Detection, Identification and Management of Seed-borne fungal Pathogens on farmer saved Soybean (*Glycine max* (L.) Merrill) seeds in Ghana

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ABSTRACT

Soybean is infected by a wide range of diseases, many of which are seed-borne. Infection by seed-borne pathogens leads to seed rot, low seed germination, low seedling vigour and reduced plant growth as well as marketability. This study investigates seed-borne fungal pathogens that are associated with farmer saved soybean seeds and identify best seed treatment for controlling them to enhance seed quality. Seed-borne fungal pathogens on farmer-saved seeds of soybeans was investigated by examining a total of eleven (11) seed samples from two districts (Saboba and Yendi) in the Northern region and one from CSIR-Savanna Agricultural Research Institute (CSIR-SARI). A total of nine fungi genera were identified to be associate with the soybean seeds including pathogenic *Cercospora spp., Alternaria spp., Fusarium spp., Macrophomina phaseolina* and saprophytic *Aspergillus flavus, Aspergillus niger, Penicillium spp., Curvularia spp.* and *Rhizopus stolonifer. Rhizopus stolonifer* (21.5%) and *Aspergillus niger* (7.0%) revealed the highest and lowest prevalence respectively. Treatment of soybean seeds with Monceren GT 390 FS, Insector T 45, Garlic extract and Neem seed extract over a period of 90 days resulted in a decrease in fungal prevalence as well as improved seed germination and seedling vigour. Pot experiment conducted to determine pathogenicity of *Microphomina phaseolina, Cercospora spp., Aternaria spp.* and *Fusarium spp.* proved to be pathogenic. These findings indicate that farmers saved soybean seeds in Ghana is fungal infected and seed treatment alleviates the destructive effect of these microbes, thus enhancing seed quality and promoting food security in Ghana.

Keywords: Pathogens, Seed-borne, pathogenicity

1.0 INTRODUCTION

Soybean (Glycine max (L.) Merrill) is an important legume crop, cultivated in the tropical, subtropical and temperate climates (Shurtleff and Aoyagi 2007; IITA, 2009). The world production is 318.95

million metric tonnes with 89% from Argentina, Brazil, United States and China (FAO, 2014). In Africa, South Africa is the leading producer of 948,000 MT followed by, Nigeria (679,000), Zambia (214,179), Malawi (120,903) and Zimbabwe (74,951) (FAO, 2014). Moreover, soybean production in Ghana is mainly in the Northern, Upper West, Upper East, Central and Volta regions where Northern region is the major production area (Lawson et al., 2008) which contributes to about 77% of the national production (SRID, 2012). Soybean is an excellent source of major nutrients, about 40% protein, 30% carbohydrate, 20% oil and varying levels of vitamins and minerals, including calcium, folic acid, and iron (Sauvant et al., 2004; Lakshmeesha et al., 2013).

Locally, it can be processed into many food products such as wean mix, soy 'khebab', 'apapransa', soymilk, 'koose', stew, and 'tubani' (Mbanya, 2011). However, like other grain legumes, its production is significantly constrained by abiotic and biotic stresses. Among these stresses, diseases are the major limiting factor against the vast global production of which many are seed-borne (Lakshmeesha et al., 2013). More than 100 pathogens including over thirty fungi and six bacteria are known to be seed-borne pathogens of soybean (Kulik and Sinclair, 1999a; Roy et al., 2000). Infection by seed-borne pathogens results in seed rots, poor germination, reduction in seedling vigour, plant growth and crop productivity (Kubiak and Korbas, 1999; Dawson and Bateman, 2001; Akranuchat et al., 2007). Several studies have reported species of seed-borne pathogens that are associated with soybean in different ecological zones and different approaches have been employed globally to mitigate the destructive effect of these pathogens and ensure quality yield (Ramannuj et al., 2014; Oladimeji et al., 2016). Efforts made include breeding for disease resistant varieties, use of biological agents, crop management, and the use of seed treatment. Moreover, in Ghana, not much studies have been reported on seed-borne fungal pathogens that are associated with soybean. In view of this, investigating and providing information on seed-borne fungal pathogens that are associated with farmer saved soybean seeds and identifying the best seed treatment in controlling them will enhance seed quality, thus promoting global food security. This study aims to detect and identify seed-borne fungal pathogens associated with farmer saved soybean seeds and asses the effect of different seed treatments on fungal growth, seed germination and seedling vigour.

2.0 MATERIALS AND METHODS

2.1 Experiment site and Seed Collection

The research was conducted at the Seed Laboratory of Ghana Seed Inspection Directorate, Pokuasi, Plant Pathology Laboratory and the Research field of Department of Crop Science, University of Ghana, Legon.

Soybean seeds were randomly sampled from farmers within ten communities in two districts in the Northern region of Ghana. These communities include Nalog Tindando, Gbadagbam, Garimata Yankazia, Zang, Kanisheigu, Zangban, Gumbaliga and Sunsong-Gbung. A check sample was obtained from the CSIR-Savanna Agricultural Research Institute. Seeds were kept in plastic bags and stored in a cold room at a temperature of 4°C prior to seed health test and treatment application.

2.2 Isolation and detection of Fungal Pathogens

Isolation of seed-borne fungal pathogens was performed using the blotter and agar plate methods described by ISTA (2007) and Mathur and Kongsdal, (2003) respectively with slight modification. For the blotter method, three sets of blotter papers were moistened with a sterilized distilled water and lined in 90 mm diameter petri plates. Seeds were surface sterilized with 1% sodium hypochlorite for 1 minute and rinsed three times with sterilized distilled water. A Completely Randomized Design (CRD) was used with four replications. 200 seeds from each sample were used with 50 seeds per replicate. Ten seeds were plated per petri plate using a pair of forceps and incubated at 24 ± 2°C for 7 days. Fungi isolates that associates with the seeds were culture onto a Potato Dextrose Agar (PDA) medium and incubated 24 ± 2°C for 7 days depending on the sporulation nature of the fungi. Fungi isolates were sub-culture thrice to obtained pure culture for easy identification. Culture morphologies such as the colour, shape and growth rate were also used in the identification. Each isolate was prepared on a slide, examined under a compound microscope and identity confirm with the aid of a mycological literature.

2.3 Pathogenicity test

Pathogenicity test was conducted for four fungal isolates to confirm their identity following Koch's postulate. Spore suspension of Cercospora spp., Alternaria spp., Fusarium spp., and Macrophomina phaseolina were prepared from two weeks old sporulated culture and inoculated onto a four weeks old soybean plants. Control plants were sprayed with distilled water and disease development was observed after 2 weeks.

2.4 Seed Treatment

Highly fungi infected seed samples were treated with Monceren GT 390 FS, Insector T 45, Neem seed (Azadirachta indica) extract and Garlic (Allium sativum) extract (Table 1). Neem seed extract was prepared following modification of method described by Adjei (2011). Garlic extract was prepared by blending gloves to form a paste. Fifty percent (50%) concentration of both garlic and neem extracts were prepared by adding fifty grams of the

the paste to 100 ml of distilled water and filtered with four layers of clean cheese cloth into a conical flask.

Table 1: Treatments composition and application rate

Treatments	Active ingredient	Rate of application
Insector T 45	Imidacloprid 350g/kg + Thiram 100g/kg	5g/kg
Moncern GT 390 FS	20% Imidacloprid and 20% Pencycuron	2.5 ml/kg
Neem seed extract	Azadirachtin	100 ml/kg
Garlic extract	Allicin	100 ml/kg
Untreated control	N/A	N/A

2.5 Treatment application

Seed treatment with garlic and neem seed extracts were performed by applying 10 mL extract to 100 g seeds in a zip lock bag, shaken vigorously for evenly distribution and incubated for 20 minutes. The seeds were dried under shade on a surface transparent plastic bag for 30 minutes. A 2.5 mL of Monceren GT 390 FS was used for seed treatment at a rate of 1 kg of the seed weight, and 5 g of Insector T 45 was used to treat 1 kg of seeds. Both treated and untreated seeds were kept in a zip lock bags and stored in a cold room at 4°C over a period of three months. Seeds were assessed monthly for a three months period. The prevalence of fungal pathogens were calculated using the formula below:

$$\text{Percentage prevalence of individual fungal} = \frac{\text{Number of infected seeds by individual fungi}}{\text{Total number of seeds planted}} \times 100\%$$

2.6 Seed germination and seedling vigour Assessment

Germination and seedling vigour test were conducted before and after seed treatments. Germination test was performed following the sand method (ISTA, 2007) by sowing 400 seeds in seed trays with 100 seeds per replicates and grown in a growth room with temperature of 22°C. Germination count, dead seeds as well as normal and abnormal seedlings were evaluated on the 8th day and germination percentage determined using the formula below.

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$$\text{Germination percentage (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds planted}} \times 100\%$$

For vigour test, 10 seedlings were randomly selected from each treatment and their lengths measured. Seedling vigour index was calculated using the formula below described by (Oshone et al., 2014; Khan et al., 2015). Seedling vigour index = Mean seedling length (cm) × Germination percentage (%).

2.7 Statistical analysis

Data obtained including incidence of seed-borne fungi, germination percentage and seedling vigour were subjected to analysis of variance (ANOVA) using GenStat (12th Edition) and the differences between means were separated using the Least Significance Difference (LSD) at 5% level of probability. Data on fungi prevalence were Arcsine transformed to stabilize the variance before analysis.

3.0 RESULTS

3.1 Detection and identification of Seed-borne Fungal Pathogens on Soybean Seed Samples obtained from CSIR-SARI, Saboba and Yendi District of Ghana

In order to identify the fungal isolates that associates with saved-soybean seeds, sample seeds were plated on a moist blotter paper and PDA. A total of nine seed-borne fungal pathogens were isolated from the soybean seed samples obtained from the different locations (Table 2; Table 3, Figure S1). Among these nine fungi isolated, four are pathogenic fungi species (*Cercospora* spp., *Alternaria* spp., *Fusarium* spp., *Macrophomina phaseolina)* and five are saprophytic species (*Aspergillus flavus, Aspergillus niger*, *Penicillium* spp., *Curvularia* spp. and *Rhizopus stolonifera)* (Figure S1) suggesting that both class of fungi are associated with the soybean seed samples. Moreover, In Saboba district, three (*Rhizopus stolonifer*, *Fusarium* spp. and *Aspergillus niger*) were isolated from all five seed samples obtained in all the communities (Table 2). However, Penicillium spp., Cercospora spp. and Macrophomina phaseolina were isolated from seed samples from four seed samples and Alternaria spp. Curvularia spp. and Aspergillus flavus were isolated from three seed samples. Furthermore, seed health test conducted on seed samples obtained from Yendi district revealed that 6 fungal pathogens including Rhizopus stolonifer, Fusarium spp., Aspergillus flavus, Aspergillus niger, Macrophomina phaseolina, and Curvularia spp. were associated with all the seed samples (Table 3).

Table 2: Prevalence of Fungal Pathogens on Seed Samples obtained from Saboba District and CSIR-SARI

Fungal Pathogens (%)	Communities					
	Nalong	Tindando	Gbadagbam	Garimata	Yankazia	CSIR-SARI
Alternaria spp.	10.7	9.0	12.2	-	-	11.7
Aspergillus flavus	9.0	-	10.5	9.8	-	11.5
Aspergillus niger	10.5	12.6	12.0	7.8	9.8	9.6
Cercospora spp.	9.0	10.5	-	9.3	11.9	9.8
Curvularia spp.	9.8	9.0	-	12.6	-	-
Fusarium spp.	14.1	12.8	13.2	14.6	15.2	9.8
Macrophomina phaseolina	-	13.4	12.0	8.5	12.2	9.0
Penicillium spp.	12.0	7.0	9.0	-	9.9	-
Rhizopus stolonifer	17.4	17.9	18.4	17.8	21.5	16.9
LSD ($p<0.05$)	2.9	3.9	3.2	5.7	4.3	3.0

Moreover, In Saboba district, three (Rhizopus stolonifer, Fusarium spp. and Aspergillus niger) were isolated from all five seed samples obtained in all the communities (Table 2). However, Penicillium spp., Cercospora spp. and Macrophomina phaseolina were isolated from seed samples from four seed samples and Alternaria spp. Curvularia spp. and Aspergillus flavus were isolated from three seed samples. Furthermore, seed health test conducted on seed samples obtained from Yendi district revealed that 6 fungal pathogens including Rhizopus stolonifer, Fusarium spp., Aspergillus flavus, Aspergillus niger, Macrophomina phaseolina, and Curvularia spp. were associated with all the seed samples (Table 3). Besides, Cercospora spp. was isolated from three seed samples, while Penicillium spp. was isolated from two seed samples (Table 3) and seed samples obtained from CSIR-SARI were found to be associated with seven fungi pathogens (Table 2). Also, there were significant ($p<0.05$) difference among fungal prevalence which suggest that fungi prevalence is community specific. However, there were no differences among prevalence of *Cercospero spp., Alternaria spp., Fusarium spp., Macrophomina phaseolina, Aspergillus flavus, Aspergillus niger.*

The highest fungal prevalence was revealed on *Rhizopus stolonifer* (16.9%), Alternaria spp. (11.7%), *Aspergillus flavus* (11.5%). *Fusarium spp.* and *Cercospora spp*. also with prevalence of 9.8% each, while Aspergillus niger, and *Macrophomina phaseolina*, had 9.6% and 9.0% respectively. However, *Penicillium spp.* and Curvularia spp. fungal pathogens were not associated with the seed sample.

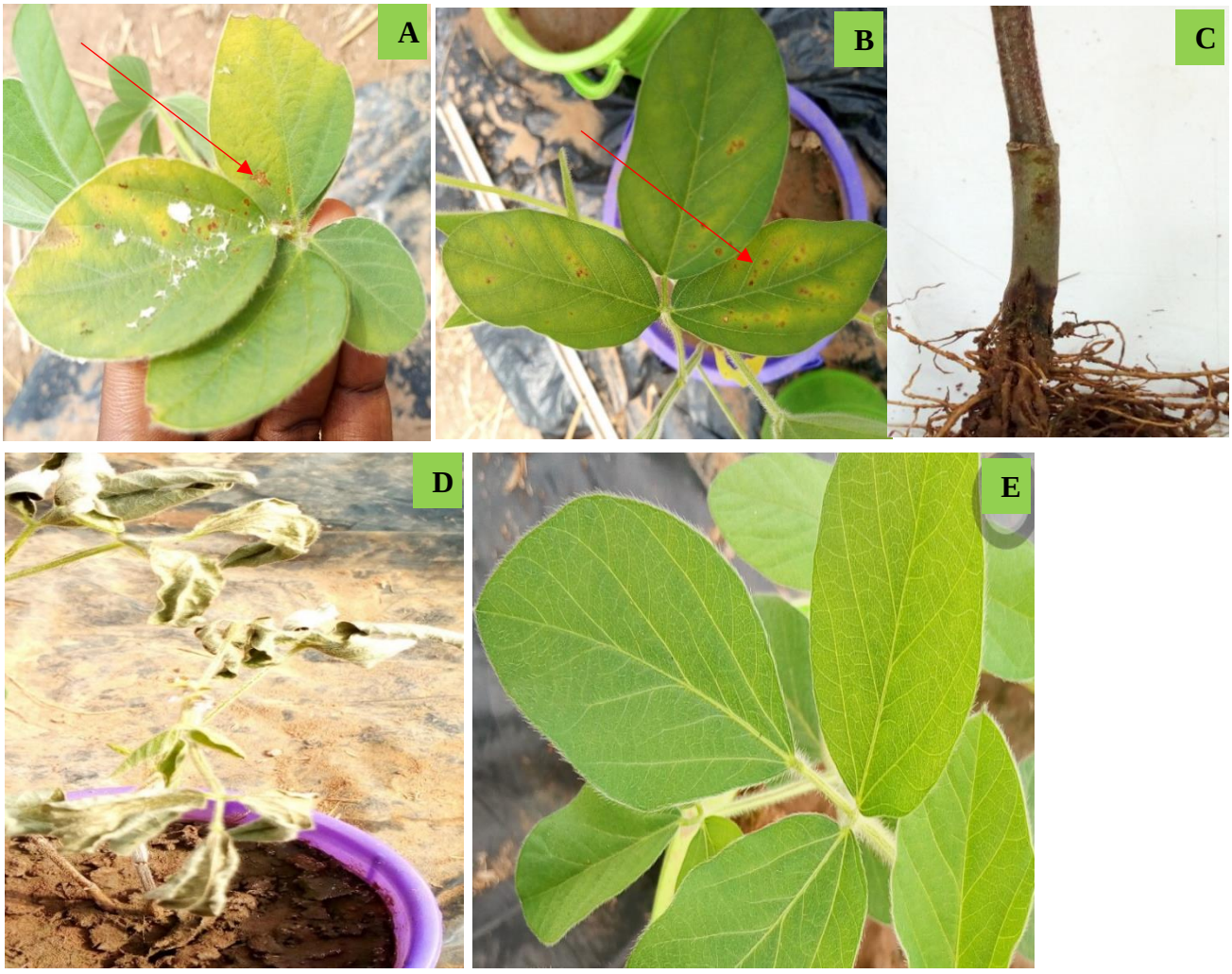

Figure 1: Soybean seedlings showing infection of the various pathogenic fungi: (A) Leaf spot (*Cercospora* spp.), (B) Leaf spot (*Alternaria* spp.), (C) rot (*Macrophomina phaseolina)*, (D) Post-emergence death (*Fusarium* spp.), (E) Negative control. The red arrows indicate symptoms of the various isolates.

3.2 Pathogenicity Test of Four Fungal Isolates on Soybean Plants

To confirm whether the isolated pathogens infect soybean plants, a 4-week-old soybean plants were inoculated with four different fungal isolates. The pathogenicity test revealed that all inoculated plant developed disease symptoms a week after inoculation compared with the control (Figure 1). Re-isolation tests revealed that, inoculated fungal isolates were the causal organism of the observed disease symptoms on the plants as all plants samples had a degree of chlorosis, lesions (spot) and wilt.

Table 3: Prevalence of Fungal Pathogens on Seed Samples obtained from Yendi District

	Communities				
Fungal Pathogens (%)	Zang	Kanisheigu	Zangban	Gumbaliga	Sunsong-Gbung
Alternaria spp.	14.2	13.2	8.5	-	10.5
Aspergillus flavus	12.9	12.2	9.9	9.8	11.5
Aspergillus niger	13.5	8.5	10.7	7.0	12.0
Cercospora spp.	11.4	8.1	-	10.5	-
Curvularia spp.	9.8	12.6	10.7	13.2	9.3
Fusarium spp.	11.4	12.0	11.9	12.6	15.2
Macrophomina phaseolina	12.0	10.5	7.8	12.9	13.2
Penicillium spp.	14.1	10.5	-	-	-
Rhizopus stolonifer	18.8	17.9	18.4	19.7	17.8
LSD ($p<0.05$)	3.0	5.7	5.6	4.0	4.5

3.2 Effect of Seed Treatments on Seed-borne Fungal Pathogen

To further evaluate the effect of various treatments on the identified seed-borne fungal pathogens, seeds were subjected to different treatments. Seed treatments were able to reduce fungal prevalence in the seed sample when treated and stored over a period of 90 days. There was a significant ($p<0.05$) differences among fungi prevalence when seeds were treated with Monceren GT 390 FS and stored over a period of 90 days (Table 4). Moreover, the prevalence of *Alternaria spp., Aspergillus flavus, Aspergillus niger, Cercospora spp. Curvularia spp., Macrophomina phaseolina* and *Penicillium spp.* did not show any significant difference and

Table 4: Prevalence of Fungal Pathogens after Treatment and Storage for 90 days

	Seed Treatments				
Fungal Isolates (%)	Monceren GT 390 FS	Insector T 45	Neem Seed Extract	Garlic Extract	Untreated Seeds
Alternaria spp.	4.1	5.3	5.3	9.1	19.4
Aspergillus flavus	4.1	4.1	5.3	7.8	16.9
Aspergillus niger	4.1	4.1	4.1	6.6	16.4
Cercospora spp.	4.1	5.3	5.3	6.6	16.4
Curvularia spp.	4.1	4.1	4.1	7.4	17.4
Fusarium spp.	5.3	4.1	6.6	7.8	16.3
Macrophomina phaseolina	4.1	4.1	6.6	5.3	18.4
Penicillium spp.	4.1	5.3	4.1	5.3	16.9
Rhizopus stolonifer	10.7	9.1	7.8	11.5	21.5
LSD ($p<0.05$)	1.5	2.1	3.1	3.8	2.4

their prevalence were reduced to 4.1% each compared with untreated seeds (Table 4). *Fusarium* spp. and *Rhizopus stolonifer* also revealed a prevalence of 5.3% and 10.7% respectively, while the untreated had 16.3% and 21.5% respectively (Table 4). Furthermore, seed treated with Insector T 45 over a period of 90 days recorded a significant ($p<0.05$) reduction on fungal prevalence. The prevalence of *Aspergillus flavus, Aspergillus niger, Curvularia* spp., *Fusarium* spp. and *Macrophomina phaseolina* were reduced to 4.1% each compared with the untreated seeds (Table 4), while prevalence of *Rhizopus stolonifera* was reduced from 21.5% in untreated seed to 9.1%. Although, there was no significant ($p>0.05$) difference among fungal prevalence when seeds were treated with neem seed extract for a period of 90 days, the prevalence of *Aspergillus niger*, *Penicillium* spp. and *Curvularia* spp. were reduced to 4.1% each compared with the untreated seeds which observed an increase prevalence of 16.4%, 16.9% and 15.4% respectively (Table 4). Similarly, *Alternaria* spp., *Aspergillus flavus* and *Cercospora* spp. revealed a prevalence of 4.1% each, while *Fusarium* spp. and *Macrophomina phaseolina* both observed a prevalence of 6.6% (Table 4). Seeds treated with garlic extract and stored

for a period of 90 days revealed no significant ($p>0.05$) difference among fungi prevalence (Table 4). Both *Macrophomina phaseolina* and *Penicillium* spp. revealed the least (5.3%) prevalence, while the untreated seeds revealed 18.4% and 16.9% respectively.

3.3 Effect of Seed Treatments on Germination and Seedling Vigour of Soybean Seeds

To determine the efficacy of seed treatment on the seed health, germination and seedling vigour test were perform prior to treatment and after 30, 60 and 90 days of seed treatment and storage. Results obtained showed a significant ($p<0.05$) difference on germination percentage and seedling vigour among the seed samples. In the Saboba district, germination percentage of seed samples obtained from Nalong, Tindando and Yankazia did not show any differences (Table 5). The highest germination was revealed on seed samples obtained from Garimata (79.8%) while Nalong and Yankazia revealed the least with 76.8% each (Table 5). Similarly, there were differences among seed samples obtained from the Yendi district (Table 5). Seed sample obtained from Zangban observed the highest germination (78%), while the lowest was observed on seed sample obtained from Zang with 71.3%. With respect to seedling vigour, seed samples obtained from Tindando (1114.3) and Yankazia (1034.2) in the Saboba district recorded the highest and lowest seedling vigour respectively. However, Zangbang (1092.0) and Zang (892.6) in the Yendi district recorded the highest and lowest seedling vigour respectively.

Table 5: Germination Percentage and Seedling Vigour on Seed Samples obtained from CSIR-SARI, Saboba and Yendi Districts

Locations	Communities (Samples)	Germination Percentage (%)	Seedling Vigour Index
	Nalong	76.8	1047.7
	Tindando	77.3	1114.3
Soboba	Gbadagbam	78.0	1092.1
	Garimata	79.8	1098.6
	Yankazia	76.8	1034.2
	Zang	71.3	892.6
	Kanisheigu	75.0	1020.0
Yendi	Zangban	78.0	1092.0
	Gumbaliga	73.3	1062.2
	Sunsong-Gbung	74.3	1007.9
CSIR-SARI	CSIR-SARI	78.8	1145.8
LSD ($p<0.05$)		1.4	45.7

The various seed treatment showed an increased in seed germination and seedling vigour for the period 30, 60 and 90 days of treatment and storage (Figure 2, Figure 3). However, there was a significant ($p<0.05$) increase in germination and seedling vigour when treated and stored over a period of 90 days. Monceren GT 390 FS seed treatment increased seed germination from 60.8% in untreated seed to 85% (Figure 2), while seedling vigour increased to 1360.1 (Figure 3). Furthermore, Insector T 45 seed treatment was able to increase seedling vigour from 732.2 in untreated seeds to 1360.1. Neem seed extract revealed an increase in germination and seedling vigour. Germination was increased to 75% after 90 days of seed treatment and storage. Meanwhile, seedling vigour was increased to 1107.5 (Figure 3). It was observed that seed treated with garlic extract and stored for a period of 90 days obtained a high germination of 75.5% (Figure 2) and seedling vigour of 1070.2 (Figure 3).

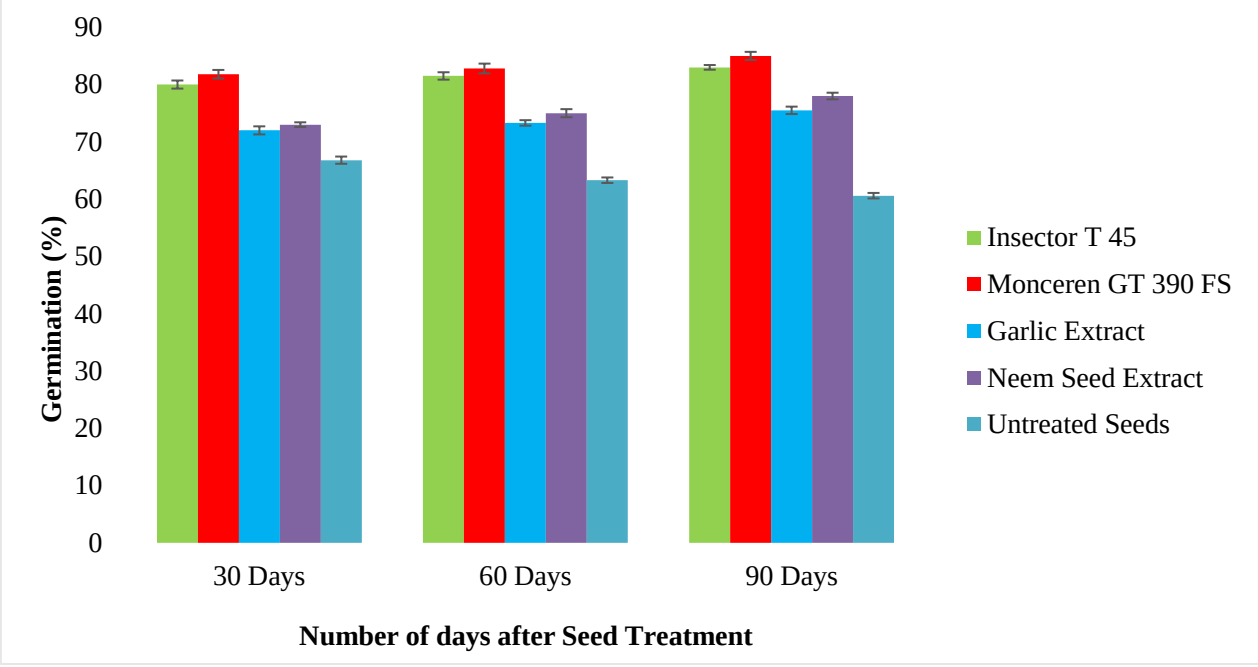

Figure 2. Effect of seed treatments on germination after storage for 90 days period

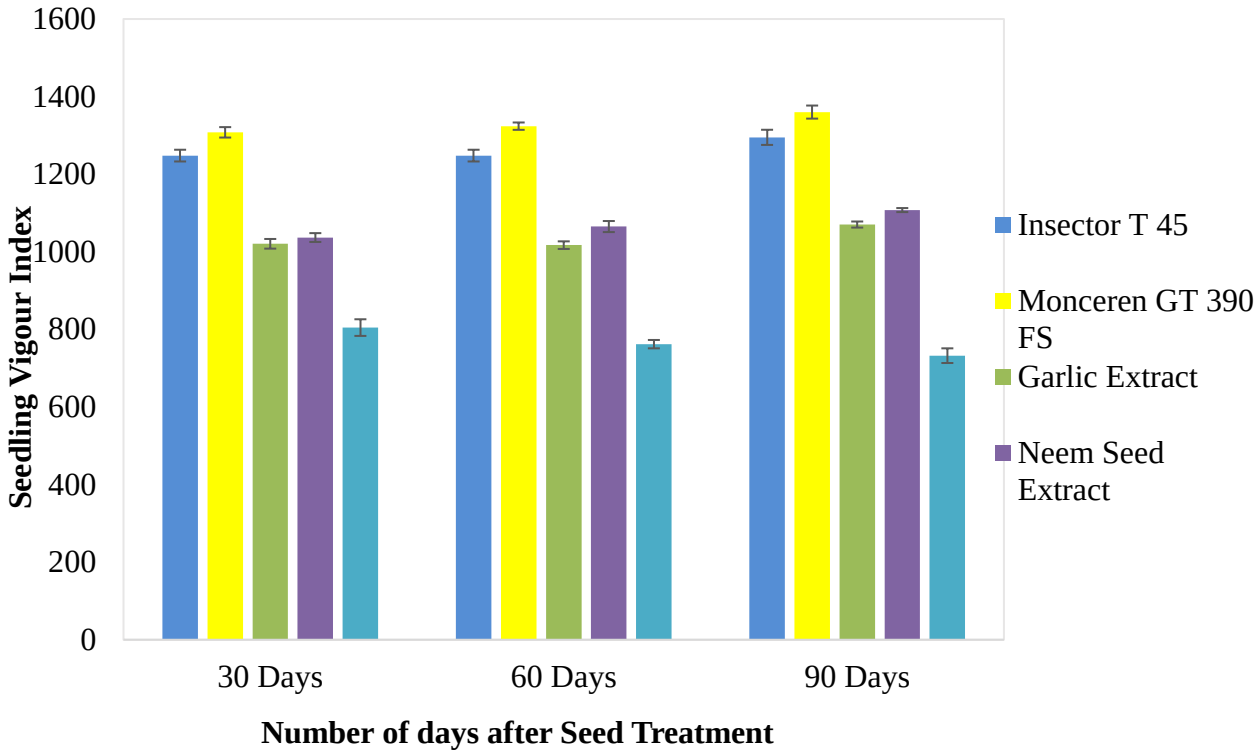

Figure 3. Effect of seed treatments on seedling vigour after storage for 90 days period

4.0 DISCUSSION

Soybean production is significantly constrained by diseases of which seed-borne pathogens are a threat to the attainment of food security in sub Saharan Africa (Lakshmeesha *et al.*, 2013; Gao *et al*., 2014). Seed quality and health examination indicates that all eleven seed samples were found to be associated with eight fungal genera, comprising of four pathogenic; *Cercospora* spp., *Alternaria* spp., *Fusarium* spp., *Macrophomina phaseolina*, and five saprophytic *Curvularia* spp., *Aspergillus niger*, *Aspergillus flavus*, *Rhizopus stolonifer* and *Penicillium* spp. *Rhizopus stolonifera*. Moreover, prevalence of fungal pathogen varied across location except for *Fusarium* spp. and *Aspergillus niger* which were found on all seed samples obtained from the different locations suggesting that the prevalence of fungi pathogen is location specific and that trade and/or transport of infected seeds could enhance the prevalence pathogens in new areas (Anderson *et al*, 2004). This variation could be as a result of the different farming practices which contributes to increase in pathogens (Pickett and Pruitt, 1989).

The prevalence of Rhizopus stolonifer, Penicillium spp., Aspergillus flavus, Aspergillus niger and Curvularia spp. could be attributed to poor storage and environmental conditions (Gupta et al., 1993, and Anwar et al., 1995). The fungal pathogens that were isolated from the seed samples have been reported to be associated with soybean seeds by several authors (Moss and Smith, 2006; Shovan et al., 2008; Ramesh et al., 2013; Ibrahim, 2015; Venugopal et al., 2015). Furthermore, all seed treatments suppress the prevalence of the pathogens that were associated with soybean seeds. Reduction in fungal prevalence by the individual seed treatment could be attributed to the effect of the active ingredients in reducing the primary source of disease inoculum in the seeds (Amare et al., 2014).

Seed treated with fungicide revealed a reduction in pathogen severity (Scherm et al., 2012). This show the effectiveness of the various treatments because each treatment was able to reduce the fungal population which suggest that these chemicals can prolong the storability of soybean seeds. Interestingly, seed samples treated with Monceren GT 390 for a 90-day period was very effective in reducing Alternaria spp., Aspergillus flavus, Aspergillus niger, Cercospora spp. Curvularia spp., Macrophomina phaseolina and Penicillium spp. to a lower prevalence due to the active ingredient (Imidacloprid and Pencycuron) inhibiting fungal inoculum (Table 4). All fungal pathogens were reduced to a lower prevalence when seeds were treated with Insector T 45 (Table 4) which could be due to the effect of the active ingredient (Imidacloprid and Thiram) in reducing fungal inoculum. Thiram has been reported to be effective in suppressing pre- and post–emergence of damping-off where cultivars were artificially inoculated with Fusarium graminearum Group 1 (Lamprecht et al., 1990). Similarly, Solanke et al., (1997) detected that pre- and post-emergence mortality caused by Aspergillus spp., F. moniliforme, Curvularia lunata, A. alternate and Penicillium spp. were controlled using thiram. Interestingly, combination of thiram and procloraz application suppresses mycelial growth of some Fusarium species (Song et al., 2004) and that of thiram and carboxim effectively reduced Gaeumannomyces zeae (Southwell et al. 2003). In this study, neem seed extract was effective in managing the fungal pathogens that were isolated (Howlader, 2003).

The reduction of these pathogens could probable be the azadirachtin property in the neem seed extract proven to inhibit the incidence of Fusarium moniliforme and other seed-borne fungal infections in sorghum (Masum et al., 2009). Similarly, Macrophomina phaseolina associated with seeds were controlled with neem extract (Dubey *et al*., 2009; Javaid and Saddique, 2011). Mondall *et al*., (2009) also reported that seed treatment with garlic extract, neem, gagra, vatpata, Bishkatali leaf extracts reduced seed-borne prevalence and increased germination percentage of wheat seeds.

Germination and seedling vigour analysis that

Germination and seedling vigour analysis revealed that all seed treatment improved seed germination and seedling vigour compared with untreated seed (Figure 2, Figure 3) due to the effectiveness of the active ingredients in the treatments (Mancini and Romanazzi, 2014). Higher germination recorded could be an indication that the treatments protected the seedling against phytopathogens (Taye et al., 2013). Increased in seed vigour index in treated seeds of tomato, rice, castor and chickpea seeds has been reported (Jamadar and Chandrashekar, 2015; Patil et al., 2015). Germination percentage and seedling vigour of seeds treated with Monceren GT 390 FS increased compared with the untreated at the end of the 90 days. This increment observed could be attributed to the reduction in fungal prevalence by the seed treatment. Similarly, Patil et al. (2015) and Sivparsad et al. (2014) reported that seed treatment of sesame and chickpea seeds increased germination percentage similar to that observed in this current study. Seeds infected with pathogens may result in poor seed germination and seedling vigour as untreated seeds are mostly infected with pathogens resulting in poor seed germination and seedling vigour. Besides, seed-borne pathogens such as Curvualria lunata and Fusarium spp. have been reported to cause reduction in seed germination in pearl millet cultivar (Ijaz et al., 2001). Seed treated with Insector T 45 resulted in significant increase in germination and seedling vigour compared to untreated seeds. Seed treated with thiram has proved to be effective in improving maize viability and emergence (Pinto, 1997) and increased germination and emergence of pea seeds by 33% and 29% respectively (Xue, 2003). High germination percentage and emergence were recorded on maize seeds treated with combined thiram and carboxim (Southwell et al., 2003). Seed treatment with garlic extract enhanced effective control seed-borne fungal pathogens and improved germination from 60.65% in untreated to 75.5% in treated seeds as well as seedling vigour. Garlic contains an active ingredient called allicin, which serves as a precursor for biosynthesis of sulphur compounds including ajoene, allyl sulfides, and vinyldithiins (Koscielny et al., 1999). Scientifically, garlic has been confirmed as a natural antibiotic, antiviral and antifungal agent (Michelle, 2003). Allicin in garlic extract suppress rice seed-borne pathogens (Mansur et al., 2013) and application of garlic extract reduced Bipolaris sorokiniana and Drechslera tritici-repentis infection on two wheat cultivars (Perelló et al., 2013). Mansur et al. (2013) reported an increase germination of rice seeds treated with garlic extract from 67.68% in untreated to 91.67% in treated seeds. However, low germination percentage recorded on untreated seeds could be as a result of the high fungal infection (Islam and Monjil, 2016a). The pathogenicity test revealed that four fungal isolates inoculated onto the plants proved to be pathogenic as all inoculated plants developed

disease symptoms of the individual fungal isolate. Re-isolation of fungal pathogens from diseased plants confirmed that these fungi were the causal organism of the observed symptoms. Soybean plants inoculated with Macrophomina phaseolina developed symptoms such as leaf spots, wilting and defoliation. Lower stems of infected plants also showed brown lesions, with black strips when cut open. Similar results have been reported, when pathogenicity test was conducted on adzuki bean with isolate of Macrophomina phaseolina (Gupta and Chauhan, 2005; Sun et al., 2015; Mishra, 2017). The adzuki bean showed symptoms of leaf chlorosis, wilting, stunted growth, withering, dried leaves as well as dark microsclerotia on the stem. Also, plants inoculated with isolate of Alternaria spp. exhibited symptoms of chlorosis, dark brown lesions on the leaves and ultimately infected leaves withered and dropped. Similar result was reported by Carla (2013), who observed brown spot and small brown lesion on the basal leaves of potato plants when inoculated with Alternaria alternate resulting in premature leave drop. Nayyar et al., (2017), also observed similar result on sesamum indicum. A. solani has been reported to cause premature defoliation when the entire leaf lamina became necrotic, even in the absence of petiole lesions (Vloutoglou and Kalogerakis, 2000). A. macrospora has been reported to cause premature defoliation which affected yield (Spross-Blickle et al., 1989). Furthermore, plants inoculated with inoculum of Cercospora spp. showed symptoms of leaf chlorosis and lesions on the leave surfaces resulting in wilting and defoliation. This study agrees with reports by Poornima (2010), who observed symptoms of brown to dark brown spots on the upper leaves of Betavulgaris during a pathogenicity test conducted using isolates of Cercospora beticola. In the same way, Lartey et al. (2005) reported similar symptoms on safflower. Plants inoculated with Fusarium spp. showed high incidence which resulted in complete wilting by the end of the study period. Besides, inoculated plants exhibited foliar symptoms including chlorosis, wrinkling and defoliation as well as infected roots had rot resulting in complete wilting and drying up of the plants. These results suggest that these pathogens are indeed associated with soybean seeds although further studies in required to elucidate how these pathogens infect the seed.

5.0 CONCLUSION

All eleven seed samples were found to be associated with eight fungal genera, comprising of four pathogenic; Cercospora spp, Alternaria spp., Fusarium spp., Macrophomina phaseolina, and five saprophytic; Curvularia spp., Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer and Penicillium spp Rhizopus stolonifera and their prevalence vary from location to location. Treatment of seed with chemicals and botanicals significantly reduced the fungal prevalence as well as enhanced germination and seedling vigour of treated seeds samples, thus enhancing seed quality and promoting

the attainment of food security in Ghana.

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