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By

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Research Article

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ABSTRACT

Piper guineense, Monodora myristica and Xylopia aethiopica seeds extracts were evaluated in the laboratory for their effects on the incidence of the seed-borne mycoflora of cowpea (Var. IAR-48) and germination of the treated seeds. The cowpea seeds were stored for 0-3 months in air-tight containers inside dark cupboards in the laboratory. On the last day of each storage period, the seeds were coated with thin films of the different plant extract powders and thereafter, they were plated and incubated at 27°C for 5 days in Petri dishes containing moistened filter papers. The experiment was a 4 x 5 factorial laid out in completely randomized design (CRD). The fungal organisms isolated from the incubated seeds were Fusarium sp., Mucor sp., Aspergillus spp., Colletotrichum sp., and Curvularia sp. All the extracts with the exception of X. aethiopica were significantly (p<0.05) superior to benlatein reducing the incidence of the seed-borne fungi associated with the cowpea seeds, however the germination of the treated seeds was highest in Piper guineense dressed seeds (21.30%). In the overall, the mean incidence of the seed-borne mycoflora of the treated cowpea seeds decreased as storage period increased while germination also decreased with increasing storage period. Hence, these plants extract powders especially Piper guineense could be used in IDM programmes, for seed-dressing of cowpea for increased productivity.

Keywords: Piper guineense, Monodora myristica, Xylopia aethiopica, Seed-borne fungi, IDM, Storage duration.

INTRODUCTION

Cowpea (Vigna unguiculata L. Walp) (Fabaceae), a leguminous annual crop is one of the most important food and forage legumes in the semi-arid tropics of Asia, Africa, Southern Europe, USA, and the Americas (Singh, 2005; Timko et al., 2007). The crop is thought to have originated from Africa with center of speciation in the Transvaal region of South Africa (Aveling, 2007). Cowpea contributes significantly to farmers’ income and diets, its grain very highly nutritious and free from metabolites; it provides a good supplement for scarce animal protein for 110 million consumers of the crop in the third world economies of the tropics and sub-tropics (Timko et al., 2007; SADAFF, 2009). Being a dual- purpose crop, it can be used in farming systems as a forage or cover crop to suppress weeds, control erosion and attract beneficial insects (Summerfield and Roberts, 1985; Valenzuela and Smith, 2002). Besides, cowpea also plays a major role in farming systems in the fixation of nitrogen through symbiosis with nodular Bradyrhizobium species of bacteria, fixing on the average 240kgN/ha per annum (FAO, 1994; Awurum, 2000; Aveling, 2007).

Worldwide statistics reveal that 4.5 million metric tonnes of cowpea grains were produced annually on 12-14 million ha of farmland; and 64-70% of the production obtained in West and Central Africa (Timko et al., 2007; SADAFF, 2009). Singh et al. (2002) reported that Nigeria is the world’s largest producer of the crop with 2.0 million metric tonnes of grain from 5 million ha of farmland per annum.

Cowpea is seed established and is vulnerable to attack by fungal pathogens in storage and at all stages of growth of the crop. Seed-borne fungi associated with cowpea include Macrophomina, Colletotrichum, Fusarium, Aspergillus, and Curvularia species. These organisms have been implicated in low seed viability and germination, seedling mortality and yield reductions (Singh and Allen, 1979; Emechebe, 1981; Richardson, 1990; Abdelmonen and Rasmy, 2000) and they present such symptoms as shrunken seeds, seed rot, necrosis, reduced seed size and other physiological alterations in affected seeds(Shetty, 1992).
Control of these seed-borne diseases is by means of chemical seed treatments using broad-spectrum fungicides such as thiram and captan. However, these chemicals have been banned in the USA and Europe due to mammalian toxicity (Taylor and Harman, 1991), thus necessitating search for alternatives (Gurjar et al., 2012). One viable alternative in recent times is the use of extracts of higher plants in plant pathology and plant disease management (Amadioha, 2001; 2003; Awurum et al., 2005; Okwu et al., 2007). These extracts have been reported to possess fungicidal properties by many workers (Awurum et al., 2005; Okwu et al., 2007); and deemed to be sources of less phytotoxic, eco-compliant and cost effective chemicals (Amadioha, 2002; Enyiukwu and Awurum, 2011).

Considering the fact that Xylopia aethiopica, Piper guineense and Monodora myristica are used extensively in African cuisines and ethnomedicine, this present study aimed at evaluating the effectiveness of duration of storage, followed by treatment with extracts from these spice plants for the management of seed-borne mycoflora of cowpea.

MATERIALS AND METHODS

Sources of seeds and plant materials

The seeds of cowpea variety IAR-48 were obtained from the Research and Training Unit of the College of Crop and Soil Sciences, Michael Okpara university of Agriculture, Umudike and the plant materials Piper guineense, Monodora myristica and Xylopia aethiopica seeds obtained from Umuahia main market, were used in the study. The mean ambient parameters of the study area were 2163mm of rainfall, temperature 20-30 °C and relative humidity 57-87% per annum.

Preparation of plant extracts and culture medium

Seeds of Xylopia aethiopica, Monodora myristica and Piper guineense were sorted and cleaned, thereafter they were milled separately into powder, using a hand-milling machine (Model; Corona Lavesh 250). The powders thus obtained were stored in air-tight bottles in dark cupboards. Then 200g of fresh peeled Irish potato was boiled in 1L of water contained in 2L flask for 1h. The broth was filtered through double-folds of sterile cheese cloth, made up to 1L with sterile distilled water to which 20g agar and 15g glucose were added and modified with 20mg of Gentamicin (an antibiotic to kill or suppress any bacteria contaminating the medium). The flask was stoppered with foiled cotton wool and then autoclaved at 15 psi (120° C, 152 cmHg) for 30 minutes to sterilize the PDA medium from contaminating fungal propagules.

In vivo experiment

Cowpea seeds (Var.IAR-48, moisture content 11.30%) were stored for periods of 0-3 months respectively in dark cupboards in the laboratory. On the last day of each storage period, the seeds were dressed separately with different powders of the plant extracts by coating them with films of the extracts. The seeds were dressed with the powder at a concentration of 100g/kg seed while benlate was at 1g/kg of the seeds. The control consisted of uncoated seeds. At the end of each test period, the seeds were then plated in Petri dishes by blotter method and incubated for 5 days at 27°C, and observed for germination and mycelial growth. Ten (10) cowpea seeds were plated per Petri dish and replicated 5 times. The whole experiment was repeated thrice giving 150 seeds per treatment. The Petri dishes were arranged in 4 x 5 factorial, in a completely randomized design (CRD). At the end of 5 days, counts of germinated seeds were taken per plate and mean of replicates recorded. Records of the seed-borne fungi that grew out of the seeds and sporulated were also taken.

Pure cultures of the mycoflora associated with the cowpea seeds were obtained by repeated sub-culturing of the organisms on solidified 20ml PDA in Petri dishes. Using a sterile needle, bits of each seed-borne fungus were transferred on glass slides, stained with a drop of lactophenol in cotton blue, teased gently and covered with cover slips. The slides were fixed by gently passing over a spirit lamp flame, then mounted on the stage of a microscope, observed and the identity of the various mycoflora confirmed through their spore characteristics by the aid of fungi identification manual by Barnett and Hunter (1983).

The percentage incidence of the seed-borne fungi on the incubated cowpea was assessed as:

\[
\text{% incidence} = \frac{\text{Number of seeds infected}}{\text{Total number of seeds examined}} \times 100/1
\]

The percentage germination of incubated cowpea seeds was also assessed as:

\[
\text{% germination} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds examined}} \times 100/1
\]
Statistical Analysis

Data collected from this study were analyzed by analysis of variance (ANOVA) using the general linear model procedure in SAS system (2008 version) at 5% level of significance. Means were separated and compared using Fisher's Least Significant Difference (LSD) at probability of 0.05.

RESULTS

The results show that the fungal organisms *Fusarium*, *Mucor*, *Aspergillus*, *Colletotrichum* and *Curvularia species* were associated with the seeds of cowpea (var. IAR-48). It indicated that the mean disease incidence of the treated seeds decreased with increasing storage period while germination decreased accordingly (Table 1). The mean fungal load decreased from 59.9% at 0 month to 25.5% after 3 months of storage while the germination profile of the treated seeds decreased from 18.50% to 13.40% respectively.

<table>
<thead>
<tr>
<th>Duration of contact in storage (months)</th>
<th>Mean disease incidence (%)</th>
<th>Mean germination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>59.90</td>
<td>18.50</td>
</tr>
<tr>
<td>1</td>
<td>31.70</td>
<td>17.30</td>
</tr>
<tr>
<td>2</td>
<td>27.50</td>
<td>15.70</td>
</tr>
<tr>
<td>3</td>
<td>25.50</td>
<td>13.40</td>
</tr>
<tr>
<td>LSD</td>
<td>3.54</td>
<td>2.25</td>
</tr>
</tbody>
</table>

The results presented in Table 2 also show that the different plant materials had significant effects on the seed-borne mycoflora as well as the germination of the treated seeds. All the plant extracts significantly (P< 0.05) reduced the incidence of the seed-borne fungi better than the control. It depicted that *Piper guineense* extract was statistically superior to benlate in reducing the disease incidence of the treated seeds. *P. guineense* effectively reduced the fungal load on the treated seeds from 53.20% in the control to 27.30%. In like manner, *P. guineense* extract treated cowpea seeds germinated better than benlate treated seeds. In addition, germination percentage obtained from *P. guineense* treated seeds (21.30%) was significantly (p<0.05) greater than all other treatments. (Table 2).

<table>
<thead>
<tr>
<th>Plant materials</th>
<th>Seed-borne disease incidence (%)</th>
<th>Germination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>53.20</td>
<td>14.00</td>
</tr>
<tr>
<td>Benlate</td>
<td>35.50</td>
<td>17.30</td>
</tr>
<tr>
<td>Xylopia aethiopica</td>
<td>32.80</td>
<td>16.20</td>
</tr>
<tr>
<td>Monodora myristica</td>
<td>30.80</td>
<td>14.20</td>
</tr>
<tr>
<td>Piper guineense</td>
<td>27.30</td>
<td>21.30</td>
</tr>
<tr>
<td>LSD</td>
<td>3.96</td>
<td>2.52</td>
</tr>
</tbody>
</table>

DISCUSSION

The result of this study showed that species of *Fusarium*, *Aspergillus*, *Colletotrichum*, *Mucor* and *Curvularia* were associated with the seeds of cowpea variety IAR-48. This finding agrees with the earlier works of Awurum and Enyiukwu (2013), Awurum and Uchegwu (2013) who also found these mycoflora associated with the seeds of this cultivar. The decrease in percent incidence of seed-borne fungi observed in this study with increase in storage duration may be because the organisms are externally borne on the seeds testa than internally in the seed endosperm; or as a result of inter-species competition for space and nutrients and/or due to parasitism amongst the organisms (Suprapta, 2012). However the decrease in germination of the stored seeds with increasing duration of storage may have been as a result of deposits of toxins and metabolites from the organisms. For instance, *Colletotrichum* and *Fusarium* have been implicated with seed rot and deterioration of several seeds while toxins from *Aspergillus spp.* were underpinned for inhibiting germination of pea seeds (Shetty, 1992). The longer duration of contact of the seeds with these toxins explains the reason for the observed decrease in germination as storage period increased. Extracts from *Eucalyptus globulus*, *Tridax procumbens*, *Acalypha ciliata*, *Azadirachta indica*, *Vernonia amygdalina*, *Zingiber officinale* and *Garcinia kola* have been reported by many workers as fungitoxic to
species of Aspergillus, Penicillium, Fusarium and Pythium causing storage and root rots of cowpea (Suleiman and Emua, 2009; Onyeani et al., 2012; Mogle, 2013). Findings from this present investigation corroborate their reports. This study also indicated that with the exception of X. aethiopica, all the test extracts were superior to benlate in fungitoxicity. This is also in agreement with the findings of several investigators. Okwu and Njoku (2009) found Afronumium melegueta extracts superior to benlate in checking Sclerotium rolfsii (basal stem rot) of cowpea while Awurum and Ucheagwu also reported the superiority of Piper guineense, Monodora myristica and Xylopia aethiopica extracts in reducing the incidence of the seed-borne fungi of cowpea more than benlate on treated seeds. Our present study however, does not agree with earlier reports by Amadioha and Obi (1998) where in field trials Xylopia aethiopica was superior to benomyl in reducing the incidence and severity of Colletotrichum lindemuthianum induced anthracnose of cowpea. This may be due to the fact that the extract powder in this present study did not stay in contact long enough with the test seeds to exert substantial fungitoxicity. Furthermore, the finding of this work does not agree with Awurum and Enyiukwu (2013), who also reported that cowpea dressed with aqueous extract of Piper guineense failed to germinate. The better germination profile obtained in this present study may have been due to differences in the method of seed dressing employed by the investigators; whereas the cowpea seeds in this present study were coated with thin film of the powders of the extracts, Awurum and Enyiukwu (2013) immersed the seeds in the aqueous extracts medium. It follows perhaps, that in the aqueous medium the active ingredients may still be locked up and released slowly in the solid state. It may also be explained as being a result of long exposure of the seeds for up to 8h in solution of the active principles. Plants contain potent antimicrobial and fungitoxic compounds. Spice plants for example are known to contain terpene-rich essential oils. SKG (2012) noted that the ability of extracts of Melaleuca alternifolia to control fungus-induced Sigatoka disease of plantains were due to terpenoids such as α-terpen-4-ol and α-pinene amongst 98 other biologically active principles in the oil. The presence of α-pinene and phellandrene have been reported in P. guineense, and may explain its high fungitoxicity (Enyiukwu and Awurum, 2013).

In conclusion therefore, these plant tissues extracts (Piper guineense, Monodora myristica and Xylopia aethiopica) are possible sources of potent fungicides for dressing cowpea seeds by resource-poor farmers in low input cowpea production systems against seed-borne mycoflora including species of Aspergillus, Colletotrichum, Fusarium, Curvularia and Mucor in order to obtain cleaner seeds required for enhanced productivity of the farmers.

REFERENCES


