with the aid of compound microscope and identification guide (27, 28).

Pathogenicity test
Healthy yam tubers were washed with tap water, rinsed with distilled water and surface sterilized with 5% sodium hypochlorite. Cylindrical discs were removed from the tubers with a sterile 5 mm cork borer. A disc of a five days old culture of *B. theobromae* was transferred into holes created in the tubers; petroleum jelly was used to completely seal the holes. The same procedure was used for the control except that discs of uninoculated PDA were placed in the holes created in the tubers (29). After incubation period of 14 days at ambient room temperature (30±5°C) the tubers were examined for infection and disease development.

Evaluation of Dual culture on agar plates
The antagonistic activity of *T. harzianum* against pathogenic fungi was performed on Potato Dextrose Agar (PDA) on Petri dishes by the dual culture method (30). The mycelial plugs (5 mm diameter) of 5-day old *T. harzianum* and *B. theobromae* were placed on the same dish 6 cm from each other. Isolate of test *T. harzianum* was plated same time with *B. theobromae*, two days before *B. theobromae* and two days after *B. theobromae*. Paired cultures were incubated at ambient room temperature (30± 5°C) for 192 hours. Dishes inoculated only with test pathogens served as controls.

Measurement of radial mycelial growth
Measurement of radial mycelial growths of *B. theobromae* in dual culture with *T. harzianum* and in control plates were done after two days of inoculation on a 24 hour interval beginning from the 72nd hour up to the 192nd hour of incubation at ambient room temperature (30 ± 5°C). Percent Growth Inhibition (PGI) of *B. theobromae* was also calculated as described by (31).

\[
PGI (%) = \frac{R - R_1}{R} \times 100
\]

Where,
PGI = Percent Growth Inhibition
R = the distance (measured in mm) from the point of inoculation to the colony margin in control plate,
R<sub>1</sub> = the distance of fungal growth from the point of inoculation to the colony margin in treated plate in the direction of the antagonist.

Effectiveness of *T. harzianum* in controlling *B. theobromae*
The percent growth inhibition was determined as a guide in selecting the minimum inhibition concentration
(MIC) that will be effective in controlling the rot-causing fungi. Antagonist was also rated for inhibitory effects using a scale by Sangoyomi (32).

$\leq 0\%$ inhibition (not effective),

$>0 - 20\%$ inhibition (slightly effective)

$>20 - 50\%$ inhibition (moderately effective),

$>50 - <100\%$ inhibition (effective)

$100\%$ inhibition (highly effective)

**Experimental Design and Data Analysis**

Completely Randomized Design (CRD) with three replicates as described by (33) was used. Test of variance was calculated using Analysis of variance (ANOVA) and statistical F-tests were evaluated at $P \leq 0.05$. Differences among treatment means for each measured parameter were further separated using fishers least significance difference (LSD) to determine levels of significance according to (34).

**RESULTS**

**Isolation of *B. theobromae***

*B. theobromae* was isolated and identified as one of the causal agent of white yam tuber rots in storage. On potato dextrose agar, the cultures of this fungus were initially white to dirty white fluffy mycelia and feathery, becoming grey and eventually black (Figure 1). The underneath plate showed black colour radiating uniformly from the point of inoculation. Observation under the microscope showed that the pathogen possess simple short conidiophores with dark and ovoid 2-celled conidia.

![Figure 1](image1.png)

**Figure 1**: Pure culture of *B. theobromae* growing on Potato dextrose agar (left); Photomicrograph structure of *B. theobromae* mycelial ($\times 10$) (right)
Pathogenicity Test

Pathogenicity test conducted confirmed that *B. theobromae* obtained from rotted yam tubers was able to induce infections on healthy looking yam tubers. Symptoms of decay were seen on the re-inoculated yam tubers as dry black rot. The controls were however, not infected.

Evaluation of Dual culture method on agar plates

The results showed a behaviour differing according to each time of introduction of antagonist-pathogen combination. It was observed that *T. harzianum* could inhibit the growth of *B. theobromae* on Potato Dextrose Agar medium in the dual culture (Figure 2, 3 and 4). It showed that when the mycelium of both the cultures came in contact with each other the hyphae growth of the pathogenic fungus were found to be inhibited by the hyphae of *T. harzianum*. The results for each combination of antagonist and pathogen with respect to time of introduction of the antagonist are summarized (Figure 2, 3 and 4). *T. harzianum* showed parasitic behaviour against *B. theobromea* (Figure 2, 3 and 4) by growing over the hyphae of the pathogen and degrading it. The dual culture plates showed initial rapid growth of the pathogen which stopped at the point of contact with the antagonist. *T. harzianum* over grew the pathogen resulting into complete degradation of the fungus and sporulation of the antagonist over the entire plate. It further revealed that the percentage growth inhibition of *B. theobromae* increased steadily from 27.80% at 72 hours to 66.14% at 192 hours respectively when the antagonist and the pathogen were introduced same time. When the antagonist was introduced 2 days before the arrival of the pathogen, the inhibition of the pathogen rose from 36.10% at 72 hours to 85.92% at 192 hours. The trend was similarly observed to increase from 66.42% at 72 hours to 71.26% at 192 hours when the antagonist was introduced two days before the pathogen in culture plates. The results of dual culture indicated that *T. harzianum* significantly (*P* ≤ 0.05) inhibited the growth of *B. theobromae* at varying degrees across duration of incubation (Table 1). Mean variation of percentage growth inhibition of *B. theobromae* tested at three different times of introduction of *T. harzianum* also significantly (*P* ≤ 0.05) inhibited the growth of *B. theobromae* (Table 1).
Figure 2: Dual culture of *T. harzianum* and *B. theobromae* on potato dextrose agar inoculated same time (Th×path) (left) and pure culture of *B. theobromae* on potato dextrose agar as control (right)

Figure 3: Dual culture of *T. harzianum* and *B. theobromae* on potato dextrose agar (left); *T. harzianum* was introduced 2 days before inoculation of *B. theobromae* (2dbi) and pure culture of *B. theobromae* on potato dextrose agar as control (right)

Figure 4: Dual culture of *T. harzianum* and *B. theobromae* on potato dextrose agar (left); *T. harzianum* was introduced 2 days after inoculation of *B. theobromae* (2dai) and pure culture of *B. theobromae* on potato dextrose agar as control (right)
Table 1: *In vitro* Percentage Growth Inhibitions (PGI) of *B. theobromae* by time of Introduction of *T. harzianum*

<table>
<thead>
<tr>
<th>Duration of Incubation</th>
<th>Time of Introduction of <em>T. harzianum</em> and Percentage Growth Inhibition (%)</th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td>ThXPath</td>
<td>Th2dbiPath</td>
</tr>
<tr>
<td>72 Hrs</td>
<td>27.80±11.20&lt;sup&gt;d&lt;/sup&gt;</td>
<td>36.10±11.50&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>96 Hrs</td>
<td>38.61±4.76&lt;sup&gt;c&lt;/sup&gt;</td>
<td>67.66±3.58&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>120 Hrs</td>
<td>47.50±2.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.70±2.10&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>144 Hrs</td>
<td>50.93±2.75&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>76.89±2.17&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>168 Hrs</td>
<td>59.34±0.80&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>83.16±1.34&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>192 Hrs</td>
<td>66.14±0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.92±0.83&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>LSD</em></td>
<td>16.02</td>
<td>15.72</td>
</tr>
<tr>
<td><em>Mean</em> (LSD= 9.27)</td>
<td>48.38±3.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70.24±4.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means on the same column with the same superscript are not statistically significant (*P*≤ 0.05)

Means on the same row (for Mean) with the same superscript are not statistically significant (*P*≤ 0.05).

*Th*<sub>x</sub>*path* = *T. harzianum* introduced same time with pathogen; *Th2dbiPath* = *T. harzianum* introduced 2 days before inoculation of pathogen; *Th2daipath* = *T. harzianum* introduced 2 days after inoculation of pathogen.

**Effectiveness of *T. harzianum* in controlling *B. theobromae***

*T. harzianum* was tested at three different levels on *B. theobromae* for effectiveness as highly effective, effective, moderately effective, slightly effective and not effective across the treatments. The results showed that *T. harzianum* introduced 2 days before inoculation of *B. theobromae* significantly (*P*≤ 0.05) reduced growth (70.24%) compared with that introduced 2 days after inoculation of *B. theobromae* (70.19%) with the least inhibition (48.38%) recorded when both the antagonist and the pathogen were introduced same time. Effectiveness levels of *T. harzianum* were moderately effective to effective and significant (*P*≤ 0.05) across treatments (Table 2).

**Measurement of mycelial radial growth**

Radial mycelia growth of dual culture and the alone culture were measured. It was found that the alone culture grew much faster than the dual culture in all the treatment. *B. theobromae* grew fast and covered the entire plate...
for each treatment within 192 hours of incubation (Figure 5).

**Table 2:** Effectiveness of *T. harzianum* in controlling *B. theobromae* at different times showing percentage growth inhibition and minimum inhibition concentration

<table>
<thead>
<tr>
<th>Time of Introduction of <em>T. harzianum</em></th>
<th>Percentage Growth Inhibition (PGI)</th>
<th>MIC (%)</th>
<th>Level of Effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>ThXPath</td>
<td>48.38±3.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;20-50</td>
<td>Moderately Effective</td>
</tr>
<tr>
<td>Th2dbiPath</td>
<td>70.24±4.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;50-&lt;100</td>
<td>Effective</td>
</tr>
<tr>
<td>Th2daipPath</td>
<td>70.19±0.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;50-&lt;100</td>
<td>Effective</td>
</tr>
</tbody>
</table>

Th XPath = *T. harzianum* introduced same time with pathogen; Th2dbipath = *T. harzianum* introduced 2 days before inoculation of pathogen; Th2daipath = *T. harzianum* introduced 2 days after inoculation of pathogen; MIC = minimum inhibition concentration (%); ≤ 0% inhibition (not effective); >0-20% inhibition (slightly effective); >20-50% inhibition (moderately effective); >50-<100% inhibition (effective); 100% inhibition (highly effective).
DISCUSSION

*T. harzianum* showed ability to inhibit growth of *B. theobromae* when they were paired together in culture. The results of this study revealed that the antagonistic fungus (*T. harzianum*) has a high inhibitory effect against the test fungus (*B. theobromae*) with a
several biological mechanisms of action like mycoparasitism, competition for space and nutrients between the pathogen and antagonist as reported (35, 36, 19). This is because the *T. harzianum* grew faster in the dual culture plates compared with the *B. theobromae* and parasitized on it.

The results of dual culture also indicated that the different treatments of *T. harzianum* inhibited the growth of *B. theobromae* at varying degrees like the results obtained by Gwa and Ekefan (12), and Gwa and Nwankiti(13) who inhibited the growth of *Fusarium moniliforme* and *Colletotrichum spp* respectively using *T. harzianum* in vitro. The aggressive competition with the pathogen caused twisting, air bubbling and disintegration of the pathogen hyphae suggesting enzymes action like the result obtained by Lorito et al. (37). In addition, it may also produce antifungal phenolic compounds as reported by Saba Banday et al., (35) and Anita et al., (38). Metcalf et al. (39) demonstrated possible role of chitinolytic and/or glucanases enzymes in bio-control by *Trichoderma*. These enzymes function by breaking down the polysaccharides, chitin, and glucans that are responsible for the rigidity of fungal cell walls, thereby destroying cell wall integrity limiting the growth of the pathogen.

The direct confrontation of *T. harzianum* against test fungus *in vitro* on PDA medium showed that when the mycelium of both cultures met each other the hyphae growth of *B. theobromae* were found to be inhibited by the hyphae of *T. harzianum*. Kullnig et al. (40) and Brunner et al. (41) previously demonstrated that before mycelial of fungi interact; *Trichoderma* sp. produces low quantities of extracellular exochitinases. The diffusion of these enzymes dissolves cell fragments of host cells. These cell fragments in turn induce the production of further enzymes and trigger a cascade of physiological changes, stimulating rapid and directed growth of *Trichoderma* sp. (42). The microscopic observation of *T. harzianum* mycelial showed that they entangled the hyphae fragments of *B. theobromae* and eventually lysed them.In culture, all the three treatments of *T. harzianum* grew faster than *B. theobromae*. The inoculation of the test antagonists two days before the pathogen was done because there are no biocontrol agents that have enough competitive ability to displace an already established pathogen. The time lapse allows adequate increase in cell concentration and subsequent colonization by antagonist before inoculation of the pathogen (43, 44). The ability of antagonist to proliferate
within a short period of favourable environmental conditions before they encounter plant pathogen is an important factor as more rapid growth and sporulation of fungi from biocontrol formulations may superficially enhance efficacy in the field. The antagonistic effect of *T. harzianum* *in vitro* on the pathogen growth was observed to increase with the days of incubation. This may probably be because of increase in the production or concentration of the antifungal metabolites (45). Minimum inhibition concentration (MIC) showed that *T. harzianum* introduced 2 days before inoculation of *B. theobromae* was more effective in controlling the pathogen than introducing the antagonist same time with the pathogen as well as introducing it 2 days after the pathogen. This shows that introducing the biological antagonist prior to the arrival of pathogen has proven more effective than after the pathogenic organism has already attacked the host.

**CONCLUSION**

*T. harzianum* is a good antagonist for biological control of *B. theobromae* due to the different mechanisms of actions the fungus employs in inhibiting the growth of the pathogen. It should therefore be applied prior to the arrival of the pathogen for it to be more effective in controlling it.

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Effect of Exposure Duration on Seed Treatment Potentials of Some Botanicals Against Seed-Borne Microbes of Onion (*Allium cepa* L.)


Department of Crop Science, Faculty of Agriculture, University of Benin, Benin City.

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Summary

This study evaluated the effect of exposure duration on the efficacy of some botanicals (*Azadirachta indica*, *Moringa oleifera*, *Ocimum graticium* and *Vernonia amygdalina*) against bacterial and fungal species associated with onion (*Allium cepa*) seed as compared to copper oxide as a check treatment. Seeds used were obtained from Kano, Kebbi and Yobe States of Nigeria. The experiment was laid out in a Completely Randomized Design (CRD), replicated three times. Microbial species associated with seeds of onion were isolated using the direct plating method. Identification was done using cultural and morphological characteristics and biochemical test for fungal and bacterial isolates respectively. Matured plant leaves were harvested, air dried for 72 hours and blended to fine powder using an electric blender. Five grams of each botanical and copper oxide (powder) were put in separate sterile Petri dishes to which 3 g of *A. cepa* seeds was added and properly mixed and for 1, 30, 60 and 120 minutes. At the end of each exposure duration regime, thirty seeds of *A. cepa* were directly plated on potato dextrose agar (PDA) and nutrient agar (NA) respectively and closely observed for microbial growth. Data collected on percentage infection were first transformed to arcsine values and then analyzed with descriptive analysis and analysis of variance (ANOVA). Significantly different means were separated with Duncan multiple range test (DMRT) at 5% level of probability using the GenStat 12th edition statistical software package. Six fungal species (*Aspergillus flavus, Aspergillus niger, Rhizopus stolonifer, Rhizopus spp., Mucor spp., Fusarium spp.,) and five bacterial species (*Bacillus spp., Erwinia spp., Pseudomonas spp., Staphylococcus*
spp., *Flavobacterium* spp.) were isolated from onion seeds obtained from the sampled locations. In decreasing order, mean percentage fungal infection for sampling locations were Yobe > Kebbi > Kano (100%, 96% and 73% respectively). Bacterial infection of seeds was Kebbi > Yobe > Kano (78.8%, 67.7% and 63.3% respectively). *M. oleifera* and *V. amygdalina* showed highest fungicidal potentials of 44.20 and 44.40 each at 120 minutes of exposure. Highest bactericidal potential for *M. oleifera* (36.47) and *A. indica* (39.57) were observed at exposure duration of 1 minute and 30 minutes respectively. There were significant differences between copper oxide (CuO) and botanicals evaluated, with CuO proving superior with 100 % fungal and bacteria growth inhibition at all exposure duration.

**Key words:** *Allium cepa*, fungicidal, non-chemical seed treatment, percentage infection, botanicals.

**ONION** (*Allium cepa* L.) is a plant species of the family of Alliaceae, genus *Allium* (6). The plant is believed to have originated from Asia and Middle East. Cultivation of onion in Nigeria is done mostly in the Northern States. Cultivation of onion provides a source of income to farmers, source of food or flavoring agent and serves as feed ingredient to livestock. It is also believed to provide health benefits to consumers such as purification of blood, improving respiratory difficulties, preventing cancer and indigestion (13). Production of onion is limited by pest and disease attacks. Some of such diseases are caused by pathogenic microbes which may be present in or on seeds, bulbs or soil. The presence of microbes on seeds have been reportedly responsible for reduced seed viability, pre and post emergence mortality, black mold, neck and basal rot, and poor health of seed in many crops including onion (4, 8). To improve onion production, the availability of healthy, microbe free seeds is invaluable. This can be achieved by the application of appropriate seed treatment prior to planting. Previous studies have reported high fungicidal and bactericidal properties in aqueous and methanolic extracts prepared from *Azadirachta indica*, *Moringa lucida*, *Occimum basilicum*, *Tagetes erecta*, *Vernonia amygdalina*, *Alium cepa*, *Alium sativum* and *Jatropha caucus* (2, 9, 12). Hence, this study evaluates the effect of exposure duration on seed treatment potentials of selected botanicals (*Azadirachta indica*, *Moringa oleifera*, *Occimum graticum* and *Vernonia amygdalina*) against microbial species associated with *A. cepa* seeds from Kano, Kebbi, and Yobe States of Nigeria.
MATERIALS AND METHODS

Experimental location/source of materials

A. cepa seeds were collected in sample bags from ‘Karfi’, ‘Gafhiwa’ and ‘Yauri’ markets in Kano, Kebbi and Yobe States respectively and transported to the Crop Science Laboratory, University of Benin where they were prepared for microbial isolation. Plant materials -leaves of A. indica and V. amygdalina, were obtained from the Faculty of Agriculture, University of Benin, while fresh O. graticimum, and M. oleifera were purchased from ‘Uselu’ Market, Benin City.

Sterilization

Glass wares were sterilized in an electric oven (Memmert UNB 400, made in Germany) at 160 °C for 2 hours. Metal wares and inoculating needles were sterilized by flaming to red-hot using a spirit lamp. The working area was disinfected by swabbing with 70% ethanol. Seeds were surface-sterilized by dipping in 10% sodium hypochlorite for 30 seconds. After which they were rinsed in three changes of sterile distilled water and placed on a sterile filter paper to dry out. Media were prepared and sterilized at 121 °C/15 Psi for 15 minutes.

Media preparation and microbial isolation

Media (Nutrient Agar and Potato Dextrose Agar) were prepared according to manufacturer’s instruction and sterilized immediately after in an electric autoclave (Hirayama HV-110, made in Japan) as described earlier. The media were allowed to cool down to about 35 °C and then transferred to the laminar flow where 20 ml of media (NA/PDA) were poured into Petri-dishes and allowed to gel. Microbial isolation was done using the direct plating method (10, 12). Sterile forceps were used to pick seeds from the lot and placing gently on already gelled media (NA and PDA). The cultures were incubated at 32 ± 2 °C for 24 hours for bacteria and 28± 2 °C for 72 hours for fungi.

Microbial identification

Bacterial identification was done by a series of appropriate biochemical tests. Fungi identification was done by viewing prepared slides under a light microscope at 40X. Slides were prepared by taking a little pinch of the mycelia mass from pure culture with the aid of a sterile needle and placed on the slide; a drop of lacto-phenol blue was added to it for staining. A little teasing with the needle was done to dislodge the spore. A slide cover was placed over the prepared slide and
mounted on a light microscope (2). Identification was done based on cultural and morphological characteristics exhibited by the various fungi isolates and compared with the description of fungal identification keys (5).

**Preparation of botanicals/seed treatment**

Fully matured leaves of plant materials of *A. indica*, *M. oleifera*, *O. graticimum*, and *V. amygdalina* were harvested and air dried for 72 hours. Dried leaf material was then blended to fine powder using an electric blender. Five (5) grams of each botanical and copper oxide (powder) were put in separate sterile Petri-dishes. Three (3) g of *A. cepa* seeds was added to Petri-dishes already containing botanicals. Thirty seeds of *A. cepa* were then carefully picked with a sterile forcep and put into plates containing botanicals and mixed properly. Seeds were subjected to exposure duration of 1 minute, 30 minutes, 60 minutes and 120 minutes. Sterilized forceps were used to gently pick seeds from the treatments (leaf powder) and gently placed on already gelled media and closely observed for microbial growth.

**Experimental design, data collection and analysis**

The experiment was laid out in a Completely Randomized Design (CRD) replicated three times. Data collected on percentage infection were first transformed to arcsine values and analyzed with descriptive analysis and analysis of variance (ANOVA) using the GenStat (General Statistics) 12th edition statistical software package. Observations for percentage infection were recorded at 24 and 72 hours for bacterial and fungal isolates respectively. Significantly different means were separated with Duncan multiple range test (DMRT) at 5% level of probability.

**RESULTS**

Table 1 presents fungal and bacterial species isolated from onion seeds from different sample locations. Fungal species isolated from ‘Karfi’ market in Kano State were *A. niger* and *A. flavus*. *Rhizopus* spp., *Rhizopus stolonifer* and *Aspergillus niger* were isolated from samples obtained from ‘Yauri’ market in Kebbi State. While *Rhizopus* spp, *Aspergillus niger*, *Fusarium* spp and *Mucor* spp were isolated from seeds obtained from ‘Gafhiwa’ market in Yobe State. Bacterial isolates associated with seeds of *A. cepa* included *Erwinia* spp., and *Bacillus* spp. from seeds obtained in Kano; *Pseudomonas* spp., and *Bacillus* spp. from seeds sourced from Kebbi and *Flavobacterium* spp., *Pseudomonas* spp. and *Staphylococcus* spp. from onion seeds collected from Yobe.
Mean percentage fungal and bacterial infection based on sampling locations is summarized in figure 1. In decreasing order, mean percentage fungal infection were Yobe > Kebbi > Kano (100%, 96% and 73% respectively). Bacterial infection of seeds was Kebbi > Yobe > Kano (78.8%, 67.7% and 63.3% respectively).

Table 2 shows the effect of exposure duration on seed treatment potentials of selected botanicals against fungal species associated with *A. cepa* seeds. *M. oleifera* and *V. amygdalina* showed highest fungicidal potentials of 44.20 and 44.40 each at 120 minutes of exposure. *O. graticimum* had highest fungicidal potentials at 60 minutes (49.93). *A. indica* showed highest treatment potentials at 1 minute of exposure (46.37).

Table 3 shows the effect of exposure duration on seed treatment potentials of selected botanicals against bacterial species associated with *A. cepa* seeds. Highest bactericidal potential for *M. oleifera* (36.47) was observed at exposure duration of 1 minute. *V. amygdalina* and *O. graticimum* showed highest bactericidal potentials (41.40 and 39.57 respectively) at 120 minutes exposure duration. *A. indica* showed highest bactericidal potentials (39.57) at exposure duration of 30 minutes.

**DISCUSSION**

Six fungal and five bacteria species were isolated from seeds of *A. cepa* obtained from three states in Nigeria. Fungal and bacterial species isolated from onion seeds were similar to those isolated from seeds of citrus species, *Gambeya albida*, and *Dialium guineense* (1, 12, 13).

The plant leaf powder (botanicals) prepared from four different plant species evaluated at different exposure times showed varied level of microbial growth inhibition against fungal and bacterial species associated with seeds of *A. cepa*. These findings are in agreement with previous studies where levels of inhibition expressed by a botanical extract was shown to be dependent on the plant species from which such extract was made (9). Some researchers have posited that other factors such as the solvent used in the preparation of the extract, age of plant, plant part used, method of extraction, and time of harvesting the plant materials are also determining factors on the level of bioactivity expressed by the extract (2, 3, 11).

Table 1: Fungal and bacterial species isolated from onion seeds (*Allium cepa*) from different locations in Nigeria

<table>
<thead>
<tr>
<th>Location (State)</th>
<th>Microbes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Market</th>
<th>Fungi</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Karfi’ Market (Kano)</td>
<td><em>Aspergillus flavus</em></td>
<td><em>Erwinia</em> spp.</td>
</tr>
<tr>
<td></td>
<td><em>A. niger</em></td>
<td><em>Bacillus</em> spp.</td>
</tr>
<tr>
<td></td>
<td><em>Rhizopus</em> spp.</td>
<td></td>
</tr>
<tr>
<td>‘Gafhiwa’ Market (Kebbi)</td>
<td><em>Rhizopus stolonifer</em></td>
<td><em>Bacillus</em> spp.</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus</em> niger</td>
<td><em>Pseudomonas</em> spp.</td>
</tr>
<tr>
<td>‘Yauri’ Market (Yobe)</td>
<td><em>Rhizopus</em> spp</td>
<td><em>Flavobacterium</em> spp</td>
</tr>
<tr>
<td></td>
<td><em>Fusarium</em> spp</td>
<td><em>Staphylococcus</em> aureus</td>
</tr>
<tr>
<td></td>
<td><em>Mucor</em> spp</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus</em> niger</td>
<td><em>Pseudomonas</em> spp</td>
</tr>
</tbody>
</table>

Figure 1: Percentage fungal and bacterial infection of onion seeds from three locations of Nigeria.

Table 2: Effect of exposure time on the efficacy of some botanicals for the control of seed-borne mycoflora of onion

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Exposure duration (Minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Initial</td>
<td>56.97&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>CuO</td>
<td>00.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>M. oleifera</em></td>
<td>55.17&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>V. amygdalina</em></td>
<td>63.03&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>O. graticimum</em></td>
<td>55.27&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>A. Indica</em></td>
<td>46.37&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means with the same letters along the column are not significantly different at 5% level of probability.
Table 3: Effect of exposure time on the efficacy of selected botanicals for the control of seed-borne bacterial species of onion

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Exposure time (Minutes)</th>
<th>1</th>
<th>30</th>
<th>60</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td></td>
<td>55.86d</td>
<td>55.86c</td>
<td>55.86d</td>
<td>55.86c</td>
</tr>
<tr>
<td>CuO</td>
<td></td>
<td>0.00a</td>
<td>0.00a</td>
<td>0.00a</td>
<td>0.00a</td>
</tr>
<tr>
<td>M. oleifera</td>
<td></td>
<td>36.47b</td>
<td>37.80b</td>
<td>42.47b</td>
<td>41.97b</td>
</tr>
<tr>
<td>V. amygdalina</td>
<td></td>
<td>54.47cd</td>
<td>56.83c</td>
<td>51.23cd</td>
<td>41.40b</td>
</tr>
<tr>
<td>O. graticimum</td>
<td></td>
<td>59.67d</td>
<td>54.13c</td>
<td>47.43bc</td>
<td>39.57b</td>
</tr>
<tr>
<td>A. Indica</td>
<td></td>
<td>49.80c</td>
<td>39.57b</td>
<td>40.03b</td>
<td>40.93b</td>
</tr>
</tbody>
</table>

Means of the same letters along the column are not significantly different at 5% level of probability.

There was observable decline in the percentage infection on seeds of A. cepa as the exposure time was increased from 1 minute to 120 minutes with M. oleifera and A. indica extract showing good inhibition potential exposure times of 120 minutes. Aside from exposure duration, other studies show that concentration of extract applied plays a key role in its growth inhibitory potential (7, 12).

The copper oxide (CuO) used as a check treatment significantly and effectively controlled all fungal and bacterial species associated with seeds of A. cepa with 100% fungal and bacteria growth inhibition at all exposure duration.

CONCLUSION

Five fungal -Aspergillus niger, A. flavus, Rhizopus stolonifer, Fusarium spp., and Mucor spp. and bacterial species -Bacillus spp., Erwinia spp., Flavobacterium spp., Pseudomonas spp., and Staphylococcus spp. were associated with seeds of A. cepa obtained from Kano, Kebbi and Yobe states of Nigeria.

The exposure duration significantly influenced the microbial growth inhibition potential of A. indica, M. oleifera, O. graticimum and V. amygdalina against microbes associated with A. cepa seeds. The growth inhibition potential of each extract was also influenced by the plant species used. Dusting onion seeds with M. oleifera leaf powder for...
120 minutes or *M. oleifera* at exposure duration of 1 minute is recommended if non-chemical control of seeds borne microbes of fungi and bacteria of onion is desired.

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Response of Roselle (*Hibiscus sabdariffa* Linn.) Cultivars to Insect Pest Infestation and damage in Maiduguri Area of Borno State

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Summary

Insect pest damage is major problem to sustainable production of roselle in the study area. Field trials were conducted at the teaching and research farm of the University of Maiduguri in 2014 and 2015 cropping seasons to determine the reactions of six roselle cultivars to insect pest infestation and damage. The six-cultivar treatments were evaluated in a randomized complete block design (RCBD) with three replications each. Data were collected on plant height, leaf area, fruit diameter, 100-seed weight and dry calyx yield, insect pest population, percentage leaf damage, percentage calyx damage and percentage calyx loss. The results showed that roselle cultivars responded differently to insect pest attack. Bakin sobo and Farin sobo cultivars, which are high yielding cultivars, tolerated higher population of insect pests. Purtu cultivar supported less population of insect pests and produced lower yield. Bakin sobo and Farin sobo cultivars can be cultivated in the area for higher calyx yield. The cultivars can also be crossbred to produce varieties with much desired qualities of resistance to pests and high yield.

**Key words:** Insect pest damage, roselle cultivar reaction, insect pest control, calyx yield, Sudan savanna

**ROSELLE** (*Hibiscus sabdariffa* Linn.) is an annual herbaceous shrub of the family Malvaceae. The crop is commonly called roselle, sorrel, red sorrel, Jamaican sorrel, Indian sorrel, karkade, among others, in different countries [1]. *H. sabdariffa* is a native of Asia or Tropical Africa [2]. It is grown mainly for its leaves, calyx, fibre and seeds [3]. Approximately 15,000 metric tons of calyces enter international trade each year with
China and Thailand the major producers, Germany and the USA the major importers [4]. In Africa, Benin, Sudan, Cote D’Ivoire, Ghana, Niger, Burkina Faso and Nigeria are the major producers of Roselle [5]. In Nigeria, the various genotypes grown are named after the colour of the calyx or by local native names. Roselle production is mainly in the Guinea and Sudan savanna zones of the country where red and green calyx genotypes are prevalent [6]. Production of roselle in Nigeria is mainly for local consumption. The leaves are used extensively as potherbs, fodder, medicine and fibre, but most importantly; the swollen calyces are products of international commerce. Roselle is used for the treatment of various ailments including hypertension, leukaemia, pyrexia and liver damage [7, 8, 9]. The calyces are rich in acid and pectin. Analysis of calyces has shown the presence of crude protein and minerals such as iron, phosphorus, calcium, magnesium, manganese, aluminium, sodium and potassium. Mucilage, calcium citrate, ascorbic acid, niacin, riboflavin, gossypetin, hibiscin chloride are also present in calyces [10, 1, 11].

One of the constraint to its sustainable production is insect pests damage to the leaves and calyces. Although [1, 4] reported that insect pests damage in roselle is a minor problem, the damage is significant as reported by [4] and [11]. Roselle is attacked by a spectrum of insects at different phenology of the crop under different ecologies. However, [12] and [13] reported Podagrica spp, Earias insulani, Aphis gossipii, Empoasca spp, Oxycarenus hyalinipennis, and Bemisia tabaci, as insect pests of roselle. Damage by these pests to the leaves, calyces and seeds of roselle can lead to economic loss if measures are not taken to curb it. O. hyalinipennis nymph and adult feed on the calyx and seed of roselle causing significant damage and loss of seeds. Roselle leaf damage by M. goldingi in the study area cause serious economic loss to farmers. In this regard, roselle farmers mostly employ the use of synthetic insecticides such as Deltamethrin and Cypermethrin to control the pests. However, the negative impact of synthetic insecticides on non-target organism, environmental contaminations and development of resistant strain has triggered the search for better alternatives.

Information on the response of different roselle genotype to insect pests attack is limited in the area. Several genotypes of roselle are grown in Nigeria and most of the differences are based on the colour of the calyx and plant height. [14] reported that there are three common cultivars of
roselle grown in Nigeria. Two of these cultivars have red calyces while one has green calyces. However, research to determine response of such cultivars to insect pests for possible utilization of such character in pest management is limited. Therefore, the objective of the study is to determine the responses of roselle cultivars to insect pests.

**MATERIALS AND METHODS**

**Experimental Site**

The experiments were conducted at the Teaching and Research Farm and Entomology Laboratory of the Department of Crop Protection, Faculty of Agriculture, University of Maiduguri (11° 51” N and 13° 51” E), Nigeria during the rainy seasons of 2014 and 2015.

**Source of Seeds**

The six roselle cultivars are Bakin Sobo, Farin Sobo, Jan Sobo, Subu, Purtu, and Mbirjinga. They were obtained from Bauchi, Lassa and Borno State Agricultural Development Project (BOSADP).

**Treatments and experimental design**

The treatments were six roselle cultivars replicated three times each. Each treatment was allocated to a plot of 4 m x 4 m size with 0.5 m within replicate and 1 m between blocks. The treatments were laid in a randomized complete block design (RCBD). Seeds were sown at 50 x 50 cm spacing with 36 stands per plot. Thinning was done two weeks after planting to two plants per stand and six stands per row in each plot. Weeding was done at 4 and 6 weeks after crop emergence [15].

**Data collection**

Insect sampling: Ten plants were randomly sampled and tagged from each plot for insect sampling. Sampled insects were collected in sampling bottles and then preserved in 70% ethanol and later identified at the insect Museum in the Department of Crop protection of the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria. The number of *Monolepta goldingi* per plot was taken by counting and recording their number on each of the ten randomly tagged plants in every plot on weekly basis, while the number of *O. hyalinipennis* was counted on the surface of each fruit of the ten randomly tagged plants in every plot on weekly basis.

Plant height was taken at fruiting from each of the tentagged plant in a plot using a measuring tape. Measurement was taken from the ground level to the growing point of the plant. Similarly, the leaf area was taken four times by measuring the length and breadth of three leaves/plant, one each from the apex, middle and the lower part of the plant for each of the tentagged plants.
in a plot using a measuring tape. Calyces were harvested from the tagged plants, dried and weighed using XL-22 model beam balance, while Vanier Callipers was used to measure the calyx diameter. A hundred-seed weight from fruits of each of the six cultivars was obtained using an electronic compact scale, Kerro BL-20001 (Japan).

**Assessment of damage on leaf and calyx**

Percentage damage on leaf and calyx was calculated using the following formulae:

\[
\text{Percentage leaf damage} = \left( \frac{\text{Total number of damaged leaves/plot}}{\text{Total number of leaves/plot}} \right) \times 100
\]

\[
\text{Percentage calyx damage} = \left( \frac{\text{Total number of damaged calyces/plot}}{\text{Total number of calyces/plot}} \right) \times 100
\]

\[
\text{Actual calyx yield (kg/ha)} = \text{Calyx per plant} \times \text{number of plants/ha}
\]

\[
\text{Calyx yield loss (kg/ha)} = \text{Attainable calyx yield} – \text{Actual calyx yield}
\]

\[
\text{Percentage calyx loss} = \left( \frac{\text{Calyx yield loss (kg/ha)}}{\text{Actual yield obtained} + \text{yield loss (kg/ha)}} \right) \times 100
\]

A leaf was considered damaged if 25% or more of its total area is damaged.

A calyx was considered damaged if 25% or more of its total area is damaged.

**RESULTS AND DISCUSSION**

The result of the study regarding the characteristics of the six cultivars used in the study is presented in Table 1. The characteristics of the cultivars differed significantly based on colour of the calyx, seed, seed shape and size. Additionally, height of the cultivars was also different as well as their leaf sizes. In the combine means of the two seasons (2014 and 2015), plant height of Bakin sobo cultivar was significantly higher than Farin sobo cultivar (Table 2.). However, the leaf areas of Subu and Purtu cultivars were significantly larger than that of Jan sobo cultivar. Bakin sobo and Jan sobo cultivars had significantly larger fruit diameter than the rest of cultivars. Mbirjinga cultivar had significantly the smallest fruit diameter. Mbirjinga cultivar had differed significantly on 100-seed weight from Farin sobo cultivar (Table 2). The six cultivars of roselle evaluated in this trial showed variation in their response to insect pests population attack at vegetative and reproductive stages. Morphologically, these cultivars differed significantly in terms of plant height, leaf area, fruits size (calyx diameter) and colour (Table 2). These morphological characters are known to influence insect behaviour toward plant host and therefore might be the reason for the
variations in their response to insect pest attack.

The population of *Monolepta goldingi* and percentage leaf damage per plot is presented in table 3. Farin sobo cultivar supported significantly higher population of *M. goldingi* while Jan sobo and Bakin sobo cultivars supported significantly lowest population of *M. goldingi* in 2014 season. However, there was no significant difference in the population of *M. goldingi* supported by the cultivars in 2015 season. Nevertheless, the combine means of both seasons show significantly higher population of *M. goldingi* on Farin sobo and significantly lower on Jan sobo. Consequently, the percentage leaf damage on Farin sobo was significantly higher than Bakin sobo in 2014 season. Similarly, in 2015 Farin sobo cultivar sustained significantly higher percentage leaf damage than Subu cultivar. The combine means of both seasons showed that Bakin sobo cultivar had significantly lower percentage leaf damage than Farin sobo and Jan sobo cultivars (Table 3). However, Jan sobo and Bakin sobo cultivars supported low population of *M. goldingi* and had sustained low to moderate levels of leaf damage. While Farin sobo cultivar supported high population of *M. goldingi* and sustained high leaf damage. The pattern of leaf damage levels sustained by each cultivar, which showed the higher the pest population the higher the damage level, is usual. Because there is a positive correlation between the population of *M. goldingi* and percentage leaf damage. Nevertheless, the differences in pest population density on each cultivar showed significant differences in insect pest preference to each cultivar. This therefore, clearly shows the involvement of an inherent characteristics possessed by the individual cultivars. The damage to leaves is of much concern if the crop is cultivated for the leaf yield otherwise, the correlation between leaf damage and calyx diameter is not significant even though it reduces dry calyx yield by a small percentage. Moreover, the calyx yield is the product of international commerce. Approximately 15,000 metric tons of calyces enter international trade each year with China and Thailand the major producer and Germany and the USA the major importers [4].

Results on population of *Oxycarenus hyalinipennis* and percentage calyx damage is presented in table 4. Bakin sobo and Farin sobo cultivars supported significantly higher population of *O. hyalinipennis* than Purtu cultivar in both seasons. The combine means of both seasons show that Purtu and Mbirjinga cultivars supported significantly lower
The percentage calyx loss and dry calyx yield presented in Table 5 showed significant difference among cultivars. Bakin sobo cultivar had significantly higher percentage calyx loss than Purtu, Subu and Farin sobo cultivars in 2014 trial. While in 2015 trial, Purtu cultivar had significantly lower percentage calyx damage than the other cultivars. Similarly, the combine means of both seasons showed that Purtu cultivar had significantly lower percentage calyx damage than rest of the cultivars. The dry calyx yield on Bakin sobo and Farin sobo cultivars differed significantly higher than on the other cultivars in 2014 trial. However, in 2015
Table 1: Characteristics of Roselle Cultivars used in the study

<table>
<thead>
<tr>
<th>Cultivar name</th>
<th>Accession number</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farin Yakuwa</td>
<td>NGR-BA-044</td>
<td>Seed diameter 3.0 mm, triangular and brighter. Calyces are white, 4-5cm long, soft and elongated (Plate 1b)</td>
</tr>
<tr>
<td>Jan Yakuwa</td>
<td>NGR-BA-046</td>
<td>Seed diameter 3.3mm, light and smaller in shape. Calyces are about 3.5cm long and reddish (Plate 1a).</td>
</tr>
<tr>
<td>Bakin yakuwa</td>
<td>NGR-BA-045</td>
<td>Seed diameter 3.4mm, rectangular, light grey. Calyces are about 6cm long and dark red (Plate 1e).</td>
</tr>
<tr>
<td>Subu</td>
<td>-</td>
<td>Seed diameter 4.0mm darker and spherical. Calyces are bright red with pointed end (Plate 1c).</td>
</tr>
<tr>
<td>Purtu</td>
<td>-</td>
<td>Seeds are large, brighter, soft and spherical. Calyces are white with grey and greenish stripes (Plate 1f).</td>
</tr>
<tr>
<td>Mbirjinga</td>
<td>-</td>
<td>Seed diameter 4.1mm, darker, oval. Calyces are 4cm long soft and light reddish in colour (Plate 1d).</td>
</tr>
</tbody>
</table>

Sources: [15]

Plate 1: Fruits and seeds of Jan sobo (a), Farin sobo (b), Subu (c), Mbirjinga (d), Bakin sobo (e) and Purtu cultivars (f)
Table 2: Mean values of agronomic parameters of the different roselle cultivars grown in Maiduguri in 2014 and 2015 cropping seasons.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant height (cm)</th>
<th>Leaf area (cm)</th>
<th>Fruits diameter (cm)</th>
<th>100-seed weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bakin sobo</td>
<td>124.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.02&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Farin sobo</td>
<td>96.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.70&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.60&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Jan sobo</td>
<td>117.27&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>54.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.10&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Subu</td>
<td>116.23&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>97.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.94&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.90&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Purtu</td>
<td>104.93&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>85.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.06&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mbirjinga</td>
<td>98.33&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>71.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.70&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SE±</td>
<td>11.98</td>
<td>13.54</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Means followed by the same letters in a column are not significantly different at the 5% level of probability according to the New Duncan’s Multiple Range Test (NDMRT).

Table 3: Effects of roselle cultivars on the number of *Monolepta goldingi*, percentage leaf damage and percentage plant damage in Maiduguri.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of <em>M. goldingi</em></th>
<th>Percentage leaf damage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2014</td>
<td>2015</td>
</tr>
<tr>
<td>Bakin sobo</td>
<td>10.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Farin sobo</td>
<td>21.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.66&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Jan sobo</td>
<td>9.67&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Subu</td>
<td>15.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.66&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Purtu</td>
<td>12.33&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>10.66&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mbirjinga</td>
<td>11.67&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>11.33&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SE±</td>
<td>2.05</td>
<td>2.81</td>
</tr>
</tbody>
</table>

Means followed by the same letters in a column are not significantly different at the 5% level of probability according to the New Duncan’s Multiple Range Test (NDMRT).
Table 4: Effects of roselle cultivars on the number of *Oxycarenus hyalinipennis* and percentage calyx damage in Maiduguri.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of <em>O. hyalinipennis</em></th>
<th>Percentage calyx damage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2014</td>
<td>2015</td>
</tr>
<tr>
<td>Baki sobo</td>
<td>30.33a</td>
<td>30.00ab</td>
</tr>
<tr>
<td>Farin sobo</td>
<td>29.00a</td>
<td>32.33a</td>
</tr>
<tr>
<td>Jan sobo</td>
<td>25.00ab</td>
<td>24.00bc</td>
</tr>
<tr>
<td>Subu</td>
<td>24.33ab</td>
<td>25.00bc</td>
</tr>
<tr>
<td>Purtu</td>
<td>20.33b</td>
<td>21.33c</td>
</tr>
<tr>
<td>Mbirjinga</td>
<td>26.33ab</td>
<td>24.33bc</td>
</tr>
<tr>
<td>SE±</td>
<td>3.40</td>
<td>2.60</td>
</tr>
</tbody>
</table>

Means followed by the same letters in a column are not significantly different at the 5% level of probability according to New Duncan’s Multiple Range Test (NDMRT).

Table 5: Effects of roselle cultivars on percentage calyx loss and dry calyx yield in Maiduguri.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage calyx loss</th>
<th>Dry calyx yield (Kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2014</td>
<td>2015</td>
</tr>
<tr>
<td>Baki sobo</td>
<td>26.13a</td>
<td>23.93a</td>
</tr>
<tr>
<td>Farin sobo</td>
<td>17.96bc</td>
<td>25.37a</td>
</tr>
<tr>
<td>Jan sobo</td>
<td>24.03ab</td>
<td>27.43c</td>
</tr>
<tr>
<td>Subu</td>
<td>17.86bc</td>
<td>21.93a</td>
</tr>
<tr>
<td>Purtu</td>
<td>12.20c</td>
<td>12.66b</td>
</tr>
<tr>
<td>Mbirjinga</td>
<td>25.53a</td>
<td>28.00a</td>
</tr>
<tr>
<td>SE±</td>
<td>3.22</td>
<td>2.88</td>
</tr>
</tbody>
</table>

Means followed by the same letters in a column are not significantly different at the 5% level of probability according to New Duncan’s Multiple Range Test (NDMRT).
trial and the combine means of both seasons showed that Bakin sobo cultivar had the highest dry calyx yield followed by Farin sobo cultivar (Table 5). Table 6 showed the correlations of insect pests damage to leaf, calyx dry calyx and yield. The results show an increase in dry calyx yield because of increase in fruit diameter. Conversely, the fruit diameter decreases with increase in the population of M. goldingi and percentage leaf damage by a small margin. The dry calyx yield decreases significantly with an increase in percentage leaf damage. The percentage leaf damage increases with increase in the population of M. goldingi. Similarly, the percentage calyx yield loss increases with an increase in the population of O. hyalinipennis. Damage to calyces of all the cultivars by O. hyalinipennis was significant. This confirms the reports of [12, 11] that damage by insect pests to roselle is a major problem. The two cultivars, Bakin sobo and Farin sobo were heavily attacked by O. hyalinipennis, which resulted into high calyx damage though their calyx yields were high. This is further confirmed by the positive correlations between populations of O. hyalinipennis and percentage calyces yield loss. Practically, these cultivars sustained higher calyx damage but also have higher dry calyx yield. This is mainly attributed to relatively taller nature with larger calyx diameter. Additionally, fruit diameter has positive correlation with dry calyx yield. Generally, the correlation between population of O. hyalinipennis and dry calyx yield was not significant because some damaged calyces formed part of the dry calyx yield. This however, did not lessen the importance of O. hyalinipennis as a major pest of roselle because; it has positive correlation with percentage calyx loss. Mbirjinga and Jan sobo cultivars had low calyx yield partly due to high loss of calyces caused by moderate population of O. hyalinipennis. These implies that cultivation of Bakin sobo and Farin sobo cultivars could benefit farmers especially if the calyx yield is the products of interest. Interestingly also, is that Purtu cultivar, which supported less population of O. hyalinipennis and sustained low damage to calyces that lead to low calyx loss, might have possessed a mechanism of resistance. Although the cultivar has small calyx diameter and had low calyx yield, which makes it a low yielding cultivar.
Table 6: Correlation of insect pests population, damage parameters and calyx yield of roselle.

<table>
<thead>
<tr>
<th></th>
<th>Fruits diameter</th>
<th>Monalepta goldingi</th>
<th>Oxyccarenus hyalinipennis</th>
<th>Dry calyx yield</th>
<th>% leaf damage</th>
<th>% Calyx damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. goldingi</td>
<td>-0.2600</td>
<td>0.2975</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O. hyalinipennis</td>
<td>0.2387</td>
<td>0.3918</td>
<td>0.3402</td>
<td>0.1079</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry calyx yield</td>
<td>0.3815</td>
<td>0.2563</td>
<td>0.5966</td>
<td>0.0090</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Leaf damage</td>
<td>-0.1531</td>
<td>0.5591</td>
<td>0.0881</td>
<td>-0.0970</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5442</td>
<td>0.0151</td>
<td>0.7280</td>
<td>0.0018</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Calyx damage</td>
<td>0.3000</td>
<td>0.5246</td>
<td>0.7435</td>
<td>0.8168</td>
<td>0.1468</td>
<td></td>
</tr>
<tr>
<td>% Calyx loss</td>
<td>0.2264</td>
<td>0.0254</td>
<td>0.0004</td>
<td>0.8706</td>
<td>0.5611</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1815</td>
<td>-0.2528</td>
<td>0.5099</td>
<td>-0.1413</td>
<td>-0.1245</td>
<td>0.0237</td>
</tr>
<tr>
<td></td>
<td>0.4711</td>
<td>0.3114</td>
<td>0.0306</td>
<td>0.0000</td>
<td>0.6227</td>
<td>0.9255</td>
</tr>
</tbody>
</table>

CONCLUSION

It can be concluded that roselle cultivars used in this showed differences in responses to infestation by insect pests. Bakin sobo and Farin sobo cultivars sustained higher population of M. goldingi and O. hyalinipennis and suffered higher damaged leaves, damaged calyces and loss calyces yet, they produced higher calyx yield. Purtu cultivar supported lower population of O. hyalinipennis and had lower calyx loss, but with low calyx yield. These cultivars can be crossbred to produce varieties with much desired qualities. Meanwhile, Bakin sobo and Farin sobo cultivars can be cultivated for higher calyx yield in the area.

ACKNOWLEDGEMENT

Thanks to Borno State Agricultural Development Project (BOSADP) for providing the seeds of some of the varieties used in this study.

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Management of Root-Knot Nematode (*Meloidogyne incognita*) (Kofoid And White, 1919) Chitwood, 1949 Infecting *Cucumis sativus* L. Using Crop Rotation

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¹ National Horticultural Research Institute, Ibadan. ²Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan.

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Summary

Potentials of three crops (marigold, sesame and maize) were evaluated in crop rotation with Cucumber (*Cucumis sativus* x 12 m and then further divided into four sub-plots of 2 m x 1.5 m with 1 m within and between rows. Soil RKN population for each sub-plot was estimated from 100 ml soil taken from each sub-plot. Seeds of cucumber (Marketer) were planted in each plot at a spacing of 75 cm x 50 cm. Eight weeks after planting, the experiment was terminated. Each plot was then tilled using simple implements (hoes) and the plots were planted with either African marigold, sesame “NCR-01M or maize “Oba super 1, and cucumber “Marketer” as a control. Eight weeks after planting, the experiment was terminated the soils were tilled using simple implements (hoes) and planted to cucumber “Marketer” the following planting season. The experimental layout was Randomized Complete Block Design with six replications. Data were collected on fresh shoot weight (FSW), marketable fruit yield (MFY), Gall Index (GI). Data were analyzed using descriptive statistics and ANOVA at α0.05. Cucumber planted in rotation with marigold, sesame and maize each had a 75.0% reduction in GI and fresh shoot weight increased by 60.8%, 57.2% and 57.7%, respectively. The MFY of cucumber planted after marigold and sesame increased by 96.0% each and 97.0% in Maize compared to control (36.0%). Rotating cucumber with marigold, sesame or maize reduced RKN infection in cucumber production.

Keywords: Crop rotation, Disease Management, Fruit yield, *Meloidogyne incognita*, Non-host.
CUCUMBER, *Cucumis sativus* L., belongs to the family Cucurbitaceae. It is the second most important crop of the family, after watermelon (*Citrullus lanatus*). It is believed to have originated in Northern India and has since spread throughout the world, including Southern India, South East Asia, China, Central and South America and Africa (2). Cucumber production in Nigeria is fast becoming popular since it is a useful ingredient in the preparation of salad. It also has medicinal and therapeutic values, such as in the cure of hypertension and skin diseases. It is also a valuable source of potassium, sodium, magnesium, silicon, phosphorous, chlorine and fluorine (20). Taking cucumber with vegetables, cereals, fruits, nuts and salads enhances the nutritional value of food items (2). It is widely cultivated in the Northern States of Nigeria, such as Kaduna, Kano, Plateau and in peri-urban sites of Lagos State. It is planted in rainy season and dry spell using irrigation or in lowland ecologies commonly known as *fadama*, characterized with residual moisture.

Despite the importance of this crop in the diets of Nigerian consumers, pests and diseases constitute major threats to its production. Cucumber is one of the most susceptible hosts of root-knot nematodes, *Meloidogyne* spp. (5). Nematode, such as sting nematodes (*Belonolaimus* spp.) occasionally cause some losses in cucumber production (16). These nematodes alone rarely cause death but can predispose the plants to infection by other pathogens, such as fungi and bacteria, which may eventually lead to wilting of crops. (1)

*Meloidogyne* spp. are notoriously difficult to control because of their wide host range and high rates of reproduction, with generation times of typically between 20 and 30 days in tropical soils, and females capable of producing a thousand eggs (16). By their activities in the roots of susceptible plants, they cause galls on roots which results in reduction in water and nutrient uptake, manifesting in yellowing of leaves and patchiness in the field, resulting in reduction in yield and quality of susceptible crops (11). *Meloidogyne* spp., are real threats to almost all vegetable crops and they have been considered as limiting factors in crop production (9).

In nematode management, the principle that guides the use of crop rotation is the reduction of populations of damaging nematode species to levels that allow subsequent crops to complete early growth before being heavily attacked (4). This can be achieved by alternating poor hosts, non-hosts or resistant crops with susceptible crops (4). The adoption of
sequential cropping in root-knot nematode control is restricted among the smallholder farms due to scarcity of arable land, coupled with market-driven demand for particular crops and/or varieties (4). More so, training is required to design and implement effective crop cycles to control pathogens such as the root-knot nematodes that have a wide host range (12, 13, 26). Previous studies have focused on plants such as Tagetes spp., (Marigold), Crotolaria spp. (Sunn hemp), Asparagus spp., Sesamum indicum (Sesame), and Azadirachta indica (neem) that are antagonistic to root-knot nematodes through release of root exudates toxic to the nematodes (13, 9, 20, 24). However, a major hindrance to their adoption into most cropping systems is low or lack of commercial value of the most intensively studied plants (11, 20). Suitability of crops incorporated into crop rotation system for nematode management is not only determined by their efficiency in nematode suppression, but also by the economic returns they bring to the farmer. Therefore, the challenge is to identify nematode-suppressive crops that satisfy the economic considerations in crop production systems. Nigerian literature is very scanty in the use of economic crops in rotation for the management of root-knot nematode which is devastating to most of the vegetables crops in the tropics. In an effort to address the above challenges, this study was conducted to investigate the management of M. incognita on cucumber using some selected plants/crops that are of economic value in rotation.

MATERIALS AND METHODS

Naturally Meloidogyne incognita-infested fields at the National Horticultural Research Institute, Ibadan located at 7° 31′ N and 3° 51′ E and the Crop Garden, Department of Crop Protection and Environmental Biology, University of Ibadan located at 7° 26′30″ N and 3° 54′00″ E, were used for the 1st and 2nd trials respectively. To multiply the inoculum, the fields were planted to Celosia argentea three months before the commencement of the experiments. At each site, land measuring 12 m x 18 m was marked out and subdivided into six equal blocks of 2 m x 12 m. Each block was further divided into four equal sub-plots of 2 m x 1.5 m with 1 m within and between rows. Soil nematode populations for each sub-plot was estimated from 250 ml soil taken from each sub-plot. Seeds of cucumber (Marketer) were planted in each plot at a spacing of 75 cm x 50 cm. The experimental layout was Randomized Complete Block Design with six replications. One week after
germination, four plants from each plot were tagged and data were recorded weekly on vine length and number of leaves. Forty five days after germination, fruit harvesting started and frequently done as required wherein number of marketable fruits, number of non-marketable fruit and fruit weights were recorded. Two months after planting, the experiment was terminated, and the following data were recorded: fresh shoot weight (g), fresh root weight (g), root galling was quantified using the scale of 0-5 according to Makete, 2000(14) as shown viz:

0 = No gall; 1 = 1-10% of the root system galled; 2 = 11-35% of the root system galled; 3 = 36-65% of the root system galled; 4 = 66-90% of the root system galled; 5 = More than 90% of the root system galled. Nematode population in 100 ml soil and eggs/juveniles in 10 g of the root system were also estimated. Each sub-plot was tilled using hoes the plots were planted with African Marigold, Sesame “NCR-01M”, Maize “Oba super I” and cucumber “Marketer” as a control. Weeds were regularly controlled, and the plants watered when necessary. The experimental design was randomized complete block design with six replications. Four plants from each sub-plot were randomly selected three months after planting, they were uprooted and washed free of soil. Data on fresh root weights, gall index (Scale 0-5) and nematodes population in the 250ml of soil were recorded. Immediately after termination of the experiment, the soils were tilled using hoes and planted to cucumber “Marketer” the following planting season as described above. Growth parameters (Vine length, number of leaves) were monitored for six weeks. Two months after planting data were taken on fresh shoot weight, number of fruit harvested by plant ( Marketable and unmarketable fruits), galling index (0-5 scale), soil nematode population in 100 ml soil, eggs in 10g of roots

Statistical Analyses

All data obtained were statistically analyzed using the SAS version 9.0 (2000) statistical package and the means were separated using the Least Significant Difference (LSD) at a probability level of 5%.

RESULTS

Growth parameters obtained from planting cucumber in rotation after resistant crops differed significantly (P<0.05) among the treatments (Fig. 1). The cucumber vine lengths arising from the various treatments recorded between one and four weeks After Germination (WAG) were not significantly different (P≤0.05) from one another (Fig. 1). However, from 4WAG, the highest mean vine length
was occurred in cucumber grown after African marigold but not statistically higher than the ones grown after sesame and maize (Fig. 1). The least vine length was recorded from the cucumber grown after cucumber (Fig. 1). The second trial followed the same trend. However, at 6WAG the cucumber grown after Marigold also had the highest vine length which was not significantly different from the one grown after Sesame. The least vine length was also recorded from the cucumber grown after cucumber but not significantly different from the one grown after maize (Fig. 1).

Number of leaves produced by cucumber grown after resistant crops also differed significantly (P≤0.05) (Fig. 2). There was no significant difference from the leaves produced by cucumber grown after resistant crops from one to three WAG (Fig.2). The highest mean number of leaves was recorded from cucumber grown after Marigold and this was not significantly different from the number of leaves produced by cucumber grown after sesame and maize (Fig. 2). However, the least number of leaves was recorded from the cucumber grown after Marigold which was significantly different from the one grown after sesame and maize (Fig. 2). The least number of leaves was also recorded from cucumber grown after sesame (Fig. 2).

Number of leaves produced by cucumber grown after resistant crops also differed significantly (P≤0.05) (Fig. 2). There was no significant difference from the leaves produced by cucumber grown after resistant crops from one to three WAG (Fig.2). The highest mean number of leaves was recorded from cucumber grown after Marigold and this was not significantly different from the number of leaves produced by cucumber grown after sesame and maize (Fig. 2). However, the least number of leaves was recorded from the cucumber grown after Marigold which was significantly different from the one grown after sesame and maize (Fig. 2). The least number of leaves was also recorded from cucumber grown after sesame (Fig. 2).
**Figure 1:** The Vine length of cucumber grown after Marigold, Maize, Sesame or cucumber in rotation at different weeks (a) 1st and (b) 2nd trials.
Figure 2: The Number of leaves of cucumber grown after Marigold, Maize, Sesame or Cucumber at different weeks (a) 1\textsuperscript{st} and (b) 2\textsuperscript{nd} trials.

The lowest significant mean gall index came from the cucumber grown after marigold, sesame and maize while the highest significant mean gall index was recorded from the cucumber grown after cucumber in rotation (Table 1). The highest fresh root weight was recorded from the cucumber root grown after cucumber which was significantly different from the cucumber root grown after marigold, sesame and maize (Table 1). The highest number of eggs of root knot nematode extracted was recorded from the cucumber roots grown after cucumber while the least was recorded from the cucumber root grown after Marigold which was not significantly different from the cucumber grown after sesame and maize (Table 1), second stage juveniles (J2) and total nematode population (TN) in the soil followed the same trend. The highest J2 and TN were recorded from the plot that was grown with cucumber after cucumber in rotation while the least were recorded from the plot grown with cucumber after marigold in rotation which was not significantly different from the plots that maize and
sesame were planted (Table 1). The Second trial also followed the same trend (Table 1).

The highest shoot weight was recorded from the cucumber grown after marigold but not significantly different from the cucumber grown after sesame and maize (Table 2) while the least shoot weight of cucumber was recorded from the cucumber grown after cucumber in rotation (Table 2). The highest marketable fruit yield (10.8 t/ha) was recorded from cucumber grown after maize which was not significantly different from the cucumber grown after sesame (8.5 t/ha) and marigold (6.5 t/ha). Least marketable fruit yield (0.9 t/ha) of cucumber was recorded from the plot grown with cucumber in rotation. The highest non-marketable fruit yield (1.87 t/ha) of cucumber was recorded from the plot grown to cucumber in rotation. The highest marketable fruit yield was recorded from the cucumber grown after sesame in the second trial which was not significantly different from the cucumber grown after maize and marigold (Table 2). The least marketable fruits yield was also recorded from the cucumber grown after cucumber in rotation in the second trial (Table 2).

**Table 2:** The Shoot weight, Marketable fruit yield and Non-Marketable fruit yield of Cucumber grown in rotation with Marigold, Maize, Sesame and Cucumber.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Season I 1st Trial</th>
<th>Season II</th>
<th>Season III</th>
<th>Shoot weight (g)</th>
<th>Marketable Fruit yield t/ha</th>
<th>Non-Marketable fruit yield t/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cucumber Marigold</td>
<td>Cucumber</td>
<td>Cucumber</td>
<td>204.0±30.75</td>
<td>6.5±0.99</td>
<td>1.30±0.14</td>
<td></td>
</tr>
<tr>
<td>Cucumber Maize</td>
<td>Cucumber</td>
<td>Cucumber</td>
<td>187.1±29.56</td>
<td>10.8±2.85</td>
<td>1.10±0.25</td>
<td></td>
</tr>
<tr>
<td>Cucumber Sesame</td>
<td>Cucumber</td>
<td>Cucumber</td>
<td>189.2±25.15</td>
<td>8.5±1.08</td>
<td>0.87±0.11</td>
<td></td>
</tr>
<tr>
<td>Cucumber Cucumber</td>
<td>Cucumber</td>
<td>Cucumber</td>
<td>80.0±33.40</td>
<td>0.9±0.33</td>
<td>1.87±0.31</td>
<td></td>
</tr>
<tr>
<td>LSD≤0.05</td>
<td></td>
<td></td>
<td>28.0</td>
<td>5.1</td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

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DISCUSSION

The results of this study show that the various non-host crops grown before cucumber are effective in reducing the root galls and nematode population compared to the cucumber that was grown after susceptible crop (Cucumber) this is in collaborate with the study conducted by Otipa et al. (17) that damage by Meloidogyne spp. was significantly reduced in tomato planted in rotation with sweetcorn or in sweetcorn with Tagetes patula, Crotalaria juncea, Sorghum bicolor and Asparagus sp. in the field. Gallling and nematode multiplication were highest on cucumber followed by cucumber. This implies that cucumber was a better host for M. incognita than any of the other crops African marigold, sesame or maize. However, gall indices and J2 number on cucumber after cucumber were higher than on cucumber after African marigold sesame or maize. Cucumber grown after marigold in rotation also reduced the population of Meloidogyne incognita in the soil and increased the yield of cucumber this might be as a result of the roots of these attractive flowering plants contain chemicals that kill nematodes which act as trap crops.

African marigold produced a bioactive compound like α-therthienyl which is very toxic to nematodes and other soil pathogens like as fungi, bacteria, and insects (7, 23, 8). Also, once Meloidogyne spp. juveniles entered T. erecta roots they were unable to fully develop (19). It was also reported that Marigold can suppress 14 genera of plant-parasitic nematodes, especially root-knot nematodes (Meloidogyne spp.) and lesion nematodes (Pratylenchus spp.) (8). Different species and cultivars of marigold vary in their ability to suppress nematode populations. Cucumber grown after sesame in rotation also reduced the gall index and root damage caused by M. incognita, this is in collaboration with Starr and Black (24) that cotton grown after sesame in rotation effectively controlled peanut root-knot nematode (Meloidogyne arenaria) and southern root-knot nematode (M. incognita). This implied that rotating poor host plants with susceptible varieties of cucumber is an effective M. incognita nematode control strategy that improves cucumber growth, quality and yield. Sesame (NICRIBEN-01M) that was used in the experiment was resistant to Meloidogyne incognita this was supported by Atungwu et al (4) that Sesame (NICRIBEN-01M) inhibited M. incognita reproduction by 74–76%, and no significant damage to the roots of the plants.

This study has confirmed the resistant status of S. indicum variety, NICRIBEN-01M, African marigold and Maize ‘Oba super 1’ which were used in rotation with susceptible crops Cucumber cv. Marketer can manage M. incognita infestation. Rotation strategies are usually based on resistance, susceptibility, or tolerance of crops to the predominant species of plant parasitic nematodes in a specific area. To be effective, the resistant crop component, as
The preceding crop must necessarily prevent damage to the following crop by suppressing populations of the nematode pests with consequent improvement in the yield of the crop to justify its economy as a crop protection tactic (4).

The use of poor-host or resistant crops in rotation with cucumber is a promising strategy for the management of *Meloidogyne incognita* below economy threshold and there will be increased in quality and yield. Marigold, sesame or maize can be planted in rotation with cucumber to reduce damage due to root-knot nematodes.

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Predator - Host Distribution and Abundance of Aphidophagous Green Lacewing (Neuroptera: Chrysopidae)Larva on Three Vegetables in the Sudano-Sahelian Savanna Agro-Ecological Zone of Nigeria

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Summary

This study assessed host distribution and abundance of the predatory green lacewing (Neuroptera: Chrysopidae) larva (GLL) in three vegetables commonly cultivated in Maiduguri/Sudano-Sahelian Savanna agroecological zone (SAEZ), Nigeria. Survey was carried out during the cropping season in 2015 and 2016, each year in 24 farmers’ fieldscultivated with Cucumis sativus L., Citrullus lanatus (Thunb.) Matsum. and Nakai) and/or Amaranthus hybridus L. GLL were recovered preying on Aphis gossypii Glover in C. sativus and C. lanatus (family, Cucurbitaceae) and Aphis craccivora Koch in A. hybridus (family, Amaranthaceae). In both 2015 and 2016, mean abundance of aphids per leave was significantly higher in C. sativus (116.22-89.17), followed by C. lanatus (62.13-48.55) than in A. cruentus (22.23-13.34). Mean abundance of GLL per leave, 0.02/0.01 in 2015 and 0.01 in 2016, was not significantly different among host plants. The ratio of GLL to A. gossypii seasonally ranged from 1:5811-1:8917 in C. sativus and 1:6213-1:4855 in C. lanatus, whilst predator ratio to A. craccivora ranged from 1:1334-1:2223 in A. cruentus. Using pooled data, predator-prey ratio was lowest in A. cruentus (1:1779), followed by C. lanatus (1:5534) and highest in C. sativus (1:6846). In conclusion, seasonal abundance and predator-prey ratio of GLL in farmers’ fields within the SAEZ is extremely low. Thorough understanding of their impact in biological control of aphid pests in this region further requires information on prey consumption efficiency and functional response pattern of all effective Chrysopid species and stages, or the effect of
varied prey consumption and climate on their development/survival and effective habitat management that enhance their survival and predatory function.

**Keywords:** Green lacewing larva, prey, *Cucumis sativus, Citrullus lanatus, Amaranthus hybridus*, predator, Sudano-Sahelian Savanna

Aphids, being soft-bodied, are subject to predation by insects in different orders including members of the family Chrysopidae (Neuroptera), commonly called ‘green lacewings’. Chrysopidae contains 1200-2000 species, largely cosmopolitan, in 85 genera (17). The most important and abundant aphidophagous predator species belong to the genera *Dichochrysa (> 130 species), Chrysopa (> 50 species and some subspecies), Ceraeochrysa (> 46 species)* and *Chrysoperla (> 36 species)* Steinmann(3, 4, 17). Adult green lacewings are not predaceous, but valuable pollinators that feed on nectar and pollen from flowers, and also aphid honeydew (2, 5, 24). The flattened, alligator shaped, grayish-brown larvae, sometimes called ‘aphid lions’ or ‘aphid wolves’, primarily feed on aphids voraciously, and additionally feed on mites, insect eggs and other small and soft-bodied insects when aphid populations drop too low (2, 5). The aggressive larvae and adults (to a limited extent) of some species (i.e., *Chrysoperla rufilabris* (Burmeister) search out and seize prey using their pincer-like jaws, prior to injecting digestive enzymes and sucking out the internal fluids. Hatched larvae quickly search for prey to consume, walking up to 7 miles and from plant to plant if leaves are touching (27). Larvae develop through three instars in 2-3 weeks, before pupating in a spherical silken cocoon, usually attached to the vegetation. Each larva is capable of devouring > 200 aphids or insect eggs per week (7). Their developmental time is not affected by relative humidity in the range of 20-80% (22, 33). Adults may live for 1-3 months depending on temperature, humidity and quality of food sources (7). Adults perform straight downwind dispersal flights of up to 40 km per night into and to colonize neighbouring or distant fields (9, 36). Depending on mating activity and food quality, fecundity can be high with as many as 200 eggs laid at a time, and 600 eggs laid in all (21, 23, 36, 39, 40). Eggs are laid on foliage near colonies of aphids to facilitate rapid and successful larval search for prey. Females uniquely place their eggs, singly or in small clusters, at the top of a hair-like filament to keep them out of reach and reduce predation and parasitism. Larval tolerance or resistance to low
rates of some insecticides such as azinophosphomethyl (Guthion), dimethoate (Cyon), trichlorfon (Dylox), carbaryl (Sevin), permethrin, rotenone, and ryania (11, 25, 35), aids green lacewings maintain their populations in spite of insecticidal use to mitigate pest density beyond an established action threshold. The population and efficiency of green lacewings can be enhanced in small scale production systems by manipulating the environment through use of food supplements (i.e., flowering plants with easy access to nectar, or homemade honeydew), attractants/artificial foods (i.e., Wheast®, or Bug Chow®/Pro®), crop inter-planting (i.e., with plants in the carrot and sunflower families) and provision of hibernation boxes (13, 38). Several species of green lacewings such as *Chrysoperla carnea* (Stephens) or *C. rufilabris* are mass-reared in commercial insectaries and available to purchase as eggs or larvae for use in augmentation releases to control aphid pests in field/garden and greenhouse crops (16, 19, 26, 27, 28). The efficacy of green lacewing might be affected by either or more of several factors including pest and crop type or distribution (in plant and field), weather, number of predators or stage released, ant interference and predator-prey ratio (7). The predator is documented to play a significant role in suppressing aphid pest populations in a wide range of agroecosystems worldwide (6, 18, 24, 30, 31, 32). All positive traits make this predator a valuable component, compatible with most other components of Integrated Pest Management (IPM) programs for managing aphid pest problems. At least 14 major species of aphids are problematic to many vegetables and crops in Africa (14, 20, 30). Nonetheless, data on biological control of aphid pests in agricultural crops by this beneficial aphidophagous predator is non-existent in Nigeria and most other African countries. This study therefore determined the host distribution and seasonal abundance of green lacewings in three fruit/leafy vegetables commonly cultivated in Maiduguri, Sudano-Sahelian Savanna agroecological zone, Nigeria.

**MATERIALS AND METHODS**

Farmers’ fields were sampled during 2015 and 2016 cropping season in Maiduguri, Sudano-Sahelian Savanna agroecological zone, Nigeria. Maiduguri, the capital of Borno state, is 354 meters above sea level, and lies between latitudes 11°45’ N to 11°51’ N and longitudes 13°2’ E to 13°9’ E. Climate of the area is generally semi-arid, with mean annual rainfall, humidity and temperature respectively 553 mm, 45.2% and 32° C (1). Mean
monthly minimum temperature is lowest, 13.5°C, during the Harmattan period when northeasterly winds are strongest in December to January and highest, 24.7°C, in April to May before onset of rains in April/May. Mean monthly maximum temperature is lowest, 31.3°C, during peak rainy period in August and highest, 40.2°C, prior to the onset of rains. Twenty-four farmers’ fields, at least 1 km apart, were randomly selected and sampled at two weeks interval across each cropping season (15). Each sampled farmers’ field was planted with *Cucumis sativus* L., *Citrullus lanatus* (Thunb.) Matsum. and Nakai) and/or *Amaranthus hybridus* L. in intercrop with cereals, legumes and/or other vegetables. Fifty leaves per crop type were randomly inspected during nine visits made to each farmers’ field. Visual sampling of both aphids and green lacewings was carried out during morning hours (Fig. 1), 6:00 to 10:30 am, when the insects were relatively inactive. Very small insects were counted with the aid of magnifying glass. Kruskal-Wallis test ($\alpha = 0.05$) or one-way analyses of variance was performed using SAS (2011) to estimate significant differences in prey and predator density between plant hosts during different years.

Plate 1: Prey: *A. gossypii* feeding in (a) *C. sativus* and (b) *C. lanatus* in the field, and *A. craccivora* feeding in (c) *A. hybridus*. Predator: Some recovered green lacewing (d) larva and (e) adults on *A. hybridus* leaves.
RESULTS

In total, 2970 leaves were inspected, at 990 leaves each for *C. sativus*, *C. lanatus* and *A. hybridus*. Green lacewing larvae were encountered preying on two aphid species (Table 1): 1) *Aphis gossypii* Glover in two host plants, *C. sativus* and *C. lanatus*, belonging to the same family Cucurbitaceae, and 2) *Aphis craccivora* Koch in one host plant, *A. hybridus*, belonging to another family Amaranthaceae. During 2015 and 2016, mean abundance of aphids was significantly higher in *C. sativus* (*A. gossypii*: 116.22 in 2015 and 89.17 in 2016) followed by *C. lanatus* (*A. gossypii*: 62.13 in 2015 and 48.55 in 2016) than in *A. cruentus* (*A. craccivora*: 13.34 in 2015 and 22.23 in 2016) (Table 2). The mean abundance, 0.02/0.01 in 2015 and 0.01 in 2016,

Table 1: Prey to green lacewing larvae in three vegetables in farmers' fields during 2015 and 2016 cropping season in Maiduguri

<table>
<thead>
<tr>
<th>Predator family</th>
<th>Aphid prey</th>
<th>Host plant</th>
<th>Species</th>
<th>Family</th>
<th>Species</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chrysopidae</td>
<td><em>A. gossypii</em></td>
<td>Cucurbitaceae</td>
<td><em>C. sativus</em></td>
<td><em>A. hybridus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>A. craccivora</em></td>
<td>Cucurbitaceae</td>
<td><em>C. lanatus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Mean prey and predator abundance per leave in three vegetables in farmers' fields during 2015 and 2016 cropping season in Maiduguri

<table>
<thead>
<tr>
<th>Host plant</th>
<th>Prey</th>
<th>Predator</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Species</td>
<td>Prey</td>
</tr>
<tr>
<td>N</td>
<td><em>A. gossypii</em> (a)</td>
<td>116.22±9.56</td>
</tr>
<tr>
<td></td>
<td><em>A. gossypii</em> (b)</td>
<td>62.13±16.24</td>
</tr>
<tr>
<td></td>
<td><em>A. craccivora</em> (c)</td>
<td>13.34±2.12</td>
</tr>
</tbody>
</table>

\[ H = 4.17 \]
\[ P = 0.005 \]

\[ H = 6.00 \]
\[ P = <0.013 \]
N, number of field observations; \( H \), Kruskal-Wallis statistic; \( P \), Probability value; Asterisk, indicates significant pairwise comparison (Wilcoxon rank-sum test: \( \alpha = 0.05 \)); ns, non-significant of green lacewing larvae was however not significantly different in these host plants. In \( C. sativus \) the ratio of green lacewing larvae to \( A. gossypii \) ranged from 1:5811 in 2015 to 1:8917 in 2016 (Table 3). Also, in \( C. lanatus \) the ratio of green lacewing larvae to \( A. gossypii \) ranged from 1:6213 in 2015 to 1:4855 in 2016, whilst in \( A. cruentus \) the ratio of green lacewing larvae to \( A. craccivora \) ranged from 1:1334 in 2015 to 1:2223 in 2016. The total ratio of green lacewing larvae to \( A. gossypii/A. craccivora \) during the cropping season of both years, 2015 and 2016 (pooled data), was lowest in \( A. cruentus \) (1:1779), followed by \( C. lanatus \) (1:5534) and highest in \( C. sativus \) (1:6846).

Table 3: Predator-prey abundance ratio (pooled data) in three vegetables in farmers’ fields during 2015 and 2016 cropping season in Maiduguri

<table>
<thead>
<tr>
<th>Host plant</th>
<th>Prey</th>
<th>Ratio (GLL:Ag/Ac)</th>
<th>2015</th>
<th>2016</th>
<th>2015 + 2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C. sativus )</td>
<td>( A. gossypii )</td>
<td>1:5811</td>
<td>1:8917</td>
<td>1:6846</td>
<td></td>
</tr>
<tr>
<td>( C. lanatus )</td>
<td>( A. gossypii )</td>
<td>1:6213</td>
<td>1:4855</td>
<td>1:5534</td>
<td></td>
</tr>
<tr>
<td>( A. cruentus )</td>
<td>( A. craccivora )</td>
<td>1:1334</td>
<td>1:2223</td>
<td>1:1779</td>
<td></td>
</tr>
</tbody>
</table>

GLL, Green lacewing larva; Ag, \( A. gossypii \); Ac, \( A. craccivora \)

DISCUSSION

These results provide the first record on green lacewing activities in Nigeria, and particularly the Sudano-Saharan Savanna agroecological zone. Results, at family level, indicate that green lacewing larvae predate on two aphid species, \( A. gossypii \) and \( A. craccivora \), in three vegetables, \( C. sativus \), \( C. lanatus \) and \( A. hybridus \), commonly grown in this region. However, predator abundance relative to its aphid prey is seasonally too low. Consequently, predator-prey ratio is extremely wide, and incapable of providing sufficient predation against aphid pests in these vegetables. The
predator-prey ratio generally documented for effective suppression of aphid pest populations by green lacewing larvae falls between 1:1 and 1:50. *Chrysopa pallen* (Rambur) eggs released at the predator-prey ratio of 1:4 on red pepper or aubergines and 1:3 on cucumber effectively suppressed the populations of *A. gossypii* (12). These authors further observed that release of second stage larvae of *C. pallen* at the predator-prey ratio of 1:30 and 1:20 satisfactorily controlled *A. gossypii* on red pepper and cucumber or tomato, respectively. Carrillo*et al.* (7) similarly reported that multiple or inundative releases of green lacewing larvae or eggs at a predator-prey ratio of between 1:3 and 1:5 in greenhouses provides good to excellent control of aphid pests. Studies in Finland using *C. carnea* against bean and green peach aphids on parsley achieved control with a predator-prey ratio of 1:27 (230 eggs/m²) (27). The same author further showed that fall cabbages with waxy aphids require a predator-prey ratio of 1:1 for 74% control, and 1:25-50 for 50% control. Three times multiple or inundative releases of eggs and early instar of *C. carnea* larvae at 1:1 predator-pest ratio in a Soviet experiment, one week after the first release, yielded fewer aphids (98.5%) and other pests (thrips 95.6%, bollworm eggs 100% and young bollworms 50%). Abundance of all these pests, in fields without green lacewing releases, increased (aphids 180%, thrips 160%, bollworm eggs 150%, young bollworms 230% and spider mites 240% (27).

Low predator-prey ratio in this study is not strange, but rather a reason, like in various agroecosystems globally, for augmentation releases of green lacewings. Aphidophagous green lacewings are an extremely important predator/biological control agent worldwide. Augmentation releases of *C. carnea* and *C. rufilabris* against aphid or other pests in North America have been reported on many crops including cotton, soybean, potato, broccoli, sugar beet, apple, pear, grape and various ornamentals (7). The introduction of *C. pallens* eggs, three successive times, against *A. gossypii* on red pepper, aubergines and cucumber was reported by GeonHwi (12) to be as effective as four applications of insecticides from mid-June to late-September. However, to exert control in a short period of time, the larval stage should be used for augmentation releases. For example, releases of green lacewing second instar larvae successfully controlled the green peach aphid in peppers, tomato and eggplant in several countries (6). Nevertheless, in greenhouses, the interval between predator releases for optimum pest
control varies depending on the pest species, environmental conditions and potential for crop damage (7). Low predator-prey ratio in parts of this agroecological zone hence provides a lucrative business opportunity for commercial mass rearing of green lacewings to supply eggs, larvae and adults of the predator for use by farmer in biological control of aphid and other crop pests in the region and beyond. Furthermore, enhancement of predator efficiency through attraction and conservation of green lacewings to and in farmer’s fields in this region becomes paramount. Emigration of green lacewings strongly depends on the presence of sugar sources/adult food and density of aphids/other larval food (9). Migration of adult lacewings toward sugar sources including nectar, pollen or insect honeydew before laying eggs should be mitigated by providing proper habitats and food that encourage the predators to remain and reproduce. Green lacewing adults live longer and lay more eggs when provided these sugar sources (24, 27). Consequently, spraying field crops with Wheast powder (an artificial diet) mixed with sugar and water considerably increases egg laying (27). Sugar water simulates aphid honeydew to quickly increase green lacewing visitation. Also, very light/low level pest infestations that make honeydew (i.e., whiteflies, mealybugs, aphids) can be tolerated to induce upwind flights of green lacewings into crop fields. Particularly, pea aphid honeydew and ice plant pollen are excellent egg laying stimulants to some species of green lacewings such as *C. rufilabris* (27). Flowering plants such as sunflower and corn borders provide shelter and dew water to green lacewing larvae and adults on very hot days, while cover crops, weeds, dead leaf litter and bark are important hibernation sites (27). In Germany, growers buy red or brown painted shelters stuffed with straw to attract overwintering lacewings (27). Importantly, to compliment the above deliberate efforts in conserving green lacewings and enhancing their effectiveness, pesticides application during active lacewing stages should be avoided. Commercial supply of artificial foods and honeydew substitutes for green lacewing attraction and rearing to farmers and insectaries further provides a profitable business opportunity in the region.

Under increased aphid prey density, the green lacewing has been noted to produce Holling’s Type II functional response curve; whereby, total prey consumption increases with prey density, though consumption rate per predator decreases. The above pattern was particularly observed by
GeonHwi (12) in larvae and adults of *C. pallens* in response to the densities of *A. gossypii*. Yet, the prey preference of a predator often directly affects the control efficiency of its various prey (37). Shrestha and Enkegaard (32), in a laboratory experiment observed that 3rd instar larvae of *C. carnea* had significant preference for lettuce aphids, *Nasonovia ribisnigri* (Mosley) at two ratios (10 aphids:80 thrips, and 65 aphids:25 thrips), but no preference for the same prey and western flower thrips, *Frankliniella occidentalis* (Pergande) at other test ratios (25 aphids:65 thrips, 45 aphids:45 thrips, and 80 aphids:10 thrips). Though linear relationship between preference index and prey ratio was not significant, the intercept of linear regression was significant, indicating an overall preference of *C. carnea* for lettuce aphids with a value of 0.651. It is imperative to further note that aphid prey consumption differentially affects green lacewing development and survival. Chen and Liu (8), for example, found that though percentage consumption of *Lipaphis erysimi* (Kaltenbach) (23.3%/30.1%) by first and second instar larvae of *C. rufilabris* in the laboratory was significantly higher than those of *A. gossypii* (12.1%/15.7%) and *Myzus persicae* (Sulzer) (11.4%/13.1%). Despite this, all *C. rufilabris* larvae fed the former prey species died prematurely whereas all those fed the latter two prey species developed to adulthood. More still, developmental duration of *C. rufilabris* larvae was observed to be significantly shorter when fed *A. gossypii* (18.0 days) than *M. persicae* (19.2 days).

**CONCLUSION**

Results show that green lacewing larvae in the Sudano-Sahelian Savanna agroecological zone prey upon *A. gossypii* in two fruit vegetables, *C. sativus* and *C. lanatus*, and *A. craccivora* in one leafy vegetable, *A. hybridus*. Abundance of green lacewing larvae and predator-prey ratio in farmers’ fields in this region is extremely low. Control efficiency of green lacewing larvae can be enhanced through sufficient augmentation or releases of the predator, and effective habitat management or environment manipulation. To thoroughly understand the impact of green lacewings in controlling aphid pests in this agroecological zone, further studies are needed to establish the: i) Prey consumption efficiency and functional response pattern of all effective species and stages, ii) Effect of varied prey species consumption on their development and survival, and iii) Effective habitat management that
increases their survival, fitness and predation rates.

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Effect of Mulching on Infestation of Sweet Pepper 
(*Capsicum annuum*) by Whiteflies and Aphids

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Summary

The trial was to study the effect of different mulching materials on whitefly, aphids’ population and agronomic characteristics of sweet pepper (*Capsicum annuum*). Sweet pepper production is constrained by insect pests which causes some diseases. A field experiment was conducted at the vegetable field, National Horticultural Research Institute (NIHORT) (7°24’ N and 7°39’ E longitude 3°50 and 3°52’ N), Idi-Ishin, Ibadan during the 2016 cropping season. The mulching materials used were (1) White plastic mulch (2) Black plastic mulch (3) Yellow plastic mulch (4) Neem leaves (5) Tithonia leaf and (6) Neem leaf + Tithonia leaf (7) Bare soil as control. The experimental field contained twenty one plots using a randomized complete block design in three replications. Parameters measured were disease incidence, severity, whitefly population, aphid population, plant height, stem girth, number of fruits and fruit weight (in grams). The white plastic mulch recorded the least disease incidence (34.43), while bare topsoil plots recorded the peak incidence (100.0). Average severity also followed similar trend as the disease incidence. The reflective white plastic mulch significantly recorded the least severity rate (1.27) as against the bare topsoil (5.32). Whiteflies (*Bemisia tabaci*) population was reduced to (0.33) under the white plastic mulch followed by the neem mulch (1.00) and Neem+tithonia mulch (1.67). Aphids (*Aphis gossypii*) population was reduced by white plastic mulch (0.83). Neem and Neem+ tithonia mulch significantly supported the growth and fruit yield as compared to all other treatments. Data generated in this study have shown that white plastic mulch may be more effective in suppressing pest populations of pepper, but neem mulch may provide better organic manure when decomposed and also as refuge for the natural enemies, it is therefore recommended as an integral option for pest management in pepper production.

Key words: *Pepper, Mulch, Neem, Aphids, Whiteflies.*
INTRODUCTION

PEPPER (*Capsicum annuum* L.) belongs to the family Solanaceae and has always been one of the most important vegetables consumed worldwide, after tomatoes and onions (1). It belongs to the family Solanaceae and was believed to have been introduced by Columbus from the new world (20). Africa’s production of green pepper was 7.70 million tons per year both dry and green fruit from 0.89 million hectares (2). It is one of the most important vegetables grown in most parts of subhumid and semi-arid tropics (2). The species encompasses a wide variety of shapes and sizes of peppers, both mild and hot, ranging from bell peppers to sweet peppers. Sweet pepper may be used fresh or dried as a culinary spice, added to teas, or taken in capsules to reap its many medicinal benefits. These peppers contain high amounts of vitamin C and vitamin A. These vitamins are best obtained from the fresh fruits. Other beneficial bioactive components of the Sweet pepper are the content of flavonoids, alkaloids and tannins (7). Vitamin C has many beneficial effects on the immune system. It helps in repairing damaged brain tissues, reduced risk of oxidative stress, pediatrics asthma, cancer and improved bone health (7). Capsicum works as mucilage when ingested by increasing the production of gastric mucus (21).

*Capsicum annuum* is attacked by many pathogens but heavy losses are caused by viruses. Aphids and whiteflies are by far the most important insects transmitting plant viruses, several viral disease cause mosaic, mottle, leaf curl, leaf roll, bushy stunt and necrosis symptom (26). The leaf curl disease of peppers (chilli) caused by leaf curl virus is transmitted by viruliferous whitefly, *Bemisia tabaci* (26). Surface Mulching either with synthetic or organic waste has been reported to stimulates the microbial activity in soil through improvement of soil agro-physical properties (29). Mulching also minimizes the use of N fertilizer (12), warms the soil and improves the soil physical condition (14,15), and suppresses weed growth (18,10,16,) and could account for increased yield (27, 25,19).

The objective of this work therefore is to determine the effect of different mulch materials on population of aphids and whiteflies, viral incidence and severity on sweet.

MATERIALS AND METHODS

The experiment was carried out at the vegetable experimental field, National Horticultural Research Institute (NIHORT), (7°24”N and 7°39” E longitude 3° 50 and 3°52”N), Idi-Ishin,
Ibadan during the 2016 cropping season. Twenty one beds each measuring 1.5m × 1.5m with 1m between and within rows were made manually with hoe after ploughing and harrowing, each bed representing a plot. The experiment was laid out in a randomized complete block design (RCBD) with three replications. The treatments were randomized in all plots. Arrangement of the treatment can be seen in Table 1. The organic mulch materials were applied at the rate of 3 kg per plot corresponding to 10t/ha, plastic sheet of 1.5m x 1.5m was used as synthetic mulch. Each plot represented different treatment and each treatment was replicated three times.

Agronomic data such as plants height, number of leaf and stem girth were collected fortnightly while, fruit and yield data were recorded as at when due. Five tagged plants were randomly sampled in each treatment for the population of whitefly, *Bemisia tabaci* and aphids using the scale below. Aphid and whitefly population was counted fortnightly on five tagged plants and the population was rated using the following scale of 0-3

0 =None  
1 =Slight less than 50  
2 =Moderate 50-100  
3 =Severe >100.

Some distinct virus symptoms such as mosaic, leaf mottles, curling, malformation, plant stunting and premature fruits abortion were some of the features considered during data collection. Disease incidence and average severity were recorded fortnightly. Disease incidence was estimated using the following formula

\[
\text{Virus incidence (\%) = \frac{T \times 100}{D}}
\]

Where,  
T= Total number of plants in a plot.  
D=Total number of diseased plants in a plot

The Disease severity was assessed using a 1-5 Scoring system (3), where:

1 = Symptomless plants (No visible symptom observed).

2 = Mild symptoms (mosaic, mottle, leaf curl, leaf reduction, puckering on at least 25 % of the total plant leaves).

3 = Moderate symptoms (mosaic, mottle, leaf curl, leaf reduction, puckering on at least 50 % of the total plant leaves)

4 = Severe symptoms (mosaic, mottle, leaf curl, leaf reduction, puckering on the entire plant leaves)

5 = Severe symptoms (mosaic, mottle, leaf curl, leaf reduction, puckering on the entire plant leaves) plus stunting of the plant. The average severity is the average of all scored plants which is a reflection of the damage caused by the viral infection. The average severity
was calculated using the formula described below;

$$\text{Average severity} = \frac{ds_1 + ds_2 + ds_3 + \ldots + ds_n}{Ndn}$$

Where, $ds$ = Severity of diseased plants.

Data collected were subjected to analysis of variance (ANOVA) using Statistical analytical software (SAS). And means separated using least significant difference (LSD) at 5% level of probability.

Plate 1: Different mulching materials (organic and synthetic)

RESULTS

Mulching materials effect on population of aphids (*Aphis gossypii*) and white flies (*Bemisia tabaci*).

Major insect pests observed on the *C. annuum* crop during the study period were aphids (*Aphis gossypii*) mostly located on the under surface of leaves, either singly or in colonies, white flies (*Bemisia tabaci* (Genn.)), and variegated grasshoppers (*Zonocerus variegatus*). Table 2 shows the effect of the different mulching materials on disease incidence, average severity, whiteflies and aphids population. The white plastic mulch recorded the least disease incidence (34.43) and was significantly different from all other mulching materials. Neem plot (50.13) also helped reduced disease incidence and was significantly lower than disease incidence recorded on bare topsoil plots (100.0). Average severity also followed similar trend as the disease incidence, the reflective white plastic mulch significantly recorded lower severity rate (1.27) as against
the bare topsoil (5.32). Whiteflies (Bemisia tabaci) population was significantly reduced to (0.33) under the white plastic mulch followed by the neem mulch (1.00) and Neem+tithonia mulch (1.67). Aphids (Aphis gossypii) population was significantly reduced by white plastic mulch (0.83), the population recorded was significantly different from the population on all other treatments, while the un-mulched plots had more aphid population on the growing pepper plants resulting into higher disease incidence and severity.

**Mulching materials on the growth parameters and yield of C. annuum:**

The following growth parameters (number of leaves, stem girth, number of fruits and fruit weight) were accessed to determine the effect of both synthetic and organic mulch. Data on the mean plant height is presented in Table 3. It revealed that there was significant difference due to various mulch treatments. The maximum mean plant height (18.47 cm) was recorded in treatment 4 (Neem mulch) which was at par with white plastic mulch (16.47 cm) and Neem+ tithonia (16.57 cm) and was significantly different from the other treatments. The least plant height (9.97 cm) was recorded on plots without mulch (Bare topsoil). The mean number of leaves and stem girth (cm) was significantly increased under neem mulch cover (47.33 and 10.50 cm) and was significant over other treatments. The yellow, white and black mulch produced similar number of leaves and was highly significant from the number of leaves produced by plants under no mulch condition (19.00). From the data obtained and trend of the result, there is a significant relationship between higher numbers of leaf (47.33) and the number of fruit (101.17) produced by plants on plots mulched with Neem. The weight of the harvested fruits also significantly vary from each other, the neem mulched plants produced heavier fruits (250 grms). Other treatments such as Neem+tithonia (194.63 grms), tithonia (183.10 grms) and white plastic mulch (188.07 grms) also produced marketable fruits and was significantly different from fruits on control plots (98.90 grms).

**Table 1:** Arrangement of treatments used

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mulch</th>
<th>Variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>Plastic mulch</td>
<td>White</td>
</tr>
<tr>
<td>Two</td>
<td>Plastic mulch</td>
<td>Black</td>
</tr>
<tr>
<td>Three</td>
<td>Plastic mulch</td>
<td>Yellow</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease incidence (%)</th>
<th>Average severity</th>
<th>Whitefly population</th>
<th>Aphids population</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>34.43±3.35</td>
<td>1.27±0.38</td>
<td>0.33±0.58</td>
<td>0.83±0.76</td>
</tr>
<tr>
<td>Black</td>
<td>78.30±2.61</td>
<td>3.52±0.62</td>
<td>3.00±1.00</td>
<td>5.00±1.00</td>
</tr>
<tr>
<td>Yellow</td>
<td>90.33±1.19</td>
<td>4.13±0.36</td>
<td>4.17±0.76</td>
<td>6.83±1.04</td>
</tr>
<tr>
<td>Neem</td>
<td>50.13±4.67</td>
<td>1.73±0.54</td>
<td>1.00±0.04</td>
<td>1.33±0.58</td>
</tr>
<tr>
<td>Tithonia</td>
<td>63.27±3.79</td>
<td>2.26±0.25</td>
<td>2.33±1.04</td>
<td>3.33±0.58</td>
</tr>
<tr>
<td>Neem+tithonia</td>
<td>60.27±1.42</td>
<td>2.09±0.13</td>
<td>1.67±0.58</td>
<td>6.83±0.76</td>
</tr>
<tr>
<td>No mulch</td>
<td>100.00±2.29</td>
<td>5.32±0.48</td>
<td>8.33±1.53</td>
<td>9.67±0.58</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>4.76</td>
<td>0.61</td>
<td>1.32</td>
<td>1.49</td>
</tr>
</tbody>
</table>

Each value represents the mean ± standard deviation

**Table 2:** Effect of different mulching materials on disease incidence, average severity, white fly and aphid population on sweet pepper

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plt.hgt</th>
<th>No.lf</th>
<th>Stm.ght</th>
<th>No.Frt</th>
<th>Wt.Frt</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>16.47±1.90</td>
<td>39.46±1.89</td>
<td>7.60±0.62</td>
<td>6.67±1.53</td>
<td>188.07±1.81</td>
</tr>
<tr>
<td>Black</td>
<td>13.90±0.17</td>
<td>36.17±8.61</td>
<td>6.73±1.50</td>
<td>5.83±0.58</td>
<td>161.74±1.41</td>
</tr>
<tr>
<td>Yellow</td>
<td>13.40±2.15</td>
<td>35.67±4.37</td>
<td>6.73±2.32</td>
<td>4.00±1.00</td>
<td>128.23±2.14</td>
</tr>
<tr>
<td>Neem</td>
<td>18.47±1.91</td>
<td>47.33±9.29</td>
<td>10.50±1.23</td>
<td>101.17±1.26</td>
<td>250.50±2.01</td>
</tr>
<tr>
<td>Tithonia</td>
<td>12.47±2.40</td>
<td>32.17±3.75</td>
<td>6.53±1.21</td>
<td>6.17±0.76</td>
<td>183.10±2.72</td>
</tr>
<tr>
<td>Neem+tithonia</td>
<td>16.57±2.38</td>
<td>43.33±12.21</td>
<td>8.70±1.01</td>
<td>8.33±0.76</td>
<td>194.63±2.51</td>
</tr>
<tr>
<td>No mulch</td>
<td>9.97±1.70</td>
<td>19.00±1.00</td>
<td>5.43±1.00</td>
<td>3.33±0.58</td>
<td>98.90±3.36</td>
</tr>
<tr>
<td>LSD(0.05)</td>
<td>3.20</td>
<td>12.72</td>
<td>2.56</td>
<td>1.81</td>
<td>3.97</td>
</tr>
</tbody>
</table>

Each value represent the mean ± standard deviation NB: Plt.hgt= plant height, No.lf= Number of leaf, Stm.ght= Stem girth, No.Frt= Number of fruit, Wt.Frt= Weight of fruit.
DISCUSSION

This study shows that different types of mulches have different beneficial effects in decreasing aphid and whitefly populations and improving the fruiting response of sweet pepper. That fewer aphids recorded on the white plastic mulch plot compared to other mulches and bare soil agrees with earlier reports on the insect-repellent characteristic of this polyethylene film (11,13, 22,23). Therefore, clear mulch may be useful for sweet pepper production where aphids are a problem because they might induce a direct damage by the feeding behaviour (11) and also, they could be vectors of viruses during the growing season (5,30).

The results obtained in this study reveals that Neem, Neem+tithonia and white plastic mulch increased plant height, foliage and resulting into good yield of sweet pepper after it must have decomposed. This is in agreement with previous findings of Hudu et al. (2002). (4). Low values obtained in white plastic mulch for disease incidence, average severity, whitefly and aphids population could be as a result of the reflective nature of the white plastic mulch. Reflective plastic mulch has been shown to help control whitefly and aphid. This control is due to the fact that reflective plastic mulch reflects ul-traviolet (UV) wavelengths, unlike black or clear plastic mulches. (30) reported that the onset of virus disease symptoms was delayed by 3 to 6 weeks in plants grown over reflective plastic mulch which was critical for normal flowering and fruiting. In addition, reflective plastic mulch delayed and reduced the severity of silverleaf whitefly infestations in squash, pumpkins and cucumber (30).

The neem plant (Azadirachta indica A. Juss), has long been recognized for its properties against insects and flies. They are known to be a natural bio-pesticide with a high rate of insect repellant. (17) observed that azadirachtin operates at the cellular level of insect by disrupting protein synthesis and secretion events and at the molecular level by altering transcription process. This makes them act as bio-pesticides and therefore tends to control insect pests, especially aphids and whiteflies.

The high values observed on the control plot (No mulch) in this study for disease incidence, average severity, whitefly and aphid population justifies the fact that mulches reduce whiteflies and aphids borne viruses. This result agrees with the work of (6) who reported that whitefly and aphids was reduced by 59% when plants are mulched with transparent polythene sheet. (8) reported significant reduction in thrips population and has been attributed it to mulch color effect on the vector, due to a modification of the light environment around the plant.

The reduction in virus disease incidence in plots with neem and neem+tithonia could be responsible for the increased fruit weight and
yield. (24) reported that mulching reduces the deterioration of soil by way of preventing runoff and soil loss, minimizing weed infestation, checks the water evaporation and improve fertility leading to increase yield.

CONCLUSION
This study has explored the possibility that though mulches might control insect pests and increase yield, however the type of material should be taken into consideration. Data generated in this study have shown that white plastic mulch may be more effective in suppressing pest populations of pepper, but neem mulch may provide better organic manure when decomposed and also as refuge for the natural enemies, it is therefore recommended as an integral option for pest management in pepper production. More studies should be conducted to establish the relationship of mulches to insect pest control in general.

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Effect of Soil Solarization on Weed Seed Emergence, Microbial Diversity and Population of an Ultisol in the Forest Zone of Nigeria

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Summary
A field experiment to evaluate the effect of soil solarization on weed seed emergence and microbial composition of soils was conducted in the forest agro-ecological zone of Nigeria. The trial, which comprised three treatments, was laid out in a randomized complete block design with seven replications. Treatment consisted of transparent polythene films (TPF) and black polythene film (BPF), each having a thickness of 0.05 mm and a control without solarization. The solarization materials were installed over each experimental unit based on treatment and held in place with pegs for three months of experimentation. 200 g of composite soil samples were collected at 30 days' interval and taken to the laboratory where serial dilution was done on soil samples to determine microbial load and further sub-culturing was done to isolate and identify microbial species present. A screen house analysis was conducted to determine the effect of solarization duration and type of materials on weed seed emergence. Data were collected on soil minimum and maximum temperatures, soil moisture content, weed, and soil microbial diversity and population. Results showed that soil temperature followed the trend of TPF (38.0) > BPF (37.1) > Control (33.8). Moisture content was comparable for TPF (8.80) and BPF (9.90) but was significantly higher than control (6.90). Weed emergence count was highest in the first month of solarization and then decreased subsequently. The number of emerged weeds was lower for TPF compared to control in the first month of solarization; at other months of measurement the differences were insignificant. There was no significant effect of solarization on soil microbial composition in the three months of solarization. Initial weed count strongly associated with weed count in first month of solarization.
Similarly, weed count in first month of solarization strongly associated with weed count in second month of solarization. However, correlations between moisture content in third month of solarization and minimum and maximum temperatures were strong and positive. Strong association was recorded among temperatures at the various times of measurement.

Keywords: Solarization, weed seed bank, soil microbial diversity, soil microbial population.

Soil solarization is a nonchemical, hydro-thermal technique which alters the micro-climate of soil by capturing and restricting radiant heat energy to the top soil, resulting in higher temperature and relative humidity that when maintained for adequate period of time causes biocidal or biostatic responses in soil biomes (18). Soil solarization has the potential to be an indispensable tool in modern agriculture as it has been shown to control soil inhabiting organisms (fungi, bacteria, and nematodes) that are deleterious to farming activities (20). The advantage of solarization also includes its ability to control weeds (12). These weeds often compete with crops for environmental resources (light, moisture, soil nutrients) and may serve as alternate hosts of plant pathogens. Solarization has also been noted for its plant growth promoting potentials as plants cultivated on solarized soils have been reported to grow faster. This is often attributed to the faster breakdown of organic materials in the soil, releasing nutrients such as nitrogen, calcium and magnesium (10, 21).

The deleterious effects associated with the dependence on the application of synthetic pesticides (11, 19, 14) coupled with the call for organic agriculture by the ‘World Food Summit for All’, 1996, and the International Conference on Agriculture and Food Security, 2007, has necessitated the gradual return to nonchemical methods for the control of pest and weed (9, 4). Thus, this paper reports the effect of soil solarization on weed seed bank and soil microbial composition in an Ultisol.

MATERIALS AND METHODS

Experimental Site

Field experiment, screen house experiment and laboratory analysis were conducted during the months of May to August 2016, at the Experimental Farm of the Department of Crop Science, University of Benin, Benin City (06° 20 ‘E, 5° 39’E; 78 m asl), Nigeria. Benin City is situated in the Rainforest zone of Nigeria. The soils in the study site are under laid by sands, clayey sands and discontinuous clay sequences of Benin formation of
the Niger Delta Basin classified as ultisols (14, 24). This region is characterized by two distinct seasons—
the rainy and the dry season. The rains begin in March/April and end in October/November with a little dry 
spell in August usually referred to as “August Break”. In Benin City, rainfall is of high intensity with an 
average annual temperature of 26.1 °C (7).

**Treatments and Experimental Design**

The trial was laid out based on a randomized complete block design comprising three treatments (black 
polythene, transparent polythene and no polythene of 0.05 mm thickness) which were replicated seven times.

**Bed Preparation**

Study beds were prepared manually using hoe to a dimension of 2.5 m x 2 m with 1 m between each bed. Beds 
were sufficiently irrigated using watering cans to 60% moisture content after which polythene sheets were 
immediately used to cover the soil for the duration of the experiment {90 days} and held in place using pegs.

**Data Collection**

Data was collected on the following: soil temperature, soil moisture content, weed seed emergence, 
microbial diversity and microbial population.

**Soil temperature**

Thermometers calibrated 0 –100 °C were inserted at a depth of 12 cm on each bed. Soil temperature readings 
were taken in the morning (9 am) and evening (4 pm) daily for each bed.

**Moisture content**

Two hundred gram (200 g) of soil samples were initially (immediately after bed were prepared but before 
solarization materials were installed) collected from 5 different spots on a bed from all 21 beds at a depth of 0-12 
cm and bulked. Similar methods were subsequently used to collect soil samples from each treatment plot at 
the end of 30, 60 and 90 days of solarization (DOS). Soil moisture content was determined using the 
difference in initial weight of soil and the weight of soil when constant weight has been attained after drying 
in the oven at 90 °C. Percentage moisture content was estimated following the formula as shown below
(16):

\[
MC = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100
\]

Where: MC =moisture content

Initial weight = weight of soil at start of trial

Final weight = weight of soil at indicated sampling period

**Weed emergence count**

Two hundred (200) gram of homogenized soil was collected at 0,
30, 60, and 90 DOS. Soil samples were put in plastic pots and labeled according to plot and treatment numbers on the field and arranged on a raised platform in the Screen house. Pots were watered daily and monitored for weed seed emergence at 7-day intervals over a four-week period. After the first two weeks these soils were overturned to expose the buried weed seeds and for a new flush of weeds to emerge. Emerged weeds were uprooted after each count was done. Total number of emerged weeds in a plot was recorded.

Microbial diversity and population

Isolation and estimation of microbial population was done by the pour plate and serial dilution method (13). Isolation of fungal and bacterial isolates was done on Potato Dextrose Agar (PDA) and Nutrient Agar (NA), respectively. Inoculated plates were then incubated at 37 °C for 24 hours for nutrient agar and 48–72 hours for potato dextrose agar at 25 °C. After incubation discrete colonies on NA plates and PDA plates were counted and expressed in cfu/g. Bacteria identification was carried out using a series of appropriate biochemical test such as: Gram staining, catalase test, oxidase test, coagulase test, urease test, citrate test, indole test, etc. Fungi identification was carried out by preparing slides from pure culture of isolates and viewing under a light microscope at x40 magnification. Cultural and morphological characteristics exhibited by the various fungi isolates were compared with the description in fungi identification keys (5).

Statistical analysis

Data collected were subjected to analysis of variance using SAS. Means were separated using the least significant difference (LSD) at 5% level of probability. Pearson correlation coefficient was done to determine the relationships among parameters measured.

RESULTS

Effects of soil Solarization on Soil Temperature

There were significant differences in soil minimum and maximum temperatures among treatments at all sampling periods (Table 1). In first, second and third month of solarization, except for minimum soil temperature at third month of solarization when TPF and BPF had similar soil temperature TPF had the highest minimum and maximum temperatures followed by BPF and then control.

Effects of Soil Solarization on Soil Moisture Content

The effects of solarization on soil moisture content are presented in Table 2. In the first month of solarization, moisture content of soil
was comparable across all treatments. In the second month of solarization, the control had the highest moisture content followed by BPF and then TPF; while at the third month of BPF and TPF had similar soil moisture contents but each significantly higher than the control.

**Table 1:** Effects of soil solarization on soil minimum and maximum temperatures at Benin City, 2016 wet season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Temperature (°C)</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M1</td>
<td>M2</td>
</tr>
<tr>
<td>Black polythene</td>
<td></td>
<td>31.4b</td>
<td>30.9b</td>
</tr>
<tr>
<td>Transparent polythene</td>
<td></td>
<td>32.0a</td>
<td>31.6a</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>29.3c</td>
<td>28.7c</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>0.582</td>
<td>0.454</td>
</tr>
</tbody>
</table>

*Within a column, means with the same letter are not significantly different at P=0.05 (LSD*0.05).*

M1=first month of solarization  
M2= second month of solarization  
M3=third month of solarization

**Table 2:** Effects of soil solarization on soil moisture content at Benin City, 2016 wet season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture content (%)</th>
<th>Initial</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black polythene</td>
<td></td>
<td>9.20a</td>
<td>13.50a</td>
<td>8.90b</td>
<td>9.90a</td>
</tr>
<tr>
<td>Transparent polythene</td>
<td></td>
<td>9.20a</td>
<td>11.90a</td>
<td>7.85c</td>
<td>8.80a</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>9.30a</td>
<td>13.10a</td>
<td>10.70a</td>
<td>6.90b</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>0.637</td>
<td>4.771</td>
<td>0.983</td>
<td>1.585</td>
</tr>
</tbody>
</table>

*Within a column, means with the same letter are not significantly different at P=0.05 (LSD*0.05).*

M1= first month of solarization  
M2= second month of solarization  
M3= third month of solarization
Effects of Soil Solarization on weed Emergence Count

Except in the first month of solarization, differences in treatment did not result in significant in weed emergence count at other sampling periods (Table 3). In the first month of solarization, only TPF resulted in significantly lower weed emergence count compared with control. Weed emergence count declined with each increase in duration of soil solarization.

Effects of Solarization on Microbial Diversity and Population

During the period of solarization, 13 fungal species including soil inhabiting species such as Aspergillus spp., Fusarium sp., and Verticillium sp. and Mucor sp. were isolated, and four bacterial species - Azotobacter sp., Pseudomonas sp. Bacillus sp., and Staphylococcus sp. were present (Table 4). While these four bacterial microorganisms were prevalent at all sampling stages in all treatments, with the fungal isolates, all of them occurred in all treatments and sampling stages, however isolates such as Aspergillus tamari, Rhodotorula sp., Candida sp., Cladosporium sp., Verticillium sp. and Aspergillus niger occurred less in second and third month compared to the first. There were no significant differences in soil fungal and bacterial populations due to soil solarization at the different sampling periods. (Table 5).

Correlation among attributes

The relationship among weed seed bank, moisture content, and soil temperature is summarized in Table 6. Initial weed count strongly associated with weed count in first month of solarization. Similarly, weed count in first month of solarization strongly associated with weed count in second month of solarization. Initial moisture content related strongly with moisture content in second month of solarization. Moisture content in second month of solarization had strong and negative correlations with both minimum and maximum temperatures at the various times of measurement. However, correlations between moisture content in third month of solarization and minimum and maximum temperatures were strong and positive. Strong association was recorded among temperatures at the various times of measurement.
### Table 3: Effects of soil solarization on weed emergence count in Benin City, 2016 wet season.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weed emergence count (No./200 g soil)</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black polythene</td>
<td>53.3a</td>
<td>74.1ab</td>
<td>41.9a</td>
<td>33.7a</td>
</tr>
<tr>
<td>Transparent polythene</td>
<td>44.7a</td>
<td>69.4b</td>
<td>36.9a</td>
<td>36.0a</td>
</tr>
<tr>
<td>Control</td>
<td>48.0a</td>
<td>83.0a</td>
<td>44.0a</td>
<td>44.7a</td>
</tr>
<tr>
<td>LSD</td>
<td>13.091</td>
<td>12.977</td>
<td>13.026</td>
<td>15.508</td>
</tr>
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</table>

Means with the same letter are not significantly different at P=0.05 (LSD\(_{0.05}\)).

M1= first month of solarization

M2= second month of solarization

M3= third month of solarization.

### Table 4: Effects of soil solarization on soil microbial diversity at Benin City, 2016 wet season.

<table>
<thead>
<tr>
<th>Type of organism</th>
<th>Isolated microorganism</th>
<th>Month</th>
<th>C</th>
<th>BPF</th>
<th>TPF</th>
<th>C</th>
<th>BPF</th>
<th>TPF</th>
<th>C</th>
<th>BPF</th>
<th>TPF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fungi</td>
<td><em>Saccharomyces</em> sp.</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus tamari</em></td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Rhodotorula</em> sp.</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium chrysogenum</em></td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium</em> sp.</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Candida</em> sp.</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Mucor</em> sp.</td>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td><em>Cladosporium</em> sp.</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td></td>
<td><em>Verticillium</em> sp.</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td><em>Fusarium</em> sp.</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td></td>
<td><em>Cryptococcus</em> sp.</td>
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<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 5: Effects of solarization on microbial population (Cfu/ g).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Month</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M1</td>
<td>M2</td>
<td>M3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fungi</td>
<td>Bacteria</td>
<td>Fungi</td>
<td>Bacteria</td>
</tr>
<tr>
<td>Black polythene</td>
<td>4.5a</td>
<td>8.1a</td>
<td>6.3a</td>
<td>4.6a</td>
</tr>
<tr>
<td>Transparent polythene</td>
<td>3.9a</td>
<td>13.9a</td>
<td>5.8a</td>
<td>5.6a</td>
</tr>
<tr>
<td>Control</td>
<td>4.5a</td>
<td>9.5a</td>
<td>6.7a</td>
<td>4.1a</td>
</tr>
<tr>
<td>LSD</td>
<td>1.27</td>
<td>5.87</td>
<td>2.78</td>
<td>1.79</td>
</tr>
</tbody>
</table>

*Means with the same letter are not significantly different at P=0.05 (LSD0.05)

* Values in Table are expressed in 10^7

M1= first month of solarization, M2= second month of solarization, M3=third month of solarization
Table 6: Correlation among attributes

<table>
<thead>
<tr>
<th>TRIBUTE</th>
<th>IWCI</th>
<th>WC1</th>
<th>WC2</th>
<th>WC3</th>
<th>IMC</th>
<th>MC1</th>
<th>MC2</th>
<th>MC3</th>
<th>TMIN1</th>
<th>TMIN2</th>
<th>TMIN3</th>
<th>TMAX1</th>
<th>TMAX2</th>
<th>TMAX3</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td></td>
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<tr>
<td>WC1</td>
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<tr>
<td>WC2</td>
<td>0.48*</td>
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</tr>
<tr>
<td>WC3</td>
<td>0.17</td>
<td>0.45*</td>
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<td></td>
<td></td>
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<tr>
<td>IMC</td>
<td>0.19</td>
<td>0.01</td>
<td>0.02</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC1</td>
<td>0.22</td>
<td>-0.05</td>
<td>-0.25</td>
<td>-0.10</td>
<td>-0.18</td>
<td>-0.20</td>
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<tr>
<td>MC2</td>
<td>-0.14</td>
<td>-0.40</td>
<td>-0.22</td>
<td>-0.31</td>
<td>-0.07</td>
<td>-0.08</td>
<td>-0.76**</td>
<td>0.50*</td>
<td></td>
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<td>-0.10</td>
<td>-0.11</td>
<td>-0.74**</td>
<td>0.51*</td>
<td>0.97**</td>
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<tr>
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<td>-0.32</td>
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<td>-0.12</td>
<td>-0.07</td>
<td>-0.73**</td>
<td>0.39</td>
<td>0.95**</td>
<td>0.96**</td>
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<td>-0.06</td>
<td>-0.37</td>
<td>-0.22</td>
<td>-0.31</td>
<td>-0.01</td>
<td>-0.10</td>
<td>-0.74**</td>
<td>0.49**</td>
<td>0.97**</td>
<td>0.97**</td>
<td></td>
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<td>TMIN3</td>
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<td>-0.32</td>
<td>-0.34</td>
<td>-0.04</td>
<td>-0.12</td>
<td>-0.69**</td>
<td>0.54*</td>
<td>0.93**</td>
<td>0.97**</td>
<td>0.94**</td>
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<tr>
<td>TMAX1</td>
<td>0.01</td>
<td>-0.22</td>
<td>-0.35</td>
<td>-0.08</td>
<td>-0.10</td>
<td>-0.74**</td>
<td>0.51*</td>
<td>0.96**</td>
<td>0.98**</td>
<td>0.93**</td>
<td>0.99**</td>
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<tr>
<td>TMAX2</td>
<td>0.05</td>
<td>-0.35</td>
<td>-0.22</td>
<td>-0.35</td>
<td>-0.08</td>
<td>-0.74**</td>
<td>0.51*</td>
<td>0.96**</td>
<td>0.98**</td>
<td>0.93**</td>
<td>0.99**</td>
<td>0.97**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMAX3</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

WC1, weed count in first month of solarization; WC2, weed count in second month; WC3, weed count in third month; MC1, moisture content first month; MC2, moisture content in second month; MC3, moisture content in third month; TMIN1, minimum temperature in first month; TMIN2, minimum temperature in second month; TMIN3, minimum temperature in third month; TMAX1, maximum temperature in first month; TMAX2, maximum temperature in second month; TMAX3, maximum temperature in third month.

**DISCUSSION**

TPF solarized plots had higher average temperature than the BPF and control. This may be because of the greenhouse effect created by the TPF where solar irradiation is allowed into soil surface, but the resultant heat is trapped between the top soil and the TFP. In addition, the water vapours that accumulated on the inner surface of the polythene sheets further enhanced the greenhouse effect, resulting in higher soil temperatures (7), based on these, transparent polythene was observed to be is better suited to achieving the necessary temperature increment required for successful solarization exercise. The lower soil temperature recorded in BPF solarized plots may be attributed to the intrinsic nature of black colored objects to absorb solar radiation, thus reducing its ability to heat up the soil. The variation in the soil temperature influenced by the TPF over BPF shows that the colour of solarization material is an important factor for the effectiveness of any solarization exercise. This is confirmed by the findings of other studies (21) where it was concluded that highest soil temperature was obtained under transparent polythene film. Other factors affecting effectiveness of
solarization includes the thickness of materials used. The results from studies conducted by other workers (3) indicated that thinner transparent polyethylene films (1 to 1.5 mm) were more effective in solar heating of soil than thicker film (2 to 6 mm) or black polyethylene film. The use of black polythene as mulch materials may be put to use in ways other than solarization such as to shade out established light-dependent species and suppress seed germination until the seeds naturally degrade in the soil and are unable to germinate.

Solarization did not effectively control weed abundance throughout the study period. This may be due to the increment to sub lethal soil temperature inadequate to destroy the seed bank. This agrees with the findings of studies where it was observed that solarization was not effective at controlling perennial weeds (10). Other research found that while solarization initially resulted in greater plant cover, repeated solarization of a site reduced native and overall plant diversity more than the various herbicide and tilling treatments (17). The study however revealed that TPF has the potential to significantly reduce the number of emerged weeds when compared with control in the first month of solarization).

Solarization did not significantly reduce soil microbial population. This could be because the soil temperature which ranged between 31.6 – 38 °C was within the tolerable temperature limit for most mesophilic soil microbes (23). Considering its low effectiveness, especially in areas with high rainfall and low irradiation, soil solarization have been used in combination with other measures to control soil borne microbes. Researchers have also reported that soil solarization used alongside soil amendment with Clove (Syzygium aromaticum) significantly reduced microbial load of Fusarium oxysporum over an 8-week period (1). Another potential modification of solarization aimed at improving effectiveness is the use of the double-tent technique (6) which can cause an additional 10 °C increment above that of a single sheet. There was highly significant negative correlation between moisture content at the second month of solarization and temperature (minimum and maximum), indicating that a decline in moisture content resulted in upward fluctuation in temperature. However, at the third month of solarization, there was a significant positive correlation between moisture content and soil temperature. This agrees a study (2) where the effect of increasing soil moisture on soil temperature, soil reflectance and soil heat storage were evaluated. The results obtained showed that that energy absorption increases as the moisture content increases, resulting in a higher heat storage capacity at higher moisture content.

**CONCLUSION**

Solarization using TPF and BPF during the months of June to August in a Rainforest zone of Nigeria
increased the mean soil temperature, but the increase did not result in a decline in soil weed seed bank or microbial population as the temperature still fell within tolerable levels. Although there was increase in emerged weeds across treatments in the first month of solarization, the number of emerged weeds was lower in TPF plots compared to control. Further studies of soil solarization in the Rainforest zone of Nigeria should be carried out during the dry and sunny seasons (November – March) so as to delineate best weather conditions for effective soil solarization.

REFERENCES


Infection of Termite Scarified Groundnuts by *Aspergillus* Section *flavi* and Contamination by Aflatoxin

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Summary

Groundnuts are invaded by members of *Aspergillus* section *Flavi* (*Aspergillus flavus*, *A. parasiticus*, *A. tamari*, *A. caelatus* and *A. alliaceus*) of which *A. flavus* and *A. parasiticus* sequester carcinogenic toxins known as aflatoxins. These aflatoxins producing fungi infect groundnut pods/seeds during maturation with the highest fungal population and aflatoxin contamination occurring in damaged seeds. One hundred and fifty six groundnut samples comprising termite scarified and uncompromised pods were collected from farmer’s fields in Zamfara, Katsina and Kano states, Nigeria. Groundnut seeds were found to be infected by *A. flavus* as well as *A. niger*. The highest incidences of *A. flavus* and *A. niger* were seen to occur in damaged seeds obtained from Kano (47%/10%) and Katsina (10%/9%) states. The undamaged seeds had a lower incidence of the fungi in Kano (38%/0%) and Katsina (7%/7%) Aflatoxin B₁ concentrations between the scarified and undamaged seeds were statistically insignificant. Mean aflatoxin B₁ concentration in scarified seeds across the three states was higher (19.65 µg/kg) than in the undamaged seeds (4.21 µg/kg). Ninety two per cent of samples had aflatoxin B₁ concentrations below the 20 µg/kg regulatory limit in Nigeria. The colonization of groundnuts by soil inhabiting aflatoxigenic *A. flavus* is enhanced by termite damage to the pods. This leads to an increase in the synthesis and concentration of the carcinogenic aflatoxin B₁ in infected seeds.

Key words: Groundnuts, *Aspergillus flavus*, Termites, Scarification, Aflatoxin B₁
GROUNDNUT (*Arachis hypogaea* L.) is the sixth most important oil seed crop in the world. It contains 48-50% oil, 26-28% protein, 11-27% carbohydrate, minerals and vitamin (15). Nigeria is the third highest producer of groundnut in the world with a production of 3,413,100 tons after China and India with yields of 16,481,700, 6,557,000 tons respectively in 2014 (7). In Nigeria, the crop is grown throughout the country except for the riverine and swampy areas. Groundnut is either cultivated sole or in mixtures with other crops like maize, sorghum, millet or cassava. The leading producing states are Niger, Kano, Jigawa, Zamfara, Kebbi, Sokoto, Katsina, Kaduna, Adamawa, Yobe, Borno, Taraba, Plateau, Nasarawa, Bauchi, and Gombe States (16). Groundnut has contributed immensely to the development of the Nigerian economy. From 1956 to 1967, groundnut products including cake and oil accounted for about 70% of total Nigeria export earnings, making it the country’s most valuable single export crop ahead of other cash crops like cotton, oil palm, cocoa and rubber (9). Presently, it provides significant sources of cash through the sales of seeds, cakes, oil and haulms (24). Confectionary products such as snack nuts, sauce, flour, groundnut butter and cookies are made from high quality nuts of the crop. In Northern Nigeria, apart from being consumed whole, groundnuts are processed into or included as an ingredient in a wide range of other products which includes groundnut paste which is fried to obtain groundnut cake (*kuli kuli*), salted groundnut (*gyada mai gishiri*), a gruel or porridge made with millet and groundnut (*kunun gyada*), groundnut candy (*kantun gyada*) and groundnut soup (*miyar gyada*) (15).

Groundnuts are invaded by members of *Aspergillus* section Flavi (*Aspergillus flavus*, *A. parasiticus*, *A. tamari*, *A. caelatus* and *A. alliaceus*) of which *A. flavus* and *A. parasiticus* are aflatoxigenic. Aflatoxins are naturally occurring toxins produced by these fungi and there are several types (B1, B2, G1, and G2) produced, of which aflatoxin B1 is the most toxic (21). Soil is a source of primary inoculum for *Aspergillus flavus* and *A. parasiticus*, fungi that produce highly carcinogenic aflatoxins in groundnuts. Aflatoxins have been shown to lead to aflatoxicosis in man and animals (12) which leads to an increase in the incidence of human hepatocellular carcinoma by acting in consonance with hepatitis viruses (1). Aflatoxigenic fungi commonly invade groundnut seeds during maturation, and the highest concentrations of aflatoxins are found in damaged seeds. This damage could be caused by
foraging activities of termites, millipedes and the scarabeid beetle larvae.

We hypothesise that scarification by termites of groundnut pods increases the surface area for infection by *A. flavus* and subsequent accumulation of aflatoxins in the seeds. The current study was conducted to provide a basis for a better understanding of the symbiosis between termite damage and *Aspergillus* sp. colonization of groundnuts as it affects aflatoxin contamination. Mature groundnut samples consisting of both termites scarified and undamaged pod were collected from farmer’s fields across three groundnut growing states in the northwest of Nigeria (Kano, Katsina and Zamfara). *A. flavus* was isolated from the samples and the seeds were subjected to enzyme linked immunosorbent assay to determine concentrations of aflatoxin B₁.

**MATERIALS AND METHODS**

**Sample collection**

Groundnut pod samples were collected from termite infested farmer’s fields Kano and Katsina state during the 2016 harvest season. Groundnut pods were then sorted into scarified and non-scarified portions, shelled and stored separately in paper bags at 4°C for further analysis.

**Fungi isolation and Identification**

One hundred (100) seeds were surface sterilized in 1% sodium hypochlorite for three minutes and rinsed with distilled water three times. Seeds were then plated on Potato Dextrose Agar (supplemented with streptomycin) and isolates belonging to *Aspergillus* section Flavi were isolated. Subcultures were done to obtain pure cultures (3). After 5 days unilluminated at room temperature (27°C - 31°C), isolates were classified on the basis of colony characteristics and conidial morphology. One representative sample from each state so identified, was sent to the Centre for Agriculture and Bioscience International (CABI), Surrey, United Kingdom for confirmation.

**Aflatoxin Quantification**

A blender was used to crust to powder 100 g of groundnut kernels. Twenty grams of this powder was mixed in 100 ml 70% methanol (v/v- 70 ml absolute methanol in 30 ml distilled water) containing 0.5% KCl and the mixture was blended until the mixture was thoroughly homogenized. The extract was transferred to a conical flask, sealed with parafilm and put on a mechanical shaker for 30 minutes at 300 rpm. The mixture was then filtered through a Whatman No. 4 filter paper, and the filtrate was stored at 4°C till needed for analysis.
filtrate was then diluted as appropriate, loaded alongside standards of aflatoxin B₁ and read off an Enzyme Linked Immunosorbent Assay (ELISA) reader. To estimate lower levels of AFB₁ (< 10 µg/kg), prior to ELISA a simple liquid-liquid clean up and concentration procedure was adopted. Twenty milliliter of methanol extract, 10 ml distilled water and 20 ml chloroform were mixed in a separating funnel. After vigorous shaking for one minute, collect the lower chloroform layer was collected and evaporated to near dryness in water bath at 60°C. To the residue 4 ml PBS-Tween containing 7% methanol was added and used for analysis by ELISA.

Data analysis
Analyses were performed with SAS (version 9.1.3, SAS Institute Inc., Cary, NC). Analysis of variance was performed on all data with the general linear model (GLM). Least significant difference (LSD) test was performed to compare treatment means at the 5% level.

RESULTS
Isolation and Identification of Fungi
Aspergillus flavus and Aspergillus niger were the two species isolated and identified from collected samples (Tables 1 and 2). Termite scarified samples showed a higher percentage occurrence of these fungi as compared to the non-scarified samples. Isolates presented with the characteristic dark green colony colouration with the reverse side being hyaline. Microscopic examination revealed the globose, ellipsoid and slightly spherical conidia.

Aflatoxin Quantification
Statistical analysis of Aflatoxin B₁ levels show no significant difference between the termite scarified groundnut pods/seeds and non-scarified obtained from Kano state (Table 3). There also presents no statistical significance in aflatoxin B₁ quantity between the local government areas sampled. There are however clear differences in the mean values between scarified (30.41 µg/kg) and non-scarified (5.76 µg/kg) seeds. Aflatoxin B₁ mean concentrations also differed across the local government areas considered with Dawakin Tofa having the highest (30.60 µg/kg) followed by Dawakin Kudu (12.72 µg/kg) and Minjibir (7.95 µg/kg) having the least (Table 3).

Aflatoxin B₁ levels, under statistical scrutiny (Table 4), show no significant difference between the termite scarified groundnut pods/seeds and un-scarified pods/seeds obtained from Katsina state within and across the two-year sample period. There is no statistical significance in aflatoxin B₁
quantity between the local government areas sampled. There are however clear differences in the mean values between scarified (6.05 µg/kg) and non-scarified (0.67µg/kg) seeds. Aflatoxin B$_1$ mean concentrations also differed across the local government areas considered with Rimi having the highest (6.39µg/kg) followed by Batagarawa (3.44 µg/kg) and Rimi (0.26 µg/kg) having the least (Table 4).

Higher percentage of the groundnut samples (95.2 %) assayed for aflatoxin contamination fell within the Nigerian permissible limit of 20 µg/kg in Katsina state. Kano state shows a higher percentage of samples (90 %) containing the toxin to be below the Nigeria limit. Katsina (82.7%) showed levels lower than the European Union’s set limit of 4 µg/kg with Kano (32%) having the lowest percentage in that range. AFB$_1$ maximum and minimum varied between the states with Kano (438.3 µg/kg and 0.1 µg/kg) showing the highest, followed by Katsina (46.2 % and 0.0 µg/kg) (Table 5).

Table 1: Percentage incidence of *Aspergillus* species associated with groundnut seed samples from Kano and Katsina states.

<table>
<thead>
<tr>
<th>S/N</th>
<th>State</th>
<th>Isolated Fungi</th>
<th>A. <em>flavus</em> (%)</th>
<th>A. <em>niger</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SC</td>
<td>NSC</td>
</tr>
<tr>
<td>1</td>
<td>Kano</td>
<td>47</td>
<td>38</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Katsina</td>
<td>10</td>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>

SC = Scarified seeds  
NSC = Non-scarified seeds

Table 2: Identification of isolates by Centre for Agriculture and Bioscience International (CABI), Surrey, UK

<table>
<thead>
<tr>
<th>S/N</th>
<th>Sample Number</th>
<th>IMI Number</th>
<th>Identification and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>K1</td>
<td>*506178</td>
<td><em>Aspergillus flavus</em></td>
</tr>
</tbody>
</table>

Identification was made by macroscopic and microscopic analysis of subcultures prepared on diagnostic media. This isolate was identified as *Aspergillus flavus*. Morphology of colonies and sporulating material matched species descriptions provided in published taxonomic keys (e.g. Klich M. (2002) Identification of Common *Aspergillus* Species pp 46-47. Central Bureau voor Schimmel cultures,
Identification was made by macroscopic and microscopic analysis of subcultures prepared on diagnostic media. This isolate was identified as *Aspergillus flavus*. Morphology of colonies and sporulating material matched species descriptions provided in published taxonomic keys (e.g. Klich M. (2002) Identification of Common *Aspergillus* Species pp 46-47. Central Bureau voor Schimmel cultures, Utrecht, Netherlands).

Representative samples from each state were positively identified as *Aspergillus flavus*.

NB: K1 – Kano isolate; K2 – Katsina isolate; K3 – Zamfara isolate

**Table 3:** Aflatoxin B₁ quantification of groundnut samples collected in Kano State

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aflatoxin Content (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damage Level</td>
<td></td>
</tr>
<tr>
<td>Scarified</td>
<td>30.41a</td>
</tr>
<tr>
<td>Un-scarified</td>
<td>5.76a</td>
</tr>
<tr>
<td>SE±</td>
<td>14.70</td>
</tr>
<tr>
<td>State (Kano)</td>
<td></td>
</tr>
<tr>
<td>Dawakin Kudu</td>
<td>12.72a</td>
</tr>
<tr>
<td>Dawakin Tofa</td>
<td>30.60a</td>
</tr>
<tr>
<td>Minjibir</td>
<td>7.95a</td>
</tr>
<tr>
<td>SE±</td>
<td>18.0</td>
</tr>
</tbody>
</table>

SE = standard error. Means followed by the same letter(s) within the same column are not statistically different at 5% level of probability.
Table 4: Aflatoxin B\textsubscript{1} quantification of groundnut samples collected in Katsina State

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aflatoxin Content (µg/kg)</th>
<th>Damage Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scarified</td>
<td>6.05a</td>
<td></td>
</tr>
<tr>
<td>Un-scarified</td>
<td>0.67b</td>
<td></td>
</tr>
<tr>
<td>SE±</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>State (Katsina)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Batagarawa</td>
<td>3.44a</td>
<td></td>
</tr>
<tr>
<td>Charanchi</td>
<td>0.26a</td>
<td></td>
</tr>
<tr>
<td>Rimi</td>
<td>6.39a</td>
<td></td>
</tr>
<tr>
<td>SE±</td>
<td>2.08</td>
<td></td>
</tr>
</tbody>
</table>

SE = standard error

Means followed by the same letter(s) within the same column are not statistically different at 5% level of probability.

Table 5: Distribution of aflatoxin contamination (AFB\textsubscript{1}) on groundnut in three North-western states of Nigeria

<table>
<thead>
<tr>
<th>State</th>
<th>Number of Samples</th>
<th>Number of samples with aflatoxin B\textsubscript{1} in range (µg/kg)</th>
<th>Max AFB\textsubscript{1}</th>
<th>Min AFB\textsubscript{1}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>≤ 4 % 4-15 % 15-20 % &gt;20 % (EU) % 4-15 % (EU, Australia, Canada) % 15-20 % (Nigeria, USA) % &gt;20 %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kano</td>
<td>50</td>
<td>16 32</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Katsina</td>
<td>52</td>
<td>43 82.7</td>
<td>4</td>
<td>7.7</td>
</tr>
</tbody>
</table>

DISCUSSION

The incidence of Aspergillus flavus on groundnuts obtained from Kano and Katsina states show that the pre-harvest colonization of groundnuts with the fungus is common. A. flavus is a cosmopolitan filamentous, saprophytic fungus that frequently infects oil-rich seeds of various crop species during pre- and post-harvest periods (5). Although A. flavus is often described as a storage mold, it has been widely accepted that the infection with this fungus occurs in the field (20). Experiments showed a higher incidence of A. flavus on seeds
obtained from termite damaged (scarified) groundnut pods with Kano state showing a higher incidence across the two damage levels (scarified and non-scarified) Research has shown that wounding of pods/seeds leads to an increase in infection by A. flavus (10). The lower incidences of colonization in viable non-scarified (undamaged pods/seeds) gives credence to earlier research showing the importance of damage in the systematic invasion by soil fungal fauna (2). Pre-harvest contamination of groundnuts with aflatoxins produced by colonising A. flavus is a global problem which pertains to human and animal health and food safety. The fungus is known to sequester mycotoxins such as the highly toxic carcinogens known as aflatoxins (B₁, B₂, G₁ and G₂) cyclopiazonic acid and aflatrem which have been implicated in both acute and chronic toxicity (aflatoxicosis) in both animals and humans (14). Aflatoxicosis in humans presents as acute liver damage and cirrhosis, tumorigenesis and teratogenic effects (13).

The detection of Aflatoxin B₁ indicates the toxigenicity of the Aspergillus flavus isolated from groundnut pods irrespective of their damage levels. This assertion finds literary concurrence with Fakruddin et al., (6) who characterized A. flavus isolated from groundnuts as aflatoxigenic producing aflatoxin B₁, Nyirahakizimana et al., (17) who reported the occurrence of A. flavus and the subsequent aflatoxin contamination in raw and roasted groundnuts and Ouattara – Sourabie et al., (18) who examined and showed the aflatoxigenic potential of A. flavus isolated from groundnut seeds using fluorescence and high performance liquid chromatography methods. A. flavus is an opportunistic fungus which produces aflatoxin as a secondary metabolite in the seeds of groundnuts both before and after harvest (11).

Concentration of aflatoxin B₁ determined using enzyme linked immunosorbent assay revealed higher concentrations of detected aflatoxin in seeds from scarified pods than seeds from non-scarified pods across the two states sampled. Statistical analysis shows no significant difference in mean concentration values but direct comparison of means show a marked difference in toxin concentrations between scarified and non-scarified seeds. Secondary metabolites such as tannins, waxes, amino compounds and structural features in the groundnut seed coat have been implicated in resistance to invasion by A. flavus and A. parasiticus (19, 25). Loss or reduction of these structural and biochemical structures due to damage
caused by termites or other soil borne arthropods or nematodes lead to the higher incidences of *A. flavus* and concurrent concentrations of aflatoxin B₁. Increased colonization of damaged groundnuts by *A. flavus* has been documented (23, 10). This research has shown an increase in colonization of damaged groundnut seeds by aflatoxigenic *A. flavus*. The differential quantities of aflatoxin B₁ between scarified and non-scarified seeds correlates with the recorded increased infection by *A. flavus* in these seeds.

Ninety per cent of samples assayed from Kano state and 96.2 % of samples assayed from Katsina state showed concentrations of aflatoxin B₁ less than or equal to 20 µg/kg. These satisfy the Nigerian regulatory limit of 20 µg/kg (22). Due to the toxic and carcinogenic properties of aflatoxins, only extremely low levels of aflatoxins in foods and feeds is allowed (8). Many countries have enforced or recommended aflatoxin permissible limits for food produce/products which range from zero detectable to 50 µg/kg (13). Countries have multiple limits depending on the utilization of the produce, the stringent limits pertaining to human consumption and exports, and the highest to commercial products. These restrictions result in a increased cost of meeting the standards – including cost of testing, rejection of shipments and even eventual loss of admissibility into foreign markets (4).

Good agricultural practices at both pre-and post-harvest stages of groundnut including storage are available to prevent aflatoxin build up in groundnut. Management of termites and other soil borne arthropods in groundnut fields will reduce the incidence of *A. flavus* and aflatoxin contamination.

**CONCLUSION**

*Aspergillus flavus* colonizes groundnut seeds with increased populations isolated from seeds that were damaged by termites. The *A. flavus* specie isolated was shown to be aflatoxigenic as evident in the detection of aflatoxin B₁ in all groundnut samples analysed. The damage of seeds not only leads to an increase in population densities of *A. flavus* but also the concurrent increase in aflatoxin B₁. Aflatoxin B₁ concentrations levels in groundnut seeds at or immediately after harvest is lower than the regulatory standards of Nigeria of 20 µg/kg. Although these levels are permissible, increase in concentrations may occur in storage if adequate control of the fungus is not ensured.
REFERENCES


In Vitro Evaluation of Selected Fungicides Against Curvularia geniculata on Digitaria iburua Kippist Stapf in Riyom, Plateau State

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Summary

Five fungicides at varying concentrations (0 x; 0.5x; 1x and 1.5x where x is manufacturer’s recommended rate) were evaluated in vitro, against Curvularia geniculata isolated from Fonio. Seven days old culture was placed on Petri dishes containing Potato Dextrose (PDAs) amended with fungicides at varying rates and mycelial growth measured at two days intervals starting from 2 days to 12 days after inoculation (DAI). On the 14 DAI, conidia were counted using a haemocytometer. The inhibitory effect on mycelial growth and sporulation significantly varied amongst the fungicides and rates with the highest effect recorded with Mancozeb while the least was from Thiomethoxan+Metalaxyl-M+ Difenoconazole although it was not different from Carbendazim, Benomyl and Carbendazim+ Mancozeb.

Keyword: Curvularia geniculata, Fonio, Fungicides, in Vitro, Mycelia, Conidia, Inhibition

FONIO or hungry rice (Digitaria exilis and Digitaria iburua), belongs to the family Poaceae (25; 13). Fonio is short seasoned, fast growing and thrives in marginal sandy and rocky soils of the Sahel with possibility of two or three crops per year hence serving as "gap crop" during months of low food supply (12). Fonio, locally named acha is tolerant to drought and flood; and mostly grown in the central and northern states of Nigeria such as Bauchi, Kaduna, Kebbi, Nassarawa and Plateau States (3). Among the cereals, fonio is the most nutritious with its grain
containing 7% crude protein, 9.8% leucine, 5.6% methionine and 5.8% valine more than other cereals like sorghum, rice, wheat or barley (14; 28; 29). Belton and John (4) also reported fonio to be richer in magnesium, zinc, iron, manganese, Calcium, phosphorus, thiamine, riboflavin and lower in glycemic index than other cereals. The methionine and cysteine in fonio are suppliers of sulphur, amino acid and other compounds required for normal body metabolism and growth (7; 4). The world three largest Fonio (acha) producers are Guinea, Nigeria and Mali with production of 429 000, 165 000 and 45 000 t from 200 000, 90 000 and 26 000 ha, respectively (8). Fonio which is traditionally consumed as stiff or thin porridge, couscous, or mixed with other flours to make bread also has medicinal values particularly in the management of diabetes and new lactating mothers (13; 1). Although both fonio species have shown low susceptibility to insect pests and diseases (29;16), the fungi Phyllachorasphearosperma, Puccinia cahuensis and Helminthosporium sphave been reported on the crop in Benin republic (11). Similarly, Helminthosporim sp., Fusarium sp. and Curvularia sp. have been reported on Digitaria iburua on NCRI Research farm Riyom (18; 20). High incidence of Brown leaf spot disease was also reported on Digitaria iburua and D. exilis at Badeggi (17). The symptoms of Brown leaf Spot caused by Curvularia geniculata on fonio are brown irregular lesions about 0.01 mm – 0.5 mm on the leaf lamina and leaf sheath, it later coalesces to form large lesions resulting into blight on the leaf margins and wilting of leaf tips (Plate 1 and 2). However there is no documented research works conducted on the control or management of brown leaf spot of fonio in Nigeria. In view of this, the choice of synthetic chemicals over other alternatives was preferred because chemicals are effective and provides a cheaper and reliable source for control of plant pathogenic fungi (27). Many in vitro studies have demonstrated that some fungicides restrict or prevent the growth of fungal pathogens (15; 21). In view of the nutritional importance of the crop and the effect of fungal diseases on the crop, there is a need to identify management options for disease associated with this important and relatively new crop. The aim of the study was to determine the in vitro effects of selected fungicides on the mycelial growth and sporulation of Curvularia geniculata isolated from fonio.
MATERIALS AND METHODS
The experiment was conducted at the Mycopathology laboratory of Crop Protection Department Ahmadu Bello University, Zaria in 2015. Diseased fonio leaves were brought to the laboratory washed and sterilized using 5% sodium hypochlorite and rinsed three times with sterile distilled water. Leaf samples were placed on Petri dishes with Potato Dextrose Agar amended with Streptomycin. Petri dishes were placed in an incubator at 28°C and observed for mycelia growth. Five fungicides (Table 1) were evaluated at four concentrations (1.5 x, 1.0 x, 0.5 x, and 0x where x is manufacturer’s recommended formulation rate). The required quantities of fungicides were weighed separately, dissolved in 5 ml of ethanol, made up to 100 ml with freshly prepared Potato Dextrose Agar with Streptomycin (PDAs) cooled to 45°C and dispensed into 9 cm diameter Petri dishes at 20ml/plate rate. Upon solidification (24 hours after pouring), using a 0.5 cm cork borer, 7 day old Curvularia geniculata pure culture was inoculated centrally in the labeled Petri dishes laid in completely randomized design (CRD) with each fungicide making a treatment replicated five times (a Petri dish representing a replicate) and incubated at 28°C ± 2°C. Radial mycelial growth was measured at two days interval (2, 4, 6, 8, 10 and 12 days after inoculation (DAI)) along two perpendicular lines on each petri dish underside. At 14 DAI, for each treatment, a stock conidial suspension was prepared by bulking its 5 petri dishes from which 10 ml was pipette into 90 ml. With the aid of a haemocytometer, conidia were counted four times for each treatment and using the formula of Booth (5) below, total conidia/ml was obtained.

\[
\text{Conidia/ml} = \frac{n}{256} \times 4 \times 10^6
\]

Where: \( n \) = number of conidia counted in the chambers, 256 = constant volume obtained from 16x16, 4x10^6 = constant. The Experiment was repeated twice.

Data collected were subjected to statistical analysis using (25) and means that were significant were compared using the Student Newman Keuls (SNK) test at 5 % level of significance.

RESULTS
The effect of varying rates of fungicides on the mycelial growth and sporulation were highly significant (P≤0.01) (Table 2, Plates 1, 2 and 3). Thiomethoxan + Metalaxyl-M + Difenconazole (Apron plus) treated plates recorded significantly the highest mycelial growth (2, 4, 6, 8, 10 & 12 DAI), and there were no
significant differences among the rates as the days of inoculation increases. Followed by Carbendazim (Forcelet), Benomyl (Benomy), Carbendazim + Mancozeb (Funguforce) and mancozeb (Z Force), respectively except at 2 and 4 DAI, Funguforce and Z Force did not differ significantly (Table 2). Mancozeb which had the highest inhibitory effect on mycelial growth also induced significant inhibitory effect on sporulation in all the DAI compared to all other tested fungicides which did not differ from each other (Table 2). The mycelial growth and sporulation were significantly decreasing with increase in fungicide rate, which was highest at 0 x and least at 1.5x concentration (Table 2).

Fungicides x rates (F x R) interaction was highly significant for mycelial growth at all the DAI and sporulation (Table 3). The inhibitory effect of each fungicide was significantly increased with increase in the concentration/ rate with 1.5x recording the least conidia count and mycelial growth all through although by 8DAI, the 0 x treated plates and those treated with Thiomethoxan + Metalaxyl-M + Difenonazolole (Apron plus) at all tested rates were statistically not different from each other as well as at 10 and 12 DAI (Table 3). Similarly Carbendazim at 0 x and 0.5 x was not significantly different from Thiomethoxan + Metalaxyl-M + Difenonazolole. Mancozeb (Z Force) which induced highest inhibitory effect on this pathogen recorded least mycelia growth at all tested rate although it was statistically similar with Carbendazim + Mancozeb (Funguforce) at all rates at 2 and 4 DAI. Z Force also completely inhibited sporulation at all rates (0.5x, 1.0 & 1.5 x).
Table 1: Fungicides evaluated *in vitro* against *Curvularia geniculata*

<table>
<thead>
<tr>
<th>Trade name</th>
<th>Active ingredient</th>
<th>Formulation</th>
<th>Manufacturer</th>
<th>Recommended rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apron Plus</td>
<td>20% Thiomethoxan + 20% Metalaxyl-M + 2% Difenoconazole</td>
<td>42% SD</td>
<td>Sygenta</td>
<td>10g /4kg seed</td>
</tr>
<tr>
<td>Forcelet</td>
<td>50% Carbendazim</td>
<td>50% WP</td>
<td>Jubaili</td>
<td>1.5 Kg / 200L / ha</td>
</tr>
<tr>
<td>Benomy</td>
<td>50% Benomyl</td>
<td>50% WP</td>
<td>Jubaili</td>
<td>2.5 Kg / 400L / ha</td>
</tr>
<tr>
<td>Z Force</td>
<td>80% Mancozeb</td>
<td>65% WP</td>
<td>Jubaili</td>
<td>2.5 Kg / 400L / ha</td>
</tr>
<tr>
<td>Funguforce</td>
<td>12% Carbendazim + 53% Mancozeb</td>
<td>65% WP</td>
<td>Jubaili</td>
<td>2.5 Kg / 400L / ha</td>
</tr>
</tbody>
</table>

Table 2: Effects of varying fungicides rates on mycelial growth and conidia sporulation of *Curvularia geniculata*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mycelial growth at days after inoculation (DAI)/ cm</th>
<th>Conidia count (4x10^6)/mls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trade name</strong></td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Thiomethoxan + Metalaxyl-M + Difenoconazole</td>
<td>2.26a</td>
<td>4.35a</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>1.92b</td>
<td>3.72b</td>
</tr>
<tr>
<td>Benomyl</td>
<td>1.24c</td>
<td>2.22c</td>
</tr>
<tr>
<td>Mancozeb</td>
<td>1.00d</td>
<td>1.68d</td>
</tr>
<tr>
<td>Carbendazim+ Mancozeb</td>
<td>1.06d</td>
<td>1.68d</td>
</tr>
<tr>
<td><strong>E±</strong></td>
<td>0.030</td>
<td>0.118</td>
</tr>
<tr>
<td>NK</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Rate (R)</td>
<td>2.80a</td>
<td>5.26a</td>
</tr>
<tr>
<td>.5</td>
<td>1.30b</td>
<td>2.06b</td>
</tr>
<tr>
<td>.0</td>
<td>1.06c</td>
<td>1.78b</td>
</tr>
<tr>
<td>.5</td>
<td>0.92d</td>
<td>1.82b</td>
</tr>
<tr>
<td><strong>E±</strong></td>
<td>0.027</td>
<td>0.106</td>
</tr>
<tr>
<td>NK</td>
<td>**</td>
<td>**</td>
</tr>
<tr>
<td>Interaction (F x R)</td>
<td>**</td>
<td>**</td>
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</tbody>
</table>
Table 3: Fungicide x Rate interaction effect on mycelial growth of *Curvularia geniculata*

<table>
<thead>
<tr>
<th>Fungicides</th>
<th>Rates</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiomethoxan + Metalaxyl-M + Difenconazole</td>
<td>0</td>
<td>2.83ab</td>
<td>5.36a</td>
<td>8.05a</td>
<td>9.00a</td>
<td>9.00a</td>
<td>9.00a</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>2.11de</td>
<td>3.49de</td>
<td>7.34b</td>
<td>9.00a</td>
<td>9.00a</td>
<td>9.00a</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>2.14cd</td>
<td>3.96de</td>
<td>7.28b</td>
<td>9.00a</td>
<td>9.00a</td>
<td>9.00a</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>1.95e</td>
<td>4.57bc</td>
<td>7.32b</td>
<td>9.00a</td>
<td>9.00a</td>
<td>9.00a</td>
</tr>
<tr>
<td>Benomyl</td>
<td>0</td>
<td>2.69b</td>
<td>5.25a</td>
<td>7.97a</td>
<td>9.00a</td>
<td>9.00a</td>
<td>9.00a</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1.11f</td>
<td>1.74g</td>
<td>2.97e</td>
<td>4.38c</td>
<td>6.20c</td>
<td>6.38c</td>
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<tr>
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<td>0.67g</td>
<td>1.41g</td>
<td>1.65f</td>
<td>2.55e</td>
<td>4.11e</td>
<td>4.25e</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0.50g</td>
<td>0.50h</td>
<td>1.10g</td>
<td>1.94f</td>
<td>3.21g</td>
<td>3.45g</td>
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<tr>
<td>Carbendazim</td>
<td>0</td>
<td>2.80ab</td>
<td>5.23a</td>
<td>7.98a</td>
<td>9.00a</td>
<td>9.00a</td>
<td>9.00a</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>2.29cd</td>
<td>4.08cd</td>
<td>6.05c</td>
<td>6.78b</td>
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<td>9.00a</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.48e</td>
<td>2.52fg</td>
<td>3.58d</td>
<td>4.46c</td>
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<td>8.60b</td>
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<tr>
<td></td>
<td>1.5</td>
<td>1.14f</td>
<td>3.06ef</td>
<td>3.02e</td>
<td>3.52d</td>
<td>5.87d</td>
<td>5.75d</td>
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<tr>
<td>Mancozeb</td>
<td>0</td>
<td>2.74abc</td>
<td>5.25a</td>
<td>7.92a</td>
<td>9.00a</td>
<td>9.00a</td>
<td>9.00a</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.50g</td>
<td>0.50h</td>
<td>0.50i</td>
<td>0.50h</td>
<td>0.50j</td>
<td>0.50j</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.50g</td>
<td>0.50h</td>
<td>0.50i</td>
<td>0.50h</td>
<td>0.50j</td>
<td>0.50j</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0.50g</td>
<td>0.50h</td>
<td>0.50i</td>
<td>0.50h</td>
<td>0.50j</td>
<td>0.50j</td>
</tr>
<tr>
<td>Carbendazim + Mancozeb</td>
<td>0</td>
<td>2.93a</td>
<td>5.24a</td>
<td>7.87a</td>
<td>9.00a</td>
<td>9.00a</td>
<td>9.00a</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.50g</td>
<td>0.50h</td>
<td>1.27g</td>
<td>1.95f</td>
<td>3.67f</td>
<td>3.87f</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.50g</td>
<td>0.50h</td>
<td>0.72h</td>
<td>1.15g</td>
<td>1.90h</td>
<td>2.17h</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>0.50g</td>
<td>0.50h</td>
<td>0.54i</td>
<td>1.10g</td>
<td>1.36i</td>
<td>1.58i</td>
</tr>
<tr>
<td>SE±</td>
<td>0.061</td>
<td>0.237</td>
<td>0.065</td>
<td>0.084</td>
<td>0.049</td>
<td>0.045</td>
<td>1.350</td>
</tr>
<tr>
<td>SNK</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>
Plate 1: Mycelial growth of *Curvularia geniculata* on different fungicides at 0.5 x mg a.i/ mls at 7 days after inoculation (DAI)

**KEY:**

- x- Manufacturers Recommended Rate
- A- Carbendazim + Mancozeb
- B- Mancozeb
- C- Carbendazim
- D- Thiomethoxan + Metalaxyl-M + Difenoconazole
- E- Benomyl
- F- Control
Plate 2: Mycelial growth of *Curvularia geniculata* at 1.0x mg a.i / mls fungicide at 7 days after inoculation (DAI)

**KEY:**

x- Manufacturers Recommended Rate  
A - Carbendazim + Mancozeb  
B - Mancozeb  
C- Carbendazim  
D- Thiomethoxan + Metalaxyl-M + Difenconazole  
E- Benomyl  
F- Control
Plate 3: Mycelial growth of *Curvularia geniculata* on different fungicides at 1.5 x mg a.i/ mls at 7 days after inoculation

**KEY:**

x- Manufacturers Recommended Rate  
A- Carbendazim + Mancozeb  
B- Mancozeb  
C- Carbendazim  
D- Thiomethoxan + Metalaxyl-M + Difenoconazole  
E- Benomyl  
F- Control
DISCUSSION

In this study, all tested fungicides significantly reduced pathogen development except Thiometoxan + Metalaxyl-M + Difenoconazole with Mancozeb being the best. The high efficacy of Mancozeb, Carbendazim and Benomyl, was reported on Physoderma maydis, causal organism of maize brown spot where sporangia germination was completely inhibited (23). Similarly, Benomyl at 5, 10 and 20 g was effective in controlling Stenocarpella maydis on maize in vitro (21). Also Gholve et al. (10) reported Mancozeb at 500, 1000 and 1500 ppm was effective in controlling the mycelia growth of Alternaria macrospora on cotton. In a similar report, Mancozeb at 1000, 1500, 2000, 2500, 3000 and 3500 ppm completely inhibited the growth of Alternaria solanii irrespective of the concentration (10). Mancozeb also gave a significant reduction in sporulation of Alternaria solanii on Apple irrespective of concentration (9). Mancozeb also gave a significant reduction in sporulation (27; 30; 31). Obagwu (23) reported Mancozeb to be effective in the in vitro control of brown blotch of Bambaranut caused by Colletotrichum capsici. In this study the poor inhibitory effect of Thiometoxan + Metalaxyl-M + Difenoconazole (Apron Plus) on mycelial growth and sporulation was observed. Report has shown Metalaxyl could not inhibit the mycelial growth of some isolates of Phytophthora infestans of Potato in Serbia (7). Also Metalaxyl could not inhibit the mycelial growth of Bremia lactucae downy mildew on Lettuce cv Salinas (31). In this experiment Carbendazim + Mancozeb gave better inhibitory effect on mycelial growth than Carbendazim alone. This is in line with Mamza et al. (18; 19; 20) who reported the inhibitory effect of Carbendazim and Mancozeb on mycelial growth and sporulation of Fusarium pallidoroseum on Castor. Similarly, Alberto, et al. (2) conducted an experiment on Collectotrichum gleosporiodes (Penz.) where Carbendazim gave a significant reduction in mycelial growth. Meanwhile, in another experiment Carbendazim + Mancozeb, Mancozeb, Carbendazim, Ziram, Metalaxyl + Mancozeb on Alternaria leaf blight of sunflower, reduced mycelial growth of A. helianth at higher concentrations (500 and 1000 ppm) as compared to untreated however, Carbendazim was found to be more effective (22).

CONCLUSION

This study indicated the suppressive effect of various fungicides on mycelial growth and conidia count of Curvularia geniculata on fonio. The inhibitory effect varies with various
fungicides. However, the inhibition in mycelial growth increased with the increase in the concentration of different fungicides. All fungicides tested effectively reduced pathogen development when compared with the control with the exception of Thiomethoxan + Metalyxl-M + Difenconozole (Apron Plus) which could not inhibit mycelial growth and conidia sporulation of Curvularia geniculata at all concentrations, while Mancozeb at all rates was highly effective. Meanwhile this is the first time fungicides is being evaluated on Curvularia geniculata isolated from fonio in Riyom, there is a need to further evaluate the fungicides under field conditions for effective management of the pathogen.

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