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EFFECT OF DIFFERENT STORAGE MATERIALS AND CONDITIONS ON SEED-BORNE FUNGI ASSOCIATED WITH COWPEA, VIGNA UNGUICULATA L. WALP

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SUMMARY
The effect of different storage materials and conditions on seed-borne fungi associated with cowpea, Vigna unguiculata L. Walp was evaluated in this study. Ife brown variety of cowpea seeds of 10g were weighed into five different storage materials: jute bag, envelope, polythene bag, muslin cloth and rubber plastic. These were stored in Refrigerator and ambient temperature; subjected to 4°C and 27 ± 2°C respectively, and taken for seed borne fungi identification at 1, 2, 3, 4, 5 and 6 months. The effect of seed-borne fungi on viability of the seeds was determined. The experiment was laid out with 3 replicates in 2 x 5 factorial in Completely Randomized Design (CRD). Data collected were subjected to analysis of variance using SAS package and means separation by Duncan Multiple Range Test. It was observed that seeds stored in refrigerating had significantly higher germination compared to ambient condition, while incidence of fungi increase as period of storage increases in ambient condition compared to refrigerating condition that decrease as the period of storage increases irrespective of the materials. The results showed that the storage materials and conditions influenced viability and seed-borne fungi in cowpea seed. The most effective among the storage materials is polythene bag at 4°C. Cowpea seeds were associated with many fungi in ambient condition which led to reduction in seed viability. It is therefore important to store cowpea seeds in polythene bag in a
refrigerating condition in order to maintain the seed viability and prevent establishment of seed-borne fungi on cowpea seeds.

**Keywords:** fungi, cowpea, storage conditions, storage materials, seed viability

**COWPEA** (*Vigna unguiculata* (L.) Walp) commonly known as bean in Nigeria, is an herbaceous plant belonging to the Leguminosae. Cowpea is the most important legume in the diet of the population and also the cheapest source of protein to most of the people in Nigeria. It provides a rich source of proteins and calories as well as minerals and vitamins. A cowpea seed consists of 25% protein and is low in anti-nutritional factor (18).

One major limitation to cowpea is disease attack; cowpea suffers most from diseases from its vegetative phase of its growth to its harvesting period and in storage. Cowpea production in Nigeria is constrained to a large extent by infection of a wide range of diseases. These diseases of cowpea induced by viruses, bacteria, fungi, nematodes and adverse environmental conditions have brought about the use of pesticides usually in large quantities in an effort to combat them and preserve the cowpea seed for future purposes. The cost of these pesticides is making cowpea production prohibitive.

Among the numerous pathogens affecting cowpea, fungi are the most numerous. The field fungi of cowpea invade seeds and the various parts of the plants on the field. High moisture in seeds which usually occurs as a result of wet weather before or during harvesting, lack of drying facilities and improper transportation and storage aids the proliferation of mycoflora and leads to deterioration of the seeds. However, it is observed that storage fungi affect the seeds in storage even under a very low moisture content compared with the field fungi which required high moisture content in seed. Field fungi of cowpea are *Alternaria* species, *Fusarium* species, *Aspergillus* species and *Penicillium* species. The major seed borne fungi of cowpea are *Aspergillus candidus*, *Aspergillus flavus*, *Aspergillus tamari*, *Aspergillus niger* and *Aspergillus fumigatum* (16).

Seed-borne pathogens of cowpea may be carried either inside the seed in form of mycelia, on the surface of seeds in forms of spores or mixed with seeds as modified structure such as Sclerotia or as contaminants (2). However, their role is either
unknown or significant affecting germination of seedlings (15). Seed-borne fungi pathogens may cause damaged such as seed abortion, shrunken, seed rot, sclerotisation, seed necrosis, seed discolouration and reduced germination and vigour, failure to control such diseases may result in low yield (22). Shetty (20) reported that seed borne fungi such species of *Fusarium*, *Aspergillus*, *Penicillium* and *Diplodia* cause seed decay, discolouration and reduction of seed germinability. In Nigeria, the fungi deterioration of cowpea stored under different storage materials and various conditions were assessed and it was found that *Fusarium* species and *Aspergillus* species was the most frequent and prevalent mould (16).

Cowpeas’ susceptibility to diseases from its vegetative phase to storage exposes the seeds to various infections by fungi pathogens and this resultantly affect the viability of the seeds. Storage materials and conditions are major determinants of the safe keeping of seeds for any planting season and for consumption. Different storage materials and conditions which include room temperature, cold room and refrigerator have been used to determine the longevity of cowpea seeds without spoilage and disease infection (13). Some species of fungi which include *Fusarium* spp, *Aspergillus* spp, *Penicillium* spp and *Drechslera* spp are able to survive in infected cowpea seed in storage and maintain its viability for over five years (10).

Therefore, the identification of the appropriate storage materials and conditions shall provide information on storage systems for high viability and prevention of the growth of seed-borne fungi associated with cowpea. The objective of this study was to determine the effects of different storage materials and different storage conditions on seed-borne pathogens of cowpea seeds. Also, to determine the viability of seeds packed in these storage materials and conditions.

**MATERIALS AND METHODS**

Ife brown variety of cowpea seeds which were harvested a month prior to the time of this study; were collected from the Seed store of Institute of Agricultural, Research and Training, Moor Plantation, Ibadan, Nigeria. The experiment was carried out at the Pathology laboratory of the Institute. Prior to storing the seeds in the different materials and conditions; Potato dextrose agar (PDA) of 9.8g was weighed into a conical flask containing 250ml sterile distilled water, then sterilized for 15 minutes
at 1.05 kg/cm² (121°C) in an autoclave.

A 0.3 g of streptomycin was added to the cooling PDA and then poured into Petri dishes. Seeds were surface sterilized and 10 seeds (with average weight of 2.4g) were placed each into the four PDA Petri dishes, to determine the mycoflora on the seeds before storage. These were incubated for 4 days for the growth of seed-borne fungi. These organisms were then sub-cultured to get pure cultures of the organism. The slides were prepared for identification of the organisms.

Germination percentage = \[
\frac{\text{Total number of seedling that emergence}}{\text{Total number of seeds}} \times 100
\]

Ife brown cowpea seeds of 10 g each were weighed into five different storage materials which include: Jute bag, Envelope, Polythene bag, Muslin cloth and Rubber plastics. These were stored on the Cold room shelf (4°C) and at ambient (room) temperature (27 ± 2°C). These were replicated three times. These were stored individually in the materials and under the two conditions for 1, 2, 3, 4, 5 and 6 months (i.e. for a storage condition, each material was used to pack differently for the 6 months and replicated three times). The packed seeds in the refrigerator and at ambient temperature were stored and taken for seed borne fungi isolation and identification as well as viability test at 1, 2, 3, 4, 5 and 6 months.

**Detection of Seed-Borne Fungi**

Ife brown cowpea seed of 10g were removed from each of the storage materials at the two storage conditions (refrigerator and ambient temperature). The seeds were surface- sterilized in 0.5% NaOCl for 5 minutes, rinsed in 3 changes of sterile distilled water, towel dried and plated on freshly prepared PDA amended with streptomycin (of 0.3g/L) in 9 cm Petri dishes, to prevent bacterial growth and incubated for 5 days. Sub-culturing
was done to get pure culture of seed borne fungi which were examined microscopically and identified using a fungi compendium as a guide (Barnett and Hunter, 1998).

**Statistical Collection and Analysis**

Data were collected include: germination count, isolate(s) identification, fungi colony were collected. The experiment was laid out in three replicates in a 2 x 5 factorial in Completely Randomized Design (CRD). The data collected were subjected to analysis of variance using SAS package (2001), and the means separation was done using Duncan Multiple Range Test.

**RESULTS**

**Occurrence of Some Fungi Found in Cowpea Seeds Stored in Different Storage Materials and Conditions**

It was observed that *Aspergillus* occurred in both storage conditions, but most frequently in ambient condition when compared with the refrigerating condition. The seeds stored in muslin cloth and jute bag had differences species of *Aspergillus* which include *Aspergillus flavus, Aspergillus niger, Aspergillus tamari, Aspergillus candidus, Aspergillus parasiticus and Aspergillus terreus*. While fungi observed in seeds stored in refrigerating conditions have *Aspergillus* species but not as much as in that of ambient condition. The other seed-borne found were *Alternaria* spp., *Fusarium* spp., *Tighemiomycetes*. It was also observed that the occurrence of fungi in cowpea seeds at refrigerating condition were not as much as that of ambient condition. Table 1 shows the list of seed-borne fungi isolated from cowpea seeds stored with polythene bag, jute bag, muslin cloth, rubber can and envelope at ambient and refrigerating conditions after 6 months of storage.
Table 1: Fungi present in cowpea seeds stored using different storage materials and conditions at 6 months after storage

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Refrigerating condition</th>
<th>Ambient condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P.bag</td>
<td>J.bag</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>A. niger</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. parasticus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A. tamari</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A. candidus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A. terreus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alternaria spp</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>F. equiseti</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F. semitectum</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F. solani</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tiekheimomycetes</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Absence of organisms = -, presence of organisms = +, P. bag = Polythene bag, J. bag = Jute bag, R. can = Rubber can, Enve = Envelope, M. cloth = muslin cloth

Incidence of Fungi in the Different Storage Materials Under the Ambient and Refrigerating Condition

The percentage incidence of fungi in the control was more than when the two storage conditions were used. Aspergillus species was found to occur most in the control with 45.5% incidence. Several fungi such as Alternaria species, Fusarium species, Thallospora and Tiekheimomycetes (Table 2) were identified in the cowpea seeds at different incidences. The presence of these organisms indicated that cowpea seeds are vulnerable to many infections which reduces the seeds quality thereby affecting the germinability of the seeds. The percentage of Aspergillus species, Fusarium species, Alternaria species and other seed-borne fungi constantly increase and became major contaminants on the seeds in
control and ambient condition while the percentage incidence of fungi on seeds at refrigerating condition decreases. Therefore, the distribution of isolated organism from cowpea seeds depend on the storage material.

Table 2: Incidence (%) of Fungi in the different storage materials at ambient and refrigerating condition

<table>
<thead>
<tr>
<th>Fungi isolated</th>
<th>Refrigerating condition</th>
<th>Ambient condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. bag</td>
<td>J. bag</td>
<td>R. can</td>
</tr>
<tr>
<td>Aspergillus species</td>
<td>22.7</td>
<td>18.2</td>
</tr>
<tr>
<td>Alternaria</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chalara</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fusarium</td>
<td>0</td>
<td>9.1</td>
</tr>
<tr>
<td>Thalassospora</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tieghemomyces</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>22.7</td>
<td>27.3</td>
</tr>
</tbody>
</table>

P. bag = Polythene bag, J. bag = Jute bag, R. can = Rubber can, Enve = Envelope, M. cloth = muslin cloth

Effect of Different Storage Materials and Conditions on Viability of Cowpea Seeds

The cowpea seeds stored in Polythene bag at refrigerating condition had the best average germination percentage of 92.2% while the ones stored inside Jute bag, muslin cloth and Envelope in ambient condition had 0% germination, no germination at all at 6th month after storage (Table 3). The different storage materials at ambient condition have detrimental effect on the viability of cowpea seeds because the germination count, especially at 3months (12 WAS) obtained were not significantly different when comparing the storage materials at ambient condition, and the values reduced with increase in storage duration.

At refrigerating condition, there were no significant differences in germination counts among the storage materials except at 16 and 24
WAS. All storage materials were significantly higher than control except rubber can and Polythene bag at 16 WAS and Jute bag at 24 WAS. Seeds stored in all storage materials at refrigerating condition had significantly higher values of germination as from 12 WAS except rubber can at 16 WAS.

**Table 3:** Effect of storage material and conditions at varying storage duration on germination count (%) of cowpea seeds

<table>
<thead>
<tr>
<th>Storage materials</th>
<th>Germination percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4WAS</td>
</tr>
<tr>
<td>Polyethylene bag (A)</td>
<td>100.0a</td>
</tr>
<tr>
<td>(R)</td>
<td>100.0a</td>
</tr>
<tr>
<td>Jute bag (A)</td>
<td>100.0a</td>
</tr>
<tr>
<td>(R)</td>
<td>100.0a</td>
</tr>
<tr>
<td>Rubber can (A)</td>
<td>90.0a</td>
</tr>
<tr>
<td>(R)</td>
<td>96.0a</td>
</tr>
<tr>
<td>Muslin cloth (A)</td>
<td>90.9a</td>
</tr>
<tr>
<td>(R)</td>
<td>100.0a</td>
</tr>
<tr>
<td>Envelope (A)</td>
<td>100.0a</td>
</tr>
<tr>
<td>(R)</td>
<td>96.7a</td>
</tr>
<tr>
<td>(C)</td>
<td>80.0b</td>
</tr>
</tbody>
</table>

Means in the same column followed by same letter are not significantly different by Duncan Multiple Range Test at 0.05 level of significance.

A = Ambient condition, R= Refrigerating condition, C = Control (seeds kept in an open tray at ambient temperature), WAS= Weeks after storage

**Frequency of Seed-Borne Fungi Due to Each Storage Material and Condition of Cowpea**

The frequency of seed-borne fungi on cowpea in response to the storage materials and conditions were taken at 1, 2, 3, 4, 5 and 6 months separately. At 6 months, irrespective of the storage conditions, *Aspergillus* spp occurred most in control with 100% incidence (Table 4), followed by. Several fungi including species of *Fusarium, Alternaria, and Tiegemomyces* were identified on cowpea seeds occurred at different
incidences. Incidence of *Aspergillus* species, *Fusarium* species, *Alternaria* species and other organisms constantly increase and became contaminants on the seeds at both conditions while the occurrence of fungi in seeds in refrigerating condition decreases (Table 3).

In refrigerating condition, *A. flavus* occurred only in the polythene bags while *A. niger* occurred in all the storage materials used except in the muslin cloth. No incidence of *A. fumigatus* at refrigerating condition but was found in all the storage materials used except in jute bag and muslin clot. For the prevention of any specie of Aspergillus, it is better to store cowpea seeds in muslin cloth especially at ambient condition. Envelope had the highest number of species of Aspergillus especially when stored at ambient condition. *F. semitectum* and *F. solani* were not observed at refrigerating condition in all storage materials, but present at ambient condition.

Aspergillus species had the highest incidence both at refrigerating and ambient conditions. No Fusarium specie was found on the cowpea seeds when stored in polythene bags at refrigerating condition.
### Table 4: Occurrence of fungi observed on the cowpea seeds stored in different materials and conditions at 6 months of storage

<table>
<thead>
<tr>
<th>Fungi isolated</th>
<th>Refrigerating condition</th>
<th>Ambient condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P.b ag</td>
<td>J.ba g</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>A. niger</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>A. parasiticus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A. tamari</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>A. candidus</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>A. terreus</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Subtotal</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Alternaria spp</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>F. equiseti</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>F. semitectum</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F. solani</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Subtotal</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Tieghemimycye</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

P. bag = Polythene bag, J. bag = Jute bag, R.can = Rubber can, Enve = Envelope, M. cloth = Muslin cloth

There were significant differences on the fungi isolated after varying time of storage of cowpea seeds at the two storage conditions. The incidence of fungi in ambient condition increased generally with the time of storage, particularly the seeds stored in jute bag (Table 5). At refrigerating condition, the incidence of fungi decreased with storage time irrespective of the storage materials. There was decrease in seed-borne fungi as the storage time progressed in the refrigerator for all seed lots irrespective of the materials. Ife brown cowpea seeds stored in Muslin cloth recorded 10.00 value at 24 weeks after storage compared to other seed lots.

Table 5: Effect of storage materials and conditions at varying storage period on incidence of fungi on cowpea seeds.

<table>
<thead>
<tr>
<th>Storage material</th>
<th>Incidence of fungi on cowpea seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4WAS</td>
</tr>
<tr>
<td>Polythene bag</td>
<td>9.00a</td>
</tr>
<tr>
<td>(R)</td>
<td>5.0a</td>
</tr>
<tr>
<td>Jute bag</td>
<td>7.67a</td>
</tr>
<tr>
<td>(R)</td>
<td>7.00a</td>
</tr>
<tr>
<td>Rubber can</td>
<td>8.00a</td>
</tr>
<tr>
<td>(R)</td>
<td>6.33a</td>
</tr>
<tr>
<td>Muslin cloth</td>
<td>8.67a</td>
</tr>
<tr>
<td>(R)</td>
<td>5.33a</td>
</tr>
<tr>
<td>Envelope</td>
<td>9.0a</td>
</tr>
<tr>
<td>(R)</td>
<td>5.67a</td>
</tr>
<tr>
<td>(C)</td>
<td>6.00a</td>
</tr>
</tbody>
</table>

Means in the same column followed by same letter are not significantly different by Duncan Multiple Range Test at 0.05 level of significance. A = Ambient condition R= Refrigerating condition, C = Control, WAS= Weeks in storage.
DISCUSSION

Several authorities in the field of Seed Science have identified seed viability and seed-borne pathogens as components of any assessment of seed quality. In this study, considerable variations occurred among the storage materials and between storage conditions for seed viability and associated seed-borne fungi. The significant differences observed in the storage materials and conditions were mostly due to the fact that Cowpea seeds undergo various modifications from germination to development on the field, maturation and storage.

The occurrence of *Aspergillus spp* in the two storage conditions showed that the storage conditions along with the materials used to store the seeds may not be the idea for the healthy preservation of the seeds. This is supported by the report of (8) who stressed that due to bad storage conditions and structures, cowpea attracts a number of fungi during storage, under hot and humid conditions, a number of storage fungi mainly belonging to *Aspergillus* and *Penicillium* species get associated with the grains. The predominant fungi storage species also mentioned by (18) include *Aspergillus niger*, *Aspergillus fumigatum*, *Aspergillus flavus*, *Aspergillus sadowl*, *Aspergillus tamari*, *Pencillium islandicum* and *Pencillium cycloplum*.

When rubber plastic can was used to store the seeds in refrigerating and ambient conditions, it had highest number of seed-borne fungi which indicates that the material used is not ideal while the seeds stored in polythene bag in the refrigerator had the least number of fungi. This implies that to store cowpea seeds under refrigeration, they must be kept inside polythene bags. At ambient condition, muslin cloth and envelopes are better to use as the incidence of fungi in the two materials were not significantly different from each other. It is important to note that polythene bags and rubber can should not be used to store seeds at ambient condition. The heat built up in these materials may have made the seeds wet and this is conducive for the growth of seed-borne fungi such as *Aspergillus spp*, *Alternaria spp* and *Fusarium spp*. Bretag et al., (6) reported *Aspergillus flavus* as a common fungus from mouldy legume and found *Alternaria alternaria* as the predominate fungus followed by *Aspergillus flavus* and *Fusarium Species*.

Storage fungi are usually not present in large quantities before harvest. Contamination occur through small
quantities of spores infecting the cowpea grain as it is going into storage from the harvest handling and storage equipment or from spores already present in storage structures under high temperature and moisture this small amount of inoculum can increase rapidly (1). It was observed that as from 12 WAS at ambient condition, seeds stored inside envelope, jute bags and muslin cloth lost their viability while the seeds kept under refrigerating condition irrespective of materials used to store the seeds had above 70% germination and least germination was observed in the rubber cans used. This buttresses the observation that rubber can used in this study was not suitable for storing cowpea seeds in the refrigerator because it had the highest number of fungi growing on the seeds. These fungi contaminated the seeds and hindered its viability. Lopez and Christensen (11) reported that infected seed samples lost their ability to germinate within few weeks or months especially in case of unhealthy seed usually invaded by storage fungi. The natural contamination of seeds with seed-borne fungi plays a vital role in deterioration of seed quality (1). All species of *Fusarium* genera reduced emergence and root growth move than shoot growth, whereas *Fusarium oxysporum* reduced shoot growth more than emergence (6, 9). *Fusarium equiseti* was the most sever pathogen among the fusaria and was also very common in seed samples.

At 24WAS, polythene bags and rubber cans were the best materials for storing seeds, these maintained the viability of the seeds under refrigerating condition. Christensen (7) reported that viability of mould free grain was not at all affected by the length of storage, the temperature and moisture content. At 4 weeks after storage there are no significant differences in seeds viability and seed-borne fungi compared to 24 weeks after storage in certain materials and conditions. It was review from the study that cowpea seeds differ in their seed viability response to period of storage and conditions.

Incidence of fungi on seed gradually increase with increased in storage days at ambient condition irrespective of the material used for storage. A sharp decline in viability and incidence of fungi after 24 weeks of storage was observed suggesting that Cowpea seed stored under ambient conditions should not exceed 6 months for the maintenance of maximum seed viability irrespective of the storage materials. Variation occurs in germination among the cowpea seeds at each
storage conditions and materials at ambient condition. Superior germination was obtained at refrigerating conditions irrespective of the materials.

It was observed that seed stored in refrigerating condition have higher germination comparing to ambient condition while the incidence of fungi increase as the period of storage increases in ambient condition comparing to refrigerating condition that decrease as the period of storage increases irrespective of the materials.

The results suggested that the storage materials and conditions influence viability and seed-borne fungi in cowpea seed. The most effective among the storage materials is polythene bag at both conditions. Cowpea seeds are associated with many fungi in ambient condition which leads to reduction in seed viability and caused increase in infestation of seed-borne fungi.

CONCLUSION

Irrespective of the storage conditions and materials used to store cowpea seeds, the storage fungus *Aspergillus* species were found in abundance in all. Polyethylene bag is better storage material than rubber plastic at refrigerating and ambient conditions though with incidence of fungi while in ambient condition, muslin cloth and envelopes had lowest fungi incidence. Seeds stored in refrigerating condition had higher germination than the ambient, while the incidence of fungi increases in the ambient condition but decreases with the period of storage.

It is important to store cowpea seeds in polythene bag in a refrigerating condition in order to maintain the viability and minimize or eliminate the establishment of seed-borne fungi.

REFERENCES


13. Lopez-Velasco, G. 2010. Molecular characterization of spinach (Spinacia oleracea) microbial community structure and


EVALUATION OF COWPEA GENOTYPES FOR THEIR REACTIONS TO STEM ROT INDUCED BY RHIZOCTONIA SOLANI (KUHN)

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SUMMARY

*Rhizoctonia solani* is one of the pathogens that infect cowpea causing stem and root rot, seedling decay and damping-off complex which result in low yield. This study aims to evaluate some cowpea genotypes for their reactions to *Rhizoctonia solani*. Six improved cowpea varieties (SAMPEA 7, SAMPEA 8, SAMPEA 9, SAMPEA 10, SAMPEA 11 and SAMPEA 12) two accessions (IAR–00–1074 and IAR–07–1050) and four local varieties (Biu local, Maifarinhanci, Kanannado and Dansokoto) were evaluated in the glasshouse and on the field for their reaction to *R. solani* infection. *R. solani* was multiplied on millet grain and used to inoculate the cowpea genotypes in both glasshouse and field studies. Symptoms of disease incidence and severity were observed on all the 12 cowpea genotypes inoculated with *R. solani*. In the glasshouse, SAMPEA 10 recorded the highest incidence (100 %) and SAMPEA 9 recorded the highest severity (62.86 %). In the field however SAMPEA 10 recorded the highest incidence (75.41 %) and severity (66.19 %). Dansokoto recorded the lowest incidence (8.33 and 21.67 % for glasshouse and field respectively) and severity (6.67 and 17.14 % for glasshouse and field respectively). Correlation analysis showed that stem rot incidence and severity significantly resulted in reduced number of pods and grain yield. It is thus concluded that the local varieties evaluated are more resistant to the disease and as such breeding program should focus on improving the desired characteristics of the local varieties.

Keywords: Screening, stem rot, cowpea, genotypes, fungi
Stem rot constitute a constraint to cowpea production in Nigeria (16). The disease was reported in Sri Lanka (5), Tanzania, Benin, South Africa (2) and Nigeria (14, 16) to be caused by a complex of fungi including species of *Pythium*, *Phytophthora*, *Sclerotium*, *Rhizoctonia*, *Macrophomina* and *Fusarium*. Stem rot is characterized by reddish brown lesions on the stem at soil level which may extend upward to about the fifth node but rarely to the growing tip. The lesions may girdle the stem at the first node or a little below it. The leaf petioles at the nodes and the basal portion of some side shoots are also invaded by the fungus. Yield loss of 11 – 40 % caused by stem rot induced by *Pythium aphanidermatum, Sclerotium rolfsii* and *Fusarium solani* at Moor plantation, Ibadan was reported (14). According to Isubikalu et al. (6), most farmers have some knowledge on methods of controlling insect pests but have little knowledge of controlling cowpea diseases (6). The most frequently used control measure against pests and diseases is by synthetic pesticides usage whose intensive and indiscriminate use in agriculture has caused many problems to the environment. Such problems include water, soil, animals and food contamination; poisoning of farmers; elimination of non-target organisms; and selection of pest and weed tolerant to certain pesticides (11). Alternative control measures of plant diseases include cultural control, biological control, regulatory control and the use of crops resistant to certain pests and diseases. The use of resistant crop varieties is the most economical, safest and one of the most effective means of managing plant diseases in crops (12). It is therefore important for scientists to give appropriate research attention to screening and breeding cowpea varieties resistant to stem rot to minimize losses caused by the disease in a manner that will not pose threat to the environment, human and beneficial animals. This study aims to evaluate some cowpea varieties reaction to stem rot so as to serve as a source of materials towards breeding for resistance.

**MATERIALS AND METHODS**

The experiment was conducted in Samaru Zaria (11° 12’ N 07° 37’ E.). Six improved cowpea varieties (SAMPEA 7, SAMPEA 8, SAMPEA 9, SAMPEA 10, SAMPEA 11 and SAMPEA 12), two accessions (IAR–00–1074 and IAR–07–1050) and four local varieties (Biu local, Maifarinhanci, Maifarinhanci,
Kanannado and Dansokoto) were obtained from the Legumes and oil seed programme of Institute for Agricultural Research (IAR), Ahmadu Bello University Zaria. The culture of *Rhizoctonia solani* used was obtained by isolation from diseased cowpea stem using standard procedure of disinfecting and plating on Potato Dextrose Agar (PDA). The fungus was identified using cultural and microscopic characteristics based on the manual by Watanabe (18). Identity of the isolate was confirmed by the Centre for Biosciences and Agriculture International (CABI), United Kingdom.

Glasshouse Evaluation

The cowpea genotypes were evaluated for their reactions to the causal organism in the glasshouse. Seeds were sterilized in 1% Sodium hypochlorite (NaOCl) for 2 minutes, rinsed twice in SDW and sown at three seeds per pot. Pots were arranged in Completely Randomised Design with four repetitions. Each genotype constitutes a treatment and is planted in two pots per repetition. The plants were inoculated with *Rhizoctonia solani* growing on millet grain (50 g/pot) one week after sowing (WAS). Disease incidence and severity on the plants were recorded weekly at 2, 4 and 6 WAS. Disease incidence was calculated as percentage of diseased plants in each repetition.

Individual plants were scored for disease severity based on a modified scale of 0 - 7 by Fernando and Linderman (5) where

0 = No visible symptoms.
1 = Lesions at base of stem (≤ 15 % stem length); leaves pale green
2 = Lesions advanced upward (16 - 30 % stem length); leaves wilting
3 = Lesions advanced upward (31 - 45 % stem length); leaves wilted
4 = Lesions advanced upward (46 - 60 % stem length); stem rotting at base
5 = Lesions advanced upward (> 60 % stem length); stem rotten at base
6 = Plant rotten, wilted and death
Disease severity percentage was determined using the following formula

\[
\text{Disease severity (\%)} = \frac{\text{Sum of disease rating}}{\text{Total number of plants rated} \times \text{Highest rating}} \times 100
\]

Using the severity values, the genotypes were grouped into five reaction groups based on a modified scale of Adejumo et al. (3) where varieties with severities (\%) of

\[
\begin{align*}
0 \quad &= \quad \text{Immune} \\
1\, -\, 15 \quad &= \quad \text{Highly resistant} \\
16\, -\, 30 \quad &= \quad \text{Moderately resistant} \\
31\, -\, 45 \quad &= \quad \text{Moderately susceptible} \\
> 45 \quad &= \quad \text{Highly susceptible}
\end{align*}
\]

Pod number and grain yield were recorded for each repetition and expressed per plant.

**Field Evaluation**

The 12 cowpea genotypes were also evaluated under field conditions in 2014. The genotypes were arranged in a Randomized Complete Block Design (RCBD) with three replications. In a replicate, each variety was sowed on 12 m² plot using three seeds per hole and an intra and inter row spacing of 20 cm and 7 cm were maintained. Plots were separated from each other by one bare, 75 cm weeded ridge while replicates were separated from each other by 1 m wide margin. One week after sowing, the top soil of each stand was inoculated by incorporating 50 g of millet seed inoculum while sterilized millet seeds were incorporated in control plots. Disease incidence and severity on 6.0 m² net plots were recorded at 2, 4 and 6 WAS. Cowpea pods were harvested at maturity and data on pod number per plot was collected at harvest and grain yield per plot was obtained after threshing and expressed per hectare. Varieties were separated into different resistant groups using the scale described above.

**Statistical Analysis**

The data generated were subjected to analysis of variance (ANOVA) procedure of SAS and means were separated using the Duncan Multiple Range Test (DMRT) at 5 % level of significance.
RESULTS

Disease incidence and severity in the glasshouse

The incidence of stem rot on cowpea genotypes inoculated with *Rhizoctonia solani* in the glasshouse significantly varied (Table 1). At 2 Weeks after Sowing (WAS), highest disease incidence was recorded on SAMPEA 10 which did not differ significantly from that of Biu local and IAR-07-1050. Dansokoto, IAR-00-1074, SAMPEA 11 and SAMPEA 12 were disease free. At 4 WAS, Biu local and SAMPEA 10 recorded the highest incidence (91.67%) which did not differ significantly from that of IAR-07-1050. Dansokoto recorded the lowest incidence (7.5%) which did not significantly differ from that of IAR-00-1074, SAMPEA 8 and SAMPEA 11.

Table 1: Disease incidence and severity of stem rot on cowpea genotypes inoculated with *Rhizoctonia solani* in the glasshouse

<table>
<thead>
<tr>
<th>Variety</th>
<th>Disease incidence (%)</th>
<th>Disease severity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2WAS</td>
<td>4WAS</td>
</tr>
<tr>
<td>SAMPEA 10</td>
<td>75.00a</td>
<td>91.67a</td>
</tr>
<tr>
<td>IAR-07-1050</td>
<td>70.83a</td>
<td>87.50a</td>
</tr>
<tr>
<td>Biu local</td>
<td>54.17a</td>
<td>91.67a</td>
</tr>
<tr>
<td>SAMPEA 8</td>
<td>23.33b</td>
<td>15.00cd</td>
</tr>
<tr>
<td>SAMPEA 9</td>
<td>20.83b</td>
<td>45.83b</td>
</tr>
<tr>
<td>Kanannado</td>
<td>15.00b</td>
<td>37.50bc</td>
</tr>
<tr>
<td>SAMPEA 7</td>
<td>8.33b</td>
<td>30.17bcd</td>
</tr>
<tr>
<td>Maifarinhanci</td>
<td>7.50b</td>
<td>33.33bc</td>
</tr>
<tr>
<td>SAMPEA 11</td>
<td>0.00b</td>
<td>15.83cd</td>
</tr>
<tr>
<td>SAMPEA 12</td>
<td>0.00b</td>
<td>37.50bc</td>
</tr>
<tr>
<td>IAR-00-1074</td>
<td>0.00b</td>
<td>15.00cd</td>
</tr>
<tr>
<td>Dansokoto</td>
<td>0.00b</td>
<td>7.50d</td>
</tr>
<tr>
<td>SE±</td>
<td>7.47</td>
<td>7.62</td>
</tr>
</tbody>
</table>

Means followed by similar superscript in a column are not significantly different at 5% level of significance (P ≤ 0.05) using Duncan Multiple Range Test (DMRT)

WAS = Weeks after sowing

At 6 WAS, IAR-07-1050, Biu local and SAMPEA 10 recorded the highest incidence (100%) followed by SAMPEA 9. Dansokoto recorded...
the lowest incidence (8.33 %) which did not significantly differ from that of IAR-00-1074 and SAMPEA 11.

The severity of stem rot on cowpea genotypes inoculated with *R. solani* in the glasshouse was significant (Table 1). At 2 WAS; highest severity (40.76 %) was recorded on SAMPEA 9 and lowest severity (0.00 %) were from IAR-07-1050; Biu local; SAMPEA 7; SAMPEA 11 and Dansokoto. This trend was similar at 4 WAS and at 6 WAS, Dansokoto had the lowest severity (6.67 %) and this was significantly lower than SAMPEA 11; SAMPEA 7 and Biu Local but not IAR-07-1050 at 17.4 %.

**Disease incidence and severity on the field**

The incidence of stem rot on cowpea genotypes inoculated with *R. solani* on the field also significantly varied (Table 2). At 2 WAS, highest disease incidence was recorded on SAMPEA 10 (14.79 %) which did not differ significantly from that of IAR-00-1074, SAMPEA 12, IAR-07-1050 and Biu local. SAMPEA 7, SAMPEA 8, SAMPEA 11, Dansokoto, Kanannado, Maifarinhanci and SAMPEA 9 were disease free.

At 4 WAS, SAMPEA 11 recorded the highest incidence (42.94 %) which did not differ significantly from that of SAMPEA 10, Maifarinhanci and SAMPEA 9. Dansokoto recorded the lowest incidence (21.67 %) which did not differ significantly from that of SAMPEA 8, IAR-00-1074, Kanannado, IAR-07-1050, Biu local, SAMPEA 7 and SAMPEA 9. At 6 WAS, SAMPEA 10 recorded the highest incidence (75.41 %) followed by Biu local which did not significantly differ from that of IAR-07-1050. Dansokoto recorded the lowest incidence (21.67 %) and was not significantly different from that of Kanannado (25.29 %) and SAMPEA 12 (30.28 %).

The severity of stem rot on cowpea genotypes inoculated with *R. solani* on the field also significantly varied (Table 2). At 2 WAS, highest disease severity (9.99 %) was recorded on SAMPEA 10 while Dansokoto, Kanannado, and Maifarinhanci were disease free.

At 4 WAS, SAMPEA 11 recorded the highest severity (31.43 %) which did not differ significantly from that of SAMPEA 10 (30.48 %). Dansokoto had the lowest severity (17.14 %) and did not differ significantly from Kanannado, Biu local, IAR-07-1050, SAMPEA 7, SAMPEA 8 and IAR-00-1074.
Table 2: Disease incidence and severity of stem rot on cowpea genotypes inoculated with *Rhizoctonia solani* on the field.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Disease incidence (%)</th>
<th>Disease severity</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2WAS</td>
<td>4WAS</td>
<td>6WAS</td>
</tr>
<tr>
<td>SAMPEA 10</td>
<td>14.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.90&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>75.41&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IAR-00-1074</td>
<td>6.86&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>24.95&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>48.93&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAMPEA 12</td>
<td>6.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>24.17&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>30.28&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>IAR-07-1050</td>
<td>6.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>26.23&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>57.91&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Biu local</td>
<td>4.19&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>28.97&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>64.62&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAMPEA 7</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.81&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>50.71&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>SAMPEA 8</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.43&lt;sup&gt;d&lt;/sup&gt;</td>
<td>51.21&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>SAMPEA 9</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>53.49&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>SAMPEA 11</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.74&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Maifarinhanci</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.77&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>53.49&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Kanannado</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.29&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>25.29&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dansokoto</td>
<td>0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.67&lt;sup&gt;d&lt;/sup&gt;</td>
<td>21.67&lt;sup&gt;d&lt;/sup&gt;</td>
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<tr>
<td>SE±</td>
<td>3.66</td>
<td>3.56</td>
<td>2.99</td>
</tr>
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</table>

Means followed by similar superscript in a column are not significantly different at 5% level of significance (P ≤ 0.05) using Duncan Multiple Range Test (DMRT)

WAS = Weeks after sowing

At 6 WAS, SAMPEA 10 recorded the highest severity (66.19%) which did not differ significantly from that of Biu local (62.86%). Dansokoto recorded the lowest severity (17.14%) which did not differ significantly from that of Kanannado (19.05%). The inoculated cowpea genotypes were separated into different host reaction groups based on the scale described earlier. The glasshouse evaluation result showed that SAMPEA 7, SAMPEA 11 IAR-00-1074, IAR-07-1050 and Biu local were moderately resistant; SAMPEA 8, SAMPEA 9 and SAMPEA 10
were highly susceptible; SAMPEA 12, Maifarinhanci and Kanannado were found to be moderately susceptible while Dansokoto was highly resistant. The field evaluation result however showed that SAMPEA 7, SAMPEA 8, SAMPEA 9, SAMPEA 11, IAR-07-1050 and Maifarinhanci were moderately susceptible; SAMPEA 10, SAMPEA 12 and Biu local were highly susceptible while IAR-00-1074, Kanannado and Dansokoto were moderately resistant (Table 3).

Table 3: Host reaction groups of cowpea genotypes evaluated for their reaction to stem rot in the glasshouse and on the field.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Host Reaction Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glasshouse</td>
</tr>
<tr>
<td>SAMPEA 7</td>
<td>MR</td>
</tr>
<tr>
<td>SAMPEA 8</td>
<td>HS</td>
</tr>
<tr>
<td>SAMPEA 9</td>
<td>HS</td>
</tr>
<tr>
<td>SAMPEA 10</td>
<td>HS</td>
</tr>
<tr>
<td>SAMPEA 11</td>
<td>MR</td>
</tr>
<tr>
<td>SAMPEA 12</td>
<td>MS</td>
</tr>
<tr>
<td>IAR- 00- 1074</td>
<td>MR</td>
</tr>
<tr>
<td>IAR- 07- 1050</td>
<td>MR</td>
</tr>
<tr>
<td>Biu local</td>
<td>MR</td>
</tr>
<tr>
<td>Maifarinhanci</td>
<td>MS</td>
</tr>
<tr>
<td>Kanannado</td>
<td>MS</td>
</tr>
<tr>
<td>Dansokoto</td>
<td>HR</td>
</tr>
</tbody>
</table>

MS = moderately susceptible. MR = moderately resistant. HS = highly susceptible.
HR = highly resistant
Table 4: Pod and grain yield loss on cowpea genotypes inoculated with *Rhizoctonia solani* in the glasshouse and on the field.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Glasshouse</th>
<th>Field</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pod yield loss (%)</td>
<td>Grain yield loss (%)</td>
</tr>
<tr>
<td>Dansokoto</td>
<td>3.91&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.15&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>IAR-00-1074</td>
<td>8.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.52&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kanannado</td>
<td>29.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.88&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAMPEA 12</td>
<td>25.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.62&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAMPEA 8</td>
<td>9.54&lt;sup&gt;c&lt;/sup&gt;</td>
<td>33.26&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Maifarinhani</td>
<td>25.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.19&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAMPEA 11</td>
<td>12.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.89&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAMPEA 7</td>
<td>5.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.50&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAMPEA 10</td>
<td>22.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.89&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SAMPEA 9</td>
<td>10.83&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.86&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>IAR-07-1050</td>
<td>66.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Biu local</td>
<td>70.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>68.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SE±</td>
<td>1.55</td>
<td>1.64</td>
</tr>
</tbody>
</table>

Means with the same superscript in a column are not significantly different at 5% level of significance (P≤0.05) using Duncan Multiple Range Test (DMRT).

Table 5 shows the correlation between disease incidence, disease severity, number of pods and grain weight of inoculated cowpea recorded in the glasshouse. Disease incidence was positively and not significantly correlated (0.077) with disease severity, it however correlated negatively and not significant with number of pods (-0.09) and grain weight (-0.21). Disease severity on the other hand was negatively and significantly correlated with number of pods (-0.76) and grain weight (-0.57). Number of pods correlated positively and significantly with grain weight (0.63).
Table 5: Correlation between disease incidence, disease severity, number of pods and grain weight of cowpea varieties inoculated with *Rhizoctonia solani* in the glasshouse.

<table>
<thead>
<tr>
<th></th>
<th>Disease incidence</th>
<th>Disease severity</th>
<th>Number of pods</th>
<th>Grain weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease incidence</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease severity</td>
<td>0.077</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of pods</td>
<td>-0.092</td>
<td>-0.76**</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Grain weight</td>
<td>-0.21</td>
<td>-0.57**</td>
<td>0.63**</td>
<td>1.00</td>
</tr>
</tbody>
</table>

NS = Not significant at 5%** = Significant at 1%

Table 6 shows the correlation between disease incidence, disease severity, number of pods and grain yield recorded on the field. Disease incidence correlated positively and highly significant (0.914) with disease severity, it correlated negatively and significant with number of pods (-0.42) and grain weight (-0.40). On the other hand, disease severity correlated negatively and highly significant with number of pods (-0.47) and grain weight (-0.47). Number of pods however correlated positively and highly significant with grain weight (0.67).

Table 6: Correlation between disease incidence, disease severity, number of pods and grain weight of cowpea varieties inoculated with *Rhizoctonia Solani* on the field.

<table>
<thead>
<tr>
<th></th>
<th>Disease incidence</th>
<th>Disease severity</th>
<th>Number of pods</th>
<th>Grain weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease incidence</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disease severity</td>
<td>0.914**</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of pods</td>
<td>-0.42*</td>
<td>-0.47**</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Grain weight</td>
<td>-0.40*</td>
<td>-0.47**</td>
<td>0.67**</td>
<td>1.00</td>
</tr>
</tbody>
</table>

* = significant at 5% ** = significant at 1%
DISCUSSION

It can be deduced from the result presented above that cowpea seedlings even at 2 WAS can be infected by *R. solani* with severity as high as 40%. There was also a gradual disease progression observed with time which can be attributed to inoculum build-up. The occurrence of disease at 2 WAS which is 1 week after inoculation indicates that inoculation period of *R. solani* is less than a week.

None of the cowpea genotypes tested was immune to the disease even though some varieties showed different level of resistance to the disease. The variability in resistance and susceptibility of the genotypes may be due to differences in their genetic makeup. This agrees with report by Mohammed *et al* that different reactions of cowpea genotypes to bacterial blight, rust and cowpea aphid borne mosaic virus was as a result of genotypic differences (8). Twenty cowpea genotypes tested against rust in Ghana also showed varied resistance response under field condition (17). Higher incidences and severities were recorded on the field than in the glasshouse. The differences are probably attributable to the fact that in the glasshouse experiment, the soil was sterilised and infection was only due to the fungi inoculated; in the field however as the soil was not sterilised, activities of other soil pathogens aggravated the disease making a disease complex that was more severe than single pathogen infection. Study showed that cowpea stem rot is caused by complex of soil fungi which include *S. rolfsii*, *R. solani* and *Pythium ultimum* (2). Onuorah (1973) also showed that cowpea stem rot caused by complex of soil borne fungi is more severe than that caused by single fungus (14).

SAMPEA 10, Biu local and IAR-07-1050 are the most susceptible of the 12 cowpea varieties tested. SAMPEA 12, Dansokoto and Kanannado were resistant in both glasshouse and field. With the exception of Biu local, the local varieties recorded relatively lower incidence and severity than the improved varieties, this indicates that the local cowpea varieties tested are more tolerant to stem rot than the improved varieties used in this study. According to IAR released varieties descriptors (4), SAMPEA 10 is resistant to striga/alectra and tolerant to major insect pests. SAMPEA 7 is susceptible to scab, bacterial blight, septoria leaf spot and brown blotch. It is also susceptible to beetles, thrips, pod borer, pod sucking bugs and bruchids. SAMPEA 8 and
SAMPEA 9 are tolerant to major insect pests. SAMPEA 11 is resistant to nematodes and major insect pests. SAMPEA 12 is field tolerant to major insect pests. None of the varieties was earlier evaluated against stem rot. In earlier studies conducted in Sokoto Nigeria, SAMPEA 9 and SAMPEA 11 were found to be highly susceptible to Cercospora leaf spot while SAMPEA 7 and SAMPEA 8 were moderately susceptible to the disease (9).

The result showed that number of pods produced and grain weight significantly decreased with increase in incidence or severity. This shows that cowpea stem rot caused significant yield reduction. This agrees with earlier research that reported negative and significant correlation between cowpea scab severity and grain yield in Samaru Zaria (7, 10). Soonthornpoc et al. (2000) reported significant negative correlation between yield of maize and severity of root and basal stem rots incited by *Rhizoctonia solani* in Mississippi, USA (15). Yield of cowpea was also found to be correlated negatively with damping off and brown blotch of cowpea in Zaria (13).

**CONCLUSION**

From the result of this study it can be concluded that *Rhizoctonia solani* induced stem rot causes significant reduction (17 -53 %) of cowpea yield. Both the local and improved varieties commonly used by farmers in North West Nigeria have different levels of susceptibility/resistance to the disease with more resistance on the local varieties. IAR-00-1074 and Dansokoto found to be resistant in both glasshouse and field are potential breeding materials for resistance of cowpea to stem rot. Grain yield reductions as a result of stem rot infection on cowpea ranges between 17- 53%.

**REFERENCES**


14. Onuorah, P. E. 1973 *Pythium* seed decay and stem rot of cowpea (*Vigna sinensis* [Linn.] Savi) in


EFFECTS OF TWO NIGERIAN STRAINS OF MOROCCAN WATERMELON MOSAIC VIRUS (MWMV) ON ELEMENTAL AND PROXIMATE COMPOSITION OF THE LEAVES OF CUCUMEROPSIS MANNII NAUDIN

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SUMMARY
The effects of two Nigerian strains of *Moroccan watermelon mosaic virus* (MWMV), (designated as MWMV-cor and MWMV-lag) on the elemental and proximate composition of the leaves of *Cucumeropsis mannii* were studied. Infected and healthy (control) leaf samples were obtained, pulvèrized and analyzed for elemental and proximate composition using standard methods described by AOAC. Inoculation with MWMV-cor led to significant increase (p<0.05) in Pb, Co, Fe and Ca compared to healthy sample by 64.03%, 18.18%, 12.12% and 11.99% respectively. Values obtained for Zn, Al and Mg, though higher than the healthy control, were statistically insignificant. Conversely, MWMV-cor engendered significant reduction in Cu content by 17.48%, Cd by 53.13% and Ni by 60.61% while values obtained for Mn, Na and K were comparable to the value obtained for healthy control and consequently not significantly different (p<0.05). For MWMV-lag, there were significant increases in Pb content by 39.07%, Cu by 19.54%, Co by 45.45%, Ni by 38.46% and Na by 12.18%. Values obtained for Ca, Zn and Mg, though higher, did not differ significantly (p<0.05) compared to healthy sample. On the other hand, Cd and Mn were significantly reduced by 50% and 12.41% by the virus strain while the reduction in Al and K were statistically insignificant. The content of Fe remained unaltered by MWMV-lag infection. With regard to proximate composition, there was an increase in the moisture and fibre contents of *C. mannii* when inoculated with MWMV-cor compared to healthy control while there were reductions in the amount of protein and lipid in infected leaf tissues. Inoculation of the
crop with MWMV-lag led to significantly higher protein content (33.62%) in infected leaf tissue compared with control. Conversely, the virus strain caused insignificant reduction in the moisture, lipid and carbohydrate contents in relation to the control. The implication of this study is that an increase or decrease in the mineral and proximate components of infected C. mannii by MWMV strains is an indication of altered physiology of the crop and could impact negatively on the yield performance and a lowering of potentials of seeds from such harvest for industrial use.

**Keywords:** Cucumeropsis mannii, Moroccan watermelon mosaic virus (MWMV), elemental and proximate composition.

**WHITE-SEED MELON**  
(*Cucumeropsis mannii* (Nudin) (syn = *C. edulis*), is indigenous to West and Central Africa. A member of the family Cucurbitaceae, it is a monoecious scandent herb and can grow up to 5-10 m long, climbing by simple tendrils. The stem is angular and sparsely hairy. The heart-shaped or roughly palmate leaves are up to 12 cm long and 14 cm wide. The seeds, which are milky in colour, are ovate in shape, compressed and smooth (1).

In sub-sahara Africa, *C. mannii* is prized for its oleaginous seeds that together with seeds of *Citrullus Schrad.*, and *Cucumis L.* species are used to thicken a traditional dish called “egusi-soup” in Cameroon, Nigeria and Benin Republic, and “postachie” in Cote d’Ivoire (2, 3). In Northern Ghana “egusi” oil is the second most prominent cooking oil (4). Most commonly it is dehulled and consumed as a snack (5). The kernel of the egusi-ito seed contains semi-drying oils which can be used for soap making and for illumination, with the rest of the seed fed to livestock. Another consumption of the melon is in patty form. After oil has been extracted from the seed, it is then eaten as a protein substitute. Most commonly it is dehulled and consumed as a snack (4).

There are several reports of nutritional composition of *C. mannii*. Dehulled seeds from *C. mannii* mainly have been found to consist of 40 - 44% fat/lipids and 34.5 - 43.63% protein. Carbohydrates, minerals and water amount to 16.5, 3.7, and 5.9%, respectively (6, 7). The oil of the seed has been reported to contain between 62.42 - 64.9% linoleic acid, 12.4 -15.9% oleic acid, 11.8% stearic acid and 10.27 -10.9% palmitic acid (6). Vitamins, thiamin, niacin, B1 and B2 are also prevalent in the seed (4). Notable minerals include phosphorus as the largest
mineral component, with potassium, magnesium, manganese, sulphur, calcium, iron and zinc in that order. The bulk of carbohydrates are starch and soluble sugars (1, 4). Egusi-itoo has been considered as the perfect complement to the largely starch-rich grain diet of Africa, providing a high-protein and high-energy concentrate. The seed is considered an excellent vegetable protein, and ideal for battling nutritional debilitations (4, 6). Just 100 g of seed daily is considered enough to provide essential fatty acid, amino acid and Vitamin E requirements (5).

Seed oil metallic soaps derived from C. mannii, when incorporated into paint matrix has been shown to act as catalyst, reducing the drying time (8). The potential use of the seeds as raw material for biodiesel production has also been demonstrated, as the measured fuel properties of the fatty acid methyl ester of the oil were found comparable to both the ASTMD 6751 and the EN 14214 biodiesel standards (9). The oil from the seeds has been established to be better than castor oil in methyl salicylate liniment and salycilic acid lotion formulation (10). The seeds of the crop have also been found to contain inhibitory effects on key enzymes relevant to erectile dysfunction and the used of the seeds to manage this condition has been recommended (11).

Moroccan watermelon mosaic virus (MWMV) belongs to the genus Potyvirus (family: Potyviridae) and is characterized by flexuous particles of about 730 nm in length. The virus which was first reported in Morocco (12), causing severe diseases characterized by mosaic, leaf malformation, green-vein banding, and stunting in several susceptible cucurbits, has been reported from southwest Spain (13) and Italy (14). In Africa, the virus has been reported in South Africa (15), Sudan (16), Democratic Republic of Congo (17), Tunisia (18) and in Nigeria, where two strains of the virus have been identified (19). The virus is now considered an emerging threat to commercial production of cucurbits where ever it is found.

Changes in the elemental composition of virus infected plants have been documented in literature. Shattuck (20) reported that infection of rutabaga (Brassica napus sp. rapifera inoculated at maturity with Turnip yellow mosaic virus (TuMV) led to higher N, P, Mg but a lower K content in infected leaf tissues compared to healthy controls. Noqueira et al (21) reported low levels of N and higher levels of Ca, S, and Fe as compared to the control in Citrus sinensis inoculated with
Citrus leprosis virus (CiLV) while the values obtained for P, K, Mg, Cu, Mn, Zn and Bo were comparable to healthy. A study on the mineral composition of broad bean (Vicia fabae) inoculated with Broad bean mosaic virus (BBMV) showed that infected plant contained more P and less total nitrogen and K than in healthy plant sample (22). Muqit et al (23) reported reduction in the amount of N in ash gourd (Benincasa hispida) due to infection by Bottle gourd mosaic virus (BGMV). The study by Yardimci et al (24) revealed that Alfalfa mosaic virus (AMV) caused reduction in P, Fe, Cu, Zn and Mn and increased N content compared to healthy plant samples of alfalfa. Owolabi et al (25) reported significant reductions in Mg, Fe and Ca contents with increases in P, Mn and K in Ivy gourd leaves (Coccinia barteri) infected with a Nigerian strain of MWMV. According to Shakeel et al (26), there was an increase in Mg content, a significant reduction in K content while Na content was either reduced or increased in cucumber, depending on the varieties when inoculated with Cucumber mosaic virus (CMV).

Plant virus infection has also been reported to alter proximate composition of infected plants. Nambiar and Ramakrishnan (27) have reported reduction in the carbohydrate level in pigeon pea infected with Pigeon pea sterility mosaic virus (PPSMV). Reduction in the carbohydrate content of infected leaves of papaya inoculated with Papaya ringspot virus (PRSV) was reported by (28). Sinha and Srivastava (29) have also reported lower carbohydrate content but increased protein content in mungbean plants inoculated with Mungbean yellow mosaic virus (MYMV). Cheema et al. (30) showed that protein contents in two soybean varieties increased when infected by Soybean yellow mosaic virus (SYMV). Mofunanya et al (31) have documented reductions in protein, fibre, carbohydrate, lipids and ash contents in Telfairia occidentalis inoculated with Telfairia mosaic virus (TeMV). Samples of bean plant (Phaseolus vulgaris) and sugar beet (Beta vulgaris L.) inoculated with Bean common mosaic virus (BCMV) were reported to contain more protein than healthy one (32,33).

Cucumeropsis mannii is an important vegetable crop in the Southwest and northern part of Cross River State of Nigeria. It has been used as experimental host for the two strains of MWMV in our previous studies (29,34) in which they induced severe mosaic, severe leaf malformation, reduced leaf size, severe stunting,
Sometimes culminating in growth cessation. The crop has indeed become the choice host for isolating cucurbits viruses in our researches. The focus of this study is to examine the effects of the two Nigerian strains of MWMV-cor and MWMV-lag on the biochemical and physiological changes in this economic plant with respect to mineral and proximate composition.

**MATERIALS AND METHODS**

**Source of Seeds**

Seeds of *C. mannii* used in this study were sourced from Ogoja in Cross River State, Nigeria. Authentication of seeds was carried out by Mr. Frank I. Apejoye (a taxonomist) in the Department of Botany, University of Calabar, Calabar, Nigeria.

**Strains of virus sources and preparation of virus inocula**

The two virus strains designated as MWMV-cor and MWMV-lag in this research were the isolates described by Owolabi *et al* (19), isolated from *Coccinia barteri* (Benth.) Roberty and *Lagenaria breviflora* (Hork. F) Kay respectively. The viruses were propagated and maintained on *C. mannii* in the screen house of the Department of Botany, University of Calabar, Calabar. Virus inocula were prepared by grinding symptomatic leaves in 0.5 M potassium phosphate buffer, pH 7.5, in sterile pestles and mortars.

**Experimental design and inoculation of experimental plants**

Seeds were sown in perforated polyethylene bags (16 cm diameter), each filled with about 4.2 kg of heat-sterilized loamy soil. The experiment was laid out in complete randomized design consisting of 4 replications. Each replication contained 24 seedlings, arranged in 8 rows and 3 columns. Prior to inoculation, the surface of the leaves was dusted with carborundum (abrasive of 6000 mesh). Using a table of random numbers, the inocula (MWMV-cor, MWMV-lag and control which was the buffer only) were randomly applied separately on the plants within each replication by mechanical or sap inoculation in the screen house with average temperature of 23 ± 3°C. The inoculated plants were rinsed with distilled water and left for symptom development.

**Preparation of sample before analysis**

Four weeks after inoculation when symptoms on the leaves had become severe, the infected leaves were harvested, rinsed with distilled water, shredded and oven-dried at 70°C to constant weight and pulverized in an
electric mill (National Food Grinder, Model 2008 Mk, Japan). Healthy leaf samples were similarly treated as described for infected samples. One hundred grams (100 g) were taken from the pulverized samples for elemental and proximate content determinations.

**Digestion of samples for determination of minerals**

Digestion of samples was carried out using the dry digestion method as described by AOAC (35). Two (2) g each of the powered samples was weighed into a crucible and swirled gently to mix the content. Each sample was leached with 5 ml of 20% HCl and subsequently transferred into 20 ml volumetric flask. The volume was made up to 20 ml with distilled water. A blank solution was also prepared in a similar way except for the omission of the samples.

**Determination of mineral contents**

The contents of Al, Cd, Co, Cu, Fe, Mn, Ni, Pb, Zn, Ca and Mg were determined using an atomic absorption spectrophotometer (Pye Unical SP8, Spec. UK) as outlined in AOAC (36). To 2 g sample was added 20 ml of acid mixture (65 ml conc. HNO$_3$, perchloric acid and 2 ml conc. H$_2$SO$_4$) in a digestion flask, heated gently at between 50-70$^\circ$C on a Stuart hot plate until clear digest was obtained and made up to 100 ml with deionized water. Appropriate dilutions (2.0 ppm, 4.0 ppm, 6.0 ppm, 8.0 ppm and 10.0 ppm) were made for each element. For Ca and Mg determination, strontium chloride (SrCl$_2$) was added to yield a 1500 mg/ml of Sr$^{2+}$ in the final solution. Calibration curves were prepared for each element. The contents of the elements were determined using the calibration curves drawn from data obtained spectrophotometrically using the appropriate wavelengths for each of the elements.

Na and K contents were estimated by flame photometry (Perkin Elmer Analyzer 2880 Spain). The digestion procedure was as described for atomic absorption spectrophotometry. The stock solutions for Na (1000 ppm Na$^+$) and K (1000 ppm K$^+$) were prepared by dissolving 2.542 g of NaCl and 1.907 g of KCl. Working standards (2.0 ppm, 4.0 ppm, 6.0 ppm, 8.0 ppm and 10.0 ppm) from the stock solutions were also prepared. The absorbances for Na (at 589 nm) and K (at 767 nm) were obtained using the flame photometer. Na and K concentrations were determined from the calibration curves obtained from the working standards solutions and the results were expressed in milligram per litre.
The phosphorus content of the samples was determined as described in AOAC (36). To 0.5 ml aliquot of the mineral digest was added 9.5 ml of 10% trichloroacetic acid in a 16 x 25 mm test tube. The mixture was agitated, centrifuged (Censaur 2 MSE, UK) for 5 min and then filtered through Whatman filter paper. Five (5) millilitres of the filtrate and 5 ml of the working standards prepared as previously described from P stock solution (4.324 g KH$_2$PO$_4$ in 250 ml of deionized water and made up to 1 litre) were measured into two 19 mm cuvettes to which 0.5 ml of molybdate reagent (prepared by adding 200 ml diluted water to 83 ml of conc. H$_2$SO$_4$ and 25 g ammonium molybdate tetrahydrate made up to a litre by the addition of deionized water). The cuvettes were shaken and 0.2 ml sulphuric acid reagent (0.125 g 1,2,4-aminonaphthosulphuric acid, 7.28 g sodium bisulphite and 0.25 g sodium sulphite in 50 ml of distilled water) was added, stoppered, shaken and allowed to stand for 10 min. The absorbance of the test and standards were read at 660 nm in the spectrophotometer. Phosphorus concentration was obtained from calibration curve of the standards.

**Determination of moisture content**

For the moisture content determination, a 3 g sample of infected and non-infected leaves were separately weighed into porcelain dishes and covered with lids. The dishes and contents were placed in cooled desiccators containing conc. H$_2$SO$_4$ as drying agent and then weighed. The procedures were repeated until constant weights were obtained for each sample as expressed in the relation below:

\[
\text{% moisture} = \frac{\text{loss in weight on drying (g)}}{\text{Initial weight of sample (g)}} \times 100
\]

**Determination of the crude protein**

The proximate contents (crude protein, ash, lipid, fibre moisture and carbohydrate) of the samples were determined by the methods of the Association of Official Analytical Chemists (AOAC) (36). The crude protein content was determined by the Kjeldah method. Two (2) gram sample was introduced into the Kjeldahl flask held at an angle to which 20 ml of conc. sulphuric acid was added. The flask was placed in the stand and heated over a gentle flame until the initial charring subsided after which 2 Kjeldahl C
(5.00 g K₂SO₄ and 0.100 g Cu SO₄.5H₂O) was added to the flask by sliding them carefully down the neck of the inclined flask. The content of the flask was further heated gently at first and then more vigorously until the solution turned green. It was then allowed to cool to room temperature. Thereafter, 2000 ml of distilled water was added and the flask swirled carefully to mix the content. The flask was connected to the distillation apparatus, ensuring that the delivery tube dipped below the surface of 50 ml boric acid solution (prepared by dissolving 40 g of boric acid in 800 ml of distilled water and made up to 1 litre by adding water). To this was added 2-3 drops of silicon antifoam reagent followed by 80 ml NaOH solution (1 g NaOH in 1 litre of water) and the distillation apparatus gently agitated to ensure thorough mixing. The flask was then heated. About 160 ml of distillate was obtained and this was titrated using 0.1N sulphuric acid to the blue grey end point (pH 4.5) of the indicator solution (prepared by dissolving 0.16 g methyl red and 0.083 g bromocresol green in 100 ml industrial methylated spirit). A blank experiment (without sample) was carried out in a similar manner and the titre from it was subtracted from that containing the sample to obtain the true titre. Percentage N in the sample was obtained using the formula:

\[
N\% = (T - B) \times 0.0014 \times 100/M
\]

Where
- \( T \) = ml of 0.1N sulphuric acid for sample
- \( B \) = ml of 0.1N sulphuric acid for blank
- \( M \) = weight of sample (g)

The crude protein content was then calculated by multiplying the N value by the protein factor 6.25.

**Determination of lipid content**

For the crude lipid determination, the petroleum ether extraction protocol (32,36) was used. Ten-gram of infected and non-infected samples was separately introduced into a fat extractor thimble to which 150 ml petroleum ether (boiling range 40-60 °C) was added. The thimble with its content was placed into the Soxhlet apparatus (Quickfit, England) to which a pre-weighed round bottom flask had been connected and was heated. The extraction process lasted for 8 h. The flask was disconnected and the solvent distilled off over a steam bath at 50 °C. The remaining traces of the solvent were removed with a current of air. The flask was dried to constant weight at 100 °C.
The weight of the crude lipid content was obtained as the difference between the weight of the flask before and after the extraction and evaporation of petroleum ether from the extract.

\[
\% \text{ crude lipid} = \frac{\text{weight of flask + sample (g)} - \text{wt of flask}}{\text{Weight of sample (g)}}
\]

**Determination of crude fibre content**

For crude fibre determination, two (2) gram defatted sample was weighed and quantitatively transferred into a 400 ml beaker to which 50 ml of 1.25% sulphuric acid (H\(_2\)SO\(_4\)) was added and the mixture made up to 200 ml with distilled water. This was heated to boiling for 30 min. The content of the beaker was filtered using a Buchner funnel with the aid of a suction pump. The residue was washed with hot water until it was acid free. The residue obtained after the acid digestion was quantitatively transferred into a 400 ml beaker to which 50 ml of 1.25% NaOH was added and made up to 200 ml with distilled water. The mixture was again heated for 30 min with constant stirring. The content of the beaker was filtered through the Buchner funnel and washed five times with water until free of NaOH. The residue obtained was washed twice with 95% methanol and quantitatively transferred into a porcelain crucible and dried at 100 °C. The weight of the oven-dried residue was noted and later ignited in a furnace at 550 °C. The weight of the ash left after ignition was also noted. The crude fibre content was determined from the weight loss of the crucible and its content after the ignition.

**Determination of ash**

For the ash content, 2 g of infected and non-infected samples were weighed separately into dry pre-weighted porcelain crucibles. The crucibles were placed in a muffle furnace (Gallenkamp, Muffle Furnace Size 2, UK) and ignited for 24 h at 550 °C to eliminate the organic components and until completely ashed to gray white. The crucibles and their contents were cooled in desiccators and later weighed. The difference in weights before and after incineration was reported as the ash content and expressed as percentage thus:

\[
\% \text{ ash} = \frac{\text{weight of dish with ash} - \text{wt of dish}}{\text{weight of dish with ash}} \times 100
\]
weight of dish and sample – wt of dish

**Determination of carbohydrate content**

The carbohydrate content in the various samples was carried out by subtracting the combined values of crude protein, fibre, lipid and ash from the total dry matter.

**Statistical Analysis**

The data obtained were analysed using the Student T-test. Mean values were compared at 5% confidence limit to determine whether differences were significant. Values presented were means of three replicates.

**RESULTS**

The results of MWMV-cor infection on the mineral content of *C. manni* are presented in Table 1. The results showed there was a significant increase (p<0.05) in Pb, Co, Fe and Ca contents when compared to the corresponding controls. Mean values obtained for these elements were 13.50 ± 0.05, 0.13 ± 0.00, 13.23 ± 0.00 and 825.35 ± 0.66 mg/l while the controls were 8.23 ± 0.01, 0.11 ± 0.00, 11.80 ± 0.00 and 737.00 ± 0.57 mg/l respectively. Percentage increases ranged from 11.99 for Ca to 64.03 for Pb above the values obtained for the controls. Though the values obtained for Zn, Al and Mg were higher compared to the controls, they were however, statistically insignificant (p<0.05).

Conversely, the virus strain engendered significant reduction in the amount of Cu, Cd and Ni with mean values of 3.45 ± 0.00, 0.15 ± 0.00 and 0.13 ± 0.01 mg/l respectively compared to the corresponding controls with mean values of 4.18 ± 0.57, 0.32 ± 0.03 and 0.33 ± 0.00 mg/l. Percentage reductions were as high as 53 for Cd and 60.61 for Ni. Values obtained for Mn, Na and K were comparable and consequently not significantly different compared to healthy controls.

Table 2 showed the results of MWMV-lag infection on the mineral composition of *C. manni*. Except for Cd, Al, Mn, and Fe, there was an increase in the amount of all the other elements in the infected leaf tissue compared to the controls. The contents of Pb, Cu, Co, Ni, and Na (with means values of 11.32 ± 0.05, 5.20 ± 0.01, 0.16 ± 0.00, 0.18 ± 0.01 and 1154 ± 0.33 mg/l respectively while the corresponding values for the controls were 8.14 ± 0.13, 4.35 ± 0.30, 0.11 ± 0.00, 0.13 ± 0.00 and 1029.33 ± 0.33 mg/l) were significantly higher (p<0.05) in infected leaf tissues compared to the controls. Percentage increases were
as high as 39.7 for Pb, 38.46 for Ni and 45.5 for Co. The values obtained for Ca, Zn and Mg, though higher compared to the corresponding controls did not differ significantly (p<0.05). Infection by the virus significantly reduced the amount of Cd and Mn with a mean value of 0.13 ± 0.01 and 7.20 ± 0.01 compared to the controls that had mean values of 0.26 ± 0.00 and 8.22 ± 0.23 with percentage reductions of 50 and 12.41. The reductions caused by the virus in the amount of Al and K were statistically insignificant while the amount of Fe was unaffected by the virus infection.

Table 1: Effect of Moroccan watermelon mosaic virus (Coccinia strain) infection on elemental composition of Cucumeropsis mannii

<table>
<thead>
<tr>
<th>Element</th>
<th>Infected samples (mg/l)</th>
<th>Healthy samples (mg/l)</th>
<th>% Diff^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead (Pb)</td>
<td>13.50 ± 0.05*</td>
<td>8.23 ± 0.01</td>
<td>64.03</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>3.45 ± 0.00*</td>
<td>4.18 ± 0.57</td>
<td>17.48</td>
</tr>
<tr>
<td>Cobalt (Co)</td>
<td>0.13 ± 0.00*</td>
<td>0.11 ± 0.00</td>
<td>18.18</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>0.15 ± 0.00*</td>
<td>0.32 ± 0.03</td>
<td>53.13</td>
</tr>
<tr>
<td>Nickel (Ni)</td>
<td>0.13 ± 0.01*</td>
<td>0.33 ± 0.00</td>
<td>60.61</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>13.23 ± 0.00*</td>
<td>11.80 ± 0.00</td>
<td>12.12</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>825.35 ± 0.66*</td>
<td>737.00 ± 0.57</td>
<td>11.99</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>11.25 ± 0.01†</td>
<td>10.50 ± 0.01</td>
<td>7.14</td>
</tr>
<tr>
<td>Aluminium (Al)</td>
<td>106.67 ± 0.36†</td>
<td>101.30 ± 0.05</td>
<td>5.30</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>8.47 ± 0.01†</td>
<td>8.70 ± 0.15</td>
<td>2.64</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>518.00 ± 0.57†</td>
<td>514.70 ± 0.88</td>
<td>0.64</td>
</tr>
<tr>
<td>Sodium (Na)</td>
<td>1015.33 ± 0.66†</td>
<td>1016.33 ± 0.33</td>
<td>0.10</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>1127.66 ± 0.88†</td>
<td>1136.33 ± 0.66</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Values presented were means ± SD of three determinations.

* Significant; † = not significant (p<0.05).

a = values were obtained by expressing the difference between the values of the control and the infected as a percentage of the control.
### Table 2: Effect of Moroccan watermelon mosaic virus (Lagenaria strain) infection on elemental composition of *Cucumeropsis mannii*

<table>
<thead>
<tr>
<th>Element</th>
<th>Infected samples (mg/l)</th>
<th>Healthy samples (mg/l)</th>
<th>% Diffa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead (Pb)</td>
<td>11.32 ± 0.05*</td>
<td>8.14 ± 0.13</td>
<td>39.07</td>
</tr>
<tr>
<td>Cooper (Cu)</td>
<td>5.20 ± 0.01*</td>
<td>4.35 ± 0.30</td>
<td>19.54</td>
</tr>
<tr>
<td>Cobalt (Co)</td>
<td>0.16 ± 0.00*</td>
<td>0.11 ± 0.00</td>
<td>45.45</td>
</tr>
<tr>
<td>Cadmium (Cd)</td>
<td>0.13 ± 0.01*</td>
<td>0.26 ± 0.00</td>
<td>50.00</td>
</tr>
<tr>
<td>Nickel (Ni)</td>
<td>0.18 ± 0.01*</td>
<td>0.13 ± 0.00</td>
<td>38.46</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>11.84 ± 0.01†</td>
<td>11.84 ± 0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>685.67 ± 0.33†</td>
<td>755.70 ± 21.67</td>
<td>9.27</td>
</tr>
<tr>
<td>Zinc (Zn)</td>
<td>11.32 ± 0.00†</td>
<td>10.54 ± 0.04</td>
<td>7.40</td>
</tr>
<tr>
<td>Aluminium (Al)</td>
<td>94.70 ± 0.05†</td>
<td>100.73 ± 0.68</td>
<td>5.99</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>7.20 ± 0.01*</td>
<td>8.22 ± 0.23</td>
<td>12.41</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>536.00 ± 0.04†</td>
<td>513.00 ± 0.97</td>
<td>4.48</td>
</tr>
<tr>
<td>Sodium (Na)</td>
<td>1154.66 ± 0.33*</td>
<td>1029.33 ± 0.33</td>
<td>12.18</td>
</tr>
<tr>
<td>Potassium (K)</td>
<td>1197.66 ± 0.88†</td>
<td>1137.33 ± 0.66</td>
<td>5.30</td>
</tr>
</tbody>
</table>

Values presented are means ± SD of three determinations,

* Significant; † = not significant (p<0.05).

a = values were obtained by expressing the difference between the values of the control and the infected as a percentage of the control.

The results of the effects of MWMV-cor on the proximate composition of *C. mannii* are presented in Fig 1. The results showed increase in the contents of moisture and fibre. The mean values for infected samples were 82.66 ± 0.47 and 3.72 ± 0.00 respectively, while the healthy samples had values of 81.30 ± 0.49 and 3.67 ± 0.01. There were reductions in the protein, lipid and carbohydrate contents of the inoculated plants when compared with buffer inoculated sample. The results of the mean values for the infected samples were 18.96 ± 0.14,
9.09 ± 0.00 and 61.05 ± 0.15 respectively, while the corresponding mean values of 20.28 ± 0.13, 10.90 ± 0.00 and 64.91 ± 0.19 were obtained for the healthy plants. Analysis of data showed that MWMV-cor infection of *C. mannii* caused significant reduction (p<0.05) in the content of lipid (16.60%).

There was a significantly higher protein content (33.62% increase) following MWMV-lag infection of *C. mannii* compared to the corresponding healthy sample (Fig. 2). A mean value of 20.28 ± 0.13 was obtained for the infected sample while that for the healthy sample was 13.46 ± 0.01. The results also indicated that the virus caused insignificant reduction (p<0.05) in the levels of moisture (4.24 %) lipid (2.57 %) ash (3.82 %) and carbohydrate (9.79 %).

**Figure 1:** Effect of *Moroccan watermelon mosaic virus* (MWMV-cor) on proximate composition of *Cucumeropsis mannii*
DISCUSSION

The effects of two strains of MWMV (MWMV-cor and MWMV-lag) on elemental and proximate composition of the leaf of *C. mannii* were investigated. In this study, beside Zn and Mg whose contents were found to be higher in plants infected leaf by either of the virus strains, though insignificant, and Cd that was significantly reduced by the virus strains, no general trend could be inferred for the other elements. For instance, there was significant reduction in Pb, Fe, Ca and Co contents in MWMV-cor infected *C. mannii*. Though the values obtained for Zn, Al and Mg were higher compared to the controls, they were statistically insignificant. On the other hand, Cu, Cd and Ni were significantly reduced while the values obtained for Mn, Na and K were not significantly different from compared to the healthy controls. For MWMV-lag, there were significant increases in Pb, Cu, Co, Ni and Na levels in infected leaves while the levels of Zn, K, Mg, though higher, were insignificant compared to the controls. On the other hand, while the contents of Cd and Mn were significantly reduced in infected plants by the virus strains, Ca and Al were higher, though insignificantly, compared to the controls. The level

**Figure 2:** Effect of *Moroccan watermelon mosaic virus* (MWMV-lag) on proximate composition of *Cucumeropsis mannii*
of Fe apparently was not affected by MWMV-lag infection.

The results of this study seem to confirm previous observations (20, 21, 24, 25) that reactions of plants to virus infection with regard to mineral contents are influenced by plant species or cultivars and virus strains. Comparing their reports with the results obtained in this study, there was also no definitive trend regarding the composition of mineral elements upon virus infection, as reduction in the content of some minerals in one plant-virus combination may be seen to be increased in another plant-virus combination.

Data obtained in this study indicated increase in the moisture and fibre contents and insignificant reduction in the contents of protein, lipid and carbohydrate in MWMV-cor infected leaf tissues of *C. mannii* when compared to healthy controls. The value obtained for ash was comparable with that of the control. Beside protein and fibre whose contents were higher in MWMV-lag inoculated plants, the virus caused reduction in the moisture content as well as lipid, ash and carbohydrate. For moisture content, MWMV-cor infection of *C. mannii* caused increased water content just has been reported for *Tomato aspermy virus* (TAV) infection of tomato (37) while MWMV-lag caused reduction in water content, an observation similar to that of Tinklin (38) in pepper varieties infected with TMV.

The results of this study further confirm previous reports on the fate of protein content in plants following virus infection, which is either an increase or decrease depending on the plant-virus combination. White and Blakke (39) reported increased level of protein in barley infected with WSMV and BSMV separately. Similarly, Cheema et al (30) Yardimci (24), Sinha and Srivastava (29) and Kotakadi et al (40) recorded higher protein content in soybean, alfalfa, mungbean and sunflower infected with *Soybean mosaic virus* (SoyMV), AMV, *Mungbean yellow mosaic virus* (MYMV) and *Sunflower necrosis virus* (SNV) respectively, similar to the result obtained for MWMV-lag infection of *C. mannii* in this study. Conversely, infection of MWMV-cor caused a reduction in the protein content in the same plant, an observation that had been made in *Benincasa hispida* infected by *Bottle gourd mosaic virus* (BGMV) (23), *Telfairia occidentalis* (fluted pumpkin) inoculated with *Telfairia mosaic virus* (TeMV) (31) and *Tobacco mosaic virus* (TMV) infection of tomato (41). Potyviruses are known to induce proteinaceous substances
such as pinwheels, scrolls and laminated aggregates in infected plants (16, 42, 43) and these could have been responsible for the increased protein content in MWMV-lag infected C. mannii. On the other hand, reduced protein content caused by MWMV-cor may be due to some host gene being shut-off, as suggested by (44).

Reduction in the carbohydrate content recorded in MWMV-cor infection observed in this study is similar to the observation made by Singh (29) in papaya infected with PLRV, Watson and Watson (45) in sugar beet infected Beet yellow mosaic virus (BYMV) and Gupta et al (46) in soybean (root nodules) infected by Soybean mosaic virus (SoyMV). On the other hand, increased carbohydrate content caused by MWMV-lag in this study has similarly been reported in cassava infected by Cassava brown streak virus (47). With regard to the lipid content, the present study revealed that infected leaves had less in leaves infected by both strains of MWMV.

The observed decrease in lipid content correlates with those of Bhavani et al (48) and Kotakadi et al (41) who reported lower lipid contents in leaves of sunflower infected by Sunflower necrosis virus. The reduced content of lipid could be attributed to lowered synthesis or degradation of lipid.

Mineral elements, particularly micronutrients, are involved in all metabolic and cellular functions and consequently are important in plant growth and development. Their deficiencies or excesses have been reported to impair plant’s wellbeing (49, 50, 51). Several of these elements such as boron (B), chloride (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn) are essential as catalytically active cofactors in enzymes and have also been reported to modulate the activities of antioxidative enzymes associated with stress (52, 53, 54, 55). These they do, according to Bowler et al (53) by direct or indirect formation of reactive oxygen species such as superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), hydroxyl radical (OH$^-$) and singlet oxygen, causing lipid peroxidation, protein denaturation and DNA mutation. Others have enzyme-activating functions, and yet others fulfil a structural role in stabilizing proteins (56).

**CONCLUSION**

From the results of this study, it could be inferred that decrease or increase in the elemental and
proximate contents of C. mannii engendered by the MWMV strains, were a consequence of perturbation of some physiological processes (since mineral elements and the proximate components are important in biochemical and physiological processes), expressed as visible symptoms such as mosaic, reduced leaf size and severe leaf malformation and stunting of infected plants. Knowing which processes are affected and how these perturbations influence yield performance in this cucurbit needs to be investigated.

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REFERENCES


48


EFFECT OF SOWING DATE, INTRA-ROW SPACING AND BIOPESTICIDE ON THE PRODUCTIVITY OF COWPEA UNDER STRIGA INFESTATION IN KATSINA, SUDAN SAVANNA OF NIGERIA.

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SUMMARY

A field experiment was conducted during the cropping seasons of 2015 and 2016 at the Teaching and Research Farm of College of Agriculture, Hassan Usman Katsina Polytechnic, Katsina, Sudan savanna of Nigeria. The study area lies between Latitudes 12° to 12.98° N and Longitudes 7.36° to 7.60° E, 619 m above sea level. The objective of the research was to evaluate the performance of cowpea under Striga infestation as affected by sowing dates (SD), intra-row spacings (SP) and bio-pesticides (B). The experiment was laid out in Split-Split Plot Design with three sowing dates (SD1: 2nd July, SD2: 23rd July and SD3: 13th August) allocated to the main plot, three intra-row spacings (SP1: 75 x 20 cm, SP2: 75 x 30 cm and SP3: 75 x 40 cm) allocated to the sub-plot and bio-pesticides to the sub-sub plot. The treatments were replicated three times. Each plot consisted of 6 ridges of 6 m long and 4.5 m wide. All agronomic practices for successful cowpea production were observed. Data collected were subjected to the analysis of variance and significant means were compared using LSD at 5 % level of probability. The physical and chemical analysis of the soil identified the soil as sandy loam with very low organic carbon (< 4 g kg⁻¹), total nitrogen (< 0.6 g kg⁻¹) and low available phosphorus (3-7 mg kg⁻¹). Lower Striga counts were observed on cowpea sown on SD3 (126.81) per cm² which was significantly different (P ≤ 0.01) from SD1 (335.42) and SD2 (238.31) which were statistically similar. The result of the effect of intra-row spacing shows...
Spacing showed that highly significant ($P \leq 0.01$) Striga counts were obtained in SP2 (236.56) but were statistically at far with SP1 (236.06). The least Striga count was obtained in SP3 (227.92). The area under Striga number progress curve (ASNPC) showed that there was up to 6 and 5.8 Striga density per m². This implies that there is possibility of 60 and 58% cowpea yield reduction during 2015 and 2016, respectively. Managements which involve early soil testing (prior to sowing) and use of Striga resistant variety could give a better control strategy in the study area.

Keywords: Striga, gesnerioides, infestation, cowpea, productivity, Sudan, savanna

**COWPEA** (*Vigna unguiculata* L. Walp.), is a dicotyledonous, leguminous plant belonging to the family Fabaceae. It is one of the ancient crops known to man. An important crop cultivated by many small-scale farmers as subsistence crop in Africa. The crop originated from Africa and spread through Egypt and domesticated in parts of Southern, Eastern and Western Africa where a large number of primitive cultivars and semi wild forms were found (20). The crop is grown primarily as the most important grain legumes in the savanna regions of the tropics and sub-tropics in Africa, Asia and South America (1, 14, 20 28). One of the limiting factors of sustainable cowpea production is the attack by *Striga* (*Striga gesnerioides* (Wild) Vatke).

The parasitic weed severely damage cowpea plants. Early reports of *Striga* attack by past researchers described it as an obligate biotic stress, a noxious parasitic weed that attack cowpea reducing its productivity in small holder farming sector (15, 23). Zitta *et al.* (32) and Omoigue *et al.* (30) described *Striga* as root parasitic flowering plant of the family Orobonchaceae, a limiting factor to cowpea production especially in the dry savannas. The effect of this parasitic angiosperm on cowpea starts well ahead before its emergence above the ground (16). Since *Striga* is a parasitic weed the seedlings cannot sustain themselves on their own resources for long after germination. Therefore, they need to find a host root shortly after germination and the germination needs to be perfectly timed with the presence of a host root. Exogenous germination stimulants called *strigolactones* are produced by the host’s root and also by some non-host roots (usually referred to as trap crops) (e.g. *Gossypium* spp.). They are plant hormones which inhibit
shoot branching (11) but also signals to seeds of parasitic weeds such as Striga to start germination. Strigol, a synthetic compound belonging to the strigolactones, was first isolated from cotton (Gossypium sp.) and is used as a germination trigger for Striga. Being obligate in its mode of nutrition, when the seed (Striga) has started to germinate, the haustorium develops which attaches to the host plant (7). A xylem-xylem connection is created between it and the host plant, in that way the seed can withdraw water and nutrients from the host plant (15), photosynthates (organic compounds) (15, 29).

According to Amusan et al (6), yield losses as a result of Striga infestation in Africa depend on the crop cultivar, weather, and degree of infestation. Cowpea yield losses associated with Striga infestation is enormous. Depending on the extent of damage and level, Striga infestation is often more serious under low soil fertility and increased moisture stress conditions (6). According to Parker (26) problems with Striga are generally caused by low economic resources, poor soil fertility, newly infested areas due to unclean sowing materials and cropping of host crops. Emechebe et al (9) reported 100% yield loss on farmer’s fields in the northern Guinea savanna of Nigeria. Noubissie et al (23) stated that yield loss due to $S.\ gese$nerioides may be up to 70%. On susceptible cowpea cultivars (16, 17) reported yield loss reaching 100% when $S.\ gese$nerioides abundance was over 10 per plant. This research was aimed at providing a baseline study on Striga control on cowpea as affected by varying sowing dates, intra-row spacings and biosticides in the study area.

MATERIALS AND METHODS

Study Area

A field experiment was carried out during 2015 and 2016 cropping seasons in Katsina, Latitudes 12° 59’ and 12.98° 01’ N and Longitudes 7.36° and 7.60° E, 619 m above sea level in the Sudan savanna of Nigeria (19). The experiment was conducted at the Teaching and Research Farm, College of Agriculture, Hassan Usman Katsina Polytechnic during the rainy seasons of 2015 and 2016, respectively. The rainy season in the area starts from May and ends in October with mean annual rainfall of 742 mm. According to Koppen Climate Classification system, Katsina has a hot semi-arid climate. The average temperature is between 33.2- 42.2°C, average relative humidity (at 07:00 LST) is 60%. The major crops grown include maize, sorghum, millet, rice, cotton,
groundnut, sesame, soya bean and cowpea (22).

The experimental site was ploughed, harrowed and ridged using tractor. SAMPEA 7 Cowpea variety was used. Prior to planting, the seeds were dressed with Allstar® 40 SD (20% Metalaxyl, 20% limidaclorpid) at the rate of one sachet per 2.5 kg of seeds against soil-borne diseases and insect pests (25). Sowing was carried out at three weeks intervals. Three cowpea seeds were sown per hole and later thinned to two seedlings per stand (18). Single super phosphate fertilizer was applied at the rate of 25 kg P₂O₅ ha⁻¹ to each plot immediately after sowing. Mancozeb 80% as Z-force (family of ethylene Bisdithiocarbamate) at the rate of 0.33 kg a.i. ha⁻¹ was applied against fungal diseases when symptoms were observed associated by appearance of powdery substance, leaf spots, lesions and leaf curl. Weeding was carried out at 3 and 6 weeks after sowing (WAS) (3). Gap filling was done at three weeks after germination (24).

Morphological Characteristics of Test crop, SAMPEA 7

SAMPEA 7 popularly known as IAR 48 was produced from a cross between Ife Brown and Yella (a local variety introduced from Birniwa District in Jigawa State). It was developed and released by Institute for Agricultural Research, Ahmadu Bello University Zaria, Nigeria in 1986. The crop has large, light brown seeds with a rough seed coat texture. It is medium maturing (90 to 100 days) variety adapted to Savanna and forest zones. The crop has a potential yield output of 1,500 - 2,500 kg ha⁻¹. However, it is susceptible to Scab, Bacterial blight, Septoria leaf spot, Brown blotch, Beetles, Thrips, Maruca pod borer, pod sucking bugs, Bruchids and Striga (13).

A composite soil sample was collected using soil auger at the depth of 0-15 and 15-30 cm for laboratory analysis. Samples were analyzed in the Soil Science laboratory, Department of Agronomy, Faculty of Agriculture/Institute for Agricultural Research, ABU Zaria, Nigeria.

The experiment was carried out during the rainy seasons of 2015 and 2016. Split-split plot design with sowing dates (SD1; 02/07/2015, SD2; 23/07/2015 and SD3; 13/08/2015) allocated to the main plot and intra-row spacing (S1; 75 x 20cm, S2; 75 x 30cm and S3; 75 x 40 cm) allocated to the sub plot and (B0; control, B1; Neem seeds kernel extract (NKE), B2; Maruca vitrata Multi-nucleopolyhedrosis virus (MaviMNPV) suspension and B3;
Cyper diforce (30g cypermethrin + 250g dimethoate) allocated to the sub-sub-plot (a total of 36 treatments). The trial was repeated during the same period in 2016. Each plot consisted of six (6) ridges of 6 m long and 4.5 m wide. The ridges were separated by 0.75 m apart. The two middle rows (3rd and 4th) constituted the net plot, 2nd and 5th rows for destructive sampling while rows 1st and 6th constituted boarders (20). The blocks were separated by 2 m while 1 m was left between plots. A distance of 1 m was also left between main plots. Data were collected on the following parameters.

Striga Count

Striga count per m² was carried out by counting the number of Striga shoots in the net plot at 7th and 10th weeks after sowing (WAS) using a m² quadrant.

Assessment of Area under Striga Number Progress Curve

Area under Striga number progress curve (ASNPC) which is a measure of the total Striga emergence throughout the season and was calculated using successive Striga counts described by Haussmann et al. (12).

\[
ASNPC = \sum_{i=0}^{n-1} \left[ \frac{Y_i + Y_{i+1}}{2} \right] (t_{i+1} - t_i)
\]

Where:

- \(n\) is the number of dates for the Striga that emerged
- \(Y_i\) Striga count at the \(i\)th assessment date,
- \(t_i\) DAS at the \(i\)th assessment date
- \(t_0\) and \(Y_0 = 0\)

Harvesting of dried pods was carried out when about 75 % of the pods in the net plot dried. Subsequently, harvesting was carried out until all the pods were fully harvested. The pods were threshed, winnowed and grains measured using an Electronic Compact Scale (ATOM A-110 model). Plot yields were placed in large envelops/polythene bags and adequately labelled. Post-harvest operations on the yield were carried out separately for each plot. Yield per plot was extrapolated to kg ha⁻¹ (4). Data obtained were subjected to analysis of variance (ANOVA). Means were separated using LSD at \(P \leq 0.05\) probability level using SAS (27) statistical package.

RESULTS

Physical and chemical Soil Characteristics of the Experimental Site
The physical and chemical soil analysis of the experimental site during 2015 and 2016 cropping seasons is presented in Table 1. The results of the analysis showed that, the soil textural class was sandy loam, the soil pH of the location ranged from slightly acidic to neutral. The soil was characterized by low to moderately organic carbon (1.00 – 1.01%), very low total N (0.14-0.16%) and low to moderate available phosphorus (5.65-5.88 mg kg\(^{-1}\)). The exchangeable cations Ca, Mg, K and Na ranged from (0.11-2.50 mg kg\(^{-1}\)) and the soil CEC ranged from 3.01-3.02 mg kg\(^{-1}\).

**Table 1:** Physical and chemical soil characteristics of the experimental site at 0-30 cm depth during 2015 and 2016 cropping seasons

<table>
<thead>
<tr>
<th>Soil characteristics</th>
<th>2015</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical Analysis (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sand</td>
<td>71</td>
<td>74</td>
</tr>
<tr>
<td>Silt</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Clay</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Textural class</td>
<td>Sandy loam</td>
<td>Sandy loam</td>
</tr>
<tr>
<td>Chemical Analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH ratio: (1:2.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH (H(_2)O)</td>
<td>6.92</td>
<td>6.96</td>
</tr>
<tr>
<td>pH CaCl(_2) (0.01 M)</td>
<td>5.89</td>
<td>5.98</td>
</tr>
<tr>
<td>Organic carbon (%)</td>
<td>1.00</td>
<td>1.01</td>
</tr>
<tr>
<td>Nitrogen (%)</td>
<td>0.16</td>
<td>0.14</td>
</tr>
<tr>
<td>Phosphorus (mg kg(^{-1}))</td>
<td>5.65</td>
<td>5.88</td>
</tr>
<tr>
<td>Exchangeable bases (meq 100(^{-1})g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>2.42</td>
<td>2.50</td>
</tr>
<tr>
<td>Mg</td>
<td>0.35</td>
<td>0.31</td>
</tr>
<tr>
<td>K</td>
<td>0.20</td>
<td>0.11</td>
</tr>
<tr>
<td>Na</td>
<td>0.16</td>
<td>0.14</td>
</tr>
<tr>
<td>CEC (meq 100(^{-1})g)</td>
<td>3.01</td>
<td>3.02</td>
</tr>
</tbody>
</table>

Soil samples as analyzed in the Soil Science laboratory, Department of Agronomy, Faculty of Agriculture/Institute for Agricultural Research, ABU Zaria, Nigeria.
Effect of Sowing Dates, Intra-Row Spacings and Biopesticides on *Striga* Count and Cowpea performance

The result in Table 2 shows the effect of *Striga* on the performance of cowpea. Although, there was no significant difference between sowing dates and intra-row spacings on effect of *Striga* on cowpea performance in all the years and combine. Lower *Striga* counts were observed during 2016 cropping season. The least (17.22) and highest (21.14) striga counts were recorded on cowpea sown on 23\textsuperscript{rd} July (SD2) and 2\textsuperscript{nd} July (SD1), respectively. This showed that delay in sowing to SD2 reduced *Striga* density per unit metre. Similarly, lower *Striga* counts (17.89) was recorded in cowpea spaced at 75 cm x 40 cm (SP3), while the highest (19.64) was recorded on cowpea spaced at 75 cm x 20 cm (SP1). The effect of biopesticides was however, statistically similar except in Cyper diforce treated plots which recorded significantly higher *Striga* counts (552.59) during 2015 cropping seasons.

The result of interaction of sowing dates and intra-row spacings during 2015 cropping season is presented in Table 3. Varying sowing dates was observed to statistically result to similar interaction effect in all the intra-row spacings except in SD3 with SP2 and SP3 in which the interaction was highly significant. Similar interaction effect was observed among the intra-row spacings in the sowing dates. A highest (1.44) control effect was obtained by SD3 x SP2 and the least (2.44) effect was observed by SD2 x SP1.

Area under *Striga* number progress curve (ASNPC) and Cowpea yield performance

The ASNPC presented in Figures 2 and 3 during 2015 and 2016 cropping seasons showed that there was up to 6 and 5.8 *Striga* per plant, respectively. This implies that there is possibility of 60 and 58 % cowpea yield reduction. The result of the effect of varying sowing dates on total grain weight in Katsina is presented in Figure 3. There was no significant difference in yield by varying sowing dates when results of two years combined. However, varying sowing dates in 2016 significantly increased total cowpea grain weight at $P \leq 0.05$. Statistically similar yields were obtained in SD1 (148.01 kg ha\textsuperscript{-1}) and SD2 (190.83 kg ha\textsuperscript{-1}), respectively. The highest grain weight (252.60 kg ha\textsuperscript{-1}) was obtained in SD3.
Table 2: Effect of Sowing Dates, Intra-Row Spacings and Biopesticides on Striga Count on the Performance of Cowpea during 2015 and 2016 Cropping Seasons in Katsina

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2015</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined Sowing date (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD1</td>
<td>335.42</td>
<td>21.14</td>
</tr>
<tr>
<td>SD2</td>
<td>238.31</td>
<td>17.22</td>
</tr>
<tr>
<td>SD3</td>
<td>126.81</td>
<td>17.81</td>
</tr>
<tr>
<td>Mean</td>
<td>233.51</td>
<td>18.72</td>
</tr>
<tr>
<td>LSD</td>
<td>255.71</td>
<td>5.255</td>
</tr>
<tr>
<td>Intra-row spacing (cm) (SP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SP1; 75 x 20</td>
<td>236.06</td>
<td>19.64</td>
</tr>
<tr>
<td>SP2; 75 x 30</td>
<td>236.56</td>
<td>18.64</td>
</tr>
<tr>
<td>SP3; 75 x 40</td>
<td>227.92</td>
<td>17.89</td>
</tr>
<tr>
<td>Mean</td>
<td>233.51</td>
<td>18.72</td>
</tr>
<tr>
<td>LSD</td>
<td>67.131</td>
<td>3.852</td>
</tr>
<tr>
<td>Biopesticide (B)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1: Neem kernels extract</td>
<td>133.26b</td>
<td>17.82</td>
</tr>
<tr>
<td>B2: MaviMNPV suspension</td>
<td>107.22b</td>
<td>18.52</td>
</tr>
<tr>
<td>B3: Cyper diforce</td>
<td>552.59a</td>
<td>18.07</td>
</tr>
<tr>
<td>B0: Control</td>
<td>140.96b</td>
<td>20.48</td>
</tr>
<tr>
<td>Mean</td>
<td>233.51</td>
<td>18.72</td>
</tr>
<tr>
<td>LSD</td>
<td>122.17</td>
<td>4.013</td>
</tr>
<tr>
<td>Interactions SD x SP</td>
<td>**</td>
<td>NS</td>
</tr>
</tbody>
</table>
Means with the same letter(s) in the same column are not significantly different using LSD at 5 % level of probability, NS = Not significant, * = Significant at 5 %, ** = highly significant at 1 %, SD = Sowing date (SD1= 02/07/2015, SD2 = 23/07/2015, SD3 = 13/08/2015); SP = Intra-row spacing.

**Table 3:** Interaction between sowing dates and intra-row spacings on *Striga* counts on cowpea during 2015 cropping season in Katsina

<table>
<thead>
<tr>
<th>Sowing dates</th>
<th>Intra-row spacings</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SP1</td>
<td>SP2</td>
</tr>
<tr>
<td>SD 1</td>
<td>2.40^a</td>
<td>2.42^a</td>
</tr>
<tr>
<td>SD 2</td>
<td>2.44^a</td>
<td>2.22^{ab}</td>
</tr>
<tr>
<td>SD 3</td>
<td>2.28^a</td>
<td>1.44^{c}</td>
</tr>
<tr>
<td>LSD</td>
<td>0.248</td>
<td></td>
</tr>
</tbody>
</table>

Key: SP1: 75 x 20, SP2: 75 x 30; SP3: 75 x 40 cm, SD = sowing date, SD1; 02/07/2015, SD2; 23/07/2015, SD3; 13/08/2015

**Figure 1:** Area under *Striga* number progress curve for *Striga* counts per plot during 2015 cropping season
Figure 2: Area under *Striga* number progress curve for *Striga* counts per plot during 2016 cropping season

Figure 3: Grain yield of cowpea (kg ha\(^{-1}\))
SD1: 02/07/2015, SD2: 23/07/2015, SD3: 13/08/2015 during 2015 and 2016 cropping seasons
DISCUSSION

The results of the analysis of physical and chemical characteristics of the experimental site showed that these C, N and P nutrients are deficient and generally characterized by very low organic carbon (< 4 g kg\(^{-1}\)) and total nitrogen (< 0.6 g kg\(^{-1}\)) and low available phosphorus (3-7 mg kg\(^{-1}\)) according to [10]. Soil organic carbon gives an indication of soil organic matter content. This is consistent with the findings of Kamara et al. 2014 (17) who observed that low level of P and organic matter are common in sandy Sudan savanna of Jigawa state. The *Striga* infestation observed could be attributed due to low level of N and P in the soil. This finding corroborates the report of Kamara et al. (17) and Yoneyeme et al. (31) who stated that the low level of N and P could have implications for *Striga* infestation in cereal crops and that under N and P deficient conditions, cereal such as sorghum, maize and rice were reported to produce high amount of *Strigolactones* (SLs) that ultimately stimulate germination of *Striga* seeds in the soil. Lopez-Obando et al (21) reported Cook et al. (8) that ‘*strigolactones*’ came from the first identified role of these compounds as stimulants of seed germination in species of the parasitic weed such as *Striga*. Zwanenburg et al. (33) regarded SLs as germination stimulants for parasitic plants. Cowpea host reaction due to *Striga* infestation could be observed inform of stunted growth, chlorosis (yellowing), necrosis (death), delay in flowering and pod formation and overall yield reduction (17).

Furthermore, the poor performance could also be attributed partly due to heavy *Striga* infestation as well as to the activities of insect pests. These findings corroborate the findings of Kamara et al. (17) who stated that 10 *Striga* plant\(^{-1}\) can cause 100 % yield reduction in cowpea.

CONCLUSION

It can be concluded from the results obtained that *Striga* infestation on cowpea in the ecological zone constitutes threat to cowpea productivity. Varying sowing dates and intra-row spacings did not have any effect on *Striga* performance on cowpea. Sowing cowpea at 23\(^{rd}\) July and at wider spacing of 75 x 40 cm reduces effect. The variety used in this study performed below its potential yield capacity according to IAR (13). Hence, efforts should be geared towards its management in order to reduce cowpea yield loss for an overall increase in food security.
ACKNOWLEDGEMENT

The authors thanked the contributions of Hajiya Wajiha Abdullahi Mu’az, IITA, Kano Zonal office for the role she played in the determination of area under Striga number progress curve used in this research.

REFERENCES


PHYTOPHTHORA POD ROT AND THE RAGING ENEMIES OF COCOA PRODUCTION IN NIGERIA: A REVIEW

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SUMMARY

*Theobroma cacao* is of major economic importance to Nigeria and other growing countries across the tropics. Being one of the fastest selling commodities in the world, demand for its beans is very robust. But contrary to what generally operates in the global scene, cocoa production in Nigeria has in recent years decreased, plunging the country to the sixth position in the world production table. The problems of pests and diseases, significant among which is *Phytophthora* pod rot (black pod), has been said to be hugely responsible for this challenge. *Phytophthora palmivora* and the highly virulent *P. megakarya* among others, have however been discovered to be major causative agents of the disease, with the latter so far reported only in West and Central Africa. In Nigeria, *P. megakarya* has over the years replaced *P. palmivora* as the main causative agent of black pod disease. The pathogens cause brown to black lesions on infected cocoa pods at every stage of development. Loss due to black pod disease of cocoa is between 20-100% annually. The infection does not only affect the pods, it also spoils the beans. Several measures have been reportedly taken by Nigerian farmers to combat cocoa pod rot, prominent among which is the use of synthetic fungicides. This, though highly effective and reliable when used at recommended dosage, has remained hazardous and expensive. Ineffectively checked proliferation of the pathogen probably due to increasingly high cost of control, has been hugely responsible for the dwindling cocoa production levels experienced in Nigeria in recent years. Long time survival of the pathogen in the soil/debris and climatic factors has made a reasonably significant control of the disease a big challenge to Nigerian farmers. Detailed exploration of
the use of botanicals with other safe and affordable techniques would therefore help keep the problems at bay.

**Keywords:** Cocoa, pod rot, Phytophthora, production, Nigeria

**CACAO** is an important crop around the world. It is a cash crop for growing countries and a key import for processing and consuming countries. The crop is a member of the large family, Malvaceae, which is comprised of former families Sterculiaceae (cocoa and kola), Bombaceae (baobab, durian and kapok), Malvaceae *sensu lato* (cotton, hibiscus and okra) and Tiliaceae (basswood) (Ploetz, 2007). It can grow in soils ranging from acidic to slightly alkaline, with a pH of 6.5 optimal for nutrient uptake by the trees (Vanegtern *et al*., 2015). The crop flourishes well under a humid, warm and constant environmental condition of between 25 and 35°C with a wide rainfall range of between 1,000 and 3,000mm per annum or more. In Nigeria and other African countries where cocoa is grown, a minimum of 25% of clay is required for the crop to be healthy and highly productive (Opeke, 2005). In high-rainfall areas however, increased soil moisture can increase the potential for black pod rot (Vanegtern *et al*., 2015).

Cocoa remains one of the fastest selling and most desirable agricultural commodities in both the local and international markets. Demand for it is very robust, moving in tandem with the rapid growth and expansion of chocolate confectioneries and other related products. Cocoa has an added advantage because it is a perennial crop and can survive for decades. Once planted, nurtured to maturity and harvested, farmers are most likely to cash out for a very long time (PII, 2017).

Worldwide production of cocoa in the 2012/2013 season stood at over 3,575,000 metric tonnes, which was lower than the about 4,205,000 tonnes produced in 2016/17, indicating a noticeable overall increase in production. While Nigeria ranked sixth on the 2016/17 world cocoa production table, some other West African neighbours: Ivory Coast and Ghana (with the production levels of 2,010,000 and 950,000 metric tonnes) ranked first and second respectively. Ivory Coast has been said to account for more than 35% of the entire world cocoa production over the past five years (Table 1).

In Nigeria, cocoa was a major agricultural export crop and a top foreign exchange earner in the 1950s
and 60s. Prior to the discovery of crude oil in commercial quantities in the 1970s, Nigeria became the world’s second largest producer of the crop. Average cocoa production declined from 420,000 tonnes in the ‘60s to 170,000 tonnes in 1999. Production climbed to 389,272 tonnes between years 2000 and 2010, but fell back in subsequent years. Nigeria’s cocoa production status significantly reduced from 248,000 metric tonnes in the 2013/2014 season to 195,000, 200,000 and 225,000 metric tonnes in the 2014/2015, 2015/2016 and 2016/2017 seasons respectively. Some years after dropping to fourth place, Nigeria has now become the sixth largest cocoa producer in the world (PII, 2017; Table1).

Cacao is grown in fourteen out of the thirty-six states of Nigeria. The cacao growing states are grouped into three ecological zones which include, the ideal cocoa climate ecology (comprising of Ondo, Ekiti, parts of Osun and Edo States) with annual rainfall range of 2,000 to 2,500mm, the ideal cocoa soil ecology (comprising of Cross River, Akwa Ibom and parts of Abia States) with deep clayed, chocolate coloured soil and rainfall in excess of 4,000mm per annum, and the marginal cocoa ecology (comprising of parts of Oyo, Kwara, Ogun, Kogi, Delta, Adamawa and Taraba States) (Olaiya, et al., 2006).

Despite the availability of environmental conditions necessary for survival of the crop in Nigeria and some other tropical countries where it’s being cultivated, cacao productivity/production is known to be hugely faced with the menace of pests and diseases (Samuels et al., 2012; Meinhardt et al., 2008). The low and declining cocoa yields currently being experienced in the country has been alluded to pests and disease attacks as well as inconsistent production patterns. Low levels of mechanization with dependence on cutlass and hoe agriculture and ageing of cocoa fields play a role in decreased productivity, especially in the southwestern states which contribute nearly 80% of national cocoa yields. If no steps were taken, the unrelenting challenges of a weak infrastructure, corruption, political instability, poor (and expensive) access to financing and weak implementation capacity have great probability to further negatively affect the industry’s potential in the country (Adelodun, 2017; PII, 2017).

Some of the major diseases and pests affecting cocoa in the tropics include black pod (Phytophthora pod rot), cherelle wilt, frosty pod rot, cocoa swollen shoot disease, charcoal rot,
cocoa mirids, mealy bug, and cocoa pod borer. The black pod disease has been identified as the most serious disease of cocoa in West Africa, especially in Nigeria. It is mostly caused by a fungus *Phytophthora megakarya* in the region (Ali et al., 2017).

**Table 1:** Top cocoa beans producing countries in 2012/2013 – 2016/2017

<table>
<thead>
<tr>
<th>S/N</th>
<th>Country</th>
<th>Amount produced (Thousand metric tonnes)/Season</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Côte d'Ivoire</td>
<td>1,449</td>
</tr>
<tr>
<td>2.</td>
<td>Ghana</td>
<td>835</td>
</tr>
<tr>
<td>3.</td>
<td>Indonesia</td>
<td>410</td>
</tr>
<tr>
<td>4.</td>
<td>Ecuador</td>
<td>192</td>
</tr>
<tr>
<td>5.</td>
<td>Cameroon</td>
<td>225</td>
</tr>
<tr>
<td>6.</td>
<td>Nigeria</td>
<td>238</td>
</tr>
<tr>
<td>7.</td>
<td>Brazil</td>
<td>185</td>
</tr>
<tr>
<td>8.</td>
<td>Papua New Guinea</td>
<td>41</td>
</tr>
</tbody>
</table>

Source: Statista (2018)

**The Phytophthora pod rot**

Phytophthora pod rot, also known as black pod rot or black pod disease of cacao, is caused by some pathogens in the genus *Phytophthora*, literally translated as the “plant destroyer.” This is the same genus responsible for the Irish potato famine of 1845–1852. These pathogens were originally classified as fungi but have since been reclassified into the kingdom *Stramenopila* (Cook, 2018; Acebo-Guerrero, 2012). There are more than 80 species of *Phytophthora* that cause plant diseases, out of which seven have been named as causative agents of black pod disease all over the world. The seven implicated species are *P. capsici, P. citrophthora, P. megasperma, P. katsurae, P.*
palmivora, and P. megakarya. While all of these pathogens can cause black pod disease, the two major pathogens are *P. palmivora* and *P. megakarya* (Guest, 2007).

Black pod rot is the most important disease of cacao on a global scale with losses estimated at 700,000 metric tonnes in 2012 (Ploetz, 2016). *Phytophthora palmivora* is present in most of the cacao growing countries around the globe and has a broad host range (McHau and Coffey, 1994). *Phytophthora megakarya* occurs only in the countries of West and Central Africa and is considered a significant pathogen only on cacao. *P. megakarya* is the most virulent species in the *Phytophthora* genus causing 60–100% cocoa pod/crop losses if not managed (Opoku et al., 2000), whereas *P. palmivora* generally causes losses of 20–30% annually (Flood et al., 2004). Cankers caused by the pathogen may kill up to 10% of all trees each year (PII, 2017).

*Phytophthora megakarya* was first identified taxonomically as a species in 1979 (Ali et al., 2017). By the mid-1980s, the pod rot pathogen became predominant on cacao in Nigeria, Cameroon, Equatorial Guinea, Gabon, and Togo (Ali et al., 2017), was confirmed in Ghana in 1985 (Dakwa, 1987), and continues to spread (Ali et al., 2016).

*Phytophthora palmivora* is no longer routinely isolated from cacao in Cameroon and Nigeria (Nyasse et al., 1999; Ndubuaku and Asogwa, 2006; Djocgoue et al., 2007), but how *P. megakarya* has displaced *P. palmivora* from cacao in these countries is still unclear (Ali et al., 2016).

*P. palmivora* tends to have a more rapid growth rate than *P. megakarya* in culture, possibly contributing to its ability to cause accelerated necrosis in mechanically wounded cacao tissues compared to *P. megakarya* (Ali et al., 2016). In a susceptible cacao genotype, mechanical wounding is almost irrelevant for *P. megakarya* infection (Ali et al., 2016). Although cacao remains the only economically important host of *P. megakarya*, isolates of the organism were not grouped taxonomically with *P. palmivora* isolates until 1979, when it was recognized as a new species (Brasier and Griffin, 1979; Despréaux, 2004). Despréaux et al. (1987) however reported that *P. megakarya* is not saprophytically active in the absence of the host. They found the infection potential of the soil decreased rapidly with time outside epidemic periods.
Disease History

*Phytophthora megakarya* is primarily known for the damage it causes to the cocoa pod. It has also been found to subsist on cacao roots (Gregory *et al.*, 1984) and can cause dieback of seedlings (Bowers *et al.*, 2001). Since all *Phytophthora* spp. causing black pod disease of cacao in Africa were classified as *P. palmivora* prior to 1979 (Brasier and Griffin, 1979), it is unknown when *P. megakarya* was first “discovered.” The center of origin of the species is however believed to be primary forests of Central and West Africa. To date, *P. megakarya* has only been reported in these geographical regions (Nyasse *et al.*, 1999).

**Disease cycle, symptoms and signs**

*Phytophthora megakarya*, like *P. palmivora*, undergoes a series of developmental stages throughout the cocoa pod rot disease cycle (Figure 2). This includes the formation of mycelium and three main spore types, i.e., sporangia, zoospores and chlamydospores that may directly or indirectly cause infection. Primary inoculum in the form of mycelium in soil and bark cankers develop into sporangia, which germinate during wet and humid conditions to establish an infection (Luterbacher, 1994).

A successful infection results in the generation of secondary inoculum of sporangia containing motile biflagellate zoospores. The most important developmental factor in *P. megakarya* is its ability to emit zoospores earlier and also two times more than *P. palmivora*. Zoospores actively detect and swim toward cacao plant tissue to infect it (direct infection) or encyst in the absence of free water and germinate later to infect susceptible plant tissue (indirect infection). Under humid conditions, a single pod may produce up to 4 million sporangia (containing motile zoospores) that are disseminated by rain, movement of planting materials, insects and rodents, and contaminated harvesting tools and pruning implements. Chlamydospores are the principal long-term survival structures of *P. megakarya* in soils. These chlamydospores develop into mycelia and infect cacao tissue (Vanegtern *et al.*, 2015).

Black pod disease starts when the infected pod shows some little yellow spots, which eventually turn brown and enlarge to a dark brown or black lesion within five days. The lesion is fast growing and covers the entire pod after eight days of infection. The infection does not only occur on the pod surface, it also invades inside the pod, affecting the
beans. The growth of white mycelia on black pod is visible after 11 days and the sporulation is initiated. The dispersal of sporangia or zoospores through water, ants and other insects occurs at this stage and will infect other healthy pods nearby. Direct contact of a black pod with healthy pods also leads to the spread of disease (Philip-Mora and Cerda, 2009).

In addition, infected flower cushion and mummified pods are the locations for *P. palmivora* survival during dry season, where the pathogen will grow and continue to infect other developing pods. The infection occurs at any stage of pod development where it causes wilting and dying of young pods and destroys the beans of mature pods. When pods are infected (or become mummified), they can produce a massive amount of inoculum to infect other pods; it is especially abundant in the infections caused by *P. megakarya*. *P. megakarya* causes similar symptom as *P. palmivora*, but the occurrence is faster and generally produces greater number of spores (Guest, 2007; Luseni and Kroma, 2012; Opoku, *et al.*, 2000).

In addition, as *Phytophthora* can persist in soil and debris for months to several years and susceptible pods may be present on the trees most of the year, the pathogen may always be present in the canopy, ready to cause major epidemics when environmental conditions become favourable for sporulation and dispersal. In addition, factors such as rain and wind, as well as biotic elements such as ants, beetles and insects in general should be considered of importance for black pod spread (Acebo-Guerrero *et al.*, 2012).
Figure 2: Disease cycle of *P. megakarya* on cacao

In the cycle, (a) sporangiophore bearing sporangia, (b) sporangia containing zoospores, (c) zoospores being discharged from sporangium, (d) infection on cacao pod, (d) infection on tree trunk, (e) infection on leaf, (f) different levels of infection on cocoa pods, mycelia and encysted zoospores are shown.

**Source:** Akrofi (2015)

In determining the survival of *P. palmivora* and *P. megakarya* in soils, the two species were introduced into plantation soil before the dry season. *Phytophthora palmivora* could be recovered for ten months and *P. megakarya* for 18 months after the introduction. The long time survival of *P. megakarya* in soil and infected debris, and evidence of its adaptation in soil and survival on roots of cacao and other forest trees (Opoku et al., 2002) makes the control of *P. megakarya* difficult. Therefore, management of the disease should be approached from different angles (Acebo-Guerrero, 2012).
Plate 1: A cacao pod and bean mass consumed by *Phytophthora palmivora*.

Source: Vanegtern (2015)

**Mode of dispersal**

The spread of spores through air from infected pods was observed and some assumption regarding this mechanism of dispersion has been made in previous studies. It was assumed that under close canopy, less water will reach the sporulating pods to spread the inoculum. However, rain drops from leaves and branches could also splash the inoculum to the surroundings. Infected pods laying on the ground or litter could also spread the inoculum, yet greater infection was observed on pods located under infected pods hanging on the tree compared to pods at the same level with the infected pods. It was reported that splash of wind-blown droplets from the infected pods are also able to infect pods on different trees nearby. The disease spreads primarily through dissemination of sporangia via rain and wind, insects, and human transmission (Maddison and Griffin, 1981; Opoku *et al.*, 2007).

The black pod rot pathogen is dispersed by caducous sporangia, and the disease is clearly polycyclic. Sporangia form on the surface of infected pods at relative humidities in the range 60-80% RH and temperatures between 20-30°C. Sporangia can germinate directly via
a germ tube, or indirectly to release about 30 zoospores. Wet, showery conditions are essential for infection and spread. Wood (1974) has shown that long periods of relative humidity at saturation point are required for the rapid spread of disease. The theory that relative humidity is the most important climatic factor helps to explain the higher incidence in Nigeria than in Ghana, and the almost complete absence of black pod disease in Malaysia (CABI, 2017).

The spread of infection to pods above bare soil was shown to be greater relative to pods above litter. The reason for this is due to the splash of rain from bare soil spreads the inoculum to pods. However, litter under the tree prevented water droplets from splashing the soil particles as well as the inoculum beneath the litter to the above pods (Maddison and Griffin, 1981).

**Diagnosis**

*Phytophthora megakarya* can be readily isolated from diseased pods through plating onto V-8 agar or potato dextrose agar media containing antibiotics such as P10VP medium. Tissue for plating should be selected from the advancing margin of the lesion. If the fungus is sporulating on the surface of the pod, sporangia can be picked off with sterile forceps and plated directly (CABI, 2017, Plate 3a-b).

**Plate 3a-b:** Growth of *P. megakarya* on V8 agar (left) and potato dextrose agar (right)

*Source: Widmer and Hebbar (2013)*
STRATEGIES ADOPTED FOR CONTROL OF PHYTOPHTHORA POD ROT IN NIGERIA

Cultural Control

The recommended cultural practices for the control/management of black pod disease of cocoa involve adequate tree spacing (3.1m x 3.1m) and pruning. These are important for cocoa planting in order to allow in more light and air flow around the trees. Konam and Guest (2002) opined that the level of humidity that causes black pod disease would be reduced as a result. The lowered humidity would enhance a quicker drying of the pod surface. Planting under thinned jungle is commonly employed in West Africa. This, though cheap and simple, provides uneven shade which is difficult to regulate. Shade is however, critical in young trees to promote the development of the most productive canopy shape. Clear felling of jungle, followed by planting of temporary and permanent shade trees, allows more effective regulation of light (CABI, 2017).

Removal of pods with black pod symptoms should also be done to eliminate the sources of inoculum. Phytosanitary pod removal was observed to significantly reduce disease occurrences by 9–11% to 22–31%, where this practice removes the source for secondary inoculum. However, increase in disease incidence after raining season was observed to be most likely due to the spread of inoculum from survival site by the rain. The application of fungicide following sanitation is commonly performed for an effective control of disease, as sanitation practice alone would not completely eliminate the source of inoculum but still causes greater black pod incident compared to sanitation followed by at least one fungicide application (Adesiji et al., 2007; Opoku et al., 2007; CABI, 2017).

Chemical control

Fungicides have been extensively used for black pod control. In Nigeria, commercial applications began in 1953, using carbide bordeaux mixture. The application of copper fungicides has been shown to significantly reduce a great number of black pod incidences in the country. Metalaxyl (Ridomil) and cuprous oxide (Perenox) were identified to be successful in increasing the number of harvested healthy pods compared to the application of fosetyl aluminium (Aliete) and control treatment. In Togo, the use of metalaxyl and red copper oxide reduced losses from
80% to 3% and 19% respectively (Djiekpor et al., 1981). On top of that, the timing of fungicide application has some positive effect on the final pod yield where sprayed plot produced greater yield than the unsprayed plot. The application was done before August, which is before the main disease epidemic that usually occurs in September and October. The recommended standard for fungicide application to control black pod disease caused by *P. megakarya* for a season is 6 to 8 times of application in every 3–4 weeks (Opoku et al., 2007).

However, the adoption of recommended application was very low among farmers in Ghana. Therefore, an experiment with a reduced number of fungicide applications demonstrated that there was 25 to 45% reduction in disease incidence (Opoku et al., 2007). In terms of disease control and yields, sanitation and three applications of *Ridomil 72 plus* (12% metalaxyl + 60% copper-1-oxide) fungicide showed a better control compared to sanitation alone and sanitation with one or two fungicide applications. However, reduced fungicide application was shown to be significantly less effective than the recommended standard fungicide application. It was suggested that the understanding regarding the source of inoculum, the amount of infective inoculum production and how the disease is disseminated is important in order to identify the appropriate and economical method in fungicide application as well as for an effective control of the disease. For example, the application of fungicide on the trunk will help farmers to control the spread of the disease up in the canopy, as it is difficult to reach the canopy during fungicide application. This will eventually save more time, labor and cost for disease management (Opoku et al., 2007).

Researchers have proposed integrated control of the disease based on cultural and chemical treatments, plus the use of resistant planting material (CABI, 2017).

The use of fungicides and some other broad-spectrum pesticides have public health and environmental implications. Other problems associated with the use of chemical pesticides on cocoa include excessive tree height (which makes infected pods disperse inoculum from high in the canopy), high cost of chemicals, labour and poor cocoa prices (Adejumo, 2005; Akrofi, et al., 2013).

**Biological control**

Heavy application of chemical fungicide would eventually lead to the resistance of pathogens and result
in soil and water pollutions. Hence, more sustainable and environmentally friendly methods such as biological control should be established and implemented. Several species of fungi from the genera of *Trichoderma* was identified to be a beneficial endophyte, to control black pod caused by *Phytophthora* spp. An isolate of *Trichoderma asperellum* from soil was observed as a potential mycoparasite for *P. megakarya* where this fungus has the potential to reduce black pod incidence under field condition in Cameroon. It was reported that moderate black pod cases (47%) occurred in the *T. asperellum* treatment to control black pod disease compared to trees with untreated (71%) and chemical fungicide (1.73%). Another species, which is *T. virens* also has been documented to reduce some black pod incident in Peru. In Brazil, a new species known as *Trichoderma martiale* was identified as an endophyte on cocoa, which has the ability to reduce black pod symptoms caused by *P. megakarya*. This endophyte species survives on cocoa pods, and has the ability to establish a long endophytic association with the host (about 3.5 months). Despite documented research efforts in Cameroon and some other cocoa producing countries, the use of biocontrol agents has not found a solid footing in Nigeria. Nevertheless, the protection against black pod via biological control is not as effective as the control involving the use of chemical fungicides (Deberdt *et al.*, 2008; Hanada *et al.*, 2008; Krauss and Soberanis, 2002).

**Use of botanicals**

In Nigeria and many other developing countries, the use of plant species as both pesticides and local medicines has been reported (Adejumo, 2005). One of the available alternatives to the use of chemical fungicides is the use of phyto-extracts of tropical plant-source which has been observed to be eco-friendly, bio-degradable, much cheaper, available and safe. Some of the extracts have been found to be effective in the control of some plant diseases (Babalola *et al.*, 2017).

Tijani and Omondiagbe (2006) reported the use of Siam weed (*Chromolaena odorata*) among cocoa farmers in Osun State, Nigeria for crop protection. Solution made of Siam weed, alum, black soap and water- called Siam weed soap solution (SWSS) was prepared, tested and found to be preliminarily effective against fungus diseases affecting cocoa. The SWSS has also been found to be very effective in the
control of cocoa pests with no visible side effects on the crop. Siam weed soap solution has also been reported to have demonstrated prominent advantages over copper sulphate and other innovations such as kerosene soap solution, neem soap solution and tobacco soap solution against the pests and diseases of cocoa in Osun State, Nigeria.

In an in vitro study conducted by Babalola et al. (2017), cold water extracts of C. odorata was suggested as a suitable replacement (though with intermittent application) for chemical fungicide in the control of cocoa black pod disease. The authors however suggested an urgent confirmation of their findings on the field.

Adejumo (2000) had earlier reported the potentials of C. odorata and Piper guineense in controlling the disease. Tiwantiwa, a herbal plant mixture containing roots of four trees and leaves of another set of herbal plants of different known weights was developed by a peasant farmer at Akure, Nigeria in 1988 as a treatment against black pod disease of cocoa. It was evaluated both at the laboratory and on the field at the Cocoa Research Institute of Nigeria, Ibadan. Olunloyo (1994) reported 10% of the extracts as the minimum concentration at which zones of inhibition could be detected. The compound was a diterpinoid and there was no significant difference between the performance of 20% concentration of the herbal extracts and Bordeaux mixture on the field (Olunloyo, 1994).

**Resistant variety**

The use of resistant varieties in the control of cocoa black pod disease is most applicable, low cost to farmers and an attractive disease control technology. Governments and private companies in developed countries invest heavily in this and have produced most encouraging output by contributing significantly to increased productivity (Adejumo, 2005).

There is no specific variety of cocoa that proved to be outrightly resistant to Phytophthora infections and the establishment and utilization of resistant varieties will most likely depend on the region. Numerous breeding programs have been established worldwide in order to screen and test for local hybrids for disease resistance to Phytophthora spp. For example, a study in Cameroon assessed the performance of local cocoa cultivars (the southern and northern Cameroon cultivar) compared to the local and international gene bank cultivars. The local gene bank cultivar
consisted of F1 hybrid of Upper Amazon x Trinidad, and an international cultivar from Papua New Guinea, and Latin America were provided through International Cocoa Genebank, Trinidad (Efombagn et al., 2007).

**Integrated pest management (IPM)**

Adejumo (2005) clearly suggested the need for farmers to adopt an integration of all available cocoa disease control methods such as biological control, genetic and induced resistance, cultural practices, natural products (botanicals), and limited use of chemicals into a single program. In this program, reduction of the overall use of fungicides to an absolute minimum was emphasized, while maximizing their benefits. Various cultural techniques including shade reduction, regular harvesting and frequent weed control, and other agronomic practices as well as the choice of resistant cultivars could also be integrated for effective disease control. There are cooperative research efforts that include various national and international research institutes with a goal to identifying biological control strategies to be used in the integrated pest management systems to fight cocoa diseases (Adejumo, 2005).

**CONCLUSION AND RECOMMENDATION**

Highly virulent *Phytophthora megakarya* has over the years replaced *P. palmivora* as the main causative agent of black pod disease of cocoa in Nigeria. The seemingly unchecked proliferation of the pathogen is one of the major factors responsible for the dwindling cocoa production levels being witnessed in the country in recent years. Long time survival of the pathogen in the soil and debris as well as some disease-encouraging climatic factors has made a reasonably significant control of the disease a big challenge to Nigerian farmers.

Effective control of *Phytophthora* pod rot disease of cocoa in Nigeria can however be achieved through the integration of (preventive) cultural control measures with timely application (but limited use) of chemical fungicides and/or other control measures.

Since the continuous use of chemical fungicides/pesticides is hazardous, expensive and encourages the development of resistance by plant pathogens (including *Phytophthora* spp.), there is a valid need for the exploration of botanicals and other crop protection techniques which are much safer, cheaper and less dependent on chemicals.
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MOLECULAR DETECTION OF *Dioscorea alata bacilliform* Virus (DABV) ON YAM LEAF SAMPLES AND MEALY BUGS ON YAM FIELDS IN NORTH CENTRAL NIGERIA

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SUMMARY

A survey was conducted in August 2017, in five States of North Central Nigeria, namely; Benue, Kwara, Kogi, Niger and Nassarawa States, to determine the presence of *Dioscorea alata bacilliform* Virus (DBaV) from yam leaf and mealy bug insect samples collected from yam fields in the study areas. Polymerase Chain Reaction (PCR) of DaBV was conducted on both the yam leaf samples and mealy bugs insect collected. PCR amplified products were analysed using sequence and Phylogeny. The Result showed that all the leaf samples tested positive for DaBV. While Sixty-one percent (61%) of the mealy bug samples tested positive for DaBV. Group one of the sequences showed 99% nucleotide identity to themselves while another group showed 88% nucleotide identity. These two (2) groups of sequences were closely related to *Planococcus ficus* and *P. minor*. Two of the sequences (BRK1 and K1) were clustered in the same group with *Rastrococcus invadens* and *P. marginatus* respectively. The two new sequences (ZM1 and GB1) appeared to be an out group of the *Planococcus* species and shared 99% nucleotide identity to themselves. The findings of this study proved that, there is the presence of DaBV in the study areas. Therefore, continue monitoring of mealy bug populations is also required, so as to reduce or maintain its population in the surveyed areas. Further studies should
be carried out to characterize the viral diversity by sequencing for thorough understanding of more existing virus strains and other mealy bug species that are likely infecting yam in the study areas; this is for increase yam productivity in the surveyed areas.

**Keywords:** Dioscorea alata bacilliform Virus, Polymerase Chain Reaction, Electrophoresis, Mealy bugs, Sequences

YAM (Dioscorea species) belongs to the family Dioscoreaceae in the genus Dioscorea. The genus consists of about 600 species of which 60 are cultivated for food or pharmaceutical purposes (18). White yam (Dioscorea rotundata), yellow yam (Dioscorea cayenensis) and water yam (Dioscorea alata) are among the edible and economically important yam species in the world (21). Water yam (Dioscorea alata) is the most widely distributed species globally while; white yam (Dioscorea rotundata) is the most preferred (13).Yam is the second most important food tuber crop in the world after cassava, in terms of production (8), and as a major staple food, which plays an important role in food security for the growing population of sub-Saharan Africa (SSA) (16; 5). Yam provides up to 500 kcal per day for millions of people and contributes to the income generation of smallholders in major yam producing areas of SSA (20).

Nigeria is the highest world producer of yam with more than 45,004 million metric tonnes (mmt) annually with a value equivalent to US$5.7 billion (7), this is contributing significantly to 40% of Nigeria’s GDP which comes from Agricultural sector (15). Yam plays significant roles in the socio-cultural activities of Sub-Saharan Africa, especially in North central Part of Nigeria and is cultivated for food or pharmaceutical purposes (14; 6), Yam contributes more than 200 dietary calories per day for over 60 million people in Nigeria (16), it may be barbecued, roasted, fried in oil, grilled, boiled, baked, smoke, pounded into paste (fufu) or grated and made into a dessert. It may be cooked or fried with rice, beans, plantain, sweet potato, lamb, and chicken and butter nut as squash soup. It can be boiled, roasted and eaten with oil, vegetable or sauce. The tubers may be peeled and sliced into tiny pieces and dried to very low moisture contents and milled into yam flour and flakes. (16) Diseases caused by viruses, including members of the genus Badnavirus are seriously affecting yam production in Nigeria (1). This group of viruses exhibits high genetic as well as serological heterogeneity which has presented a
big challenge for developing reliable diagnostic tools for their detection (9; 7). Therefore, continuous detection of these viruses will help to determine their diversity for reliable diagnosis. Viruses are a major threat to sustainable yam production in Sub Saharan Africa (SSA) and are an impediment to the safe movement of clean planting materials (7). Estimates indicated that more than 30% of yams in SSA are lost due to viral diseases, resulting in annual losses of more than US$1.25 billion (10). These viruses are spread by sap-feeding mealy bugs (Pseudococcidae) and through perpetuation of virus infected planting materials by farmers (2). The sap-feeding insects of the family Pseudococcidae contain over 2,200 species and many are active vectors of Dioscorea alata bacilliform Virus (DaBV) (18). Presently, there is no existing data on mealy bug species that are feeding on yam in north central Nigeria, although earlier researchers reported that in south western part of Nigeria, Planococcus citri can acquire and transmit Dioscorea alata bacilliform Virus (DaBV) between diseased and healthy yam plants (17). Furthermore, several mealy bug species were reported as vectors of so many members of the genus Badnavirus in different Agricultural crop products which include Planococcus citri (Risso or citrus mealy bug), Saccharicoccus sacchari (Cockerel or pink sugarcane mealy bug), P. kenyae (Lepelley or coffee mealy bug), P. njalensis (Laing or cacao mealy bug), and Dysmicoccus boninis (Kuwana or gray sugarcane mealy bug) (8). The present research work provides the relevant information on the status of Dioscorea alata bacilliform virus (DaBV) and the mealy bug insects that are found in yam fields which serve as the potential vectors of DaBV in the study areas.

MATERIALS AND METHODS

The survey study was conducted in August, 2017 covering major yam producing regions of North Central Nigeria, including; Benue, Kogi, Kwara, Nassarawa and Niger State.

Yam Leaf Samples Collection

Leaf samples were collected based on criteria of disease severity ranking scale in which 1= symptomless, 2=mild, 3= moderate, 4 severe and 5= very severe. The samples were randomly collected from farmer’s fields. A total of 200 yam leaf samples were collected from the surveyed areas; in a quantity of 45, 35, 40, 40, and 40 each from Benue, kwara, Kogi, Nassarawa and Niger States from a total number of 26 locations including 7,4,5,5, and 5 each from
the above-mentioned states. The samples were placed in Eppendorf tubes containing Cetyl-trimethyl ammonium bromide (CTAB) buffer and kept in a room temperature in the field and in the – 20 degrees refrigerator in the molecular laboratory of KSUSTA before DNA extractions, polymerase chain reaction (PCR), and gel electrophoresis.

**Nucleic Acid (DNA) Extraction from Yam Leaf Samples**

Total nucleic acid was extracted from all yam leaf samples using an adapted CTAB protocol (12) as follows: The yam leaf tissues (~100 mg per sample) were placed in individual polythene bags (10x15 cm Polybags, UK), followed by the addition of 1 ml extraction buffer (2% (w/v) CTAB, 100 mM Tris-HCl (pH 8.0), 20 mM EDTA, 1.4 M NaCl) containing freshly added 2% (w/v) polyvinylpyrrolidone-40 (PVP-40) and 1% (w/v) sodium sulphite (Na$_2$SO$_3$). The leaf materials were then ground using a small hand roller. For each sample, crude extract (700 μl) was transferred into 1.5 ml micro centrifuge tubes, vortexed and incubated in a water bath (60°C, 30 min) for cell lysis. The extract was then first clarified by the addition of 700 μl chloroform: isoamyl alcohol 24:1 (v/v), vortexed and centrifuged (13000 rpm, 10 min) in a micro centrifuge tube. The aqueous layer was removed carefully and transferred into a fresh micro centrifuge tube. A second clarification step using chloroform: isoamyl alcohol 24:1 (v/v) was performed as above. The aqueous layer was transferred carefully into a fresh micro centrifuge tube and then mixed well with 75 μl of 5 M NaCl and 450 μl of cold isopropanol. After incubation (-20°C, 1 h), the mixture was centrifuged (4°C, 13000rpm, 10 min) to pellet the nucleic acids. The supernatant was decanted off, and the pellet was washed twice by the addition of 500 μl of 70% (v/v) ethanol, and then recentrifuged (13,000rpm, 5 min). The ethanol was carefully decanted off and nucleic acid pellets then dried using a DNA vacuum drier (Thermo Scientific, UK). The dried pellets were re-suspended in 100 μl 1x TE buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) and stored at -20°C until use. The extracted DNA samples were diluted 1:10 (v/v) in nuclease-free sterile distilled deionised water (SDW, Thermo Scientific, UK) prior to PCR.

PCR using primers set as Badna-forward 5'ATGCCITTYGGIITAAR and Badna-reverse AAYGCICC3’.
primer (RP) 5'CCAYTTRCAIACISCICCCCAIC C3'.

Mealy bug Samples Collection and Nucleic Acid Extraction
A total of 76 mealy bug samples were collected from the surveyed areas, in a ratio of 16,15,15,15, and 15 each from Benue, Kwara, Kogi, Nassarawa and Niger States, and the total DNAs were extracted from 76 mealy bugs samples using a non-destructive DNA extraction method with a Blood and Tissue Qiagen kit, following the manufacturer’s protocol. In which Individual mealy bug were placed in 1.5-ml tube and 180-µl of ALT buffer was added, followed by the addition of 20-µl of proteinase K and incubated at 56°C overnight for cell lyses. Then 200-µl of AL buffer added and then incubated at 56°C overnight for 10 mins. 200 µl of ethanol was added, after then the mixture was then transferred in to column and the centrifuged 8000 rpm for 1 min. 500 µl of AW1 buffer was then added and the centrifuged 8000 rpm for 1 min, same quantity of AW2 buffer was added and centrifuged 14000 rpm for 3 mins, 30 µl of AE buffer was then added and centrifuged 8000 rpm for 1 min, this step was then repeated to obtain very qualitative DNAs but in this step 20-µl elution buffer was added.

PCR and Gel Electrophoresis of Mealy bug DNA
A 649-bp segment of the barcode region was amplified from each mealy bug sample using primer set Badna-forward (FP) 5'ATGCCITTYGGIITIAAR AAYGCICC3' and Badna-reverse primer (RP) 5'CAYTTRCAIACISCICCCCCIACC3'. PCR thermos-cycling was done under the following conditions: Initial and Final Denaturation for 2 mins at 95°C and 94°C; Annealing for 30s, at 52°C; and Initial and final extension for 30s, at 72°C; 60s, 72°C; 5 min at 72°C; held at 4°C for about 30 cycles. PCR products were visualized in 1.5 % agarose gel stained with red safe. The gel was prepared by dissolving 1.5 grams of agarose powder in 100 ml of 0.5× TBE buffer (0.045 M Tris-borate, 0.5 mM EDTA, pH 8). The agarose-buffer solution was heated in a microwave oven for 3 minutes and was allowed to cool to 40 °C before pouring into a gel tray that was fitted with a gel comb. The gel was allowed to solidify for 20 mins before loading samples. 10 µl of the sample was loaded into separate wells on the gel, 5 µl of 5× orange G loading dye. 5 µl DNA markers (100 to 1000 base pair) were loaded into each end slots of the gel. Electrophoresis was performed at
80V for 1 h. The gel was observed under UV light. Analysis was done using PCR techniques, in which PCR amplified products were analyzed using sequence and phylogeny techniques.

RESULTS

PCR of *Dioscorea alata* bacilliform virus (DaBV) Extracted DNA

All symptomatic leaf samples showed positive bands of approximately 579 base pair (bp), indicating the presence or occurrence of DaBV in all the surveyed areas. The bands were graded from one plus (+) to three plus (+++) based on the intensity of the bands in 1.5% agarose gel electrophoresis. 64% showed weak bands and were scored one plus (+). The remaining 36% samples showed stronger bands and were scored three plus (+++). (Plate 1)

Plate 1: PCR Detection of DaBV sequences by PCR using Badna FP and Badna-RP primers. Lane M = 100bp ladder, Lane 1 = negative control (distilled water), Lane 2 = positive control and Lanes 3-15 = representative of the surveyed samples.

PCR and the Sequences of Mealy bugs Extracted DNA

PCR of 76 tested samples indicated that 56 samples showed positive bands of approximately A649 base pairs (bp). The total positive samples obtained indicated that 61% of the tested mealy bugs were able to acquire DaBV. The bands were graded from one plus (+) to three plus (+++) based on the intensity of the bands in 1.5% agarose gel electrophoresis. (Plate 2)
Plate 2: PCR of Mealy bugs on yam fields. Lane M = 1Kbp plus ladder, Lane 1-14 = representative of the surveyed samples. Sample 1 – 9, 13 and 14 were positive for the DaBV sequence whereas, samples 9 - 12 were negative of DaBV sequences. Lane 15 = negative control and Lane 16 = positive control

The PCR products of mealy bug insect vectors were sequenced and the sequences clustered into four main groups of the family Pseudococcidae namely: Planococcus ficus and minor, Rastrococcus invadens, P. marginatus and a putative new group. Group one of the sequences showed 99% nucleotide identity to themselves while another group showed 88% nucleotide identity.

These two groups of sequences were closely related to P. ficus and minor. The two sequences (BRK1 and K1) clustered in the same group with R. invadens and P. marginatus respectively. The other two new sequences (ZM1 and GB1) appeared to be an out group of the Planacoccus species and shared 99% nucleotide identity to themselves (Figure 1)
Figure 1: Phylogenetic analysis of CO1 mealybug sequences
DISCUSSION

Total extracted DNAs were screened for the presence of DaBV, all the samples tested positive bands of approximately 579 base pair. This result agrees with those reported by (19) for the detection of Badnavirus sequences in different tropical food crops such as Banana, Cacao, Pineapple, Sugarcane, Taro and Yam. Therefore, the analysis of the infected yam leaves sampled confirmed the presence of DaBV in the study areas. Many viruses have been identified in yam fields of sub-Saharan Africa (4, 3) and DaBV is among the most prevalent virus infecting yam cultivation in SSA. Similar result was also reported in Benin (4). PCR of 76 tested samples of mealy bug indicated that 56 samples showed positive bands of approximately 579bp. A segment of 649 of the barcode regions that was amplified from each mealy bug sample signified that mealy bugs are the potential vectors of DaBV in the study areas. This study agrees with the findings of (11), on the ability of Mealy bugs to acquire Badnavirus at all stage of their life using a single insect (Planococcus citri). Phylogenetic analysis carried out on the mealy bug sequences amplified proved that DaBV are found in mealy bugs and could be the potential vectors of the former in the study areas. The study agrees with that of (14) which reported the ability of mealy bugs to acquire the Badnavirus at all stages of their life cycle.

CONCLUSION

This study proved that, there is the presence of DaBV in the study areas; therefore, there is need for the establishment of more working institutions that would be involved in the distribution of improved and clean planting materials in order to improve the level of yam productivity in the study areas. Further studies should be carried out to characterize the DaBV for understanding of the existing virus strains and other mealy bug species that are infecting yam in the study areas. This finding will help in adoption of management strategies against the DaBV in the study areas through effective control of mealy bug insect’s populations, which will result in increase in income generation to the yam farmers in the surveyed states through increase in yam quantity production. Furthermore, result from this study will be of great benefit to plant virologists, entomologists, yam seeds multipliers, seed council and yam growers.
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EFFECT OF SPRAYING FREQUENCY OF DIFFERENT CONCENTRATIONS OF SPOTLIGHT ON STEM BORER INFESTATION AND GRAIN YIELD OF PEARL MILLET (Pennisetum glaucum (L.) R. BR.)

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SUMMARY

Field experiments were conducted at the Leventis Foundation/Gombe State Agricultural Training School, Tumu, Gombe State of Nigeria during 2013 and 2014 cropping seasons. Five concentrations (0.0 l/ha, 0.5 l/ha, 1.0 l/ha, 1.5 l/ha and 2.0L/ha) of spotlight were applied at three different frequencies (1, 2 and 3) on a plot size of 3.0 m x 4.0 m replicated three times. Spotlight application frequency was allocated to main plots and concentrations were allocated to subplots. Insecticide application commenced at 4 weeks after germination till harvest period. The plot size was 3.0 m by 4.0 m (12m²) with 1.0 m space between plots and 2.0 m as alley to allow for field operations. The results obtained shows that pearl millet (SOSAT C-88) plants infestation by stem borer was significantly reduced during the study period. Spotlight sprayed three times at 1.5 l/ha had proved to be effective and appropriate for reducing stem borer feeding on pearl millet growing points (leaf sheathes), stems, peduncles and found to improve the number of grains/panicle, panicle and grain weights. It is recommended that spotlight should be applied three times at 1.5 l/ha for stem borer protection on pearl millet.

Keywords: pearl millet, spraying frequency, spotlight, stem borer, infestation, concentrations

PEARL MILLET (Pennisetum glaucum (L.) R. Br.) is the most widely cultivated of all millet species and the most important millet species which account for half of the global millet production in terms of cropped area and contributes to food security in regions of Africa and Asia (11; 2). In West Africa sub region, pearl millet constitutes more than 25% of
the millet produced and it is one of the most important food grains (10; 13). It is grown as a subsistence crop in Nigeria and most it is used for human consumption (5). Apart from being a source of reliable and important food, millet stalks provide building and fencing materials for farmers (13). Millet fodder and Stover are also used as livestock feed in the growing areas (18; 11).

Among the insect pests attacking pearl millet in Africa, the lepidopteran stem borer (Coniesta ignefusalis) is by far the most dominant and most injurious stem borer (19; 8; 16). Feeding and tunneling by stem borer larvae on plants results in crop losses as a result of destruction of the growing points, early leaf senescence, interference with translocation of metabolites and nutrients that result in malformation of the grain, stem breakage, plant stunting, lodging and direct damage to millet heads (1; 8; 9; 11).

Yield reduction due to stem borers occur as a result of leaf feeding, stem tunneling, direct damage to millet grain (14). Depending on the season and nutritional status of the plant, crop yield reduction by stem borers feeding and tunneling activities in Africa can fall between 10-100% (12). In the Sudano - sahelian zone, where pearl millet is the most important cereal crop, C. ignefusalis is known to cause more damage and cause serious yield reduction (19; 16; 5). Yield loss in pearl millet depends on the time of stem borer infestation, nutritional status and season (6 and 1). Millet can be protected from stem borer infestation, damage and obtain high grain yield when the right control measure is taken between 15 and 30 days after emergence (1; 11). In view of the destructive activities of stem borer, the control of stem borer becomes important in order to have a sustainable high millet yield. Most crop farmers use synthetic insecticide for controlling field and stored pests. Synthetic insecticides are the most effective means of controlling insect pests of millet stem borers due to their fast knock down action and long-lasting effects (3; 15). However, most of the farmers either overuse or underuse the chemicals thereby, causing adverse effects on human beings, environment, animals and beneficial organisms. Moreover, resource - poor farmers do not achieve the desired insect pest control goals owing to their low financial status (5; 9; 11). Therefore, this study was conducted to determine the application frequency of different concentrations of spotlight required
for reasonable control of stem borer of pearl millet.

MATERIALS AND METHODS

Experimental Site

Field experiments were conducted at the Leventis Foundation/Gombe State Agricultural Training School, Tumu, Gombe State of Nigeria during 2013 and 2014 cropping seasons. The site is located in the Sudan Savannah ecological zone of Nigeria with an annual rainfall of between 860 - 1100 mm and a temperature range of 20 - 38 °C per annum. The rainy season usually last 120 -151 days.

Source of Experimental Materials

The millet variety used for the study was SOSAT C - 88 which is grown by most farmers in the study area and was purchased from Lake Chad Research Institute (LCRI), Maiduguri, while the insecticide, Spotlight (chlorpyrifos 25% EC + dimethoate 25% EC) was purchased from Jubaili Agrotech Nigeria Limited marketing shop in Gombe town.

Experimental Design and Field Layout

The trial consisted of five concentrations (0.0, 0.5, 1.0, 1.5, 2.0 L/ha) and three spraying frequencies (1 spray at seedling stage; 2 sprays at seedling and vegetative stages and 3 sprays at seedling, vegetative and booting stages) laid out in a split plot design replicated three times. Spotlight application frequency was allocated to main plots and concentrations were allocated to the subplots. Insecticide application commenced from 4 weeks after germination to harvest. The plot size was 3.0 by 4.0 m² with 1.0 m space between plots and 2.0 m as alley to allow for field operations.

Agronomic Practices

The experimental field was cleared of debris, harrowed, leveled manually by using hand-held hoe in order to pulverize and level the land to fine tilt. Ridges were prepared manually and spaced 75 cm. Three pearl millet seeds were sown at an intra-row spacing of 15 cm and 2.5 cm deep on 6th June, 2014 and 22nd June 2015 when the rains were established. The seedlings were thinned to 2 seedlings per stand at 3 weeks after germination (WAG). Weeding was done manually using hand-held hoe whenever weeds when necessary. A compound fertilizer, N.P.K. (15:15:15) was applied using spot application at 3 WAG.

Data Collection

The following data were collected and analyzed:
i) Number of pearl millet with perforated leaf sheathes: Ten millet plants were randomly selected, tagged and observed from each plot for the sign of leaf sheath holes (perforations) caused by stem borer larvae feeding damage during vegetative stage, and were counted and recorded.

ii) Number of pearl millet with “dead hearts”: Ten randomly selected pearl millet plants from each plot with sign of dead growing points caused by stem borer larvae feeding damage were counted and recorded.

iii) Number of pearl millet stem with holes: This was obtained by taking ten pearl millet stems at random from each plot. The infested stems were peeled and those showing sign of boring holes/tunneling were counted and recorded.

iv) The abundance of stem borers: It was obtained by randomly selecting ten pearl millet stems with holes from each plot and dissected to count and record the stem borer larvae/pupae inside the nodes, internodes and peduncles.

v) Panicle weight: Pearl millet panicles (heads) from each plot were harvested, sun dried and put in well-labeled bags and weighed using Jenway top loading balance (2000 model) scale.

vi) Number of grains per panicle: Ten randomly selected pearl millet panicles (heads) from each plot were threshed, winnowed and the grains were counted and recorded.

vii) Grain weight: The grains obtained from panicles (heads) in each plot were weighed using Jenway top loading balance (2000 model).

Data Analysis

Data collected were subjected to analysis of variance (ANOVA) using a statistical package (17) version and where there were significant differences among the treatments, the means were separated at 5 % level of significance using Duncan New Multiple Range Test (DNMRT).

RESULTS

Number of pearl millet with perforated leaf sheathes

Results presented in Table 1 showed that plots treated with spotlight at 1.5 L/ha had significantly lower (0.86) pearl millet leaf sheathes with holes followed by 2.0 L/ha while untreated
plots had the highest (1.24) leaf sheathes with holes. Results further indicated that plots sprayed three times had lower (0.57) leaf sheathes with holes than plots sprayed only once (2.46).

Table 1 also showed that spotlight application at 1.5 L/ha had the least (0.18) number of pearl millet plants with “dead hearts” dead growing points) while control (0.0L/ha) plots had the highest (2.11) dead hearts. The plots that were sprayed only once had the highest (2.14) plants with dead hearts followed by two applications (1.67) while three insecticide applications had the lowest (0.44) plants with dead hearts.

Table 1: Number of pearl millet with leaf sheath holes and dead hearts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of plants with leaf sheath holes</th>
<th>No. of plants with dead hearts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spotlight Concentrations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L/ha (SC)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>2.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.86&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5</td>
<td>1.54&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.33&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.0</td>
<td>0.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.18&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.5</td>
<td>1.24&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.14&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.0</td>
<td>0.43</td>
<td>0.46</td>
</tr>
<tr>
<td><strong>Spraying Frequency (SF)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>1.61&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.67&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>0.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>SE</strong></td>
<td>0.61</td>
<td>0.58</td>
</tr>
<tr>
<td><strong>Interaction (SC x SF)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 2 showed that there were significant differences among the
treatments. Pearl millet plants treated with spotlight at 1.5 L/ha had the least (0.91) number of plants with stem holes while untreated plots had the highest (1.79) number of plants with stem holes. When the spraying frequency was compared, three insecticide sprays had the least (1.12) number of plants with stem holes followed by two sprays (1.51) while one spray had the highest (2.18) stems with stem holes. The results also showed that there were higher (1.46) number of millet peduncles with holes in control plots while plots treated with spotlight at 1.5L/ha had the least (0.48) peduncles with holes. Plots sprayed three times with the insecticide had the least (0.33) peduncles with holes while plots sprayed only once had the highest (1.75) peduncles with holes.

**Table 2:** Number of pearl millet with stem and peduncle borer holes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of plants with leaf sheath holes</th>
<th>No. of plants with dead hearts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spotlight Concentrations L/ha. (SC)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>1.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.46&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.5</td>
<td>1.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.0</td>
<td>1.23&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.87&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>1.5</td>
<td>0.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.46&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.0</td>
<td>1.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.63&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SE±</td>
<td>0.12</td>
<td>0.09</td>
</tr>
<tr>
<td><strong>Spraying Frequency (SF)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.75&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>1.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.87&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>1.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SE±</td>
<td>0.62</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Interaction (SC x SF)</strong></td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
Effects of insecticide concentrations and frequency on the abundance of pearl millet stem borers

Results in table 3 showed that spotlight concentrations and spraying frequency reduced the abundance of stem borer on millet plants. Spotlight applied at 1.5 L/ha reduced the number of stem borer larvae feeding in the nodes, internodes and peduncle. Spotlight insecticide applied at 1.5 L/ha had the least (0.12, 1.44 and 0.28) number of larvae/pupae in the nodes, internodes and peduncle respectively. Untreated plots of pearl millet had the highest (0.6, 2.21 and 0.88) number of larvae/pupae in the nodes, internodes and peduncle in that order.

Spraying frequency also showed that the application of the insecticide three times produced the lowest (0.11, 0.31 and 0.08) larvae/pupae in the nodes, internodes and peduncle while one application produced the highest (0.72, 2.11 and 0.67) larvae/pupae in the nodes, internodes and peduncle. The result further indicated that the pearl millet stem borer preferred to attack and feed in the internodes more than the nodes and peduncles.

**Table 3:** Effect of spotlight concentrations and spraying frequency on the abundance of millet stem borers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Node</th>
<th>Internode</th>
<th>Peduncle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spotlight Concentrations L/ha.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(SC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>0.61^a</td>
<td>2.21^a</td>
<td>0.88^a</td>
</tr>
<tr>
<td>0.5</td>
<td>0.57^a</td>
<td>2.03^a</td>
<td>0.62^a</td>
</tr>
<tr>
<td>1.0</td>
<td>0.33^{ab}</td>
<td>1.67^a</td>
<td>0.40^{ab}</td>
</tr>
<tr>
<td>1.5</td>
<td>0.12^{b}</td>
<td>1.44^{ab}</td>
<td>0.28^{a}</td>
</tr>
<tr>
<td>2.0</td>
<td>0.28^{b}</td>
<td>1.59^{ab}</td>
<td>0.36^{b}</td>
</tr>
<tr>
<td>SE+</td>
<td>0.49</td>
<td>0.79</td>
<td>0.59</td>
</tr>
<tr>
<td>Spraying Frequency (SF)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Effects of spotlight concentrations and spraying frequency on panicle number, panicle and grain weight

Panicle number, panicle and grain weights were lowest (196.90, 611.70 kg and 514.13 kg) in untreated plots and highest (2298.41, 1236.31 kg and 1016.51 kg) as presented in Table 4. The results on spraying frequency indicate that plots sprayed three times produced more panicles (2318.33), panicle weight (1241.11 kg) and grain weight (1108.21 kg) than plots sprayed two times (2014.92, 714.33 kg and 608.13 kg) and one application with only 1899.64 number of panicles, 608.45 kg of panicle weight and 510.25 kg of grain weight. In all the interaction analysis, there were no significant differences between the insecticide concentrations and spraying frequency.

Table 4: Effect of spotlight concentration and spraying frequency on Number of panicle grains, panicle and grain weight

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of grains/panicle</th>
<th>Panicle weight (Kg)</th>
<th>Grain weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spotlight L/ha. (SC)</td>
<td>196.90^c</td>
<td>611.70^c</td>
<td>514.13^a</td>
</tr>
<tr>
<td>0.0</td>
<td>1037.16^b</td>
<td>669.41^a</td>
<td>561.33^b</td>
</tr>
<tr>
<td>0.5</td>
<td>2089.72^ab</td>
<td>1022.49^ab</td>
<td>862.12^ab</td>
</tr>
<tr>
<td>1.0</td>
<td>2298.41^a</td>
<td>1236.31^a</td>
<td>1016.51^a</td>
</tr>
<tr>
<td>1.5</td>
<td>1997.12^ab</td>
<td>1016.33^ab</td>
<td>891.67^ab</td>
</tr>
<tr>
<td>2.0</td>
<td>2101.51</td>
<td>624.61</td>
<td>502.40</td>
</tr>
</tbody>
</table>
DISCUSSION

Among the spotlight concentrations used during the study, 1.5 L/ha was found to be effective in reducing the number of pearl millet plants with perforated leaf sheathes, dead hearts, stem and peduncle tunneling, the number of stem borer larvae/pupae per plant and had improved the number of pearl millet grains/panicle, panicle and grain weights. This indicates that spotlight insecticide applied at 1.5L/ha was the appropriate and effective concentration for the control of pearl millet stem borer. Plots treated with spotlight at 0.5L/ha and 1.0L/ha were found not to be effective and appropriate in controlling pearl millet stem borer infestation and damage during the study period. This implies that only when spotlight is applied at the appropriate and effective concentration that will give the required control of pearl millet stem borer while application of wrong concentration will not be effective against the pest and may cause pest resistance and probably resurgence (7, 1, 16) reported that for adequate control of cereal stem borers, the appropriate chemical and concentration should be applied using the appropriate equipment. For optimum grain production in cereal growing areas, the appropriate concentration and spray regime must be adapted (12; 4). Feeding and tunneling by stem borer larvae on plants results in crop losses as a result of destruction of the growing points, early leaf senescence, interference with translocation of metabolites and nutrients that result in malformation of the grain, stem breakage, plant stunting, lodging and direct damage to millet heads (1, 8, 9, 11). Yield reduction due to stem borers occur as a result of leaf feeding, stem tunneling, direct damage to millet grain (14).
Spraying frequency also was found to have significant differences. Crops sprayed three times with the correct concentration of spotlight was found to be very effective in reducing the number of millet plants with holes, dead hearts, stem and peduncle holes, number of larvae/pupae/plants, higher panicle and grain weights during the study period. Millet plants sprayed two times and once were found not to be effective in reducing the stem borer infestation and damage. This implies that for effective millet stem borer control, three chemical sprays are recommended for maximum pearl millet production (13, 10). The more frequent the spray of this systemic, contact, stomach poison and repellant organophosphate insecticide, the more effective it is in controlling the pest because they will not allow the pest in their feeding areas to be comfortable and enjoy feeding in the plants (1, 19).

It has been reported that pearl millet growth and yield reduction result due to stem borer feeding on the growing point, early leaf senescence, interference with translocation of metabolites, malformation of the grain, stem breakage, plant stunting and lodging. Maximum pearl millet growth and yield will be obtained when millet is protected with the right insecticide sprayed at the appropriate concentration and frequency (7, 6, 10). This is because growth and yield loss in pearl millet depends on the time and duration of the infestation which needs to be targeted correctly (1).

CONCLUSION

Pearl millet protection was better when sprayed three times with spotlight insecticide at 1.5L/ha. This concentration and spraying frequency had proved to be more effective and appropriate for reducing stem borer feeding in the plants growing points (leaf sheath), stems, peduncles, interfering with nutrient and metabolites translocation, panicle and grain yield. The appropriate concentration of the insecticide sprayed at the appropriate frequency to target the stem borer time and duration of infestation is very important in obtaining maximum pearl millet growth and yield in the study area.

ACKNOWLEDGEMENT

We are grateful to the Principal and the entire management of Leventis Foundation/Gombe State Agricultural Training School for granting us the permission to conduct this research in their training farm. We also wish to thank some staff and students who assisted in weeding, fertilizer application,
insecticide spraying and data collection. God bless you all.

REFERENCES


EVALUATION OF SEED DISSEMINATION STRATEGIES OF
XANTHOMONAS AXONOPODIS PV. VIGNICOLA CAUSAL AGENT OF
BACTERIAL BLIGHT OF COWPEA

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SUMMARY
Bacterial blight is seed transmitted but the mechanism of pathogen
attachment to seed, their colonization of emerging seedling has not
been fully studied. The aim of this study was to investigate the seed
dissemination strategies of bacterial blight induced by Xanthomonas
axonopodis pv. vignicola. Experiment to determine bacteria adhesion
to seeds were conducted using twenty seeds each of Ife Brown
(cowpea), Maize, Millet and Sorghum. The seeds were surface
disinfectected by washing in 3 % sodium hypo chlorite solution for 5
minutes and was rinsed SDW. Ten seeds each of the grains were put
into a sterilized sample bottles containing 10 ml of bacterial
suspension adjusted to ca.4.5x10^7 cfu/ml. Seeds soaked in SDW was
used as control. The experiment to determine bacteria seed and
seedling colonizing pattern were conducted using one hundred seeds
of cowpea which were soaked in 100 ml of SDW containing Xav
suspension adjusted to ca. 4.5 x 10^7 cfu/ml. Five seeds were placed
in a Petri-dish containing SDW and spread filter papers. Seeds soaked
in SDW were used as control. At different times after inoculation
seeds were collected at random and ground, from which serial
dilutions were made to quantify the population of bacterial
colonizing the seeds/seedlings (cfu seed^-1). The result shows the
adhesion of Xav to host’s seed (cowpea) and non-host seeds (millet,
sorghum and maize) after 4 h of inoculation. The result shows the
population of \textit{Xav} was correspondingly increasing with the germinating seeds and growing seedling. This shows that distribution of bacterial blight is partly associated with the ability of the pathogens to adhere to seeds of both host cowpea and other seeds surfaces.

\textbf{Keywords:} \textit{Xav}, Adhesion, colonization, nonspecific, cowpea seeds, transmission.

\textbf{COWPEA} (\textit{Vigna unguiculata} L. Walp) is one of the most important legume crops grown in the tropical belt of Africa, Asia and South America (21). This crop provides food, animal feed and cash for the rural populace in addition to benefits to farmlands via \textit{in situ} de-cay of root residues and ground cover due to the spreading habit of the plant. In addition, cowpea grain provides a cheap and nutritious food for relatively poor urban communities (17). The seeds are important in diets for the high protein content providing protein to rural as well as the urban dwellers as a substitute for the animal protein (24). Worldwide and in Africa, Nigeria ranked first in both production and consumption of cowpea (1, 8). Cowpea's high protein content, its adaptability to different types of soil and intercropping systems, its resistance to drought, and its ability to improve soil fertility and prevent erosion makes it an important economic crop in many developing countries. Increased and intense cultivation of any crop often results in an increase in pest pressure especially in the humid tropics. The major economic diseases of cowpea in the humid agro ecological regions of South-Western Nigeria include brown blotch, anthracnose, Cercospora leaf spot, web blight, sclerotium stem blight (1), cowpea aphid-borne mosaic virus, black-eye cowpea mosaic virus and cowpea mosaic virus (12), root-knot nematodes (16) and cowpea bacterial blight and bacterial pustule (6, 15).

The important bacterial diseases in Nigeria, are cowpea bacterial pustule (CBP) and cowpea bacterial blight (CoBB) are caused by different strains of \textit{Xanthomonas axonopodis pv vignicola} (23). Bacterial blight occurs in all cowpea growing areas of Nigeria (7), causing severe grain yield loss of more than 64% in some areas of West Africa (19). When highly susceptible cultivars are sown, the crop may even be completely destroyed (7).

Disease incidence of cowpea bacterial blight is related to the seed borne nature of the pathogen with secondary spread occurring by wind-
driven rain (3). The bacterium can also survive in soil for up to eight months and in debris for longer periods (15). Symptoms of cowpea bacterial blight on leaves begin with small water-soaked spots, which remain small and, when the adjacent tissues die, gradually coalesce into large, irregular, brown, necrotic lesions surrounded by yellow haloes. The pathogen also invades the stem where it produces cracking with brown stripes, swelling (canker) and form dark green water-soaked patches on pods from where it enters the seeds and cause discoloration (3).

The management of the bacterial pathogen can be designed to reduce the multiplication rate at the site of infection on the host plant or to eliminate the inoculum at its source. Measures that affect pathogens at primary sources are more effective (9). It is necessary to ascertain the importance of the source in relation to the survival of the pathogen and the initiation of epidemics. A pathogen at its source is more effectively managed when detected early. Detection of the bacterium in seed and quick feedback on pathogenicity has a high impact on disease management options and cost of production. The movement of infected seed through international trade is also a major means of pathogen dispersal (20).

It has been established that CoBB is seed transmitted, and that seed-borne inoculum play a significant role in the disease epidemiology but the mechanism of pathogen attachment to seed has not been fully understood. The aim of the study was to investigate seed dissemination strategies of *Xanthomonas axonopodis* pv. *vignicola* causal agent of cowpea Bacterial Blight.

**MATERIALS AND METHODS**

**Isolation of bacteria**

Cowpea leaves showing symptoms of bacterial blight were collected from different fields. The leaves were placed in plastic bags and taken to the laboratory at the crop protection department faculty of agriculture Ahmadu Bello University Zaria. Surface sterilization with 0.5% NaOCl and rinsing in 3 changes of sterile distilled water was done thereafter 1-2 mm leaf pieces were cut at the border of diseased portion. The pieces were ground in a porcelain mortar and pestle in 0.5 ml of sterile distilled water (SDW). Loop-fulls of the resulting suspension were streaked on to nutrient agar and incubated at 28 °C for 48-72 hours. Pure cultures were made from single colonies of bacteria. The isolates were stored on
nutrient agar slants at 4°C and later used for the inoculation of the seeds as well as control.

**Bacteria adhesion to seed**

Twenty seeds each of Ife Brown (Vigna unguiculata L.), Maize (Zea mays L.), Millet (Setaria italica L.) and Sorghum (Sorghum bicola L.), were surface disinfected by washing in 3% sodium hypochlorite for 5 minutes and rinsed with SDW. Ten seeds each of the grains were put into a sterilized sample bottles containing 10 ml of bacterial suspension in SDW and concentration adjusted to ca. 4.5 x 10^7 cfu/ml. Seeds soaked in SDW were used as control. These were left on the laboratory bench for 2 h and 4 h at room temperature (28°C). After 2 h and 4 h the suspension was drained away and the seeds washed twice with SDW. Bacteria population sizes adherent on seeds were monitored by seed maceration and making serial dilutions and plating on nutrient agar to quantify the bacteria population. The percentage number of attached cells to the number of inoculated cells indicated capacities of the bacteria pathogen to the seed surface (4). Experiments were laid in complete Randomized Design (CRD) with five replications and repeated three times.

**Colonization pattern of the pathogen on cowpea seedling**

Seeds of Ife brown were surface disinfected before inoculation. One hundred seeds of surface sterilized cowpea were soaked in 100 ml of SDW containing Xav suspension adjusted to ca. 4.5 x 10^7 cfu/ml-1. After 4-5 h the seeds were removed from the suspension and dried at room temperature. Five seeds were placed in a Petri-dish containing moistened filter papers. Seeds soaked in SDW were used as control. At different times after placing seeds in the petri-dishes (24, 48 and 72 h) seeds were collected at random and ground, from which serial dilutions were made to quantify the population of bacterial colonizing the seeds (cfu seed^-1). The experiment was laid out with four replication and repeated three times. At 5, 7, and 9 days after inoculation, plants were withdrawn from the Petri dishes and were divided into: seed coat, cotyledons, hypocotyle, radicle, side roots, primar y root and the emerging leaves, each were ground and serial dilution was made to quantify the bacterial population colonizing the various parts of the emerging plant (25).

**RESULTS**

Table 1 shows the adhesion of Xav to seeds. After 2 h of inoculation,
cowpea had 100% adhesion, followed by millet (40 %), sorghum (20 %) and maize had no Xav adhesion. The Xav population was higher in cowpea, followed by millet and sorghum. At 4 h after inoculation all the seeds were attached by Xav. The highest adhesion was on cowpea (100%) followed by millet, sorghum and maize. There was however, statistical difference between Xav population on the seeds, (P<0.05). Table 2 shows the result of colonization pattern of Xav on seedling. No Xav was detected on seed coat but at 5 DAI only was Xav detected on the cotyledons. The population of Xav was higher (10^7) on radicle, side roots and primary roots while that of hypocotyle has (10^6). The population of Xav was lower on the primary root at 5 DAI, similar result was observed of Xav population at 7 DAI. The population of Xav was lower on hypocotyle (10^6) followed by emerging leaves (10^3). Figure 1 shows the result of progressive colonization of seeds/seedling over time. The population of Xav was correspondingly increasing with the germinating cowpea seedling.

**Table 1: Adhesion of Xav to Seeds in 2011 Combine Analysis of three trials**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% adhesion</th>
<th>Population (cfu/seed)</th>
<th>% adhesion</th>
<th>Population (cfu/seed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>0.00d</td>
<td>0.00d</td>
<td>40d</td>
<td>2.4x10^7a</td>
</tr>
<tr>
<td>Cowpea</td>
<td>100a</td>
<td>2.4x10^4a</td>
<td>100a</td>
<td>2.2x10^3c</td>
</tr>
<tr>
<td>Millet</td>
<td>40b</td>
<td>1.3x10^4b</td>
<td>70b</td>
<td>2.4x10^7a</td>
</tr>
<tr>
<td>Sorghum</td>
<td>20.0c</td>
<td>0.75x10^3c</td>
<td>60c</td>
<td>2.3x10^7b</td>
</tr>
<tr>
<td>Control</td>
<td>0.00d</td>
<td>0.00d</td>
<td>0.00e</td>
<td>0.00d</td>
</tr>
<tr>
<td>S. E</td>
<td>0.65</td>
<td>0.05</td>
<td>0.84</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Means in a column followed by the same letter are not significantly different at 5 % level of significance using Student-Newman-Keuls test.
Table 2: Colonization Pattern of *Xav* on Seed/Seedling (cfu/seed/seedling) in 2011 Combine Analysis of three trials

<table>
<thead>
<tr>
<th>DAI</th>
<th>Seed coat</th>
<th>Cotyledons</th>
<th>Radicle</th>
<th>Hypocotyle</th>
<th>Side roots</th>
<th>Primary root</th>
<th>Emergingleaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>-</td>
<td>2.3x10^2</td>
<td>2.3x10^7</td>
<td>1.4x10^6c</td>
<td>1.1x10^7c</td>
<td>2.6x10^7c</td>
<td>1.2x10^3c</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>-</td>
<td>2.5x10^7</td>
<td>1.8x10^6b</td>
<td>1.4x10^7b</td>
<td>2.8x10^7b</td>
<td>1.3x10^3b</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>-</td>
<td>2.7x10^7</td>
<td>1.9x10^6a</td>
<td>1.6x10^7a</td>
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<td>1.5x10^3a</td>
</tr>
<tr>
<td>S. E</td>
<td></td>
<td></td>
<td>0.020</td>
<td>0.024</td>
<td>0.020</td>
<td>0.022</td>
<td>0.20</td>
</tr>
</tbody>
</table>

Means in a column followed by the same letter are not significantly different at 5% level of significance using Student-Newman-Keuls test.

DAI = Days after inoculation; - = not detect
DISCUSSION

Bacterial adhesion is the initial step in colonization and biofilm formation and it begins with the attachment of free-floating bacteria to a surface through weak, reversible van der Waal’s forces (10). The 100% of infection on cowpea seeds at 2 h after inoculation could be partly due to high imbibition of cowpea seeds that draw the bacteria to the seeds and tissue tropism (13, 22). Though bacteria attachment to surfaces appear to be nonspecific (4), particular bacteria pathogens are known to have an apparent
preference for certain tissues over other. For example, *Salmonella mutan* is abundant in dental plaque but does not occur on epithelial surfaces of the tongue; the reverse is true for *Salmonella salivarius* which is attached in high numbers to epithelial cells of the tongue but is absent in dental plaque (22). The nonspecific adherence involves nonspecific attractive forces which allow approach of the bacterium to the eukaryotic cell surfaces. Possible interactions are (a) hydrophobic interactions (b) electrostatic attractions (c) Brownian movement and (d) recruitment and trapping by biofilm polymers interacting from fluctuating dipoles of similar frequencies (22). When the bacteria are not properly adhered or docked to the surface, it can easily slough, this explain why sorghum and maize seeds have little to no *Xav* attachment after 2 h inoculation (18, 22). The non-reversible (dock) attachment of bacteria cells to surfaces depend on surface properties of plant tissue, time, nutrient and water availability (18). The nature of the surface influence the attachment of the pathogen to the surface while rough and fleshy surface attract adhesion, the stony and leathery surface was not suitable for attachment (14). The stony and leathery surface were more pronounced on sorghum and maize seeds. The surface properties of seeds used in this work vary considerably. After 4 h of inoculation, all the seeds had *Xav* attached to it.

Individual bacterium coalesces by linking extra-cellar polysaccharides (EPS) on their cell walls. Polysaccharide chains exhibit chemical properties that make them polar and thus very “sticky”. This polarity is what leads to surface adhesions and cell cohesion and is one of the properties that make biofilms tough to remove (18). This result is in agreement with the report of Huber *et al.* (11) that many bacteria utilize sophisticated regulatory system to ensure that some functions are only expressed when a particular population density has been reached. The term “quorum sensing” has been coined to describe this form of density dependent gene.

The little or no *Xav* attachment on seed coat and cotyledon of the germinating cowpea seedling might be of the available nutrients being used up by the germinating seedling making not much nutrient for the pathogen to feed on and multiply (2). Similarly, the corresponding increase in the population of bacteria cells with the germinating and growing cowpea seedling might be as a result of high nutrient exuding from the
germinating and growing seedling (2, 25).

CONCLUSION
Essentially, the work has demonstrated that bacteria may nonspecifically attached on any surface exposed to some amount of water and nutrients. Once bacteria are attached to surface, they carry out a variety of detrimental or beneficial reactions depending on the species and on the surrounding environmental conditions. Bacterial adhesion to surfaces is the initial step in colonization and it begins with the attachment of free-floating bacteria to a surface through weak, reversible van der Waal's forces and sometimes strongly attached through biofilm formations. In addition, the study demonstrated ability of Xav to multiply as the seeds and seedling germinate and grow a strategy used by plant pathogenic bacteria to disseminate to the emerging plants. Bacteria need to be able to remain in, or in close contact to the seed, and to multiply there, before it can do harm to the germinating plant. The colonization of germinating seeds and seedlings represents a critical step in the establishment of bacterial disease; hence management strategies should aim at breaking the sequence of adhesion and colonization of the pathogen seeds and seedlings.

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chemotaxis and Biofilm formation as requirements for colonization of Roots and seeds of soyabean plants by *Bacillus amyloliquefaciens* BNM339, *Current Microbiology*. 56:625-632
INCIDENCE OF SEED-BORNE FUNGI ON SESAME (SESAMUM INDICUM L.) AND THEIR EFFECT ON GERMINATION

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SUMMARY
Investigations were conducted in the teaching and research laboratory of Department of Crop protection, Bayero University, Kano, to detect seed borne fungi and their effect on the germination of some Sesame cultivars. Standard blotter method of seed health testing was used to detect fungi from the seeds of five sesame cultivars namely E8, Ex-Sudan, Yendev-55, Ex-Katsina and Ex-Bauchi; these fungi were sub cultured in potato dextrose agar amended with streptomycin for further growth and sporulation. The effect of associated fungi on germination was evaluated using rolled paper towel method. Fungi associated with the seeds were Fusarium oxysporum, Aspergillus flavus, Aspergillus niger and Rhizopus sp. Fungal incidence varied among the cultivars and ranged from 0.00-26.00 % with E8 (0.00-4.00%), Ex-Sudan (5.00-25.50%), Yendev-55 (3.00-4.50%), Ex-Katsina (16.50-25.00%) and Ex-Bauchi (8.50-26.00%). The incidence of Aspergillus niger was higher on surface sterilised seeds while, Rhizopus sp. was more prevalent on unsterilized seeds. The germination percentage varied among cultivars, from 84.50-95.12% with E8 recording the highest.

Key words: Seed borne fungi, Sesame, Incidence and Germination.

SESAME (Sesamum indicum L.) also known as Benniseed, is one of the world’s oldest spice (common ingredient in cuisines) and important oilseed crop in Saharan Africa (12). It is reported to have originated in Africa and is considered to be one of the primeval oil seed plant brought into cultivation in various parts of the world. Nigeria is the world’s 6th largest producer of sesame, producing 461,000 metric tons.
Sesame cultivation in Nigeria is traditional to the Guinea Savanna, Sudan and Sahel agro-ecological zones, comprising Benue, Kwara, Kogi, Niger, and Jigawa states (6). Sesame seeds are a good source of protein (20%) and edible oil (50%), and contain about 47% oleic acid and 39% linolenic acid (15). Sesame seeds are used as ingredients in bread, candies, chips, and other health foods whereas sesame oil is used as cuisine oil which has brilliant permanence due to the presence of innate antioxidants, sesamol, sesamoline, and sesamin, hence known as the “king of oils” (14), it is also a raw material for the manufacturing of margarine, paints, varnishes, soaps, perfumes, pharmaceuticals, and insecticides.

Although sesame is extensively used for numerous purposes, the crop has very low yielding capacity (2). Numerous deteriorative microbes particularly fungi have created so many problems, particularly seed rot and mortality, and poor germination in sesame production and storage hence presenting a major threat to crop establishment and yield (10). These seed borne fungi may be externally borne on the seed surface or within their tissues (13). Many authors reported the incidence of *Aspergillus flavus* in sesame seeds along with other fungi (11). Other fungi viz., *Aspergillus niger*, *A. viridus*, *A. alba*, *Fusarium* sp., *Alternaria redivinca*, *A. brassicola*, *Drechslera* sp., *Curvularia* sp., *Cephalosporium* sp., *Penicillium* sp. have also been isolated from sesame (13) which have serious effects on this crop such as root rot, wilting, and damping off (7).

In Nigeria, seeds for planting are often untreated and often obtained from local markets or from farmers’ own reserve, sometimes from seed companies or from agricultural development programmes (ADPs). However, (17) indicated that, the first step towards the attainment of maximum crop yield is the use of high-quality seeds hence revealing seed borne pathogens as a factor in production. Thus, proper identification of these pathogens is vital in disease management strategies. The present work was planned to detect seed mycoflora of sesame cultivars grown in Kano (Sudan savannah ecological area of Nigeria) and their effect on germination.
MATERIALS AND METHODS

Experimental Site and Sesame Cultivars

The experiment was conducted at the Research and Teaching Laboratory of Crop Protection Department, Faculty of Agriculture, Bayero University, Kano (11° 58' N, 8° 25' E and 475m above sea level). Five cultivars of sesame seeds namely E8, Yendev-55, Ex-Sudan, Ex-Katsina and Ex-Bauchi were used for this study. E8 and Ex-Sudan were obtained from Department of Agronomy, Faculty of Agriculture, Bayero University, Kano while, Yendev-55, Ex-Katsina and Ex-Bauchi were obtained from Dawanau grain market in Kano. These seeds were kept in polyethylene bags and stored in refrigerator until when needed.

Seed testing and detection of seed borne fungi

The inoculating chamber was washed with Dettol (Chloroxylenol B. PC. 4.8 % w/v; Oleum PiniAromaticum 8.38% w/w, Isopropyl Alcohol 9.43% w/w, SapoVegetalis 5.60% w/w, Saccharum Ustumq.s.,Aqua ad 100 vols) and fumigated with a spray of 80% ethanol. Sample processing was in accordance with the International rules for seed testing (9). Four hundred (400) randomly selected seeds per cultivar were divided into two subsamples. One subsample was surface sterilized in 1% sodium hypochlorite (NaOCl) for three (3) minutes and then rinsed in three changes of sterile distilled water (SDW) while the second which served as control was rinsed in three changes of sterile distilled water (SDW) and tested using the standard paper blotter methods (10). Twenty-five (25) seeds were equidistantly arranged from each other in three (3) concentric rings in 9 cm diameter sterile plastic Petri dishes lined with SDW moistened filter paper and covered to create high humidity and stimulate fungal growth. Filter papers in the Petri dishes were kept moist as when needed periodically, while care been taken not to completely open the plates when adding the distilled water so as to avoid contamination. The experimental design used was Completely Randomized Design (CRD) in which each variety serves as treatment and repeated eight (8) times (twenty-five seeds per repetition) respectively. The same procedures were followed also for the unsterilized subsamples. These treatments were randomly arranged to avoid bias.

The Petri dishes containing the seeds were then spread out on a laboratory bench for seven (7) days at 28±2°C.
The incidence of the fungi growing on the sesame seeds was assessed between third and fifth day (however, the plates were left for additional two days before discarding) by counting infected seeds (with fungal growth) and expressed as percentage of the total seeds plated on the blotter. The frequency of isolation of a fungus was assessed by counting the number of times a particular fungus manifest per each treatment (cultivar) and expressed as per cent frequency of isolation. However, normal and abnormal seeds were counted separately and expressed as percentage of the total number of seeds per plate.

Isolation of fungi on Agar plate
Fungi growing on infected seeds based on their growth characteristics were cultured on Potato Dextrose Agar (PDA) medium amended with Streptomycin. The plates were incubated at 28± 2°C for 7 days. Growth habits of the various fungi were observed, slides were prepared for microscopic examination, and identification using the illustrated genera of imperfect fungi as a guide (3).

Germination test
Germination test was carried out using standard rolled paper towel method (10). One hundred (100) seeds of each cultivar were randomly selected and allowed to germinate between two (2) rolled papers at ambient temperature of 28±2°C in darkness for seven (7) days. After incubation, the numbers of germinated seeds were counted. The germinated seeds were graded as normal and abnormal. Normal seedlings were with developed roots and shoot and free from symptoms of infection while, the abnormal seedlings are those seedlings with either poorly developed short roots or shoot or both, and exhibiting disease symptoms.

Data Analysis
Data obtained in percentages were transformed using arc sine transformation and subjected to analysis of variance (ANOVA) using GENSTAT 17 Edition. Differences between means were separated using Student Newman Keuls (SNK) test at 5% level of probability.

RESULTS
The result shows the presence of four fungi belonging to three genera, this include Fusarium oxysporum, Aspergillus flavus, A. niger and Rhizopus sp. (Plate 1). Morphological characteristics of the fungi identified: (A) Fusarium; presence of macro and micro conidia. Macro conidia were several-celled, slightly curved or bent,
typically canoe shaped. Micro conidia were 1-celled, ovoid, borne singly. (B and C) *Aspergillus*; presence of upright conidiophores, simple, terminating in a globose ascus. Conidia were 1-celled and globose. (D) *Rhizopus*; presence of branching hyphae which is coenocytic. Sporangiospores are produced inside sporangium. Sporangiohores arise among distinctive, root like rhizoids.

Frequency of isolation on sterilized seeds was lower for all the fungi (Figure 1), *Fusarium oxysporum* had the least frequency of isolation in both sterilize and unsterilized seeds. When sterilized, frequency of isolation on seeds was highest for *A. niger* followed by *Rhizopus* sp. and *A. flavus*. On unsterilized seeds (Figure 2), however, *Rhizopus* sp. had highest frequency followed by *A. niger* then *A. flavus*.

Table 1 shows the incidence of seed borne fungi on normal and abnormal surface sterilized (1%NaOCl) and unsterilized seeds. The incidence significantly varied among cultivars both on sterilized and unsterilized, where E8 had the least fungal incidence, while Ex-Katsina and Ex-Bauchi recorded the highest. The normal seeds were significantly higher on surface sterilized E8 and Yendev-55 (22.50% and 21.50% respectively) than Ex-Sudan and Ex-Bauchi (20.50%) while, Ex-Katsina (20.12%) recorded the least normal seeds although it did not differ from Ex-Sudan and Ex-Bauchi too. The abnormal seeds (surface sterilized) were significantly higher on Ex-Katsina, Ex-Sudan and Ex-Bauchi while E8 recorded the least abnormal seeds but did not differed from Yendev-55. However, there was no significant difference observed on unsterilized seeds. The interactive effect of cultivar and treatment on the incidence of fungi is presented in Table 2. There was significant effect of treatments on sesame cultivar. The incidence was higher on unsterilized (0%NaOCl) treated Sesame cultivar than the sterilised (1%NaOCl) counterpart. Sesame cultivar shows significant differences on fungal incidence with E8 and Yendev-55 recording significantly the least incidence (2.00%) while Ex-Katsina which was statistically similar to Ex-Sudan and Ex-Bauchi had the highest (20.75%). The sterilant (1%NaOCl) used significantly reduced the incidence of the fungi.

Table 3 shows the effect of seed borne fungi on germination capacity of the Sesame cultivar, significant difference was observed among cultivars on germination, number of normal and abnormal seedlings. Cultivar E8 had the highest
germination percentage (95.12%) followed by Ex-Katsina and Ex-Bauchi while Ex-Sudan had the least germination. Yendev-55 had the highest number of normal seedlings (82.62) and least number of abnormal seedlings (9.50). This was followed by E8 while Ex-Katsina and Ex-Bauchi had the lowest number of normal seedlings and highest number of abnormal seedlings.

Plate 1: Photomicrographs of Seed Borne Fungi Detected on Seeds of five Sesame Cultivars. (A) Fusarium oxysporium (B) Aspergillus flavus (C) A. niger (D) Rhizopus sp.

Figure 1: Frequency of isolation of fungi on sterilized seeds of sesame cultivar
**Figure 2:** Frequency of isolation of fungi on unsterilized seeds of sesame cultivar

**Table 1:** Incidence of Seed-borne fungi on normal and abnormal seeds of surface sterilized (1%NaOCl) and unsterilized seeds of Sesame cultivar

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Incidence of Infected Seeds (%)</th>
<th>Fungal Incidence (%)</th>
</tr>
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<tr>
<td></td>
<td>Normal</td>
<td>Abnormal</td>
</tr>
<tr>
<td></td>
<td>Sterilized          Unsterilized</td>
<td>Sterilized          Unsterilized</td>
</tr>
<tr>
<td>E8</td>
<td>22.50a 21.38</td>
<td>2.50a 3.63</td>
</tr>
<tr>
<td>Ex-Sudan</td>
<td>20.50a-d 19.25</td>
<td>4.50b 5.75</td>
</tr>
<tr>
<td>Yendev-55</td>
<td>21.50ab 19.50</td>
<td>3.50ab 5.50</td>
</tr>
<tr>
<td>Ex-Katsina</td>
<td>20.12bd 19.88</td>
<td>4.86b 5.12</td>
</tr>
<tr>
<td>Ex-Bauchi</td>
<td>20.50a-d 19.89</td>
<td>4.50b 5.13</td>
</tr>
<tr>
<td>SED</td>
<td>0.79 1.08</td>
<td>0.79 1.08</td>
</tr>
<tr>
<td>LS</td>
<td>* NS</td>
<td>* NS</td>
</tr>
</tbody>
</table>

Figures in parenthesis are arc sine values to which SED are applicable. Means followed by the same letter(s) in a column are not significantly different.
different at 5% level of probability using Student-Newman Keuls Test (SNK). LS=level of significance, NS=not significant, *=Significant, **=highly significant.

**Table 2:** Effect of Surface Sterilizations (1%NaOCl) on the Incidence of Seed Borne Fungi on five Sesame Cultivars

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sesame cultivar (SC)</strong></td>
<td></td>
</tr>
<tr>
<td>E8</td>
<td>2.00(0.02a)</td>
</tr>
<tr>
<td>Ex-Sudan</td>
<td>15.25(0.16b)</td>
</tr>
<tr>
<td>Yendev-55</td>
<td>3.75(0.04a)</td>
</tr>
<tr>
<td>Ex-Katsina</td>
<td>20.75(0.21b)</td>
</tr>
<tr>
<td>Ex-Bauchi</td>
<td>17.25(0.17b)</td>
</tr>
<tr>
<td>SED</td>
<td>0.025</td>
</tr>
<tr>
<td><strong>Sodium Hypochlorite (SH)</strong></td>
<td></td>
</tr>
<tr>
<td>1% NaOCl</td>
<td>6.60(0.07a)</td>
</tr>
<tr>
<td>0% NaOCl</td>
<td>17.00(0.17b)</td>
</tr>
<tr>
<td>SED</td>
<td>0.016</td>
</tr>
<tr>
<td>Interaction SC X SH</td>
<td>**</td>
</tr>
</tbody>
</table>

Figures in parenthesis are arc sine values to which SED are applicable. Means followed by the same letter(s) in a column are not significantly different at 5% level of probability using Student-Newman Keuls Test (SNK). **=Highly significant. NaOCl=Sodium hypochlorite, SC=Sesame cultivar, SH=Sodium hypochlorite.
Table 3: Effect of seed-borne fungi on germination capacity of Sesame cultivar

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination (%)</th>
<th>Number of Normal seedlings</th>
<th>Number of Abnormal seedlings</th>
</tr>
</thead>
<tbody>
<tr>
<td>E8</td>
<td>95.12(1.26a)</td>
<td>71.12b</td>
<td>24.00b</td>
</tr>
<tr>
<td>Ex-Sudan</td>
<td>84.50(1.01c)</td>
<td>57.00c</td>
<td>27.50c</td>
</tr>
<tr>
<td>Yendev-55</td>
<td>91.62(1.16bcd)</td>
<td>82.62a</td>
<td>9.50a</td>
</tr>
<tr>
<td>Ex-Katsina</td>
<td>92.50(1.18bc)</td>
<td>56.25c</td>
<td>35.62d</td>
</tr>
<tr>
<td>Ex-Bauchi</td>
<td>92.62(1.18b)</td>
<td>56.25c</td>
<td>36.38d</td>
</tr>
<tr>
<td>SED</td>
<td>0.031</td>
<td>0.93</td>
<td>1.55</td>
</tr>
<tr>
<td>LS</td>
<td>**</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

Figures in parenthesis are arc sine values to which SED are applicable. Means followed by the same letter(s) in a column are not significantly different at 5% level of probability using Student-Newman Keuls Test (SNK). **=Highly significant.

DISCUSSION

The four fungal species identified have also been reported earlier in Sesame seeds in countries like Nigeria, Pakistan and India (1, 13 and 5). Most of these species identified are reputed seed borne pathogen of sesame (17). The frequency of isolation of these fungi in the samples tested revealed that Rhizopus sp. and A. niger were the most frequently detected fungi on both surface sterilized and unsterilized samples. This agrees with (5) who reported that A. niger, A. flavus and Rhizopus sp. were the most frequently isolated fungi from sesame seeds in Sialkot, Pakistan. A similar study by (13) found A. niger in high frequencies on almost all the cultivars of sesame tested. The genera Aspergillus have been reported to be present on all sesame samples and produce toxic substances in addition to deteriorative effect on seeds (11). The incidence was found to be higher on unsterilized seeds of sesame than the sterilized counterpart, with E8 having the lowest fungal incidence. This is in line with findings of (1) that reported that variety E8 has the lowest incidence of fungi. Surface sterilization of seeds with 1% NaOCl might have eliminated some of the
fungi on the seed surface, thereby reducing the fungal incidence. Report from (4) showed that 1% NaOCl treated seeds reduces the incidence of Aspergillus spp. On the effect of fungi on seed germination, the result showed that, the germination capacity of Ex-Sudan and Yendev-55 was slightly reduced. The slight reduction in germination capacity might be as a result of the deteriorative effect of some fungi present on or in the seeds samples. In a study by (16), it was reported that associated fungi decrease germination potential. Similarly, (13) reported that seed borne fungi are most disastrous as they reduce seed vigour and weaken the plant at initial stage of growth. However, this study agrees with the findings of other authors that had worked on seed health of Sesame seeds and other crops, that seeds are the major source of plant infection and disease transmission.

CONCLUSION

The results of this study indicated that there was significant variation in the incidence of the seed borne fungi associated with the different varieties tested, and the variety E8 had the lowest fungal incidence. The fungi isolated were also among the commonly reported seed borne fungi on Sesame. However, there is need for routine checks in order to find out if there are new fungal discoveries. Also, from this study, seeds that were surface sterilized with 1% NaOCl had significantly lower incidence of seed borne fungi than the unsterilized ones, hence indicating its effectiveness in reducing the seed borne fungi present on the seeds.

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EFFECTS OF SINGLE AND MIXED INOCULATIONS OF TWO VIRUSES ON GROWTH PARAMETERS OF SOME TOMATO CULTIVARS

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SUMMARY

Worldwide, Tomato is an important vegetable in the diets of many consumers. However, its production is being hampered by many plant pathogens virus inclusive. To study the effect of two viruses on Tomato cultivars, Cucumber mosaic virus (CMV), genus Cucumovirus and Tobacco mosaic virus (TMV), genus Tobamovirus were mechanically inoculated separately and in mixed infections onto two tomato cultivars; F1 Mongal and Dankukumi D1 collected from the Tissue Culture Unit of Department of Crop Protection, Federal University of Agriculture, Abeokuta. Experimental design was a completely randomised design. There were four treatments namely: the buffer-inoculated controls, CMV-inoculated plants, TMV-inoculated plants and CMV+TMV-inoculated plants. For each treatment, there were two replicates with each replicate containing 4 plants. In all a population of 48 plants per cultivar were inoculated. Leaves of plants were inoculated at 4 weeks old. Scoring of inoculated plants was done between 2 and 5 weeks after inoculation (WAI). Parameters scored were disease incidence, severity of viral symptoms, plant height, stem diameter and number of leaves per plant. Symptoms observed on inoculated tomato cultivars included mosaic, mottling and necrotic lesions. Viral disease incidence for CMV-inoculated plants ranged from 25.0 % at 2 weeks after
inoculation (WAI) to 100.0 % at 5 WAI. Severity of viral symptoms expression for Tomato cultivar Dankukuimi D1 increased from 2.0 at 2WAI to 4.0 at 5WAI respectively. In TMV-inoculated Tomato cultivars, increase in plant height of both inoculated and buffer-inoculated controls was recorded from 2 to 5 WAI, however there was a significant(p<0.05) increase in the plant height of the healthy control of Dankukumi D1 cultivar over inoculated plants. No significant difference was observed in the plant height of both control and inoculated plants of cultivar F1 Mongal.

Keywords: Tomato, virus, mosaic, mottling, Tobacco mosaic virus, inoculation

**TOMATO** is the edible, often red berry type fruit of *Solanum lycopersicum*, commonly known as a tomato plant, which belongs to the nightshade family, Solanaceae. In tropical Africa, vegetables are produced as components of prevailing farming systems including traditional shifting cultivation and associated fallow systems, home gardens, various uplands, systems, and specialized horticulture (9).

Tomato fruit can be bilocular or multilocular and is comprised of skin, pericarp, columella and locular contents. The locular cavities are filled with seeds that are surrounded with jelly parenchyma cells. Tomato dry matter normally varies between 5 and 10%, of which about 75% is soluble, and about 1 to 3% consist of skin and seeds (14).

On a worldwide scale tomato continues to increase the interest not only for the fresh market but also as component in a variety of processed foods and pharmaceutical products (4, 12, 13).

Tomato species originated in Central and South America and consumed greatly in Mexico. Many of its varieties are widely grown, sometimes in greenhouse, in cooler climate. The plants typically grow 1-3 metres and have a weak stem that often sprawls over the ground and vines over other plants. It is a perennial in its native habitat, and grown as an annual in temperate climates.

Tomato fruits are consumed fresh or incorporated in canned, frozen, preserved or dried foods. The most widely grown commercial tomatoes tend to be in the range of 5-7 cm in diameter (10).

In Nigeria, Tomato plays important role as a soup condiment as it is used in almost all homes for stew preparation, salad making and eggs. Its use is very diverse and varies
depending on occasions. However, the production of tomato in the world is being threatened by many pathogens among which are bacteria, fungi, nematodes and viruses.

The objectives of this study are to investigate the responses of two tomato cultivars to mechanical inoculations of *Cucumber mosaic virus* (CMV) genus *Cucumovirus* (cowpea strain) and *Tobacco mosaic virus* (TMV) genus *Tobamovirus* and check their levels of symptom severity and expression. Information obtained would be useful for Breeders for breeding improved Tomato varieties with durable resistance to TMV and CMV.

**MATERIALS AND METHODS**

The method of planting was a completely randomized design with Tomato plants sown as 5 pregerminated seedlings per pot but thinned to two seedlings before inoculation. Two pots (each of 2 L in capacity) were used per replicate and there were two replicates per treatment and tomato cultivar. In all, plant population was 48 tomato plants per cultivar. The two cultivars *Lycopersicon esculentum* used for this study were F1 Mongal and Dakunkunmi D1. Seeds of these cultivars were collected from the Tissue culture laboratory of the Department of Crop Production and Protection, Federal University of Abeokuta, Ogun State, Nigeria.

Tomato seedlings were inoculated when they were four weeks old. Viruses used in this study were *Cucumber mosaic virus* (CMV), genus *Cucumovirus* and *Tobacco mosaic virus* (TMV), genus *Tobamovirus*. The former was a Cowpea strain obtained from the Virology Unit of International Institute of Tropical Agriculture while the latter was collected from leaves of infected *Mucuna pruriens* collected from the wild at Kotopo area of Abeokuta, Ogun State, Nigeria.

Viruses were extracted by homogenizing the infected leaves in inoculation buffer (0.05M phosphate buffer pH 7.0) using sterilized mortar and pestle. Leaves of tomato plants were then rubbed with the extracted sap of CMV and TMV respectively. Equal volume of each of CMV and TMV inoculum were mixed to obtain mixed viral inocula. Inoculated leaves were rinsed afterwards with tap water using a wash bottle (11).

Plants were labeled and set aside for observation in screened cages. Data on viral disease incidence, severity, plant height, stem diameter and number of leaves was collected for
every plant in a replicate every week for 5 weeks.

Data Analysis

Data obtained for each parameter was subjected to analysis of variance (ANOVA) to determine level of significance (SAS version 9.0.1). Duncan multiple range test was used to separate the means so as to determine level of significance at p≤0.05.

RESULTS

Among various symptoms observed on leaves of Tomato cultivars, ‘F1 Mongal’ and ‘Dankukumi D1’ were yellowing, mosaic, necrosis and stunted growth. CMV induced leaf yellowing and necrotic lesions on leaves of ‘Dankukumi D1’, while necrotic lesions were observed on leaves of ‘F1 Mongal’ Tomato cultivars. When Tomato cultivars ‘Dankukumi D1’ were inoculated with TMV, symptoms such as chlorotic lesions and necrosis were observed. Disease incidence observed on the inoculated cultivars steadily increased, for example CMV on Dankukumi D1 had an incidence of 25.0 % at 2WAI while at 5WAI, the incidence increased to 100.0 % (Table 1). Moreover, Dankukumi inoculated with mixed inocula of CMV and TMV did not show any viral symptom at 2WAI but at 4 WAI, the incidence was 50.0 %. Throughout the period, the control plants did not show viral symptom, a disease incidence of 0.0 % was therefore recorded (Table 1).

On cultivar Dankukumi D1 inoculated solely with TMV, a disease incidence of 25.0 % was recorded at 2 WAI and this gradually increased to 100.0 % at 5 WAI. However, 50.0 % disease incidence was recorded for F1 Mongal at 2 WAI and 100.0 % at 5 WAI.

The severity of viral symptom expression for ‘Dankukumi’ tomato cultivars increased from 1.3 at 2WAI to approximately 4.0 at 5WAI when inoculated with mixed inocula of CMV and TMV. Tomato cultivar ‘F1 Mongal’ had its severity being constant at 2.0 from 2 WAI to 5WAI (Table 2).

Inoculation of tomato cultivars ‘F1 Mongal’ and ‘Dankukumi D1’ with CMV showed gradual increase in the plant height of both inoculated and control plants between 2 and 5 WAI. Plant height of CMV-inoculated ‘F1 Mongal’ and ‘Dankukumi D1’ cultivars were observed to be significantly higher than the control plants (Table 3).
Table 1: Effect of viral inoculation on Percentage disease incidence on Tomato plants.

<table>
<thead>
<tr>
<th>Virus inoculated/Plant Cultivar</th>
<th>Percentage incidence (%):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2WAI</td>
</tr>
<tr>
<td>CMV</td>
<td></td>
</tr>
<tr>
<td>Dankukumi D1</td>
<td></td>
</tr>
<tr>
<td>Control (H)</td>
<td>0.0±0.0c</td>
</tr>
<tr>
<td>Diseased</td>
<td>25.0±0.0a</td>
</tr>
<tr>
<td>F1 Mongal</td>
<td></td>
</tr>
<tr>
<td>Control (H)</td>
<td>0.0±0.0c</td>
</tr>
<tr>
<td>Diseased</td>
<td>12.5±0.0b</td>
</tr>
<tr>
<td>TMV</td>
<td></td>
</tr>
<tr>
<td>Dankukumi D1</td>
<td></td>
</tr>
<tr>
<td>Control (H)</td>
<td>0.0±0.0c</td>
</tr>
<tr>
<td>Diseased</td>
<td>25.0±0.0b</td>
</tr>
<tr>
<td>F1 Mongal</td>
<td></td>
</tr>
<tr>
<td>Control (H)</td>
<td>0.0±0.0c</td>
</tr>
<tr>
<td>Diseased</td>
<td>50.0±20.4a</td>
</tr>
<tr>
<td>CMV+TMV</td>
<td></td>
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<tr>
<td>Dankukumi D1</td>
<td></td>
</tr>
<tr>
<td>Control (H)</td>
<td>0.0±0.0b</td>
</tr>
<tr>
<td>Diseased</td>
<td>0.0±0.0b</td>
</tr>
<tr>
<td>F1 Mongal</td>
<td></td>
</tr>
<tr>
<td>Control (H)</td>
<td>0.0±0.0b</td>
</tr>
<tr>
<td>Diseased</td>
<td>25.0±0.0a</td>
</tr>
</tbody>
</table>

Means followed by the same letters along each column for each treatment are not significantly different at 5% level of probability.
Table 2: Effect of *Cucumber mosaic virus* and *Tobacco mosaic virus* on symptom severity of Tomato cultivars.

<table>
<thead>
<tr>
<th>Virus inoculated/Plant Cultivar</th>
<th>Severity scores</th>
<th></th>
<th></th>
<th></th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>2WAI</td>
<td>3WAI</td>
<td>4WAI</td>
</tr>
<tr>
<td>CMV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dankukumi D1</td>
<td>Control (H)</td>
<td>1.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diseased</td>
<td>2.0±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F1 Mongal</td>
<td>Control (H)</td>
<td>1.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diseased</td>
<td>2.0±0.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0±0.27&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TMV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dankukumi D1</td>
<td>Control (H)</td>
<td>1.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diseased</td>
<td>2.0±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.4±0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.3±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F1 Mongal</td>
<td>Control (H)</td>
<td>1.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diseased</td>
<td>2.0±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CMV+TMV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dankukumi D1</td>
<td>Control (H)</td>
<td>1.0±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diseased</td>
<td>1.3±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.0±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F1 Mongal</td>
<td>Control (H)</td>
<td>1.0±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.0±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.0±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diseased</td>
<td>2.0±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by the same letters along each column per treatment are not significantly different at 5% level of probability.
Table 3: Effect of viral inoculation on plant height of Tomato cultivars.

<table>
<thead>
<tr>
<th>Virus inoculated/Plant Cultivar</th>
<th>Plant height (cm):</th>
<th>2WAI</th>
<th>3WAI</th>
<th>4WAI</th>
<th>5WAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV</td>
<td>Control (H)</td>
<td>6.0±0.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.1±1.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.8±0.9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>16.4±1.2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diseased</td>
<td>8.4±1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.1±0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.8±2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.3±2.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>F1 Mongal</td>
<td>Control (H)</td>
<td>7.7±1.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.0±1.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.1±2.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19.5±4.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diseased</td>
<td>14.2±3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.7±4.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.5±5.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.5±8.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>TMV</td>
<td>Control (H)</td>
<td>6.0±0.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.1±1.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.8±0.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.4±1.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diseased</td>
<td>6.5±0.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.3±1.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.1±2.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11.8±2.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>F1 Mongal</td>
<td>Control (H)</td>
<td>7.7±1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.9±1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.2±2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.5±4.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diseased</td>
<td>10.0±2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.8±3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.6±4.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.5±3.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CMV+TMV</td>
<td>Control (H)</td>
<td>6.0±0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.1±1.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.8±0.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.4±1.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dankukumi D1</td>
<td>Diseased</td>
<td>8.9±0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.2±3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.9±1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.5±1.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>F1 Mongal</td>
<td>Control (H)</td>
<td>7.7±1.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.9±1.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.2±2.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>16.5±4.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diseased</td>
<td>8.9±1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.4±2.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.2±3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.1±2.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by the same letters along each column per treatment are not significantly different at 5 % level of probability.

However, in TMV-inoculated tomato cultivars, there was an increase in plant height of both inoculated and control plants from 2 to 5 WAI. At 5 WAI, there was a significant increase (p<0.5) in the plant height of the healthy control of Dankukumi D1 with mean height of 16.4±1.2 centimetres (cm) over the inoculated plants with mean height 11.8±2.3 cm while no significant difference was observed in the control and inoculated plants of F1 Mongal at 5 WAI (Table 3). Similar trends such as obtained on effect of virus inoculations on plant heights of tomato cultivars were also observed on the stem diameter parameters of
inoculated and healthy controls of ‘Dankukumi D1’ and ‘F1 Mongal’. There was a steady increase in the stem diameter of both inoculated and healthy controls of both Tomato cultivars from 2 to 5 WAI (Table 4). However, at 5WAI there was a significant increase in the stem diameter of CMV inoculated F1 Mongal over that of healthy control (p<0.05) (Table 4). This was also observed for cultivar ‘Dankukumi’ inoculated with mixed inocula.

Table 4: Effect of virus inoculation on Stem diameter of two tomato cultivars

<table>
<thead>
<tr>
<th>Virus inoculated/Plant Cultivar</th>
<th>Stem diameter (cm)</th>
<th>2WAI</th>
<th>3WAI</th>
<th>4WAI</th>
<th>5WAI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dankukumi D1</td>
<td>Control (H)</td>
<td>0.71±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.89±0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.06±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.18±0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diseased</td>
<td>0.84±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.96±0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.15±0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.45±0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>F1 Mongal</td>
<td>Control (H)</td>
<td>0.64±0.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.79±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.96±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.94±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.01±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.36±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.60±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>TMV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dankukumi D1</td>
<td>Control (H)</td>
<td>0.71±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.88±0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.06±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.18±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diseased</td>
<td>0.66±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.80±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.06±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.21±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F1 Mongal</td>
<td>Control (H)</td>
<td>0.64±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.79±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.96±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
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<td>0.94±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.01±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.36±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.60±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>CMV+TMV</td>
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<tr>
<td>Dankukumi D1</td>
<td>Control (H)</td>
<td>0.71±0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.89±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.06±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.18±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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<td></td>
<td>Diseased</td>
<td>0.89±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.10±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.33±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F1 Mongal</td>
<td>Control (H)</td>
<td>0.64±0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.79±0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.96±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td>0.76±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.85±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.11±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.26±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means followed by the same letters along each column per treatment are not significantly different at 5 % level of probability.

Effect of viruses on number of leaves per plant showed that leaves of tomato cultivar ‘F1 Mongal’ inoculated with CMV at 5WAI
increased significantly (p≤0.05) with mean number of leaves per plant of 12.38±1.0a over that of healthy controls (10.38±1.0b). Mean number of leaves of CMV-inoculated ‘Dankukumi D1’ (7.87±0.4c) were not significantly different from that of healthy controls which had 8.25±1.0c (Table 5).

**Table 5:** Mean number of leaves observed on Tomato cultivars inoculated with the two viruses.

<table>
<thead>
<tr>
<th>Virus inoculated/Plant Cultivar</th>
<th>No. of leaves per plant</th>
<th>2WAI</th>
<th>3WAI</th>
<th>4WAI</th>
<th>5WAI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dankukumi D1</td>
<td>Control (H)</td>
<td>4.00±0.2b</td>
<td>4.13±0.4b</td>
<td>7.13±0.7b</td>
<td>8.25±1.0c</td>
</tr>
<tr>
<td></td>
<td>Diseased</td>
<td>4.75±0.5b</td>
<td>5.75±0.5b</td>
<td>6.63±0.3c</td>
<td>7.87±0.4c</td>
</tr>
<tr>
<td>F1 Mongal</td>
<td>Control (H)</td>
<td>4.50±0.4b</td>
<td>5.88±0.8b</td>
<td>8.63±0.7b</td>
<td>10.38±1.0b</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.38±0.5a</td>
<td>9.00±0.6a</td>
<td>10.75±0.7a</td>
<td>12.38±1.0a</td>
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<tr>
<td>TMV</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Dankukumi D1</td>
<td>Control (H)</td>
<td>4.00±0.2b</td>
<td>4.13±0.4c</td>
<td>7.13±0.7bc</td>
<td>8.25±1.0b</td>
</tr>
<tr>
<td></td>
<td>Diseased</td>
<td>4.00±0.2b</td>
<td>5.00±0.4b</td>
<td>6.38±0.3c</td>
<td>8.25±0.3b</td>
</tr>
<tr>
<td>F1 Mongal</td>
<td>Control (H)</td>
<td>4.50±0.4ab</td>
<td>5.88±0.8ab</td>
<td>8.63±0.7a</td>
<td>10.38±1.0a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.25±0.3a</td>
<td>6.00±0.5a</td>
<td>7.88±0.4ab</td>
<td>10.13±1.0a</td>
</tr>
<tr>
<td>CMV+TMV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dankukumi D1</td>
<td>Control (H)</td>
<td>4.00±0.2b</td>
<td>4.13±0.4c</td>
<td>7.13±0.7c</td>
<td>8.25±1.0c</td>
</tr>
<tr>
<td></td>
<td>Diseased</td>
<td>5.65±0.4a</td>
<td>6.40±0.3b</td>
<td>8.25±0.5b</td>
<td>10.00±0.2b</td>
</tr>
<tr>
<td>F1 Mongal</td>
<td>Control (H)</td>
<td>4.50±0.4b</td>
<td>5.88±0.8b</td>
<td>8.63±0.7ab</td>
<td>10.38±1.0b</td>
</tr>
<tr>
<td></td>
<td>Diseased</td>
<td>5.65±0.6a</td>
<td>7.05±0.4a</td>
<td>9.63±0.4a</td>
<td>10.90±0.8a</td>
</tr>
</tbody>
</table>

Means followed by the same letters along each column per treatment are not significantly different at 5 % level of probability.
DISCUSSION

Viruses are important plant pathogens causing diseases and loss in crop yield. Plant viruses upon infection replicate at the site of infection and move to the other plants making use of the host factors. Therefore, viruses are completely depending upon their host plants to complete their cycle. Various efforts have been made to investigate the mechanisms of virus infection and their systemic spread in plants. This research work had been carried out to investigate effect of inoculation of two different viruses on growth parameters of two tomato cultivars ‘Dankukumi D1’ and F1 Mongal. The results obtained in this study are in agreement with the studies of Agrios et al. (1) on effects of *Cucumber mosaic virus* (CMV) inoculations on growth and yield of pepper.

Similar symptoms such as necrosis on leaves and mosaic as earlier reported by Mohammed (8) were observed. Observation of these symptoms on leaves of Dankukumi and F1 Mongal showed the susceptibilities of these cultivars to *Cucumber mosaic virus* and *Tobacco mosaic virus* (TMV-Mucuna strain). Incidence of CMV on tomato crops and others plants such as pepper and melon had been shown to be very high (between 30 and 100 %) in the Mediterranean Basin (7). This virus had been shown to cause a decrease in plant height by 0.97 to 30.19% in tested Tomato genotypes. The decrease in height varies with Tomato cultivars (2).

Furthermore, CMV infection on some Tomato plants had been shown to decrease relative water content of Tomato (5). A combination of *Tomato mosaic virus* (ToMV) and CMV on Tomato plants caused slight symptoms of mosaic in some tomato plants studied by Mohammed (8). The virus combinations decreased the effect of ToMV on Tomato plants. Infection with ToMV supported the symptoms of subsequent infection with CMV (8). Infection of Tomato plants by CMV and *Tomato yellow leaf curl virus* (TYLCV) was shown to significantly caused a reduction in plant height of Tomato (8).

Ability of some plants to express necrotic lesions during infection by CMV is possible due to the presence of of satellite ribonucleic acid (RNA) (14) which is the fifth RNA satellite component designated CARNA 5 (CMV-associated RNA 5) which modulate symptom severity in infected plants.

Another plant’s pathogenic virus of Tomato plants includes TMV. The
virus particles are rigid rods measuring about 200 x 18 nm, and the virus has a positive sense RNA genome that encodes at least four proteins (6). In Brazil, TMV had been found to infect Tomato plants and symptoms such as mosaic and leaf narrowing were recorded (3). Transmission of TMV is possible through rubbing and use of contaminated planting materials.

Mixed infection of CMV and TMV is possible on the field especially when an infected seed stocks or seedlings had been planted and also by mechanical rubbing during rainfall. Insects also play major roles in the transmission of CMV.

The reports on the increase in stem diameter of inoculate plants over buffer-inoculated healthy controls is in correlation with the study of Parzarlar et al. (11) who observed increase in stem diameter in TMV-infected pepper plants than the controls in Turkey. The reason for the increment in stem girth in inoculated plants could be probably because of genetic make-up of the two tomato cultivars used in this study. They are hybrid crops obtained from the Tissue Culture unit. They might have been able to become tolerant to the inoculated viruses to some extent, hence the mild severity obtained in CMV and TMV-inoculated varieties. Therefore, the responses of these Tomato plants depend on their resistant abilities to inoculated viruses.

CONCLUSION

The two tomato cultivars (F1 Mongal and Dankukumi D1) inoculated singly and in mixed infection were observed to be infected with varying degrees of symptoms and severities. Infection with single inoculations of these viruses caused increase in stem length and diameter of the two Tomato varieties. Though no spectacular losses or damage of plants were recorded, more researches are still recommended to check for the effect of these viruses on fruit yield.

REFERENCES


MORPHOLOGICAL CHARACTERIZATION OF *Trichoderma* spp. AND THEIR EFFICACY AS BIOCONTROL AGENTS AGAINST *Ganoderma boninense* *In Vitro*

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SUMMARY

*Trichoderma* species act as biological control agents against fungal plant pathogens either directly or directly. In this study, dual culture was used to assess the potential of *Trichoderma asperellum, T. harzianum, T. brevicompatum* and *T. virens* to control *Ganoderma boninense*. These species were morphologically and Potato dextrose agar (PDA) and malt extract agar (MEA) supported the highest mycelia growth. Antagonistic effects of *Trichoderma* spp. against *G. boninense* mycelia growth were evaluated using dual culture test. The results demonstrated that the percentage inhibition of radial growth (PIRG) of *G. boninense* was >75% in in-vitro assay. The highest percentage inhibition of radial (PIRG) values was observed in *T. asperellum* (84.1%), while the minimum colony overgrowth was observed in *T. brevicompatum* (76%). This study showed that *Trichoderma* spp. have a good antagonistic effect on *G. boninense* mycelia growth and *T. asperellum* has
the best potential to control the basal stem rot disease pathogen.

Keywords: Trichoderma spp., G. boninense, Dual culture, PIGR

Trichoderma is a filamentous ascomycete saprophytic fungus with a worldwide distribution [3]. They are cosmopolitan fungi found both in the tropics and temperate climates; growing on the bark of dead woods, building materials, other fungi and animals; demonstrating its high adaptability to different ecological conditions [1] [19]. They are successful colonizers of their habitats, due to their efficient utilization of the substrates available in the habitat and production of antibiotic metabolites and enzymes [18]. Their capacity to produce antibiotics, parasitize other fungi and compete with deleterious plant microorganisms have been widely studied [2]. These fungi produce a number of secondary metabolites such as non-ribosomal peptides, terpenoids, pyrones and indolic-derived compounds [2]. Many strains of Trichoderma are antagonistic to other fungi and have shown promising potentials as biological control agents of soil-borne diseases [8]. The biological control potential of Trichoderma spp. has been demonstrated on a wide spectrum of plant diseases both in in vitro and in vivo [11]. Species of Trichoderma are recognized for their antagonist ability towards various pathogens: Rhizoctonia solani, Sclerotium rofssii, Alternaria alternata, Botrytis cinerea, Fusarium solani, F. oxysporum [10]. This characteristic has promoted an increase in the interest on these fungi. T. harzianum is most widely used in biological control of many pathogenic fungi such as A. alternata, B. cinerea, Cochliobolus heterostrophus, F. oxysporum, R. solani, S. rolfsii [17]. The concern surrounding food safety and the environmental impacts on agriculture, caused by the use of classic fungicides, biological control agents have received increased recognition. The biological control agents are being used more often as a complement or replacement of fungicides [13].

Due to the needs for large scale production of Trichoderma spp. as reliable alternative for for the control plant diseases, there is need to properly characterize. Characterization of Trichoderma was based on the morphological and cultural behaviors: growth pattern and speed as well as colony’s colour and odour. The other characters studied with the aid of light
microscope include conidial size, shape and colour; conidiophores apex elongation, shape and branching pattern [18]. The objectives of this study were to morphologically characterize selected Trichoderma spp. and to determine their in vitro antagonistic activities against Ganoderma boninense which causes basal stem rot disease in oil palm [9].

MATERIALS AND METHODS

Culture of Trichoderma species

Three Trichoderma spp (T. asperellum, T. harzianum, T. virens and T. brevicompatum) isolated from different oil palm plantation soils in Malaka, Malaysia, using dilution plate method onto Trichoderma selective medium (TSM) and maintained on potato dextrose agar (PDA) at 27°C ± 2°C were studied.

Culture morphology

Seven days old Trichoderma spp cultures on PDA at 27 ± 2°C were observed under light microscope (Nikon Model Eclipse E200, Japan) at 400 magnification where conidia size (length and width) was measured as well as conidia shape and colony colour observed [4]. A minimum of 50 conidia was measured per replicate for each species. Culture slides of each species were prepared using a modified method for fungal slide culture [8]. Sterile distilled PDA (seven to eight milliliters) was poured into each sterile 90 mm plastic petri dish and allowed to solidify. A sterile 22 mm² cover glass was centered on the agar. The PDA medium (10 ml) was poured into a second 90 mm petri dish, allowed to solidify, and cut with a sterile stainless steel spatula into blocks approximately five to eight millimeters. One block was aseptically removed and placed on the cover glass. Inoculation of the agar block on one or more sides with fungal hyphae or conidia was followed by placement of a second sterile cover glass on top of it. Then the petri dish lid was replaced. The completed modified slide cultures were incubated at the 27 ± 2°C for three days. Using sterile forceps, the top cover slide was gently lifted onto a glass slide with a drop of lactophenol cotton blue and viewed microscopically at 400 magnifications.

Cultural characteristics of Trichoderma species

The colony growth rate of each specie was studied on four different types of artificial media (Potato dextrose agar (PDA), Czapex-dox agar (CDA), cornmeal agar (CMA) and malt extract agar (MEA)); PDA
at varying pH levels (5, 6.7 & 9) and on PDA at varying temperatures (20, 25, 30 & 35°C). The pH of levels were adjusted with potassium hydroxide (KOH) and set using pH meter prior to autoclaving at 121°C and 100 kPa (15 psi) above atmospheric pressure for 20 min. Mycelia plugs of six millimeters in diameter from each specie were taken from actively growing margin of a three days old cultures, placed onto petri dishes with media for the various treatments and incubated at 27±2°C for five days.

**In vitro antagonistic activity of Trichoderma spp. against G. boninense**

An agar disc (6 mm) was taken from four days old PDA plate cultures of each *Trichoderma* spp. and placed on the periphery of the PDA plates (9 cm). Another agar disc of the same size of *G. boninense* was placed opposite end the same plate, while another plate inoculated with just a *G. boninense* plug served as a positive control. Each treatment was replicated five times and incubated at 27±2°C. Antagonistic activity was determined as from four days after incubation (DAI) by measuring the radius of the *G. boninense* colony growth in the direction of the antagonist (*Trichoderma*) colony (R2) and the radius of the *G. boninense* colony in the positive control plate (R1). The experiment was repeated twice. The percentage (%) inhibition of *G. boninense* radial growth was measured at 5 days after inoculation by measuring the radial growth (mm) of the developing colony toward the antagonist until the plant pathogen (*G. boninense*) colony was completely surrounded by the antagonist (*Trichoderma* spp.). The percentage growth rate inhibition (PIRG) of pathogen was calculated according to the formula below developed by Skidmore and Dickinson [23].

\[
\text{PIRG} = \left(\frac{R1 - R2}{R1}\right) \times 100
\]

Where R1 – radius of the pathogen colony (mm) in the control plate, R2 – radius of the pathogen colony (mm) in the dual cultures plate.

**Experimental design and statistical analysis**

The experimental design used in this study was completely randomized design (CRD) in five treatments (*Trichoderma asperellum* + *Ganoderma boninense*; *T. harzianum*...
+ *G. boninense*; *T. brevicompactum* + 
+ *G. boninense*; *T. virens* + *G. boninense* alone as positive control), each treatment was replicated six times for the laboratory experiments. The data collected were analyzed using SAS software [SAS 9.4 Version Institute Inc. Cary, NC, USA], and statistical means were separated using Duncan's multiple range test (DMRT) at 5% significant level.

**RESULTS**

**Morphological characteristics of Trichoderma species**

Seven days old *Trichoderma* spp. cultures on PDA showed variations in colony, conidia size and shape (Table 1). *T. harzianum* had pale green colony while all the other species were dark green (Plate 1) colour from green, pale green, to dark green. Morphological colour changed from pale green to dark green for *Trichoderma* spp (Plate 2). Conidia of *T. asperellum* and *T. harzianum* were subglobose; *T. brevicompactum* obovoid and *T. virens* ellipsoidal (Table 1 and Plate 2). *T. asperellum* and *T. brevicompactum* produced longest and shortest conidia which ranged between 3.0 – 4.5 and 2.5 -2.5 µm, respectively (Table1). Conidia width range for *T. virens* (3.0 -3.0 µm) was the highest while *T. brevicompactum* recorded the least (2.0-2.4 µm).

Plate 1: Conidia shape of *T. asperellum* (a), *T. harzianum* (b), *T. brevicompactum* (c), and *T virens* (d)
Table 1: Morphological and cultural characteristics of *Trichoderma* species

<table>
<thead>
<tr>
<th>Fungal species</th>
<th>Conidia size range (µm)</th>
<th>Conidia shape</th>
<th>Colony colour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Length</td>
<td>Width</td>
<td></td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>2.8 - 3.4</td>
<td>2.0 - 2.9</td>
<td>subglobose</td>
</tr>
<tr>
<td><em>T. asperellum</em></td>
<td>3.0 - 4.5</td>
<td>2.2 - 3.0</td>
<td>subglobose</td>
</tr>
<tr>
<td><em>T. brevicompatum</em></td>
<td>2.5 - 2.7</td>
<td>2.0 - 2.4</td>
<td>obovoid</td>
</tr>
<tr>
<td><em>T. virens</em></td>
<td>2.9 - 4.3</td>
<td>3.0 - 4.0</td>
<td>ellipsoidal</td>
</tr>
</tbody>
</table>

Plate 2: Morphological growth and sporulation appearance of *T. asperellum* (A), *T. harzianum* (B), *T. virens* (C) and *T. brevicompatum* (D) on PDA after five days at 27+2°C

*Trichoderma* species colony growth on different media

*Trichoderma* species grew on all nutrient media tested with the fastest growth recorded in PDA and MEA (Table 2). On PDA, the mycelia growth of *Trichoderma* spp significantly varied from each other with *T. asperellum* exhibiting the highest mycelial growth (8.57 cm), while *T. brevicompatum* had the least (6.50 cm). However, there were significant differences in colony growth between the species on each medium (PDA, MEA, Czapek and CMA. Effect of the media tested showed that the nature of media affected the mycelial growth. They were found to be capable of growing on almost all the media tested with different growth. Potato dextrose agar proved to be the most suitable medium for the growth of *Trichoderma* spp. On PDA, the
growth pattern showed slight differences in the nature of the colony growth and sporulation. The colony colour changed from light green shade to green and dark green with the production of conidia. The conidia wall patterns and shape were rough, subglobose, and smooth. These observations were in consistent with the previous reports on different growing media for *Trichoderma*.

**Table 2**: *Trichoderma* species colony growth on different fungi growing media at 5 days after inoculation

<table>
<thead>
<tr>
<th>Species</th>
<th>Colony diameter (cm) on:</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PDA</td>
<td>CMA</td>
<td>Czapek agar</td>
<td>MEA</td>
</tr>
<tr>
<td><em>T. harzianum</em></td>
<td>8.17±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.43±0.25&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.52±0.40&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>7.80±0.45&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>T. asperellum</em></td>
<td>8.57±0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.80±0.34&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.03±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.90±0.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>T. virens</em></td>
<td>7.10±0.20&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.70±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.16±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.52±0.36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>T. brevicompactum</em></td>
<td>6.50±0.25&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.60±0.78&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.31±0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.11±0.20&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

± Standard deviation (SD). The values followed with the same superscript letter within a column were not significantly different (P≤ 0.05) according to Duncan's Multiple Range Test.

**Effect of temperature on the colony growth of *Trichoderma* species**

All the four *Trichoderma* species produced colony growth at different temperatures, with the maximum colony growth recorded at 25 and 30 °C. The colony growth was generally increasing with an increase in temperature but drastically decreased at 35°C (Fig. 1). At 15°C, *T. harzianum* and *T. virens* recorded significantly higher mycelia growth than *T. asperellum* and *T. brevicompactum*. However, at 20°C, *T. asperellum* and *T. virens* which did not differ from each other recorded the highest colony growth as shown in figure 1.
Figure 1: Effect of temperature on colony growth of *Trichoderma* spp on PDA. Error bars represents the Standard Error (SE) of the mean. Bars with the same letter are not significantly different according to a to Duncan's multiple range test at $P \leq 0.05$. TH: *Trichoderma harzianum*; TA: *Trichoderma asperellum*; TV: *Trichoderma virens* and TB: *Trichoderma brevicompactum*

**Effect of pH on the colony growth of *Trichoderma* species**

The pH was observed to affect colony growth of all *Trichoderma* species studied with significant variation amongst the species at various acidity levels (Fig.2). and mean growth for species grown at different pH levels 4, 5, 7, 8 and 9 were significantly different ($P \leq 0.01$). Colony growth for all *Trichoderma* spp were at pH 6, but there were significant difference ($P \leq 0.01$) between *T. brevicompactum* and three others species at pH 6. The minimum colony growth for *Trichoderma* species were recorded at pH 9.
Figure 2: Effect of pH on colony growth of *Trichoderma* spp on PDA. Error bars represent Standard Error (SE) of the mean. Bars with same letter are not significantly different according to a Duncan's multiple range tests at P ≤ 0.05. TH: *Trichoderma harzianum*; TA: *Trichoderma asperellum*; TV: *Trichoderma virens* and TB: *Trichoderma brevicompactum*

**In vitro screening of Trichoderma spp. against Ganoderma boninense**

The dual plate assays performed with four *Trichoderma* species against *G. boninense* showed varying levels of antagonistic responses against the pathogen (Fig.3 and plate 4). There were significant differences in percent growth inhibition (PIGR) between the four *Trichoderma* species studied. The PIRG values ranged from 76.0 to 84.6%, with *T. asperellum* recording the maximum (84.6%) and *T. brevicompactum* the least (76.0%) as shown in Figure 3 and Plate 4. All the *Trichoderma* spp tested clearly demonstrated high (above 50%) antifungal activities, and they may be used as antagonist against *G. boninense*. 
Figure 3: *In vitro* antagonistic activity of *Trichoderma* species against *G. boninense*. Bars with same letter are not significantly different according to a Duncan's multiple range tests at \( P \leq 0.05 \). TH: *Trichoderma harzianum*; TA: *Trichoderma asperellum*; TV: *Trichoderma virens* and TB: *Trichoderma brevihacterium*

Plate 4: *In-vitro* growth and inhibition of *G. boninense* on PDA by *T. virens* (A), *T. asperellum* (B), *T. brevihacterium* (C), and *T. harzianum* (D) and control (E) at seven days of incubation.

**DISCUSSION**

Our results showed that morphological characters of *Trichoderma* spp. studied showed close similarity in their conidia shape, colour and conidia size. Thus, making it difficult to characterize at species level based on the microscopic features. The shapes of conidia were not useful in characters for characterization of *Trichoderma* spp. because of the confusion caused by the use of different terms in different literatures to describe the shapes of the conidia. Furthermore, no systematic rule was established in defining the shapes of
the conidia. The description of the shapes of conidia may be subjective, and thus it may not be precise for characterization of *Trichoderma* species.

It was reported that different growing media used for *Trichoderma* spp. characterization based on their morphological characteristics, confirmed that PDA provides useful information on the pigment production while MEA is an appropriate medium for conidium production and observation of conidiophore branching [20]. *Trichoderma* spp. colony growth was affected by medium pH and temperature. The results showed that pH and temperature influenced the mycelial growth which clearly demonstrated the ability of *Trichoderma* spp: (*Trichoderma asperellum; T. harzianum; T. brevicompactum and T. virens*) to grow on a wide range of pH and temperature, with an optimal pH and temperature of 4 - 7 and 25-30°C respectively. The results are in conformity with Limon et al., [12], who reported that acidic pH favoured the fungal growth than alkaline pH. Singh *et al.*, [21] also confirmed that *T. atroviride* optimum soil pH is 4 -7.5 for growth and sporulation. The optimum pH for *Trichoderma* spp. growth varied between 4.6 and 5.6 depending on the strains [22].

The most favourable temperature for growth and sporulation of *T. harzianum* and *T. viride* was found in between 25- 40°C, whereas for *T. asperellum* and *T. hamatum*, it was 25-35°C. Similarly, the most favourable range of pH was in between 4.6-7.6 for all the four spp. of *Trichoderma* [26].

*Trichoderma* spp. tested have the potential as a biological control agent of *G. boninense* with all of them inhibiting colony growth by over 70%. The selection of *Trichoderma* spp. as biological control agents depends largely on their effectiveness in the *in vitro* results [5] the biological control potential of *Trichoderma* spp. could be due to production of antibiotic, secondary metabolite compounds or lytic enzymes, which contributed to direct antagonistic effect on the *G. boninense* cell wall and mycoparasitic activities [14]. The potential of *Trichoderma* in producing antibiotic and lytic enzymes capable of hydrolyzing the fungal pathogens cell wall contents [24] [13]. Many *Trichoderma* spp. are also capable of improving plant growth and disease resistance [6] [25].

Due to variable antagonistic ability of individual *Trichoderma* species, it is important that they be screened
first to select for the most active antagonist against *G. boninense*, thus *T. asperellum* can be considered as a best biocontrol agent.

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EFFECTS OF SINGLE AND MIXED VIRUS INFECTIONS ON THE PERCENT GERMINATION OF SOME COWPEA (*Vigna unguiculata* [L.] Walp) CULTIVARS

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SUMMARY

A field trial was carried out to assess the response of twenty-five cultivars of cowpea to single and mixed infections with *Blackeye cowpea mosaic virus* (BlCMV) and *Cowpea mottle virus* (CPMoV) on seed quality. The field trial was conducted at the Teaching and Research Farm of the Faculty of Agriculture, Ahmadu Bello University (ABU), Zaria, Mokwa Station (09°21'N and 5°13'5'E, 201 m above sea level) situated in the Southern Guinea Savannah agro-ecological zone of Nigeria. The seed viability test was determined at the Crop Production Laboratory, Department of Crop Production, Federal University of Technology, Minna, Nigeria. Four independent trials were conducted simultaneously, for single and mixed infections. The field was cleared, ploughed, harrowed and ridged at 0.75 m apart then marked out into plots and replications. The trial was a randomized complete block design (RCBD) replicated three times giving a total land area of 900 m². Three cowpea seeds of each cultivar were sown after dressing with Apron – star (methylthiuram + metalaxyl + carboxin) at the of rate 3.0 kg seed per 10 g of the chemical. Seeds were sown at an intra and inter–row spacing of 0.30 × 0.75 m along the ridges and later thinned to two per stand at 2 weeks after sowing (WAS). For the single virus infection, seedlings of the twenty five cultivars were inoculated at 10 days after sowing (DAS) while for the mixed virus infections,
seedlings were inoculated at 10 and 17 DAS. The results of the experiment revealed that all cultivars were susceptible to single and mixed infections of the two viruses but to seemingly different extents. The viability of seeds from single infection with CPMoV was slightly reduced in some instances, but, even when seeds viability was not much affected, test of accelerated ageing for four weeks indicated that seed vigour was seriously impaired as compared to the other three virus treatments.

**Keywords:** Blackeye cowpea mosaic virus, Cowpea mottle virus, cowpea seeds, Seed quality, Germination

**COWPEA** (*Vigna unguiculata* [L.] Walp) is one of the ancient crops known to man. Its origin and domestication occurred in Africa near Ethiopia and subsequently was developed mainly in the farms of the African Savannah (12). Today, it is widely adapted and grown throughout the world but Africa predominates in production. It is a major staple food crop in sub-Saharan Africa, especially in the dry savanna regions of West Africa (9). The seeds are a major source of plant protein and vitamins for man, feed for animals, and also a source of cash income. The young leaves and immature pods are eaten as vegetables (9). It has been estimated that the annual world cowpea crop is grown on 12.5 million hectares, and the total grain production is 3.9 million tonnes (11). More than 8 million hectares of cowpea are grown in West and Central Africa. Also, it is known that Nigeria is the largest producer with 4 million hectares accounts for 45% of the total on 1.15 million hectares annually (9). Other producers are Niger, Mali, Burkina Faso and Senegal (12). The major cowpea producing areas in Nigeria include Niger, Kwara, Kaduna, Borno, Taraba and Yobe States in the northern part while Oyo, Ogun and Ondo also produce appreciable quantities in the southern part of the country (14).

Virus diseases are considered to be a major limiting factor for the production and productivity of legumes in the tropical and subtropical countries (6). Out of more than 20 viruses reported on legumes from different parts of the world, (15) nine are known to infect cowpea naturally in Nigeria. **Blackeye cowpea mosaic virus** was first reported on cowpea in the U.S. in 1955 (4). It is distributed in all ecological zones and cowpea-
Growing areas of Nigeria. Local symptoms appear as large reddish lesions that spread along the veins, while systemic symptoms appear as severe mottle, mosaic, vein-banding, veinal chlorosis, distortion and stunting of the plant. Disease symptoms vary with virus strain and host cultivar. Incidence varies from 1-40 % on farmers’ fields. Yield losses due to the virus vary from 10-85 % on individually infected plants and vary with time of sowing. Cowpea mottle virus is a positive sense single-stranded RNA, unipartite, isometric virus, 30 nm in diameter (4). The pathogen is distributed in all ecological zones of Nigeria, particularly in the riverine areas of the middle belt which has a Southern Guinea Savanna climate and where a lot of bambara groundnut is grown. Infected plants display severe mosaic, mottling or bright yellow mosaic. Leaf distortion and reduction in leaf size sometimes leading to a witches’ broom appearance in cowpea occurs (8).

Seed-borne viruses are important for source of diseases at the beginning of production even at low rates of seed transmission (15). In addition, seed-borne viruses can aggravate other transmission methods and cause disease to spread rapidly. Seed-borne and seed transmitted viruses are also damaging to cowpea productivity owing to inherent primary inoculum and potential for their wide dispersal. Information on the possibility of seed transmission in virus infected cowpeas will be valuable to numerous cowpea farmers. Information on germination of infected seeds and survival of resulting plants, virus disease progress during the growing season, magnitude of yield loss and amount of infection in harvested seeds in replicated field experiments is required to establish acceptable threshold levels of seed-borne infections. The study is essential to develop preventive and management measures for cowpea virus diseases in Niger State. Therefore, this research aimed at examining the effects of virus infections on seed quality.

MATERIALS AND METHODS

Field trial was conducted during the 2017 wet session at the Teaching and Research farm of the Faculty of Agriculture, Ahmadu Bello University (ABU), Mokwa Station (09°21'N and 5°135'E, 201 m above sea level) situated in the Southern Guinea Savannah agro - ecological zone of Nigeria. The site used was under continuous cropping with soybean between 2012 till the commencement of the study.
Treatments and experimental design

Four independent trials were conducted simultaneously, for single and mixed infections of the two most common viruses in the study area. In each trial, 25 photosensitive and yielding cowpea cultivars under virus free conditions namely Ife Brown, IT90K – 277 – 2, IT96D – 610, IT97K – 499 – 35, IT97K – 568 – 18, IT97K – 573 – 2 – 1, IT98K – 205 –M8, IT98KD – 288, IT99K – 316 – 2, IT99K – 377 – 1, IT00K – 901 – 5, IT03K – 337 – 6, IT04K – 267 – 8, IT04K – 291 – 2, IT04K – 321 – 2, IT04K – 332 – 1, IT06K – 124, IT06K – 137 – 1, IT07K – 211 – 1 – 8, IT07K – 222 – 2, IT07K – 243 – 1 – 10, IT07K – 251 – 3 – 3, IT07K – 292 – 1 – 10, IT07K – 299 – 6 and IT07K – 318 – 33) constituted the treatments. The trial was arranged as randomized complete block design (RCBD) replicated three times giving a total land area of 900 m².

Source of inoculum and multiplication

The Blackeye cowpea mosaic virus (BICMV) and Cowpea mottle virus (CPMoV) isolates used were obtained from the Department of Crop Production, Federal University of Technology, Minna Niger State. The isolates were identified through serological test at the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The virus isolates were extracted by grinding 1g/ml of each isolate in extraction buffer containing 0.1M sodium phosphate dibasic, 0.1M potassium phosphate monobasic, 0.01M ethylene diamine tetra acetic acid and 0.001M-cystine per litre of distilled water using a pre-cooled sterilized mortar and pestle as described by Kumar (16). Two microlitres of β- mercapto-ethanol was added to the extract just before use. Cowpea seedlings were infected with BICMV and CPMoV inoculum at under sreenhouse 10 days after sowing (DAS) by rubbing the virus extracts on the upper surface of the leaves that was dusted with carborundum powder (600- mesh). The leaves of inoculated plant were rinsed with sterile distilled water. Symptomatic cowpea leaves were collected from the infected plants at 3 weeks after inoculation (WAI) and used for inoculation during the main experiment. The leaves were preserved at room temperature in airtight via bottle on silica gels covered with a thin layer of non-absorbent cotton wool.

Agronomic practices

The field was cleared, ploughed, harrowed and ridged with tractor at
0.75 m apart then marked out into plots and replications. Each cultivar was evaluated in 0.375 m ridge wide, 3 m long and 0.75 m apart giving a total plot size of 18.75 m per replicate. Cowpea seeds were sown one week after the land preparation. Three cowpea seeds of each cultivar were sown after dressing with Apron – star (methylthiuram + metalaxyl + carboxin) at the of rate 3.0 kg seed per 10 g sachet of the chemical to protect seed against soil borne pathogens. The sowing was carried out at an intra and inter–row spacing of 0.30 × 0.75 m along the ridges and later thinned to two per stand at 2 weeks after sowing (WAS). The BICMV and CPMoV infected cowpea leaves previously preserved on silica gels were used for inoculation. For the single virus infection, seedlings of the twenty five cultivars were mechanically inoculated singly with BICMV or CPMoV at 10 days after sowing while for the mixed virus infections, seedlings were inoculated singly at 10 DAS and inoculated with the second virus at 17 DAS. Weeds were manually controlled through hand weeding at 4 and 6 weeks after sowing. Insect pests were controlled by spraying D-D force (Cypermethrin plus Dimethoate) at flower initiation and pods setting. Pods were harvested at physiological maturity. The pods were processed and packaged for seed quality assessment in the laboratory.

**Assessment of Virus Infection on Seed Quality**

Seed lots from the various virus treatments were subjected to seed quality test as follows;

Germination and longevity of seeds of all the virus treatment combinations were determined by germination test after harvest and at four weeks of storage respectively at the Crop Production Laboratory, Department of Crop Production, Federal University of Technology, Minna. There were 25 seeds placed in distilled-water moistened filter paper lined in Petri-dish in three replicates. The filter papers in the petri-dishes were kept moist every other day. The petri-dishes were arranged inside the seed germination chamber. Germination counts were taken at 1, 2, 3, 4 and 5 days after sowing. Seeds were considered germinated when the tip of the radicle had grown free from the seed coat (10). Germination percentage (GPCT) was calculated as follows:

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GPCT = \frac{\text{Total number of seedlings that emerged on the final day}}{\text{Total number of seeds planted}} \times 100
\]

Cowpea seeds were also subjected to accelerated ageing tests at two and
four weeks as described by El Balla et al. (10) for vigour determination. The seeds of all the treatments were stored in open plastic plates and arranged inside an incubator at 35 °C and 86 % relative humidity. This was aimed at accelerating the ageing of the seeds so that the relative longevity of the seed samples could be determined. Twenty-five seeds from each treatment that were artificially aged in three replications were counted and placed on layer of distilled water moistened-filter paper placed in Petri-dishes over a wire mesh screen inside a growth chamber at 30 °C in the dark. Germination count was taken as described above.

Data analysis
Data were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS, 2008) to verify if there were significant differences among the cultivars. Significance was determined at 5 % level of probability. Where the F-test ratio was significant, means were separated using Student-Newman-Keuls (SNK) test.

RESULTS
Effects of single and mixed virus infections on cowpea seed quality
The study revealed significant impairments in germination before and after four weeks of storage of the 25 cultivars of cowpea both in single and mixed infections of the viruses used. The variation in seed germination of cowpea cultivars with respect to virus infections is presented in Table 1. Prior to storage of seeds, the difference between the lowest and highest mean value for seed germination was wide and significant (p<0.05). Seed germination percentage varied from 77.4 to 99.7 % for the BICMV infected cultivars, 77.4 to 98.7 % for CPMoV infected cultivars, 74.8 to 98.5 % for BICMV + CPMoV infected cultivars and 78.6 to 98.5 % for CPMoV + BICMV inoculated cultivars (Table 1). Seeds obtained from IT97K-568-18, IT04K-332-1 and IT07K-292-1-10 cowpea cultivars infected with BICMV had significantly (p<0.05) higher germination percentage of 99.7 which was statistically similar to 97.6 and 97.3 % germination obtained from seeds of cultivar IT07K-243-1-10 and IT03K-337-6 respectively. Seeds from cultivars IT90K-277-2, IT07K-211-1-8 and IT06K-124 had germination values of 94.7, 94.3 and 93.7 % respectively which were not significantly different among each other. Seeds of cultivars IT07K-251-3-3 and IT07K-222-2 had 92.3 and 92.5 % germination values respectively.
which were statistically similar while seeds from the remaining cowpea cultivars had germination percentages ranging between 77.4 and 91.3.

Furthermore, seed germinability of 98.7 % was highest in IT90K-277-2 with CPMoV infected cowpea seeds which was not significantly (p>0.05) different from seeds obtained from cultivars IT04K-332-1 (98.5 %), IT07K-243-1-10 (98.4 %), IT04K-267-8 (98.2 %) and IT96D-610 (97.7 %), while significantly lowest seed germination percentage of 77.4 was recorded in seeds of cowpea cultivar IT07K-292-1-10 (Table 1). On the other hand, co-infections of cowpea seeds significantly (p<0.05) affected seed germinability across the cowpea cultivars investigated. BICMV + CPMoV infected IT04K-332-1 exhibited the highest germination percentage of 98.5 % than all other cultivars, whereas IT96D-610 and IT97K-499-35 gave 97.6 % each. Seeds of cultivars IT07K-292-1-10 and IT97K-573-2-1 had 96.0 and 94.8 % germination respectively, while seeds of cultivar IT07K-222-2 gave in the lowest germination percentage of 74.8. Seeds obtained from cultivar IT97K-568-18 infected with CPMoV + BICMV exhibited the highest germination percentage of 98.5 before storage which was not significantly (p>0.05) different from 97.3 % obtained from seeds of IT99K-316-2. Next to these with high germination percentage of 96 were seeds obtained from IT90K-277-2, IT96D-610, IT98K-205-M8, IT98KD-288, IT04K-332-1 and IT07K-222-2 whereas the significantly lowest germination percentage of 78.6 was recorded in seeds of cowpea cultivars IT04K-321-2 and IT07K-211-1-8. (Table 1). Similarly, the difference between the lowest and highest percentage mean values for the longevity test was also wide and significant (p<0.05) when seeds were stored for four weeks.
Table 1: Cowpea seed quality as affected by single and mixed infections of *Blackeye cowpea mosaic virus* (BICMV) and *Cowpea mottle virus* (CPMoV) at Mokwa in 2017.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>BICM V</th>
<th>CPMoV V</th>
<th>BI + CP</th>
<th>CP + BI</th>
<th>Germination Test (%)</th>
<th>Accelerated Ageing Germination (%)</th>
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<tr>
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<td>4 Weeks of Storage</td>
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<tr>
<td><strong>Ife Brown</strong></td>
<td>93.5a</td>
<td>90.5c-f</td>
<td>86.7f</td>
<td>86.5gh</td>
<td>77.9a 69.0b 56.6i</td>
<td>46.2i</td>
</tr>
<tr>
<td>**IT90K – 277 –</td>
<td>94.7bc</td>
<td>98.7a</td>
<td>78.5c</td>
<td>96.0bc</td>
<td>76.6a 68.0bc</td>
<td>58.4a 51.6b</td>
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<td><strong>IT96D – 610</strong></td>
<td>87.3g</td>
<td>97.7a</td>
<td>97.6b</td>
<td>96.0bc</td>
<td>70.3b 66.5d 52.0e</td>
<td>52.0gbc</td>
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<tr>
<td>**IT97K – 499 –</td>
<td>88.0g</td>
<td>86.9j</td>
<td>97.6b</td>
<td>92.0e</td>
<td>61.5g 60.0e 55.0f</td>
<td>56.0ab</td>
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<td>**IT97K – 568 –</td>
<td>99.7a</td>
<td>91.2c</td>
<td>81.3j</td>
<td>98.5a</td>
<td>69.5b 57.2h 48.0i</td>
<td>41.2j</td>
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<td>**IT97K – 573 –</td>
<td>87.8g</td>
<td>93.4b</td>
<td>94.8d</td>
<td>94.5d</td>
<td>50.6l 57.1h 45.5i</td>
<td>35.6ma</td>
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<td>**IT98K – 205 –</td>
<td>87.6g</td>
<td>89.2e</td>
<td>77.5m</td>
<td>96.0bc</td>
<td>57.5h 70.6a 56.3b</td>
<td>41.5j</td>
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<td>M8</td>
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<tr>
<td><strong>IT98KD – 288</strong></td>
<td>91.3c-g</td>
<td>90.7de</td>
<td>82.6i</td>
<td>96.0bc</td>
<td>48.0m 62.7f 48.3gh</td>
<td>51.3ef</td>
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<tr>
<td>**IT99K – 316 –</td>
<td>92.1c-f</td>
<td>93.4b</td>
<td>85.0h</td>
<td>97.3ab</td>
<td>53.3lk 64.0ef 57.3ab</td>
<td>46.0j</td>
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<td>**IT99K – 377 –</td>
<td>88.9eg</td>
<td>90.8ed</td>
<td>85.4h</td>
<td>92.0c</td>
<td>60.0ef 60.0g 50.6f</td>
<td>31.6a</td>
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<td>**IT00K – 901 –</td>
<td>88.8eg</td>
<td>86.1i</td>
<td>81.3j</td>
<td>89.3f</td>
<td>70.6b 65.0c 52.0f</td>
<td>47.0 hij</td>
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<td>**IT03K – 337 –</td>
<td>97.3ab</td>
<td>89.4deg</td>
<td>84.6b</td>
<td>89.3f</td>
<td>50.5l 66.8ecd 46.4j</td>
<td>41.4j</td>
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<td>**IT04K – 267 –</td>
<td>92.2c-f</td>
<td>98.2a</td>
<td>81.3j</td>
<td>86.5gh</td>
<td>56.0hi 62.6f 49.5fg</td>
<td>54.6bc</td>
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<td>**IT04K – 291 –</td>
<td>87.8g</td>
<td>86.9j</td>
<td>89.3s</td>
<td>87.7g</td>
<td>54.6j 58.7g 57.4ab</td>
<td>52.0de</td>
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<tr>
<td>**IT04K – 321 –</td>
<td>90.5c-g</td>
<td>93.8b</td>
<td>85.3eh</td>
<td>78.6k</td>
<td>58.6g 56.3hi 48.0h</td>
<td>50.6e fg</td>
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<tr>
<td>**IT04K – 332 –</td>
<td>99.7a</td>
<td>98.5a</td>
<td>98.5a</td>
<td>96.0bc</td>
<td>60.0ef 53.4k 49.3l</td>
<td>48.1hi</td>
</tr>
</tbody>
</table>
Means with the letter (s) within the same column are not significantly (p ≤ 0.05) different by Student-Newman-Keuls (SNK) test

Significantly highest germination percentage of 77.9 was recorded in seeds of BICMV infected Ife Brown followed by IT90K-277-2, IT00K-901-5 and IT96D-610 with 76.6, 70.6 and 70.3 germination percentage, respectively. Seeds of cultivar IT97K-568-18, IT07K-292-1-10 and IT07K-299-6 exhibited germination values of 69.5, 64.4 and 62.1 %, respectively whereas the least germination values of 46.6 % was obtained from seeds of IT06K-124. Mean value for accelerated ageing germination (AAG) on CPMoV infected cowpea cultivars showed that seeds of IT98K-205-M8 had 70.6 % germination. This was closely followed by seeds of Ife Brown with 69 % while 68, 66.8 and 66.5 % were obtained from cultivars IT90K-277-2, IT03K-337-6 and IT96D-610, respectively. The germination capacity of 64 % was recorded from seeds of cultivars IT99K-316-2 while IT07K-299-6 and the remaining cultivars had
AAG percentages ranging from 53.4 to 62.7 % (Table 1).

For the mixed infection treatments, germination value of 58.6 % was obtained from IT90K-277-2, IT06k-124 and IT07K-292-1-10 BICMV + CPMoV infected cowpea cultivars. This value (58.6 %) was significantly ($p<0.05$) higher than the values obtained from seeds of other cultivars. Seeds from cultivars IT98K-205-M8, IT97K-499-35, IT06K-137-1 and IT07K-211-1-8 gave germination values of 56.5, 55, 54.5 and 53.4 % respectively. Seeds of cultivars IT96D-610 and IT00K-901-5 exhibited similar germination percentage of 52 while the remaining cowpea cultivars had germination percentages of between 44.0 and 50.6. Also, seed germinability of 57.3 % was highest in IT07K-292-1-10 with CPMoV + BICMV infected cowpea seeds which was statistically ($p>0.05$) similar to the performance of seeds of IT97K-499-35 with 56 %. Seeds of cultivar IT04K-267-8 and IT07K-222-2 exhibited 54.6 and 53.7 % respectively, while IT96D-610 and IT04K-291-2 had germination values of 52 % which did not differ from one another. The lowest accelerated ageing germination (AAG) percent of 31.6 was recorded in seeds of cowpea cultivar IT99K-377-1 (Table 1).

**DISCUSSION**

Germination and longevity are two major indices used for determining the performance capability of seed lot. Seed quality is influenced by the environment where it is produced. Pathogens namely virus, nematode, fungi, bacteria among others are integral components of the environment of any seed crop; failure to effectively manage their competition could mean zero harvest (1). However the imperative of understanding the impact of virus management strategies and management for quality seed production arises from the paucity of information on the agronomy of seed production (1), more so that seed production efforts are judged on the basis of quality of the produce rather than quantity. The result of this study has established a clear negative influence of virus infection on cowpea seed quality and that the differential ranking of the virus infection treatments in different seed quality test is an indication of the response of the developing seeds on the mother plant to competing virus infection situations. Differences in time of flower initiation, pod setting, seed formation and maturity to virus infections are critical factor to tropical farming. The results obtained from this study revealed that there was a variation in
germination percentage before and after four weeks of storage which is a measure of seed viability and longevity. When seed that has this trait is sown on the field for production, it exhibits a wide variation in performance after sowing due to the differences in quality (1).

It is known that cowpea seedlings are susceptible to virus infection at different stages of development (3). This is supported by the differential responses of cowpea seeds harvested from the different virus treatment seed lots in the present study. The initial general high germination percentage recorded in seeds of all treatment combinations in this study is an indication that the seeds did not exhibit dormancy contrary to what is known with most vegetable seeds when freshly harvested. This rapid germination also showed that the activities of the pathogens (viruses) on the seeds were not severe enough to impaired germination (5).

Mandhare and Gawade (17) reported that though seeds obtained from mosaic infected bean at harvest exhibited high seed germination, a significant sharp decline in germination percentage of the seeds was recorded following four weeks of storage at 32 °C and 50 % relative humidity. Following storage of seeds for four weeks in this study, a sharp decline in the germination capability of seeds of all the treatment combinations was recorded. This sharp decline in the quality of seeds is abnormal according to the normal/natural seed ageing process (13). The reason may be that the pathogen activities must have been activated which resulted in the sudden and heavy decline in the germination percentages (19). Furthermore, the variation in germination percentages amongst the cultivars and treatments as shown in this study suggest genetic superiority (5) and tolerance level of the cultivars over one another.

**CONCLUSION**

The results of the experiment revealed that all cultivars were susceptible to single and mixed infections of the two viruses but to seemingly different extent. The germination of seeds as seen from this study was generally high before storage; the high initial germination percentage was not sustained (short lived); an indication that conservation of infected seeds of all cultivars was impaired. More so, all the cowpea cultivars did not exhibit dormancy which is a problem with most freshly harvested vegetable seeds. The benefits of increased
cowpea production include improved nutrition for humans and livestock, improved soil properties and substantial opportunities for greater income. The monitoring and management of these viruses therefore is crucial to sustainable cowpea production most especially in sub-Saharan Africa. There is the need, therefore, for constant monitoring of legume fields through regular field sanitation, disease surveys to identify new and emerging viruses because these facts present a good starting point for legume virus diseases diagnosis in the study area. Finally, there is also need to ensure availability of acceptable horticultural desirable cowpea cultivars with a high level of resistance to cowpea viruses for the nation to sustain its high level of production.

REFERENCES


