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PATHOGENICITY OF SEED-BORNE FUNGI ON SOYBEAN (*GLYCINE MAX* (L.) MERRIL) IN NIGERIA

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ABSTRACT

Soybean provides an inexpensive and high quality source of protein worldwide as compared to animal protein but its production is constrained mainly by diseases which include the seed-borne diseases. Five seed-borne fungi isolated from fifteen soybean cultivars grown in the Guinea Savannah agro-ecology of Nigeria were evaluated for their pathogenicity on soybean cultivar TGX 1448-2E. The effect of the fungi was determined on seed viability, seedling biomass and plant morphology using the seedling symptom method. Translocation of the fungi in the seedlings was also determined. All the fungi reduced seed viability, mean root and shoot weight and seedling height significantly ($p<0.05$) with *Phomopsis* sp. being the most virulent. The result also showed that translocation of the fungi decreased from the root towards the upper part of the plant. The number of plants that showed symptoms of post-emergence damping off and stunting in response to inoculation with the different fungi and those with no symptom (normal seedlings) also differed significantly ($p<0.05$). Implications of the fungi on healthy growth of soybean are discussed.

Keywords: Pathogenicity, Soybean, Seed-borne fungi, Guinea Savannah, Nigeria

INTRODUCTION

Soybean is a widely cultivated food and oil crop in every continent (Anon, 2009) and highly nutritive oil seed crop (Kakade and Chavan, 2011). In sub-Saharan Africa, Nigeria is the largest producer of soybean, where the crop can be successfully grown in many states in the country, followed by South Africa (Anon, 2009). Disease is, however, one of the major constraints in soybean production worldwide. Disease reduces yield and quality of the produce (Wrather *et al.*, 2001). Yield losses in soybean have been estimated at 11% of total harvest worldwide (Hartman, *et al.*, 1999) and one of the major problems encountered include infections of the seeds caused by seed-borne pathogens thereby resulting in poor quality seeds (Okoro, *et al.*, 2004). About 30 species out of more than 135 parasitic microorganisms which includes the fungi have been reported on soybeans and are known to cause economic damage to the crop every year (Roy, *et al.*, 2000). Some of the more important diseases have been reviewed (Grau, *et al.*, 2004). The seed borne nature and seed transmission of some of the microorganisms have also been demonstrated by (Shovan, *et al.* 2008). These seed-borne organisms may cause

chemical changes and deterioration in seed contents or release mycotoxins with potentially harmful effects on humans and livestock (Chiarappa and Gambogi, 1986). In this way, the produce becomes unsuitable for human and animal consumption. Even as buffer stock, the seed-borne pathogens may be transmitted to the seedlings and cause disease and death resulting ultimately in food loss.

However, little information is available on the impact of seed-borne pathogens on growth, development and yield of soybeans in Nigeria. As an important factor in significant loss of soybean harvests, there is the need to establish the role of these seed-borne pathogens in soybean disease development and yield losses with a view to finding solutions to the problems. This is particularly important in Nigeria where the crop is just gaining popularity as a crop with the potential to solving the problem of food insecurity.

This study was therefore undertaken to determine the pathogenicity of five seed-borne fungi associated with soybeans grown in the Guinea Savannah agro-ecology of Nigeria. It is hoped that the information obtained from this study will contribute to solving the problem of yield losses due to the effects of the seed-borne pathogens.

MATERIALS AND METHODS

Source of Fungi

The fungi used for the study were isolated from fifteen soybean cultivars commonly grown in the Guinea Savannah agro-ecology of Nigeria. The isolation was carried out in the Plant Pathology Laboratory of the Department of Crop Protection Faculty of Agriculture University of Ilorin, Nigeria.

Preparation of Fungal Inocula

The method of (El-Wakil and El-Metwally, 2001) was used with slight modification. Five millimeter mycelia disc from five-day-old pure culture of each fungal isolates was transferred into 100 ml conical flask containing Potato Dextrose Broth (PDB) and incubated in the dark at $25 \pm 2\ ^{0}C$ until the entire liquid surface was covered with the hyphal mat. Fifty gram of the freshly harvested hyphal mat of each fungus was measured into 500 ml of sterile water and the mixture was blended with warring blender to produce the inoculum suspension.

Seed Inoculation and Germination Test

Seeds of soybean (*Glycine max* (L.) Merril) cultivar TGX1448-2E was obtained from Shonga soybean farm in Kwara State, Nigeria. The seeds were first surface sterilized in 0.5% solution of sodium hypochlorite (NaOCl) for 1 minute and later rinsed in several changes of sterile water. The seeds were left on sterile absorbent paper to dry. The sterile seeds were then soaked in the inoculum suspension for 6 hours and left to dry at room temperature before sowing. Seeds soaked in ordinary sterile water served as control. The treated seeds were sown in plastic pots (25 cm diameter) containing sterile top soil at the rate of thirty (30) seeds per pot. The pots were then arranged in the screen house during the entire period of the experiment. The number of germinated seeds was counted at ten days of planting.

Growing-on and Biomass test

Seed inoculation and growing-on-test procedures were carried out according to the methods of El-Wakil and El-Metwally (2001) with slight modification. Seedlings of inoculated and un-inoculated seeds of soybean cultivar TGX1448-2E were left to grow under ambient conditions in the screen house. The plants were maintained for 4 weeks during which record of symptoms of pre- and post-emergence damping off and stunted growth were made. Fresh root and shoot weights as well as height of the plants were then recorded for the seedlings inoculated with the different fungi to determine their effect on the biomass of the seedlings.

Translocation of the fungi within the seedlings

In a separate experiment, the movement of the fungi within the seedlings after emergence was

evaluated. Fresh soybean seeds were inoculated with the fungal isolates as described earlier. For each of the isolate, ten replicates were prepared and in each replicate, five inoculated seeds were planted in plastic bags. The emerged seedlings were left to grow under ambient condition with supplementary watering. Recovery of fungi from inoculated seedlings was carried out six (6) weeks after planting. Five plants were removed from each treatment and washed with running tap water. Each of the clean seedlings was then dissected aseptically into five different parts; shoot tip, upper part of stem, basal part of stem, basal part of root and the root tip. The parts were sterilized in 0.5% sodium hypochlorite for 1 minute and later plated on Potato Dextrose Agar (PDA) and incubated for 5-7 days at 25 ± 2 ^{0}C. Fungi recovered for each treatment were identified and the transmission percentage was recorded.

RESULTS

Germination Test

The result of germination test conducted on soybean (TGX1448-2E) is shown in Table1. All the fungi reduced germination percentage of the seeds ten days after planting. The reduction was significantly different ($p<0.05$) compared to the control. Seeds inoculated with *Phomopsis* sp. had the lowest germination percentage followed by those inoculated with *Curvularia lunata* while un-inoculated seeds (the control) had the highest germination percentage. Incidence of post-emergence damping off in the seedlings was highest for *Phomopsis* sp. with an average of 6.5 seedlings out of the 30 seedlings planted which was significantly different ($p<0.05$) from all the other treatments, followed by *F. oxysporum* with an average of 10 seedlings damped off while the un-inoculated seedlings (the control) showed the least post-emergence damping off. The damping-off in the seedlings caused by *A. niger, C. lunata* and *P. oxalicum* were all significantly lower than those caused by *Phomopsis* sp. and *F. oxysporum*.

Table 1: Viability of soybean cultivar TGX1448-2E 10 days after inoculating with fungal inoculum

Fungi	Mean Germination (%)
Phomopsis sp.	31.67[a]
Curvularia lunata	46.67[b]
F. oxysporum	56.67[c]
A. niger	53.89[c]
P. oxalicum	57.22[c]
Control	90.56[d]

Means followed by the same superscript are not significantly different at P=0.05 using the Duncan's New Multiple Range Test.

Growing-on test and Biomass of seedlings

At 28 days after sowing (DAS), the number of seedlings that showed symptoms of post-emergence damping off and stunting in response to inoculation with the different fungi as well as those that appeared normal differed significantly ($p<0.05$) (Table 2).

However, all the fungi inoculated seedlings displayed significant stunted growth as compared to the control (un-inoculated) seedlings. In a similar pattern to the post-emergence damping off result, *Phomopsis* sp. had the lowest number of normal seedlings at 28 days after sowing (DAS) with an average of 10.33 normal seedlings out of 30 seedlings sowed which was significantly different from those of other treatments, followed by *F. oxysporum* with an average of 17 normal seedlings. The number of normal seedlings obtained from the control treatment was significantly higher than those obtained from the fungi inoculated seeds.

Table 2: Soybean plants showing Damping off and Stunting symptoms of the fungal pathogens

Fungi	Post-emergence damping off	Stunted Seedling	Normal Seedlings
Aspergillus niger	6.50^{b}	4.17^{b}	19.33^{cd}
Curvularia lunata	7.40^{b}	3.93^{b}	18.67^{bc}
Fusarium oxysporum	10.00^{c}	3.03^{b}	17.00^{b}
Penicillium oxalicum	5.17^{b}	3.83^{b}	21.00^{d}
Phomopsis sp.	14.32^{d}	5.34^{b}	10.33^{a}
Control	0.33^{a}	0.67^{a}	29.00^{e}

Values are means of 5 replicates each of which has 30 plants. Data were taken 28 DAS
Values in the same column followed by the same superscript are not significantly different (p=0.05) using the Duncan's New Multiple Range Test.

Some of the observed symptoms are shown in plate 1. These results suggest that the tested fungi significantly ($p<0.05$) caused pre- and post-emergence damping off, seed rot, rot/decay of root tip and stunted growth of soybean.

Plate 1. Symptoms of *Phomopsis* sp. seed infection: A. Pre-emergence damping off of seed with damaged embryo. B. Pre-emergence damping off affecting the cotyledon (seed rot). C. Post emergence damping off with rot/decay of the root tip. D. Healthy seedling and the one raised from seed infected with *Phomopsis* sp.

Analysis of the effect of inoculation on the mean fresh root, shoot weights and heights of the inoculated plant showed that significant differences ($p<0.05$) existed between the fungi inocula. *Phomopsis* sp. consistently reduced mean root, shoot weights and seedling heights more than other fungi. However, the effect on mean height

was not significantly different from that caused by *A. niger* (Table 3).

Table 3: Effect of different fungi on the growth of soybean plants

Fungi	Mean root weight (g)	Mean shoot Weight (g)	Mean height (cm)
Phomopsis sp.	9.41[a]	8.96[a]	39.57[a]
C. lunata	9.75[b]	10.68[b]	40.82[b]
F. oxysporum	10.37[c]	11.18[c]	41.77[c]
A. niger	11.73[e]	11.47[d]	39.95[a]
P. oxalicum	11.60[d]	12.65[e]	41.17[b]
Control	14.26[f]	15.48[f]	52.42[d]

Means followed by the same superscript along the column are not significantly different at P=0.05 using the Duncan's Multiple Range Test Values are means of three replicates.

Translocation of fungi within inoculated plants

The degree of movement of the fungi within the plant in 42 days (i.e. six weeks) after sowing is shown in Table 4.

Table 4: Percentage recovery of fungi from plant parts 42 days after sowing artificially inoculated soybean seeds

Plant parts	Pathogens				
	Phomopsis sp.	*F. oxysporum*	*C.lunata*	*P. oxalicum*	*A. niger*
Shoot tip	6.67	20.00	0.00	13.33	6.67
Upper stem	6.67	26.67	6.67	6.67	13.33
Basal stem	26.67	46.67	6.67	13.33	13.33
Basal root	46.67	73.33	20.00	20.00	20.00
Root tip	33.33	66.67	33.00	26.67	33.33

Percentage was estimated based on the number of plant part samples manifesting fungal growth in culture

The fungi were isolated from all parts of the plant except *Curvularia lunata* that was not isolated from the shoot tip. The percentage recovery of *Fusarium oxysporum* was 66.67, 73.33, 46.67, 26.67 and 20.00 from root tip, basal root and basal stem, upper part of the stem and shoot tip respectively. Similar trend was observed for *Phomopsis* sp. and *Curvularia lunata* where the percentage recoveries were 33.33, 46.67, 26.67, 6.67, 6.67 and 33.00, 20.00, 6.67, 6.67, 0.00 respectively. The translocation of the fungi decreased from the root towards the upper part of the plant.

DISCUSSION

The observed reduced germination percentages were as a result of seed rot induced by the invading fungal pathogens. The pathogens would have caused depletion of food reserve in the seeds as also noted by (Christensen, 1980) and probably secreted toxic substances which might have resulted to weakening and eventual death of the embryo. The 'dead seeds' became vulnerable to secondary attack by decomposing saprophytic fungi especially those found in the soil. This study shows that the fungi; *Aspergillus niger*, *Curvularia lunata*, *Fusarium oxysporum*, *Penicillium oxalicum* and *Phomopsis* sp. can reduce yield of soybean by lowering

percentage germinability of the crop. *Phomopsis* sp. and *F. oxysporum* have the potential to cause the most severe damage of all the fungi.

Isolation of these fungi from soybean seeds have also been reported by Begum, Sariah, Puteh, & Zainal Abidin, 2007; Dwivedi & Gopal, 2014; Patharkar & Hedawoo, 2014; Ramesh et al., 2013. (Dwivedi & Gopal, 2014; Levic, Stankovic, Krnjaja, Bocarov-Stancic, & Ivanovic, 2012) have also specifically reported *F. oxysporum* to be the most common *Fusarium* species on Soybean seeds.

The reduced biomass of the seedlings was probably a result of the effect of the fungi on the physiological functions of the plants by causing biochemical changes, such as the reduction of carbohydrate, protein and total oil content or the increase of moisture and free fatty acid content as well as some other biochemical changes (Kakade & Chavan, 2011). (Kita et al., 2005) also reported the suppression of growth and germination of Tomato and cucumber root rot by *Rhizoctonia solani* and *Phomopsis* sp. respectively in Japan. (Dias, Urban, & Roessner, 2012) also identified *Fusarium oxysporum* to be a major pathogen of Soybean causing reduction in the yield of the plant biomass.

Phomopsis sojae and *Fusarium* spp. were found to cause more damage to both the seeds and the seedlings of soybean (Okoro, Nwankiti, & Ogunwolu, 2010). Other plant's seedlings attacked by *Phomopsis* sp. include Eggplant (Solanum melongena) (Islam & Meah, 2011),

CONCLUSION

Phomopsis sp. caused the most severe reduction of germinability and growth as well as symptom development in seedlings of inoculated soybean seeds. Recovery of the pathogens from the different parts of the plant following seed inoculation showed that the test fungi are transmitted from seed to seedlings indicating that freedom from seed-borne pathogens is an important aspect of seed quality and subsequently plant health.

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