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INFLUENCE OF TWO TROPICAL PLANT EXTRACTS ON GERMINATION AND SEEDLING GROWTH OF GROUNDNUT (*Arachis hypogeae*) SEEDS

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Abstract
Seedling growth is an important process in groundnut cultivation, and growth retardation constitutes one of the major problems. Seed health test was carried out on five local cultivars of groundnut collected from seed vendors at Ishiagu, Ebonyi State, Nigeria and the effect of two plant extracts; *Solanum torvum* and *Heliotropium indicum* as seed treatment on radicle length, plumule length, seed germination and seedling vigour was further investigated. The experiment was laid out in a completely randomized design (CRD) and replicated three times. The data collected were subjected to analysis of variance (ANOVA) and the means separated using Tukey at $p < 0.05$. Visual inspection revealed that healthy seeds varied from 79% to 97% across the cultivars. Kafachan cultivar recorded highest (13%) discoloured number of seeds (21.0%), while Nwaevu cultivar had the least abnormal seeds. *Aspergillus niger* had the highest (47%) incidence, while *Penicillium* species had the least (2%). Extracts of *S. torvum* and *H. indicum* significantly ($p < 0.05$) enhanced seedling growth across all concentration. However, extract of *H. indicum* was superior to that of *S. torvum* by increasing radicle length by 2.59cm, germination (90.19%) and vigour index (379.7) at concentration of 400ml. Extracts from *S. torvum* and *H. indicum* could therefore be used as a viable option for seed treatment and seedling growth stimulators.

Keywords: Influence, Extracts, Germination, Groundnut, Seeds

Introduction

Groundnut (*Arachis hypogeae*) is a leguminous oilseed food crop belonging to the family Fabaceae (Ajeigbe *et al.,* 2014). It is one of the most popular and universal crop cultivated in over 100 countries in six continents (Nwokolo, 1996), but mainly Asia, Africa and America with a production of 37.1million metric tons from an area of 23.1million hectares (Geleta *et al.,* 2007). Major groundnut producing countries are China, India, Nigeria, U.S.A. and Indonesia (FAOSTAT, 2014). Groundnut is a very important cash crop for small scale farmers of developing countries in Africa where it is grown over an area of 41.78ha, with an annual production of 46.887tons (FMARD, 2009). Groundnut gained more significance because of its use as dietary supplement for children on protein poor cereals-based diets and also as remedy for children with protein related malnutrition health conditions. Groundnut production serve as a source of income for mainly small scale farmers and the country also obtains foreign currency by exporting the crop (Geleta *et al.,* 2007). Groundnut is a very important component in Nigeria diet and about 5% of the estimated 58.9g of crude protein available per head per day is contributed by groundnut (Abulu, 1978). Industrial processing of oil from groundnut is done in many countries like India, Sudan, Senegal, Nigeria and Gambia (Rama *et al.,* 2000). Groundnut oil is generally used as a cooking medium and may be processed into different products. In oil extraction, groundnut cake is obtained as bye-product which contains about 43 to 65% protein and 6 to 20% fat plus some B-group vitamins depending on the method of extraction (Achaya, 1993). Despite the importance of groundnut, the average national yield of groundnut in Nigeria is very low (Ajeigbe *et al.,* 2014). This might be due to a number of biotic and abiotic factors. Among the biotic constraints, are fungal diseases affecting the production and quality of the crop (Smith *et al.,* 1992). The damages caused by fungi generally range from defoliation to reduction in pods, seeds and hulm yield (Jianmei and Ipek, 2007). Fungal diseases are wide spread and economically important disease of groundnut in Nigeria and they cause 50-65% yield loss (Hagan *et al.,* 2008 and Tekklemariam *et al.,* 1985). Groundnut diseases are highly destructive in areas that farmers do not apply any control measures like fungicides and selection of

improved varieties. As indicated by different studies in different countries, effective control of fungal disease can be achieved by applying recommended fungicides (Chen and Zhou, 2009). Significant yield improvement up to 75% was observed with fungicide application (Naab *et al.,* 2005). Synthetic fungicides that combat phytopathogenic fungi can increase crop yield and provide stability of crop production and market quality (Lucas *et al.,* 2015; Hof, 2001). Chemical method still plays a vital role in the management of plant diseases (Christopher *et al.,* 2010). However, reported applications of fungicide can lead to reduced efficacy, greater production cost and environmental pollution (Igbedioh, 1991). It can also kill or negatively affect beneficial organisms used as bio-control agents (BCA) and nitrogen fixer in the soil (Mbodi *et al.,* 1986). It is incontestable that proper seed treatment measures can substantially improve the quality of seed and significantly increase the yield (Akter *et al.,* 2015). Also, to ensure eco-friendly disease management, botanicals are used instead of hazardous chemicals (Hikal *et al.,* 2017). Hence, the need to determine the effect of the botanicals on emergence and seedling vigour of groundnut.

Materials and Methods

Experimental location

The experiments were conducted at the Plant Pathology Laboratory, Federal College of Agriculture, Ishiagu with Latitude 5°56″N and Longitude 7°41″E, Ebonyi State in the Derived Savannah Agro ecological Zone of Nigeria.

Source of the groundnut seeds and plant extracts

Five hundred grams each of local cultivar of groundnut seeds namely Nwaeffion, Kafanchan, Nwawere, Nwaevu and Oso-Oboh obtained from five different vendors in commercial markets of Eke Ishiagu in Ebonyi State, Nigeria. Turkey berry (*Solanum torvum*) and Cock"s comb (*Heliotropium indicum*) were obtained from Ngwogwo Community in Ishiagu, Ebonyi State.

Visual inspection of seeds

Each cultivar was examined by visual inspection under the stereoscopic binocular microscope for normal and abnormal seeds. Normal seeds were those with smooth shell, light carton colour without discoloration or fungal propagules. Abnormal seeds were those with malformed seed shapes, wrinkled seeds, discoloration or those with fungal propagules. Four replicate samples each of 100 seeds per cultivar were examined and result expressed in percentage.

Isolation of fungi associated with normal and abnormal seeds

Four hundred seeds from both normal and abnormal samples were surface sterilized (1% NaOcl for 5 min) and plated on blotter at the rate of 5 seeds per 9 cm-diameter Pyrex Petri dish. Three replicates (Petri dishes) were used for each sample in Completely Randomized Design. These were incubated for 5 days at 28±2°C under alternating cycles of 12 hrs daylight and 12 hrs darkness. Fungi colonies observed on seeds were identified and counted and further expressed as percentage of the total number of such seeds (normal or abnormal) plated on blotter. Germinated seeds were counted and expressed as a measure of seed viability using the formula Khan and Ungar, (1984):

$Sv = n/N \times 100$

Where Sv is % seed viability, n is the number of seeds germinated from each normal or abnormal seed type and N is the total number of seeds (normal or abnormal type) plated on blotter. Fungi mycelia were further transferred separately to PDA and inoculated for 5 days after which single spore isolation (purification) were done. Identifications were done by sub culturing fungi species from PDA for pigmentation and colony morphology, and incubated as above for 7 days for growth. Morphological identification of fungi species was done according to Barnett and Hunter (1999) and Leslie *et al.* (2005). All the isolated fungi species were identified using Olympus BX51 Digital Microscopy (Olympus Optical Co., Ltd, Japan).

Preparation of plant extract

Fully grown leaves of *S. torvum* and *H. indicum* were obtained and rinsed in 2 % solution of sodium hypochlorite for 1 min then thoroughly washed in several changes of tap water then in distilled water and were air dried at room temperature (28 ± 2 °C) for at least 2 weeks. The well dried leaves were weighed using Mettle Toledo AG balance, AB104, Switzerland into 30g then blended in Eurosonic, ES210, Great Star, Asia into powder. The weighed powder was dissolved in 200.0, 300.0 and 400.0 ml of sterile distilled water (3.0w/v), soaked for 12 hours and then sieved through three layers of cheese cloth (Yekini and Afolabi, 2020). The mixture was shaken thoroughly and filtered using Whatman No.1 filter paper. The extracts thus obtained were kept in a refrigerator at 4°±1 °C until use.

Seed treatment with plant extracts

For each treatment, 10 seeds per plate with three replications were placed on Petri dishes in a Completely Randomized Design (CRD). Selected seed samples of groundnut were treated following dipping method. The seeds were dipped in 200, 300 and 400 dilutions for 10 minutes in previously prepared *S. torvum* and *H. indicum* leaves extracts while the untreated seeds serve as the control. After 10 minutes, groundnut seeds were drained out from the Petri dishes. The treated seeds were allowed to dry up on filter paper for 10 min and were tested following the standard blotter methods (ISTA, 2001) to observe the growth of the seeds on different extracts. Germination percent was taken at 10 days after sowing using the formula according to Yekini and Afolabi, (2020).

Germination (%) = 100plated seeds Totalseeds Germinated*x*

Effects of the botanicals on the seedlings vigour

Vigour test was done according to ISTA (2001) where five seedlings from each plate were randomly selected for shoot and root length measurement at 10 days after sowing. The seedling vigour was determined following the formula of Randahawa *et al.* (1985):

Vigour index = (Mean of shoot length + Mean of root length) X % seed germination.

Statistical design and Analysis

Data collected were subjected to analysis of variance (ANOVA) using Minitab software Version 17 and the means separated using Tukey at $p \leq 0.05$.

Results and Discussion

Results

Incidence of normal and abnormal seeds of groundnut

Figure 1 shows that incidence of healthy and abnormal seeds from visual inspection of groundnut seeds. The percentage of normal seeds was higher than abnormal seeds. Incidence of normal (healthy) seeds was between 79and 97%, while abnormal seeds ranged from 03 to 13% across all cultivars. With incidence of 13.0%, Kafanchan recorded the highest discoloured seeds, followed by Akitiokpokpo (12.0%). However, Nwaevu and Oso-Oboh were free of discoloured seeds. Nwaeffion had 1% spotted and unfilled seeds, while more deformed seeds were found in Kafanchan (8%).

Isolation and identification of fungal organisms associated with five groundnut cultivars

Figure 2 shows five fungi namely: *Aspergillus niger*, *A. flavus*, *Fusarium* spp, *Penicillum* spp and *Marcrophomina phaseolina* isolated from the groundnut cultivars and identified. The frequency of the occurrence of the fungi ranged between 1 and 23. *Aspergillus niger* (4%) was highest while *Penicillium* spp (2.0 %) occurred the least. However, other occurrence of other fungi was between 10.01 % and 27.0%.

Effect of the botanicals on the radicle length, plumule length, percentage germination and vigour index

Table 1 shows the effect of plant extracts on radicle length, plumule length, percentage germination and vigour index of groundnut cultivars at three concentration levels. All extracts at varying concentrations influenced the radicle length, plumule length, seed germination and vigour index of the seeds. However, higher concentration of the extracts showed a superior impact on all the parameters. Groundnut seeds treated with 200 ml and 300 ml of extracts enhanced a significantly ($P < 0.05$) higher radicle length than untreated seeds (control). However, seeds treated with 400 ml concentrations of *S. torvum,* recorded a comparable radicle length with that of untreated seeds of groundnut (Table 1). Plumule length of groundnut seeds treated with both extracts at 200 ml concentration was significantly different from that of untreated seeds. Although, application of both extracts on the seeds at 300 and 400 ml concentrations recorded equal effect on the plumule length, it was still significantly ($P < 0.05$) different from that of untreated groundnut seeds. Both extracts induced comparable percentage germination but higher than that of untreated seeds across all concentration levels. Although, *S. torvum* extract at 200 ml enhanced higher percentage germination, it was not significantly ($P < 0.05$) different from that obtained from untreated seeds. Similarly, at 400 ml concentration, percentage germination was comparable across treated and untreated seeds. The vigour index of groundnut seeds treated with *H. indicum* at all concentrations was significantly ($P < 0.05$) higher than that due to *S. torvum*. However, all untreated seeds recorded a significantly ($P < 0.05$) higher vigour index than untreated seeds (control).

Discussion

Visual inspection of seeds groundnut showed that the five groundnut cultivars had higher incidence of abnormal than normal seeds. This might be due to longer storage duration of groundnut seeds leading to deteriorative changes attribute to storage fungi (Bhattacharya and Raha, 2002). Seed deterioration revealed a degree of discoloration, spot, unfilled or empty pods and pod or seed deformation (Chavan and Kakde, 2008). The presence of fungi which includes A. *niger*, *A. flavus, Fusarium* species, *Penicilluim* species and *Macrophormina phaseolium* underscored the level of abnormalities in the groundnut cultivars. This agrees with the earlier findings of Yekini and Afolabi (2020) that associated storage fungi promoted seed abnormalities in rice. According to Ameer *et al*. (2013), *Penicillium chrysogenum* was reported to initiate seed discoloration and deformation. High frequency of the storage fungi in the groundnut seeds is considered to be contamination. Prevalence of *A. niger* among the isolated fungi on the groundnut seeds is not strange as Aliyu and Kutama (2007) also reported *Aspergillus* species is the most frequently occurring fungi in tropical and sub-tropical countries and causes mycotoxin contamination as a result of moulding of badly stored commodities, such as groundnut, cereal and cotton seeds. Olivera *et al*. (2009) reported that groundnut could be contaminated by aflatoxins; mycotoxins produced by *Aspergillus* species. Mycotoxins are secondary metabolites in food commodities which are capable of reducing nutritional benefits threatens food safety and consumer wellness (Vishwanath *et al.,* 2009). Moreover, extracts of *S. torvum* and *H. indicum* promoted radicle length, plumule length, germination and vigour index of groundnut seeds. This is contrary to the findings of Roy *et al.* (2006) that plant extracts inhibit seedling growth and germination. The enhancement by the plant extracts might be due to inherent active allelochemicals which has been proven to stimulate seedling growth (Zhu and Wang, 2009). Allelochemical are compounds produced in plants that influence germination, growth and seedling survival (Maryshany *et al.,* 2018). The efficacy of plant extracts in enhancing growth parameters of seed cannot be over emphasized. Yekini and Afolabi (2020) reported

extracts of *Morinda lucida*, *Piper guineense* and *Xylopia aethiopica* increased seed germination and seedling vigour of rice. Furthermore, the plants used in the study have historical background of enhancing seed health and viability. Egbontan *et al*. (2013) indicated that extract of *S. torvum* increased seed germination and inhibited mycelium growth of fungi. Also, various solvent extracts of *H. indicum* has been investigated and revealed different bioactivities in animal models for medicinal purposes (Pranabesh *et al.,* 2018). Contrary to other reports that higher concentration of some extracts decreases seedling growth (Chon *et al.*, 2002), extracts of *S. torvum* and *H. indicum* increased radicle length, plumule length, germination and vigour index as concentrations were increased. This outcome further established that active ingredients in the extracts stimulate seedling growth index at higher concentrations.

Conclusion

The study revealed that seedborne fungi could be a threat to emergence and seedling growth of groundnut and seed treatment may be a quick remedy to retarded seedling growth. Seed treatment with plant extracts has showed to be promising and ecofriendly in enhancing germination, radicle length, plumule length and vigour index. Against the conventional use of synthetic chemical in seed treatment that poses danger to the environment due to its residual effects, extracts of *S. torvum* and *H. indicum* could be a viable option.

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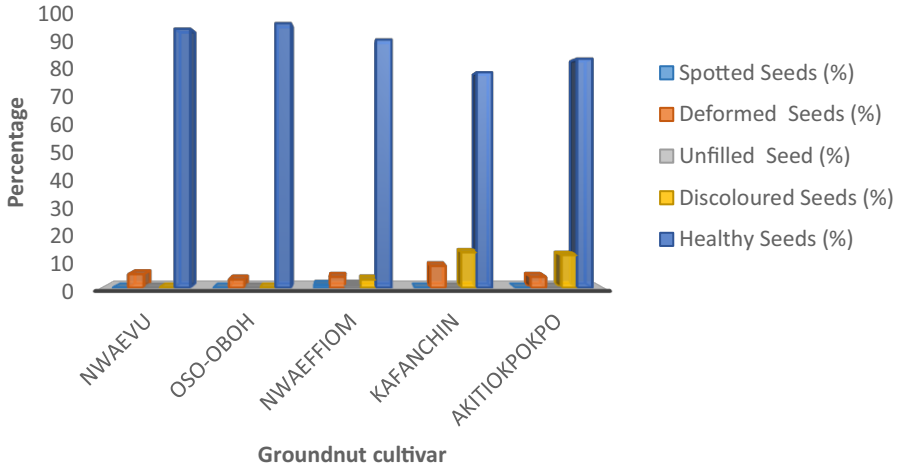

Figure 1: Incidence of normal and abnormal seeds of five groundnut cultivars

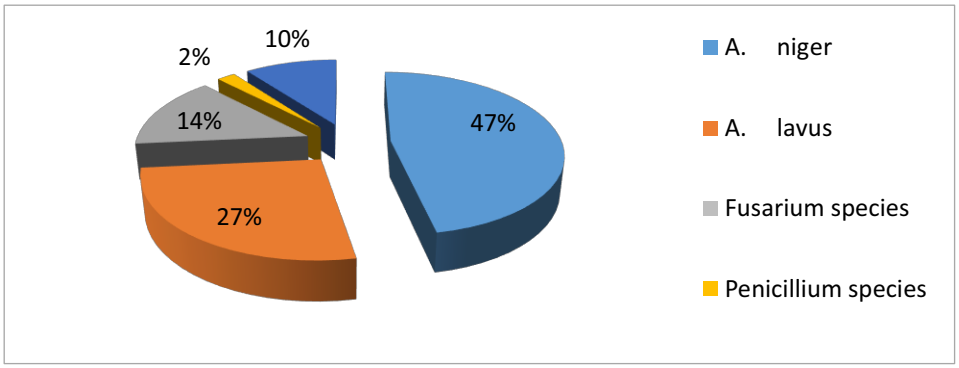

Figure 2: Frequency of fungi associated with five groundnut cultivars

Table 1: Effect of the botanicals on radicle length, Plumule length, percentage germination and vigour

	Radicle length (cm)			Plumule length (cm)			Germination (%)			Vigour index		
	200ml	**300ml**	**400ml**	**200ml**	**300ml**	**400ml**	**200ml**	**300ml**	**400ml**	**200ml**	**300ml**	**400ml**
Solanum torvum	2.77^{b}	2.39^{b}	1.97^{b}	1.85^{b}	1.54^{a}	1.63^{a}	96.69^{a}	92.79^{ab}	88.8^{ab}	447.7^{b}	364.8^{b}	322.6^{b}
Heliotropium indicum	4.15^{a}	3.03^{a}	2.59^{a}	2.30^{a}	1.68^{a}	1.62^{a}	97.91^{a}	94.19^{a}	90.19^{a}	631.4^{a}	462.9^{a}	379.7^{a}
Control	1.72^{c}	1.42^{c}	1.49^{b}	0.53^{c}	0.53^{b}	0.53^{c}	90.23^{b}	90.45^{b}	90.12^{a}	289.3^{c}	231.4^{c}	243.4^{c}
Mean	2.88	2.28	2.02	1.56	1.5	1.26	94.94	92.47	89.70	456.13	353.03	315.23
Standard error	0.27	0.17	0.20	0.13	0.13	0.13	0.89	1.09	1.14	35.9	25.7	27.7
Coefficient of variation (%)	29.27	23.98	0.13	24.37	28.59	29.8	3.52	4.53	4.92	25.26	23.55	30.46

Means in the same column with different superscripts are significantly different using Tukey at (P < 0.05)