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# EFFECT OF ASPERGILLUS SPP ON THE GERMINATION AND SEEDLING VIGOUR OF GROUNDNUT (ARACHIS HYPOGAEA L.) AND THEIR MANAGEMENT WITH HEAT STERILIZED GARLIC EXTRACT

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**ABSTRACT**

The study investigated the effect of seedborne fungi *Aspergillus flavus* and *A. niger* on the germination and seedling vigour of groundnut. The effect of heat sterilization on the efficacy of garlic extract in the management of the radial growth and sporulation of the two fungi was also assessed. Each experiment was arranged in a completely randomized design and replicated three times. Results showed that the combination of both fungi produced significantly ($p< 0.05$ %) lower germination percentage of 61.70% compared with the un-inoculated control and the germination percentage of the particular fungus. Seedling length of groundnut seedlings inoculated separately with *A. flavus* and *A. niger* were not significantly different at 4 and 6 days after planting (DAP) compared with the un-inoculated control. The combination of the two fungi significantly reduced the seedling length of groundnut at 4 and 6 DAP. The groundnut seedlings separately inoculated with *A. flavus* and *A. niger* had seedling vigour index of 1.56 and 1.38 respectively. Garlic extract significantly ($p< 0.05$ %) reduced radial growth of *A. niger* and *A. flavus* at 4 days after inoculation but had no significant effect ($p>0.05$) on the sporulation of the two fungi.

## 1. Introduction

Groundnut (*Arachis hypogaea* L.) is an annual legume which is also known as peanut, earthnut, monkey-nut and goobers (Adetumbi *et al.*, 2009). It is an outstanding food and oilseed crop eaten as boiled nuts, roasted and baked as flour (Adebisi *et al*., 2010; Radha *et al*., 2011). In Nigeria, groundnut production is majorly cultivated in the Guinea Savannah laying between $6^o$ to $11^o$ N (Audu *et al*., 2017). Benue State is among the Nigerian States within the groundnut production zone (Ani *et al*., 2013). Groundnut production in Nigeria is however declining due to contamination of seeds by *Aspergillus flavus* which is the major producer of aflatoxin in groundnut. *Aspergillus flavus* and *A. niger* are ubiquitous fungi which compete and exist together resulting in rot in stored groundnut (Ihejirika *et al*., 2005).

Although much work has been done on the investigation of fungal diseases that affect the groundnut seeds, the effect of these *Aspergillus* spp on the germination and seedling vigour of groundnut and its management with garlic plant extract has not been studied. The study investigated the effect of

*Aspergillus flavus* and *A. niger* isolated from groundnut seeds on the germination and seedling vigour of groundnut and the effect of heat sterilization on the management of the radial growth and sporulation using garlic extract.

## 2. Materials and methods

### 2.1 Experimental location

The research was conducted at the Advanced Plant Pathology Laboratory of the Federal University of Agriculture, Makurdi, Benue State. The location falls within the Southern Guinea Savanna Agro ecological zone of Nigeria on Latitude 07º45'N to 07º50'N, longitude 08º45'E to 08º50'E, 98 m above sea level.

### 2.2 Isolation of the fungal organisms

Two pathogens *Aspergillus flavus* and *A. niger* previously obtained from groundnut seeds evaluated for aflatoxin contamination were used in the study. Infected groundnut seeds were cultured to isolate fungal organisms. Infected groundnut seeds were sterilized for 1minute in 10 % Sodium hypochlorite solution after which they were rinsed in three changes of Sterile Distilled Water (SDW) and blotted dry on sterile filter papers. Potato Dextrose Agar (PDA) was prepared by adding 39 g in 1litre of Sterile Distilled Water (SDW) in a conical flask. The flask was autoclaved at 121$^{o}$C for 15 minutes. After autoclaving, the media was allowed to cool to about 40$^{o}$ C and Streptomycin Sulphate was added at the rate of 0.2 g/L. The media was then poured into 9 cm Petri dishes and allowed to solidify. The seeds were plated on PDA (ten seeds per plate).The plates were then incubated on the laboratory bench at ambient conditions of light and temperature (30 ± 2$^{o}$C) for 7 days. Pure culture was obtained by sub culturing unto fresh PDA plates.

Microscopic examination was done by examining the colony characteristics. A sterile needle was used in taking a little portion of the hyphae containing spores on the sterile glass slide stained with lactophenol cotton blue and examined under the microscope (x10 Mag.) for fungal structures (Wanatabe, 2010).

### 2.3 Effect of test fungi on the germination and seedling vigour of groundnut seeds

The effect of A. *flavus*, *A. niger* and *A. flavus*/ *A. niger* on the germination of groundnut seeds and seedling vigour was investigated. The experiment was laid out in a completely randomized design with three replicates. The plates containing the pure cultures of the fungi were flooded with 10ml sterile distilled water (SDW) and the spores dislodged using a sterile glass rod. The culture suspension was filtered through one layer of cheese cloth. The concentration of spores was determined by a haemocytometer and adjusted with SDW to 5 × $10^6$ spores/ml. The spore suspension was used as in-oculum for the experiment.

Twenty apparently healthy groundnut seeds were placed in a beaker containing 5 x $10^6$ inoculum of each fungus and in combination for 30 minutes. The inoculated seeds were removed and placed in 120cm Petri dishes containing double layer blotter moistened with 10ml distilled water. Control consisted of groundnut seeds soaked in SDW for 30minutes.The Petri dishes were incubated at ambient conditions of light and temperature (30 ± 2) for 8 days.

### 2.4 Collection and preparation of aqueous plant extracts

Fresh healthy bulbs of *Allium sativum* (white type) were purchased from Northbank market, Makurdi, Benue State, Nigeria. The plant was selected based on antifungal potential as reported by David and Arabami (2015); Ekhuemelo and Yaaju (2017). The bulbs were washed with sterile distilled water (SDW). Fifteen grams of *A. sativum* was macerated and infused in 100 ml SDW to give 15 %w/v aqueous extract, filtered through double layer cheese cloth and used for the study.

**2.5 Application of plant extract**

The aqueous extract was used in two ways. First, *A. sativum* extract was used to amend potato dextrose agar before heat sterilization (autoclaving) and secondly *A. sativum* was added to the Potato dextrose agar medium without heat sterilization (no autoclaving). In the amended medium, five (5) grams of PDA was added to the extract and the flask was autoclaved at 121$^{o}$ C for 15 minutes and allowed to cool to about 40$^{o}$ C. Stretomycin Sulphate was added at the rate of 0.2g/L and the PDA media poured into sterile 9 cm Petri dishes and left to solidify. In the second method, 5 ml of *A. sativum* extract was added to each 9 cm Petri dish of already prepared PDA containing 0.2g/L streptomycin sulphate using a sterile 5 ml needle and the plates swirled gently and allowed to solidify.

**2.6 Fungitoxicity effect of the aqueous extract of *A. sativum* on radial growth and sporulation of aflatoxigenic *A. flavus* and *A. niger*.**

The PDA amended and non-amended extracts prepared above were inoculated with 3 mm disc taken from the advancing edges of 7 days old pure cultures of *A. flavus* and *A. niger* using a 3 mm cork borer. The 2x2 factorial experiments consisting of two test fungi, heat sterilization and non-heat sterilization plant extract application methods and a control (where no extract was added) were arranged in completely randomized design and replicated three times. The plates were incubated on the laboratory bench at 30 ± 2 $^{o}$C for seven days.

Sporulation was determined by flooding the surface of each plate with 10 ml SDW and gently scraping with a sterile glass rod to dislodge the spores. Spore suspension obtained was filtered with a sterile cheese cloth into a 50 ml glass beaker and homogenized manually. The spores were counted using a Neubauer haemocytometer.

**2.7 Data Collection.**

Data on percentage germination which was the number of germinated seeds relative to the total number of seeds sown multiplied by 100 was recorded on 4, 6 and 8 days after inoculation (DAI). Seedling length was measured with a metre rule at 4 and 6 days after planting (DAP) and recorded in centimeters. Seedling vigour index was calculated using the formula of Anjorin and Mohammed (2014):

Vigour index = seedling length x percentage germination/100.

Radial growth was measured at 4, 6 and 8 DAI along two lines drawn perpendicular to each other at the reverse of each plate.

Sporulation was determined using the formula:

Number of spores per milliliter (x10$^{6}$) = average number of spores in a small square ÷ 4

**2.8 Data Analysis**

Data analysis was done using Genstat statistical package version 9. Significant treatment mean was separated using Fishers least significant difference (F-LSD) at 5% level of probability.

## 3. Results

The effect of *A. niger* and *A. flavus* on the germination of groundnut seeds is presented in Table 1. The germination percentage of groundnut seeds inoculated with *A. niger* and *A. flavus* was not significantly ($p > 0.05$) different compared with the control at 4 DAI. At 6 DAI, the un-inoculated groundnut seeds had the highest germination percentage of 80%. Groundnut seeds inoculated with *A. flavus* had germination percentage of 71.70 % although this was not significantly ($p > 0.05$) different from the percentage germination of groundnut seeds inoculated with *A. niger*. The combination of both fungi produced significantly ($p < 0.05$ %) lower germination percentage of 61.70 % compared with the un-inoculated control and the germination percentage of the particular fungus. At 8DAI, the

germination percentage of groundnut seeds inoculated with *A. niger* (66.70 %) and *A. flavus* (71.70 %) was not significantly different ($p > 0.05$) from the un-inoculated control (80.00 %).

**Table 1: Effect of fungi on Germination Percentage of groundnut seeds**

| Treatment | Germination percentage (%) | |
|---|---|---|
| | 4DAI | 6DAI |
| *A. flavus* | 66.70 | 71.70 |
| *A. flavus*/*A. niger* | 60.00 | 61.70 |
| *A. niger* | 60.00 | 66.70 |
| Control | 65.00 | 80.00 |
| LSD(0.05) | NS | 7.63 |

The effect of *A. niger* and *A. flavus* on seedling length and seedling vigour index of groundnut seedlings is presented in Table 2. Seedling length of groundnut seedlings inoculated with *A. flavus*, *A. niger* and the un-inoculated control were significantly ($p < 0.05$) different at 4 and 6 DAP. However, the combination of the two fungi significantly ($p < 0.05$) increased the seedling length of groundnut at 4 and 6 DAP.

The groundnut seedlings treated with a combination of *A. flavus* and *A. niger* had the lowest seedling vigour index of 1.10 while the un- inoculated groundnut seedlings had the highest seedling vigour index of 1.77 which was not significantly ($p > 0.05$) different from the seedling vigour index of the groundnut seeds inoculated with *A. flavus* with seedling vigour index of 1.56.

**Table 2: Effect of Fungi on Seedling Length and Seedling Vigour Index of Groundnut**

| Treatment | Seedling Length(cm) | | Seedling Vigour Index |
|---|---|---|---|
| | 4DAP | 6DAP | |
| *A. flavus* | 2.04 | 2.17 | 1.56 |
| *A. flavus*/*A. niger* | 1.55 | 1.65 | 1.10 |
| *A. niger* | 1.96 | 1.94 | 1.38 |
| Control | 2.27 | 2.21 | 1.77 |
| LSD(0.05) | 0.46 | 0.42 | 0.51 |

The interaction effects of heat application to garlic extract on the radial growth of *Aspergillus flavus* and *Aspergillus niger* isolated from groundnut seeds is presented in Table 3. The radial growth of *A. niger* isolated from groundnut seed was significantly ($p < 0.05$) higher (3.88cm) compared with *A. flavus* (2.41cm) at 4 DAI. Heat application to garlic extract had no significant effect ($p > 0.05$) on the radial growth of the two fungi compared with the control at 4 and 8 DAI. However non heat application resulted in lower radial increase.

The interaction effect of heat application to garlic extract on the radial growth of A. *flavus* and A. *niger* isolated from groundnut seeds is presented in Table 4. A significant interaction was recorded at 4 DAI. The application of heat to garlic extract significantly ($p < 0.05$) reduced the radial growth of *A. niger* (2.85 cm) compared with the control (5.90 cm) while the radial extension of *A. flavus* (3.38 cm) was significantly ($p < 0.05$) reduced without heat application to garlic extract compared with the control (4.05 cm).

**Table 3: Effect of heat application to garlic extract on the radial growth of *Aspergillus flavus* and *Aspergillus niger* isolated from groundnut seeds.**

| Treatment | Radial Growth (cm) | |
|---|---|---|
| | 4DAI | 8 DAI |
| **Fungi** | | |
| *A. flavus* | 2.41 | 7.48 |
| *A. niger* | 3.88 | 8.24 |
| LSD (0.05) | 0.54 | NS |
| **Heat application method** | | |
| Heat application | 3.35 | 8.02 |
| No heat application | 3.14 | 7.90 |
| Control | 2.95 | 7.65 |
| **LSD (0.05)** | NS | NS |

**Table 4: Interaction effect of heat application to garlic extract on the radial growth of *Aspergillus flavus* and *Aspergillus niger* isolated from groundnut seeds.**

| Fungi | Treatment | Radial Growth | |
|---|---|---|---|
| | | 4 DAI | 8 DAI |
| ***A. flavus*** | Heat application | 3.85 | 7.72 |
| | No heat application | 3.38 | 7.55 |
| | Control | 4.05 | 7.17 |
| ***A. niger*** | Heat application | 2.85 | 8.33 |
| | No heat application | 2.90 | 8.25 |
| | Control | 5.90 | 8.13 |
| LSD (0.05) | | 0.66 | NS |

**Table 5: Effect of heat application on to garlic extract on the sporulation of *Aspergillus flavus* and *Aspergillus niger* seven days after incubation at ambient conditions of light and temperature**

| Treatment | Sporulation ( $\times 10^6$ spores/ ml) |
|---|---|
| ***A. flavus*** | |
| Heat application to garlic | 19.73 |
| No heat application | 18.00 |
| Control | 17.14 |
| LSD | NS |
| ***A. niger*** | |
| Heat application to garlic | 12.70 |
| No heat application to garlic | 14.50 |
| Control | 17.90 |
| LSD | NS |

The effect of heat application to garlic extract on the sporulation of *A. flavus* and *A. niger* is presented in Table 5. The use of garlic extract with or without heat application had no significant effect on the sporulation of *A. flavus* and *A. niger* seven days after incubation at ambient conditions of light and temperature (30 ± 2$^{o}$ C).

## 4.0 Discussion

The individual test fungus had no significant effect on the germination of groundnut seeds at 4DAI but by 6DAI, the combination of the two fungi significantly lowered the germination of groundnut seeds. This is in line with the report of Anjorin and Mohammed (2014) in which combined inoculation of *Mucor racemosus* and *Rhizopus nigricans* reduced the germination of watermelon seeds. The planting of groundnut seeds infected with *A. flavus* and *A. niger* may result in lower field emergence. Adebisi and Ajala (2007) reported a correlation between germination percentage and field emergence in sesame. The lower seedling vigour index recorded in the present study when the combination of *A. flavus* and *A. niger* was applied to groundnut seeds also agrees with the report of Anjorin and Mohammed (2014) which reported significantly lower seedling vigour in watermelon seeds inoculated with a combination of *Mucor racemosus* and *Rhizopus nigricans*. Adebisi *et al.* (2010) had earlier reported that seedling vigour index and standard germination of rice seeds had a significant positive relationship with field performance traits such as field emergence, seedling establishment and seed yield per plant. Mehrotra and Aggarwal (2003) agreed that seed borne fungi could affect seed viability and severely retard seed germination.

The significant reduction in the radial growth of *A. niger* resulting from the interaction of the fungus and garlic extract subjected to heat in this study implies that garlic possessed inhibitory ingredients against the fungus at a high temperature. The antimicrobial activity of *A. sativum* has been linked to the active ingredient allicin (Analia *et al*., 2013) which is produced by the action of allinase on alliin when garlic is crushed (Rahman, 2007).

Ojo and Olufolaji (2011); Ekhuemelo and Yaaju (2017) reported 100 % inhibition of the mycelia growth of *Colletotrichum gloeosporiodes* and *Botryodiplodia theobromae* by the aqueous extracts of *A. sativum* respectively. Ahmed *et al*. (2009) reported the possibility of an increase in the solubility of active ingredients of plant material in hot water. Similarly, Abu- Gharbia *et al.* (2014) noted that the activity of *A. sativum* plant extract was not affected by different temperature ranges up to 90$^{o}$ C due to the thermostability of its phytoconstituents. However, Abu- Gharbia *et al.* (2014) observed that heating *A. sativum* plant extract beyond 120$^{o}$ C resulted in loss of antimicrobial activity due to some physical and chemical changes in the molecules of the extract during heating. Negi (2012) reported that heat treatment (sterilization) of plant materials could result in the decomposition of active phytochemicals. Conversely, Ginovyan (2017) reported that the methanolic and acetone extracts of *Sanguisorba officinalis* retained their antibacterial activity against *Escherichia coli* after treatment at 121$^{o}$ C for 30 mins.

The inability of *A. sativum* L. at 15 % w/v to inhibit the sporulation of *A. flavus* and *A. niger* in the present study, may be due to reduced toxicity. This agrees with the report of Dania and Arabami (2015) in which 15 % concentration of *A. sativum* L. did not exhibit strong toxicity on the conidia of *A. niger* and *A. flavus* associated with cowpea seeds. Kurucheve *et al*. (1997) linked lack of spore inhibition to the slow absorption of the fungi toxicants present in the extracts incorporated in the growth medium. Also Okigbo and Emoghene (2003) reported that leaf extract of *Vernonia amygdalina* Del. was metabolized for the growth and spore germination of *Mycosphaerella fijiensis* resulting in increased spore germination.

## 5.0 Conclusion

The results of the study showed that the individual inoculation of the *A, flavus and A.niger* after 4 days had no significant effect on the growth of the groundnut seedling as compared to the combined impact of *A. flavus* and *A. niger* on the growth of the groundnut seedling after 6 days. *Allium sativum* was useful in the inhibition of the radial growth but not on the sporulation of the fungi. Also heat application during media sterilization reduced the activity of garlic extract on *A. flavus*.

## 6.0 Recommendations

Aqueous garlic extract is recommended to be used in the management of *Aspergillus flavus* and *A. niger* infecting groundnut seeds.

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