UNIVERSITY OF GHANA

COLLEGE OF BASIC AND APPLIED SCIENCES

DETECTION, IDENTIFICATION AND MANAGEMENT OF SEED-BORNE FUNGAL PATHOGENS ON FARMER-SAVED SOYBEAN (*Glycine max* (L.) Merrill) SEEDS

BY

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WEST AFRICA CENTER FOR CROP IMPROVEMENT

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A THESIS SUBMITTED TO SCHOOL OF GRADUATE STUDIES IN PARTIAL FULFILMENT OF THE AWARD OF DEGREE OF MASTER OF PHILOSOPHY IN SEED SCIENCE AND TECHNOLOGY

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DECLARATION

I hereby declare that except for references to works of other researchers, which have been duly cited, this work is my original research and that neither part nor whole has been presented elsewhere for the award of a degree.

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ABSTRACT

Soybean is afected by a wide range of diseases, many of which are seed-borne. Infection by seed-borne pathogens leads to seed rot, low seedling vigour and reduction in plant growth. Seed-borne fungal pathogens on farmer-saved seeds of soybeans were 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). Appearance quality of farmer-saved soybean seed samples revealed the main categories of damaged seeds as shrivelled (ranging from 6% to 10.5%), discolored (8.3% to 12.5%), broken and cracked (5.5% to 12.5%). Four hundred seeds (400) from each seed sample were used. Seed sample obtained from Sunsong-Gbung in the Yendi district were found to have the highest proportion of discolored seeds (12.5%). Seed samples obtained from Zang district were observed to have the highest proportion (12.5%) of broken and cracked seeds. The blotter and agar plate incubation methods as described by International Seed Testing Association (ISTA) were used to identify fungi associated with the seed samples. A Completely Randomized Design with four replications was used. Two hundred (200) seeds from each seed sample were used with fifty (50) seeds per replication. A total of nine fungi were found to be associated with the soybean seeds evaluated using blotter and agar plate methods. Out of this number, four of them were identified as pathogenic on soybean and had high prevalence level before seed treatment applications as follows: *Alternaria* spp. (ranging from 9.0% to 14.2%), *Cercospora* spp. (9.0% to 11.9%), *Fusarium* spp. (9.8% to 15.2%) and *Macrophomina phaseolina* (7.8% to 13.4%). The other five fungi species were identified to be saprophytic. They had a high prevalence levels as well in the seed tested before seed treatment as follows: *Aspergillus flavus* (9.0% to 12.9%), *Aspergillus niger* (7.0% to 13.5%), *Curvularia* spp. (9.0% to 13.2%), *Penicillium* spp. (9.0% to 14.1%) and *Rhizopus stolonifer* (16.9% to 21.5%). Pathogenicity tests conducted on the four fungal isolates; *Alternaria* spp., *Cercospora* spp., *Fusarium* spp. and *Macrophomina phaseolina* to fulfil Koch's postulate proved to be positive. A Completely Randomized Design with three replications was used. Nine potted plants were used for each treatment. All soybean plants inoculated with fungal isolates developed disease symptoms one week after inoculation. Re-isolation of fungal pathogens from diseased plants confirmed they were the causal organism of the symptoms that were observed. The use of Insector T 45 (Imidacloprid 350g/kg and Thiram 100g/kg), Monceren GT 390 FS (20% Imidaclopride) and 20% Pencycuron), Garlic extract (Allicin) and Neem (Azadirachtin) seed extract as seed treatments over a period of 90 days, led to a decrease in prevalence of fungal as well as an increase in seed germination and seedling vigour compared with the untreated seeds. Seeds treated with Monceren GT 390 FS over a period of 90 days had the highest reduction in fungal as well as high germination (85.0%) and seedling vigour (1360.1). Further studies should be conducted to determine bio-efficacy of the neem seed and garlic extract which have been proved to have some degree of antifungal effect in the study. Farmers should be educated on seed treatment measures before storage to prevent the build-up of seed-borne fungi which results in higher prevalence of fungi and poor seed germination.

DEDICATION

I dedicate this thesis to my mother Mad. Margaret Owusua and aunty Mad. Elizabeth Ohene Anima for their love and support in my academic pursuit.

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LIST OF ABBREVIATION

ANOVA……………………………..Analysis of Variance

cm……………………………………Centimeter

CRD………………………………........Complete Randomized Design

CSIR……………………………........Council for Scientific and Industrial Research

FAO……………………………. …...Food and Agricultural Organization

g……………………………………...gram

ha……………………………..............Hectare

PDA………………………………….Potato Dextrose Agar

SIRD……………………………….....Statistical Research and Information Directorate

IITA………………………………….International Institute of Tropical Agriculture

ISTA………………………….............International Seed Testing Association

kg…………………………….............Kilogram

MIS…………………………………..Management Information Service

MoFA………………………………...Ministry of Food and Agriculture

ml……………………………………Milliliter

MT……………………………………Metric tonne

RH……………………………………Relative Humidity

WACCI………………………………West Africa Center for Crop Improvement

CHAPTER ONE

1.0 INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is an important legume crop, which is cultivated in the tropical, subtropical and temperate climates (Shurtleff and Aoyagi 2007; IITA, 2009). It belongs to the family Leguminosae and subfamily, Papilionoideae. Soybean was produced on 1.5% of the world's agricultural land in 2000 and an increase of 2.2% in 2012 with South America recorded about 75% of the growth (FAO, 2013). The world production is 318.95 million MT with 89% from Argentina, Brazil, United States and China (FAO, 2014). In Africa, the leading producing countries are, South Africa (948,000 MT), Nigeria (679,000), Zambia (214,179), Malawi (120,903) and Zimbabwe (74,951) (FAO, 2014).

Soybean cultivation in Ghana is mainly in the Northern, Upper West, Upper East, Central and Volta regions. In these regions, the major production occurs in the Northern region, (Lawson *et al.*, 2008), which contributes about 77% of the national production (SRID, 2012).The average yields of soybean in the Northern region ranges from 509 to 642 kg/ha, representing about 30% of the national average of 1,910 kg/ha (Dogbe *et al.*, 2013).

Soybean production in Ghana faces numerous challenges which includes; low technology adoption and inappropriate seed conditioning which affect seed quality (Mbanya, 2011). Many small-scale farmers in Ghana mostly rely on traditional methods of farming with few technological improvements which results in about 30% of postharvest losses (MoFA, 2009a).

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). It is used for food, animal feed, bio-energy source and industrial use (Myaka *et al.*, 2005) such as lubricants, emulsifiers and plasticizers (Addai and Safo-Kantanka, 2006). It also used for soil fertility management (Vanlauwe *et al.*, 2003), by fixing atmospheric nitrogen in symbiosis with *Rhizobium* bacteria for its own use with benefits to subsequent crops (Asafo-Adjei *et al.*, 2005). In Ghana, it serves as source of economical dietary protein, minerals and vitamins for both rural and urban dwellers. Locally, it can be processed into many food products such as wean mix, soy 'khebab', 'apapransa', soymilk, 'koose', stew, and 'tubani' (Mbanya, 2011).

The haulm is used to feed livestock and the cake as feed for poultry (Dugje *et al*., 2009). In Japan and other Asian countries, it has been revealed that, people who consume soybean in large quantities had reduced rate of heart disease (Reynolds *et al.*, 2006; Hirayama *et al.*, 2010). It is also used in the industries as anti-corrosion agent, core oil, and bio-fuel due to less or no nitrogen element in the oil, and as disinfectant, in pesticides, printing inks, paints, adhesives, antibiotics and cosmetics (Ngalamu, *et al*., 2012). The sale of the seeds serves as source of income to farmers and foreign exchange for the country.

Soybean cultivation is gaining popularity and acceptance among smallholder farmers in Ghana, however, average yields remain well below global averages (Dogbe *et al.,* 2013). This has be attributed to limited production area, lack of improved agronomic practices such as fertilizer application, weed control, pest and diseases control (Asafo-Adjei *et al*., 2005).

Soybean as with other grain legumes, are affected by a wide range of diseases, many of which are seed-borne (Lakshmeesha *et al.*, 2013). Soybeans are known to be infected by more than 100 pathogens and of these over thirty fungi and six bacteria are known to be seed-borne (Kulik and Sinclair, 1999a; Roy *et al.*, 2000). About 35 of them are of economic importance as seed-borne pathogens. These pathogens are borne internally or are mere contaminants. Infection by seed-borne pathogens leads to seed rots, poor germination, low seedling vigour, reduction in plant growth and crop productivity (Kubiak and Korbas, 1999; Dawson and Bateman, 2001; Akranuchat *et al.*, 2007).

Most pathogens infect seeds on the field and are able to survive and increase in population when storage conditions are favorable. Poor storage practices affect seed quality of soybean. In Ghana, most farmers obtain their seeds from seed producing farmers which are of low quality. More than 95% of farmers do not treat their seeds before storage and this results in seed deterioration (Plahar, 2006). Seed quality is maintained when the seed is stored under dry, cold favorable conditions (Vertucci and Roos, 1990; Balesevic-Tubic *et al.*, 2007; Mbofung *et al.*, 2013). Farmers in the production areas rely on their own saved seed for production and due to the inappropriate storage conditions and practices, it result in poor seed quality.

Several authors have reported species of seed-borne pathogens that are associated with soybean in different ecological zones (Ramannuj *et al.*, 2014; Oladimeji *et al.*, 2016). The use of healthy seeds is a way of preventing the spread of pathogens from one field to the other areas. Different approaches have been employed globally to reduce crop losses due to seed-borne pathogens to ensure improved yield. Efforts made include breeding for disease resistant varieties, use of biological agents, crop management, and the use of seed treatment.

Seed treatments are used to control diseases and pests that affect plant development and decrease crop yield (Buehring *et al.*, 2004; Munkvold, 2009). Successful use of chemicals and botanicals in controlling seed-borne pathogens has been revealed (Mansur *et al.*, 2013). Application of irradiation and botanicals have also proved to be effective in eliminating fungi and improving the quality of the seed (Sahu and Kar, 2009; Alpa *et al*., 2010; Ikram *et al*., 2010).

Specifically, for Ghana, not much has been reported with regards to seed-borne fungal pathogens that are associated with soybean. In view of this, there is urgent need to investigate and provide information on seed-borne fungal pathogens that are associated with farmer saved-seeds of soybean and identify the best seed treatment in controlling them to enhance seed quality.

Objectives of the study therefore are to;

i. Assess the appearance quality of farmer-saved seeds
ii. identify seed-borne fungal pathogens associated with farmer-saved seeds and determine their prevalence across locations
iii. assess the effects of different seed treatments in controlling seed-borne fungal pathogens of soybean, and
iv. determine the effect of seed treatments on germination and seedling vigour of soybean

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and Botany of Soybean

Soybean belongs to the family Leguminosae and the subfamily Papilionideae (Shurtleff and Aoyagi, 2007). It is a herbaceous annual leguminous plant which originated from Eastern Asia (Dadson and Noureldin, 2001). The plant is normally erect with height of 45-120 cm and maturity periods between 75-150 days depending on the variety (Onwueme and Sinha, 1991). The primary leaves are unifoliate, opposite and ovate (Dadson and Noureldin, 2001). The secondary leaves are trifoliolate and alternate and compound leaves with four or more leaflets are sometimes present. The root system comprises of a tap root from which a lateral root system develops (Dadson and Noureldin, 2001).

Most varieties have a main stem that branches from the lower nodes. The extent of branching depends on environmental conditions (Onwueme and Sinha, 1991). Soybean is a self-pollinating crop, with white, purple or pink small flowers which are attached to the axils of the leaves (Ngeze, 1993). The stem, leaves and pods are covered with fine tawny or grey pubescence (Onwueme and Sinha, 1991). The pod is a hairy and grows in groups of 3-5, with a length of 5-8cm which contains between 2 to 4 seeds (Rienke and Joke, 2005). Seed colour may be black, cream or brown (Chianu *et al.*, 2009) with round or oval shape depending on the variety (Van-Gastel *et al*., 1996).

2.2 Trend in Soybean Production

Over the years, there has been a tremendous increase in soybean production worldwide. In 2003, United State of America alone produced 189 million MTs (40%-45%) of the world's total soybean production (Boerma and Specht, 2004). There has been an increase in the world's production from the year 1990 to 2006 with an estimate of 107 million MTs and 229 million MTs respectively (USDA FAS, 2007). About 89% of the 229 million MTs of the soybean was produced from Argentina, Brazil, United States and China (USDA FAS, 2007).

The growth rate of soybean production worldwide is not sufficient especially, when compared to global demand for soybean. For instance, between the periods 1961 to 2003, the average global per capita consumption of soybean rose from about 8 kg to about 15.6 kg (FAO, 2005). The demand rate for soybean grows at about 10 million MTs (52%) per annum (USDA FAS, 2007). The increasing trend in demand for soybean for food, feed, oil and fuel needs is a source of concern to stakeholders in the world and calls for the adoption of realistic and more efficient measures to increase production of soybean.

2.3 Soybean Cultivation in Ghana

Northern region is the most leading soybean producing region in Ghana with about 1.4 ha average farm size and dominated by small scale farmers equipped with traditional tools and outmoded methods of production (Plahar, 2006). The production levels tend to be small because smallholder farmers are unable to adopt new technologies that will help to increased production.

There have been several interventions aimed at increasing the production for both domestic and industrial utilization of soybean in Ghana. One of them is the formation of inter-sectoral National Committee on Soybean Production and Utilization which was formed during 1980s and 1990s. It was made up of MoFA, Ministry of Health, CSIR-Agricultural-based Institutes, Universities, Food Distribution Corporations, Farmers, and Industries (Plahar, 2006).

The development of a non-shattering varieties like “Jenguma” and “Quarshie” are among the several interventions adopted by the CSIR- SARI in conjunction with the MoFA and NGOs to enhance farmer productivity of soybean (Clottey, 2003). Also, over 5,000 soybean farmers in Ghana were linked to processors (Savanna Farmers Marketing Company) and marketers, and input suppliers, respectively (Clottey, 2003).

The USAID under the Feed the Future Program in collaboration with Savanna Agricultural Research Institute, is helping researchers to develop better improved high yielding soybean varieties that would help farmers to increase their income levels (CSIR-SARI, 2014).

In Ghana, nine soybean varieties have been released and registered (Table 2.1). These varieties are mostly preferred by farmers due to their attributes such as; high yield, resistance to pod shattering, tolerance to low soil Phosphorus and ease of threshability.

Table 2. 1: Soybean Varieties Cultivated in Ghana and their Attributes

Variety	Seed Colour	Growth habit	Maturity Period (Days)	Shattering	Yield (t/ha)	Pest/Disease	Preferred Ecology
Jenguma	Cream	N/A	110-115	Resistant	2.5-2.8	Tolerant to common soybean pests and diseases	Guinea and Sudan savannahs, transitional and forest zones
Anidaso	Cream	Medium	105-115	Resistance	1.8	Not applicable	Guinea Savannah, Sudan Savannah, Transition and Coastal Savannah zones
Quarshie	Cream	N/A	110-115	Resistant	2.0-2.4	Tolerant to common soybean pests and diseases	Guinea and Sudan savannahs, transitional zone
Bengbie	Cream	N/A	100-110	Not applicable	1.8	Not applicable	Guinea Savannah, Sudan Savannah, Transition and coastal zones
Salintuya- I	Cream	Determinate	115	Not applicable	Above 2.2	Tolerant to bacterial pustule and Cercospora leaf spot	Guinea and Sudan savannahs, transitional zone
Salintuya- II	Cream	Determinate	130	Not applicable	2.8	Not applicable	Guinea and Sudan savannahs, transitional zone
Afayak	Cream	N/A	110-115	Resistant	2.0-2.4	Tolerance to common soybean pests and diseases	Guinea and Sudan savannahs, transitional zone
Songda	Cream	N/A	110-115	Susceptible	1.8-2.2	Tolerance to common soybean pests and diseases	Guinea and Sudan savannahs, transitional zone
Suong-Pungun	Cream	N/A	85-92	Resistance	1.5-1.8	tolerance to common soybean pests and diseases	Guinea and Sudan savannahs, transitional zone

*N/A: Not applicable

Source: Crop Varieties Released and Registered In Ghana. (Available from: www.csir.org.gh)

2.4 Seed-borne Fungal Pathogens of Soybean

Soybeans as with other grain legumes, are infected by several diseases, many of which are seed-borne (Lakshmeesha *et al.*, 2013). Diseases are caused by organism such as fungi, bacteria, viruses and nematode. Fungi have been reported to be the most common organisms that are associated with seeds (Neergaard, 1977). Over hundred pathogens have been reported to infect soybean; thirty fungi and six bacteria are known to be seed-borne of soybeans (Kulik and Sinclair, 1999a; Roy *et al.*, 2000). These pathogens cause diseases such as pod and stem blight, charcoal rot, purple seed stain, leaf spot, anthracnose, bacteria postule and bacterial blight.

In crop production great losses are incurred as a result of infection by seed-borne diseases (Fakir *et al.*, 2002). Seed-borne pathogens, serve as means of transferring diseases from seeds to seedlings or from seedlings to growing plants (Fakir *et al.*, 2002). It is also causes poor seed germination and viability, seedling vigour and reduction in yield (Bewley and Black, 1994; Elias *et al.*, 2004; Anjorin and Mohammed, 2009).

Several pathogens have been identified to cause diseases to soybean. Phomopsis seed decay caused by *P. longicolla* has been reported to be seed-borne pathogens of soybean (Sinclair, 1993). Infection by this pathogen causes seed coat cracking, wrinkling and white mycelium growth on the seed surfaces (Kmetz *et al*., 1974; Kmetz *et al*., 1978; McGee *et al*., 1980; Kulik and Schoen, 1981; Shortt *et al*., 1981 Mengistu and Heatherly, 2006). *P. longicolla* infection to seeds damage the cotyledonary tissues, reduce germination, and causes pre- and post-emergence damping off (Velicheti *et al*., 1992). Cercospora leaf blight, leaf spot and purple seed stain diseases are caused by the pathogen *Cercospora kikuchii* (Cai *et al*., 2009; Schuh, 1999). *C. kikuchii* infection results in low seed germination, damage of the seed coat (Velicheti *et al*., 1992) and adversely affect plants establishment (Cai and Schneider, 2008).

Macrophomina phaseolina has been reported to be seed-borne pathogen of soybean which cause charcoal rot disease. The pathogen infection result in low vigour, continuous wilt, premature drying of leaves, grayish black discoloration on stem tissue and decreased in yield (Kunwar *et al*., 1986). *Colletotrichum truncatum* infection may result in latent infection of the seed, low germination, seedling vigor, wrinkling of seeds, discoloration and small seed size (Begum *et al*., 2008).

Several seed-borne fungal pathogens have been isolated from soybean seed samples from different locations. These include; *Alternaria* spp., *Cercospora* spp., *Cheatomium globosum, Colletotrichum dematium*, *Curvularia lunata*, *Fusarium* spp. and *Macrophomina phasaelina* Shovan *et al*. (2008), detected ten fungal species to be associated with thirty three soybean seed samples in Bangladesh; and recorded *Alternaria alternate, Aspergillus niger*, *Aspergillus flavus*, *Chaetomium globosum*, *Colletotrichum dematium*, *Curvularia lunata*, *Fusarium oxysporum, Macrophomina phaseolina*, *Penicillium* spp. and *Rhizopus stolonifer*. Zad (1987), in his studies in Iran identified *Fusarium moniliforme, Fusarium oxysporum, Cercospora kikuchii, Diaporthe phaseolorum, Macrophomina phaseolina* and *Peronospora manshurica* and to be associated with soybean seeds.

A study conducted by Bhuiyan and Fakir (1982), revealed *Cercospora kikuchii, C. dematium var. truncatum, C. lindemuthianum, F. equiseti, F. oxysporum, F. solani, M. phaseolina, Myrothecium roridum* and *Phomopsis sojae* to be associated with soybean seed samples obtained from Bangladesh. These pathogens have been reported by several authors to be seed-borne (Garcia *et al*., 1991; Bhuiyan and Fakir, 1993; Gupta *et al*., 1993, Anwar *et al*., 1995). Nik (1983), in his studies identified fifteen genera of seed-borne fungi to be associated with twenty five soybean seed samples. They include *Botryodiplodia theobromae*, *Fusarium* spp., *Collectotrichum dematium*, *Macrophomina phaseolina*, *Pestalotia* spp., *Phoma sorghina*, and *Lasiodiplodia* spp.

2.5 Economic Importance of Seed-borne Pathogens

Seed-borne pathogens have negative impact on seed quality and cause diseases that significantly affect yield and marketability of seed lots (Van Gastel *et al*., 2002; Machado *et al*., 2002; Mathur and Kongsdal 2003). Damage caused by these pathogens result in premature defoliation, shriveled pods and shrunken seeds. (Katungi, 2010; Nga'yu-Wanjau, 2013), seed abortion, seed rot, seed necrosis, seedling damage and low nutritive value (Miller, 1995; Kavitha *et al*., 2005).

Infection caused by seed-borne pathogens results in reduction in germination and post-emergence damping off, normally affect seed marketability (McGee 1980; McDonald 1998). A reduction in seedling emergence of up to 30% has been reported as a result of infection by *Cercospora kikuchii* (Singh and Agarwal 1986). Gangopadhyay *et al.* (1971) recorded a 50% reduction in seedling emergence attributed to *Macrophomina phaseolina*, the causal agent of Charcoal rot. Infection of *Macrophomina phaseolina* have been reported to cause annual losses of about 30%-50% (Wrather, *et al*., 2003).

The production of different types of aflatoxins and mycotoxins on the seed by pathogens affect man and animals when such seeds are consumed. These toxins affect seed viability, germination which eventually affect yield (Marley, 1996). Production of ergot alkaloids by seed-borne mycotoxin have adverse effect on human and animals. Ergotism infection in human still occur in some developing countries (Krogh, 1988; Marley, 1996).

Contamination of food by pathogen toxins can lead to acute or chronic intoxications which may reduce life span. Pathogen infection does not only affect seed quality and yield but also as a means of transferring inoculum to different fields as a result of their ability to transmit through the seed (Shovan *et al*., 2008).

2.6 Management of Seed-borne Diseases

Management of diseases is very necessary to obtain high yield and quality seeds. Several approaches have been developed to control diseases and one of them is seed treatment application. Different approaches of seed treatment have been employed to control seed-borne diseases and includes Physical, Botanical extract, Biological and Chemical seed treatments.

2.6.1 Physical Seed Treatment

Physical seed treatment consist of heat and hot water treatment to eliminate deep-seated infections from seed (McGee, 1995). Hot water treatment is one of the old means in controlling diseases caused by seed-borne pathogens by exposing seeds to heat to eliminate the pathogens without destroying the seeds (Muniz, 2001; Floyd, 2005). Treatment with hot air is another thermotherapy ways of controlling pathogens without having any effect on the viability of the seed (Baker, 1972).

The use of aerated steam as a seed treatment at a required intensity helps to control pathogens without destroying the seed. The electrons destroy the DNA of the pathogens without damaging the embryo of the seed during seed treatment. Dry heat treatment with the use of microwave techniques have been reported to eradicate fungi and bacteria from cassava seeds (Lozano *et al.*, 1986).

Microwave heating technique has also reduced transmission of soybean mosaic virus, with little reduction in germination in seeds treated at 8.5% moisture content, but germination was considerably reduced in seeds treated at 16% (Jolicoeur *et al.*, 1982).

2.6.2 Botanical Extracts

Different species of plants are known to have antifungal and anti-bacteria properties (Hasan *et al.*, 2005; Ogbo and Oyibo, 2008; Dubey *et al*., 2009) which control seed-borne pathogens. These include tea tree, clove, peppermint, rosemary, laurel, oregano and thyme oils. Such oils have been reported to be effective against pathogens like *Ascochyta* spp., which are responsible for Ascochyta blight on *Fabaceae*, and *Alternaria* spp., which affect carrot seeds (Riccioni, 2013). Thyme oil have been found to constitutes compounds such as antifungal and thymol (ˇSegvi´c Klari´c, *et al*., 2007) which serves as antimicrobial agent in controlling seed-borne fungi and bacteria (Van der Wolf *et al*., 2008).

These plants produce various sulfur containing compounds, and some of these have been shown to have antimicrobial effects (Lanzotti, 2006). Onion seed exudates is constituted of sugars, amino acids, organic acids and phenolic compounds which can be released during seed imbibition to inhibit pathogenic fungi (Nelson, 2004) which are not able to colonise seeds (Wright, 2003).

The use of chitosan have proved to be effective in controlling pathogens in cereal crops, such as wheat (Reddy, 1999), pearl millet (Manjunatha, 2008), maize (Liz´arraga-Paul´ın *et al*., 2011) and oil-bearing crops (Nandeeshkumar, 2008) and several horticultural crops. The active ingredient in neem seeds Azadirachtin, contains antifungal properties. Aqueous extracts of *Azadirachta indiica* seeds, garlic bulbs, ginger rhizomes and basil leaves were used to control *Alternaria padwickii* in rice seeds (Shetty *et al.*, 1989).

Garlic and peppermint extract have been reported to control *Cochliobolus miyabeanus* on rice seed (Alice and Rao, 1986). Spores and mycelia growth of *Colletotrichum corchori*, *Lasiodiplodia theobromae* and *Macrophomina phaseolina* have been suppressed by the use of garlic extract (Ahmed and Shultana, 1984). *Curvularia pallescens, Chaetomium indicum* and *Aspergillus flavus* infection on maize were inhibited by the used of cassia oil and clove (Chatterjee, 1990). Lemon grass extract has been used to control *Fusarium moniliforme* and other pathogens which affect sorghum (Somda *et al.*, 2007).

2.6.3 Chemical Method

The use of chemical seed treatment can only be effective based on the product, rate of appplication, environmental conditions and the organism (McMullen and Lamey 2004; Dadari *et al.*, 2005). The common chemicals used for seed treatment include mancozeb, maneb, fludioxonil, fluoxastrobin, prothioconazole, picoxstrobin, and shavit, (Leadbitter *et al*., 1994; Morton and Staub, 2008). Primary active ingredients that have also come into common use include: carbithiins for control of rusts, smuts, and *Rhizotonia*; carboxin which is particularly effective against loose smut; benzimidazoles for control of *Leptosphearia maculans* in crucifers; metalaxyl, which is effective against P*hycomycetes* such as *Phytophthora* and downy mildews; and ethirimol and triademenol forcontrol of the powdery mildews. Other systemic chemical that has potential for use as seed treatment includes iprodone and imazilil (McGee, 1995).

Application of fungicides such as benomyl, chlorothalonil, thiobendazole, thiophanate methyl are effective for the control of soybean seed-borne pathogens like *Alternaria spp*., *C. truncatum*, *C. kikuchii* and *Phomopsis longicolla* (Sinclair, 1993). Thiram and carbendazim (1:1) application at a rate of three grams per one kilogram of seed was found to be effective in managing pathogens, as well as increasing seed germination and seedling vigour index (Koche *et al.*,2009).

CHAPTER THREE

3.0 MATERIALS AND METHODS

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

The experiment was in three phases;

1. Seed sample collection

2. Seed health testing

3. Seed treatment and storage

3.1 Seed Sample Collection

Purposive sampling technique was used to select communities and soybean farmers who have recognized disease incidence on their soybean fields. In consultation with Management Information System Officers (MIS) at the directorate of the Ministry of Food and Agriculture, a list of soybean producing districts were generated and two districts which are known to be the leading soybean producing districts in the region were selected. Section of these districts were also based on reported cases of disease infections by farmers in these areas. From each district five communities popularly known for the production of soybean were selected, and from each community one farmer was identified for the seed collection. One sample obtained from CSIR-Savanna Agricultural Research Institute (CSIR-SARI) was added to the farmers' samples making a total of eleven (11) samples (Table 3.1). Out of the ten farmers, six were males and four females.

Five kilogram primary seed sample was collected from each selected farmer. Collected samples were labelled and kept in plastic bags and tightly sealed. Seeds were stored in a cold room at a temperature of 4°C at the Crop Science Department of the University of Ghana prior to seed health test and treatment.

Table 3. 1: Source of Soybean Seed Samples

District	Community	Variety
Saboba	Nalog	Jenguma
	Tindando	Sonpongma
	Gbadagbam	Afayak
	Garimata	Jenguma
	Yankazia	Jenguma
Yendi	Zang	Jenguma
	Kanisheigu	Jenguma
	Zangban	Jenguma
	Gumbaliga	Jenguma
	Sunsong-Gbung	Jenguma
Tolon	CSIR-SARI	Jenguma

3.2 Determination of Appearance Quality of Farmer-saved Soybean Seeds from different Locations

Seed-borne infection by some pathogens may have effect on the physical quality or appearance of the seeds. In view of this, four hundred seeds were sampled from each seed sample from the 11 communities and visually examined and categorized into undamaged, shriveled, discolored, broken and cracked seeds. A Completely Randomized Design (CRD) with four replications was used. Four hundred seeds (400) from each seed sample were used with hundred (100) seeds per replication.

3.3 Identification of Seed-borne Fungal Pathogens

Seed health test was conducted on soybean seed samples to detect fungal pathogens that were associated with the seeds. This was done using the blotter and agar plate methods (ISTA, 2007; Mathur and Kongsdal, 2003) as described in section 3.3.1 to 3.3.3.

3.3.1 Blotter Method

A working table was first cleaned and disinfected with 70 % ethanol. Three sets of blotter papers were moistened in a sterilized distilled water and placed in 90 mm diameter petri plates. Seeds were surface sterilized using 2% sodium hypochlorite for 1 minute and rinsed in three changes of sterilized distilled water. A Completely Randomized Design (CRD) was used with four replications.

With slight modification of the methods described by ISTA, (2007) and Mathur and Kongsdal (2003), two hundred (200) seeds from each seed sample were used with fifty (50) seeds per replication. Seed samples were taken at random using the spoon method as described by Mathur and Kongsdal, (2003). Ten seeds were plated per Petri plate using a pair of forceps. Codes of each seed samples, plate number and date were written on the plate cover and plated seeds were incubated in the laboratory at 24 ± 2°C and 85-90% RH % for 7 days.

Plates were carefully examined after the incubation period to identify fungal pathogens found on each seed. Different fungal pathogens that were found in each petri dish were counted and recorded

(Mathur and Kongsdal, 2003; ISTA, 2007). Slide preparation of pathogens were made and examined under a compound microscope to confirm their identity with the help of mycological lbook (Illustrated Genera of Imperfect Fungi, Fourth edition)

3.3.2 Agar Plate Method

Fungal pathogens that were isolated from the blotter method were sub-cultured onto Potato Dextrose Agar (PDA) medium. A quantity of 3.9 g of dehydrated PDA was dissolved in 100 ml of distilled water and autoclave for 15 minutes at 121°C to produce the agar. Plates containing isolates were incubated at a temperature of 24 ± 2°C and 85-90% RH for 7 days. Sub-culturing was repeated until pure isolates of fungi pathogens were obtained and preserved in a Patri plates in the refrigerator.

3.4 Test for Pathogenicity of some seed-borne fungi on soybean seeds

Pathogenicity test was conducted using four fungal isolates to confirm whether these pathogens will be able to transmit disease. Soybean seeds (Jenguma variety) treated were sown in pots containing 2 kg of sterilized soil arranged in a screen house. CRD was used with three replications at 3 pots per plot. A total of nine pots were assigned to each treatment. The treatments were spore suspension of *Cercospora* spp., *Aternaria* spp., *Fusarium* spp., *Macrophomina phaseolina* and distilled water which served as a control.

3.4.1 Inoculation of Plants

Spore suspension was prepared from two weeks old culture. Inoculum was adjusted to 1 x10^5 cfu/ml and sprayed onto four weeks old plants. A quantity of 30 ml of the suspension was inoculated onto the plants, while distilled water was sprayed onto the control plants.

Plants were monitored from 24 hours after inoculation to observe symptoms development on the individual treatments. Plants with disease symptoms were sent to the laboratory and re-isolated unto PDA to confirm their identity to fulfil Koch's postulate.

3.5 Seed treatment with some fungicides on the germination and seedling vigour of soybean

3.5.1 Seed treatments

Seed sample which revealed higher pathogen infection during the blotter test was selected and treated with chemical seed dressing and plant extracts. The treatments were Monceren GT 390 FS, Insector T 45, Neem seed (*Azadirachta indica*) extract and Garlic (*Allium sativum*) extract (Table 3.2). Neem seeds were collected from the field, while the other treatments were purchased.

Table 3. 2: Application rates of different seed treatments

Treatments	Formulation	Source	Active ingredient	Rate of application
Insector T 45	Dust	Agro shop	Imidacloprid 350g/kg + Thiram 100g/kg	5g/kg
Moncern GT 390 FS	Emulsifiable concentrates	Bayer	20% Imidacloprid and 20% Pencycuron	2.5 ml/kg
Neem seed extract		Collected seeds	Azadirachtin (50%)	100 ml/kg
Garlic extract		Market	Allicin (50%)	100 ml/kg
Untreated control		N/A	N/A	N/A

3.5.2 Preparation of Plant Extracts

Neem seed extract was prepare following the procedure described by Adjei (2011) with slight modification. Seeds collected were depulped and pounded in a mortar and further ground in electronic blender to obtained paste. Fifty percent (50%) concentration was prepared by dissolving fifty grams of the paste in 100 ml of distilled water. The solution was thoroughly stirred and squeezed through four layers of clean cheese cloth into a conical flask and covered with aluminium foil to prevent contamination.

Garlic cloves were peeled, washed with distilled water and ground in electronic blender to form a paste. Fifty gram of the paste was dissolved in 100 ml of distilled water to prepare fifty percent (50%) concentration of garlic extract. The solution was squeezed through four layers of clean cheese cloth into a conical flask and covered with aluminium foil to prevent contamination

3.5.3 Seed Treatments with Synthetic fungicides and Plant Extracts

3.5.3.1 Monceren GT 390 FS (20% Imidaclopride and 20% Pencycuron) Seed Treatment Application

Twenty (20) ml of water was added to 1kg of seed in a container and vigorously shaken for a uniform spread of the water to make the seeds moist. Following the manufacturer's instruction, a quantity of 2.5 ml of Monceren GT 390 FS was added to the moist seed and vigorously shaken for 1 m and air dried under shade on a transparent rubber for 30 minutes. The treated dried seeds were kept in a zip lock bag for storage.

3.5.3.2 Insector T 45 (Imidacloprid 350g/kg and Thiram 100g/kg) Seed Treatment Application

Seed lots were treated with Insector T 45 following manufacturer's recommendation. Five (5) gram of the chemical was added to 1kg of seed in a glass jar. The bottle was shaken and rotated through 360° for 1 minute to ensure even coverage of the powder to the seed. The treated seeds were transferred into a zip lock bag.

3.5.3.3 Neem Seed Extract (Azadirachtin) Seed Treatment Application

A quantity of 10 ml of neem seed extract was added to 100g of seeds in a Zip lock bag. The seeds were vigorously shaken to obtain uniform covering of the extract. The seeds were kept in the bag for 20 minutes before being dried under shade on transparent plastic for 30 minutes. The seeds were collected into a new Zip lock bag.

3.5.3.4 Garlic Extract (Allicin) Seed Treatment Application

Ten (10) ml of garlic extract was added to 100 g of seed in a zip lock bag and vigorously shaken for evenly spread. The seeds were kept in the bag for 20 minutes before being dried under shade on transparent plastic for 30 minutes. The seeds were collected into a zip lock bags and well labelled.

3.5.4 Storage of Treated Seeds

The experiment was carried out in CRD with three replication. A quantity of 900 g of seeds were allotted to each treatment. The treatments included, Monceren GT 390 FS, Insector T 45, Neem seed extract, garlic extract and untreated seed which served as a control. Each sample was divided into three portions with 100 g seeds per replicate. Treated and untreated seed samples were stored in a cold room under a temperature of 4°C over a period of three months. One portion of each sample was taken every month without disturbing the rest of the seeds to conduct seed health, germination and vigour test.

3.5.5 Health Assessment of Treated Stored Seeds

Treated and control seed samples were assessed monthly for a period of three months to evaluate the effect of the various treatments on seed-borne fungal pathogens that were identified. The procedure described in section 3.3.1 to 3.3.3.was followed. The formula below was used to calculate percentage fungal prevalence.

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

3.5.6 Germination and Vigour Assessment of Untreated and Treated Seeds

Germination and seedling vigour test were conducted before and after seed treatments. With the sand method (ISTA, 2007), 400 hundred seeds were sown in seed trays with 100 seeds per replication. Seed trays were kept in germination room under a temperature of 22°C. Germination count, dead seeds as well as normal and abnormal seedlings were evaluated on the 8th day (ISTA (2007). Seedlings obtained from germination test were used for vigour test. Ten seedlings were randomly selected from each treatment and their lengths were measured. The formula below was used to calculate germination percentage. Seedling vigour index was calculated using the formula described by (Oshone *et al*., 2014; Khan *et al*., 2015).

$$\text{Germination percentage (\%)} = \frac{\text{Total number of germinated seeds X 100}}{\text{Total number of seeds sown}}$$

Seedling vigour index = Mean seedling length (cm) X Germination percentage (%).

3.6 Statistical Analysis of Data

Data was subjected to analysis of variance (ANOVA) using GenStat (12^{th} Edition). Where there was significant difference Means were separated using the Least Significance Difference LSD at 5% level of significance. Data on fungi prevalence were Arcsine transformed to stabilize the variance before analysis.

CHAPTER FOUR

4.0 RESULTS

4.1 Determination of Appearance Quality of Farmer-saved Soybean Seeds from different Locations

Results obtained showed that, all the eleven farmer-saved soybean seed samples obtained from the different locations contained discolored, shriveled, undamaged, broken and cracked seeds (Figure 4.1). The result showed no significant (P>0.05) difference among the seed samples with respect to discoloration. The maximum seed discoloration was observed on seed samples obtained from Sunsong-Gbung (12.5%), Zang (11.0%) and Yankazia (10.8%), while seed samples obtained from Gbadagbam (8.3%) and CSIR-SARI (9%) obtained minimum seed discoloration (Figure 4.1).

With respect to shriveled seeds, there was a significant (P<0.05) difference among the seed samples. Seed samples obtained from Garimata, Yankazia and Zangban showed no differences. Similarly, there was no differences among seed samples obtained from Nalong, Tindando, Sunsong-Gbung and CSIR-SARI. The highest and lowest shriveled seeds were revealed on seed samples obtained from Gumbaliga (10.5%) in the Yendi district and Yankazia (6.3%) in the Saboba district respectively (Figure 4.1).

Though there was significant (P<0.05) differences among the seed samples, seed samples obtained from Nalong, Tindando, Gbadagbam, Garima, Kanisheigu, Zangban, Gumbaliga and CSIR-SARI showed no differences (Figure 4.1). Seed samples obtained from Zang revealed the highest (12.5%) broken and cracked seeds, while the lowest was revealed on seed samples from Tindando, Garimata and CSIR-SARI with a percentage of 5.5% each.

There was a significant (P<0.05) difference among the seed samples with respect to undamaged seeds. However, seed samples obtained from Gbadagbam, Zangban and CSIR-SARI showed no differences. Seed samples obtained from Garimata (79.3%) and Zang (68.5%) revealed the highest and lowest undamaged seeds respectively (Figure 4.1).

Table 4. 1: Discolored, Shriveled, Undamaged, Broken and Cracked Farmer-saved Seed Samples obtained from Eleven Communities

Communities	Undamaged Seeds (%)	Discolored Seeds (%)	Shriveled Seeds (%)	Broken/Cracked Seeds (%)
Nalong	76.8	10.3	7.3	5.8
Tindando	78.5	9.3	6.8	5.5
Gbadagbam	77.3	8.3	8.3	6.3
Garimata	79.3	9.3	6.0	5.5
Yankazia	74.5	10.8	6.3	8.5
Zang	68.5	11.0	8.0	12.5
Kanisheigu	75.0	9.5	9.5	6.0
Zangban	77.8	9.5	6.3	6.5
Gumbaliga	73.0	10.3	10.5	6.3
Sunsong-Gbung	73.3	12.5	7.3	7.0
CSIR-SA	78.0	9.0	7.5	5.5
LSD	3.7	2.6	1.6	1.9

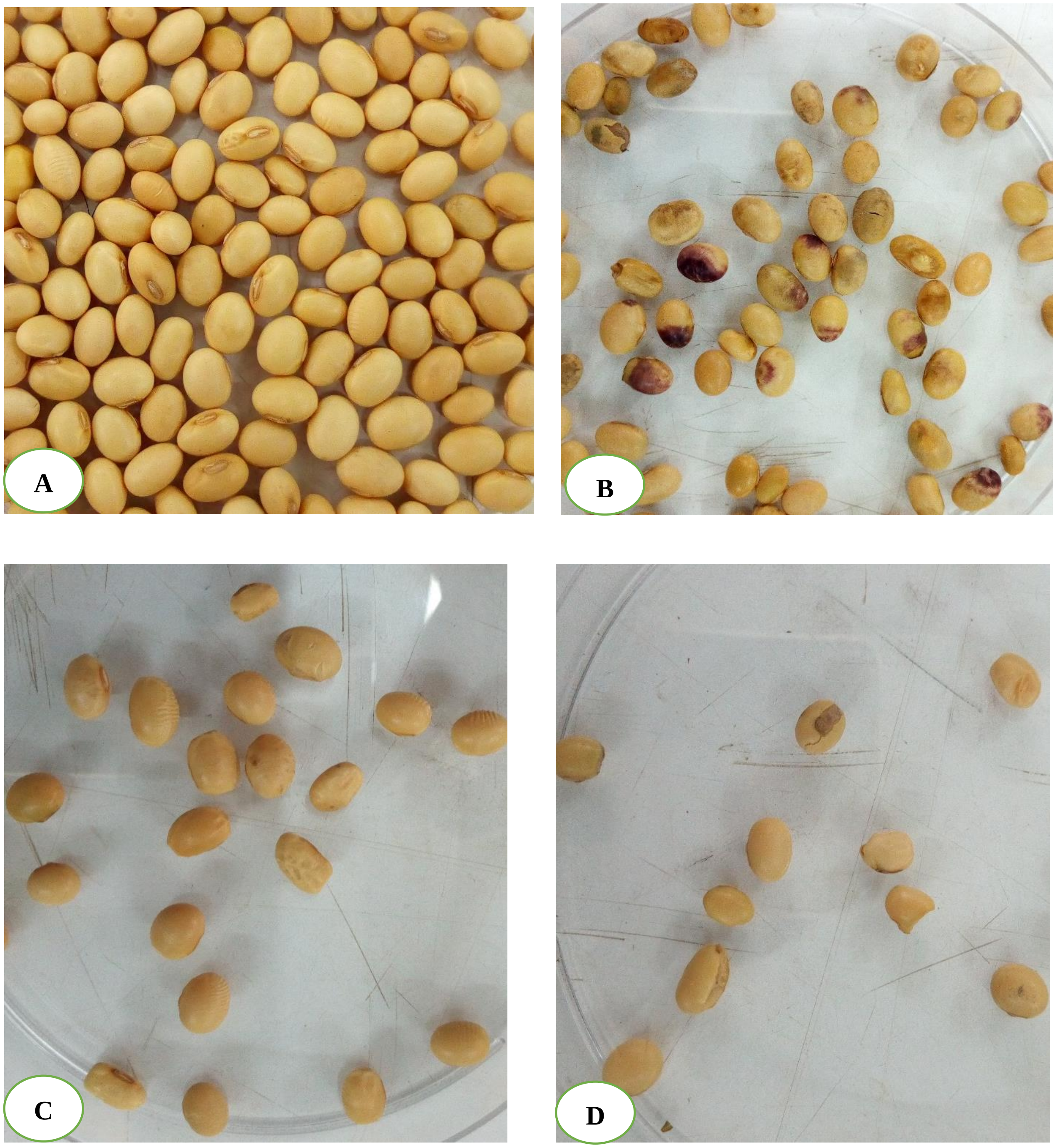

Figure 1: Dry farmer-saved seed samples showing: A. Undamaged seeds, B. Discolored seeds, C. Shriveled seeds, D. Broken and cracked seeds

4.2 Seed-borne Fungal Pathogens associated with Farmer Saved Soybean Seed Samples obtained from CSIR-SARI, Saboba and Yendi District of Ghana

4.2.1 Seed Health Test on Seed Samples before Seed Treatment

All the eleven seed samples obtained from the different locations were infected with seed-borne fungal pathogens (Table 4.2 and Table 4.3).

Table 4. 2: Mean Prevalence of Fungi Associated with Farmer-saved Soybean Seed Samples obtained from Saboba District, CSIR-SARI and Yendi District

	Communities										
Fungal Pathogens (%)	**Nalong**	**Tindando**	**Gbadagbam**	**Garimata**	**Yankazia**	**CSIR-SARI**	**Zang**	**Kanisheigu**	**Zangban**	**Gumbaliga**	**Sunsong-Gbung**
Alternaria spp.	10.70	9.00	12.20	-	-	11.70	14.20	13.20	8.50	-	10.50
Aspergillus flavus	9.00	-	10.50	9.80	-	11.50	12.90	12.20	9.90	9.80	11.50
Aspergillus niger	10.50	12.60	12.00	7.80	9.80	9.60	13.50	8.50	10.70	7.00	12.00
Cercospora spp.	9.00	10.50	-	9.30	11.90	9.80	11.40	8.10	-	10.50	-
Curvularia spp.	9.80	9.00	-	12.60	-	-	9.80	12.60	10.70	13.20	9.30
Fusarium spp.	14.10	12.80	13.20	14.60	15.20	9.80	11.40	12.00	11.90	12.60	15.20
Macrophomina phaseolina	-	13.40	12.00	8.50	12.20	9.00	12.00	10.50	7.80	12.90	13.20
Penicillium spp.	12.00	7.00	9.00	-	9.90	-	14.10	10.50	-	-	-
Rhizopus stolonifer	17.40	17.90	18.40	17.80	21.50	16.90	18.80	17.90	18.40	19.70	17.80
LSD	2.90	3.90	3.20	5.70	4.30	3.00	3.00	5.70	5.60	4.00	4.50

A total of nine seed-borne fungi were isolated from the soybean seed samples obtained from the different locations. *Cercospora* spp., *Alternaria* spp., *Fusarium* spp., *Macrophomina phaseolina*, *Aspergillus flavus, Aspergillus niger*, *Penicillium* spp., *Curvularia* spp. and *Rhizopus stolonifer* were found to be associated with the soybean seed samples.

In the Saboba district, out of the nine pathogens isolated, three (*Rhizopus stolonifer*, *Fusarium* spp. and *Aspergillus niger*) were isolated from all five seed samples obtained with an average prevalence rate of 18.6%, 14.0% and 10.5% respectively. Similarly, *Penicillium* spp., *Cercospora* spp. and *Macrophomina phaseolina* were isolated from four seed samples with an average prevalence of 9.5%, 10.2% and 11.5% respectively (Figure 4.2). However, *Alternaria* spp. (10.6%), *Curvularia* spp. (10.5%) and *Aspergillus flavus* (9.8%) were isolated from three seed samples. There was a significant ($P<0.05$) difference among the fungi prevalence with exception of fungal isolated form Kanisheigu sample (Table 4.2 and 4.3). Seed samples obtained from Tindando and Garimata communities revealed the least fungal prevalence with a mean of 11.5% each, while seed sample obtained from Yankazia revealed the highest prevalence with an average of 13.4%.

Seed health test conducted on seed samples obtained from Yendi district revealed that, fungal pathogens: *Rhizopus stolonifer*, *Fusarium* spp., *Aspergillus flavus*, *Aspergillus niger*, *Macrophomina phaseolina*, and *Curvularia* spp. were associated with all the seed samples. Besides, *Cercospora* spp. was isolated from three seed samples, while *Penicillium* spp. was isolated from two seed samples. There was significant ($P<0.05$) difference among fungal prevalence. The average prevalence of the individual fungal pathogens associated with the seed samples are: *Rhizopus stolonifer* (18.5%), *Fusarium* spp. (12.6%), *Alternaria* spp. (11.6%), *Penicillium* spp. (12.3%), *Macrophomina phaseolina* (11.3%), *Aspergillus flavus* (11.3%), *Curvularia* spp. (11.1%), *Aspergillus niger* (10.3%) and *Cercospora* spp. (10.0%) (Figure 4.2). Seed samples obtained from Zang and Zangban communities in the Yendi district revealed the highest and lowest average prevalence of 13.1% and 11.1% respectively.

Seed samples obtained from CSIR-SARI were found to be associated with seven fungi pathogens. There was a significant ($P<0.05$) difference among fungal prevalence (Table 4.2). However, there were no differences among prevalence of *Cercospora* spp., *Alternaria* spp., *Fusarium* spp., *Macrophomina phaseolina, Aspergillus flavus, Aspergillus niger* (Table 4.2). The highest fungal prevalence were revealed on *Rhizopus stolonifer* (16.9%), *Alternaria* spp. (11.7%), *Aspergillus flavus* (11.5%). *Fusarium* spp. and *Cercospora* spp. also revealed a prevalence of 9.8% each, while *Aspergillus niger, and Macrophomina phaseolina*, had 9.6% and 9.0% respectively (Figure 4.2). However, *Penicillium* spp. and *Curvularia* spp. fungal pathogens were not associated with the seed sample.

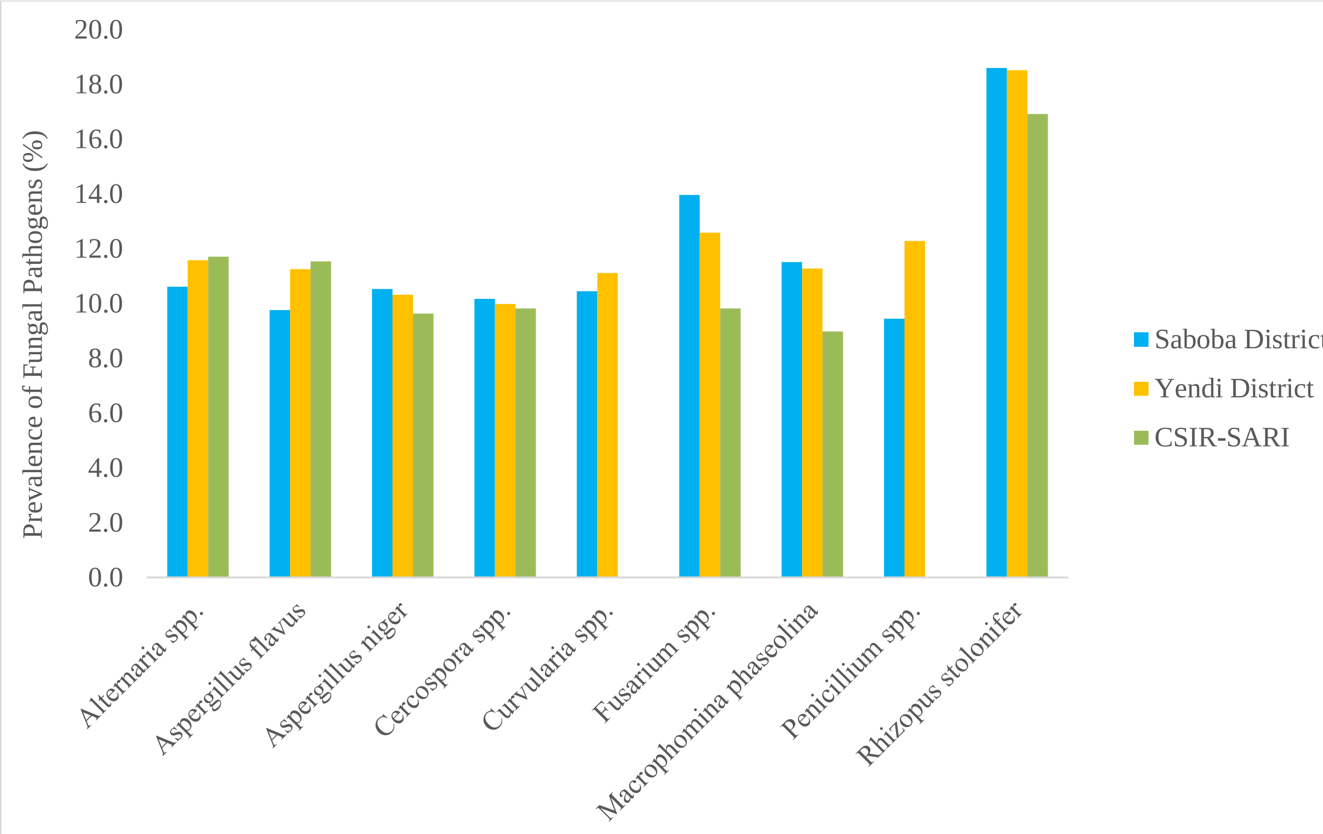

Figure 2 : Average Prevalence of Fungal Pathogens Associated with Seed Samples obtained from CSIR-SARI, Saboba and Yendi District

4.3 Seed-borne Fungi Associated with Fungicide and Plant Extract Treated Farmer-saved Soybean Seeds

4.3.1 Monceren GT 390 FS Seed Treatment

There was a significant (P<0.05) differences among fungi prevalence when seeds were treated and stored for a period of 90 days. Prevalence of *Alternaria* spp., *Aspergillus flavus*, *Aspergillus niger*, *Cercospora* spp. *Curvularia* spp., *Macrophomina phaseolina* and *Penicillium* spp. did not show any significant difference. Their prevalence were reduced to 4.1% each compared with untreated seeds (Table 4.6). *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 4.6).

There was a significant (P<0.05) reduction in fungal prevalence when seeds were treated and stored for a period of 60 days. All fungi prevalence with the exception of *Rhizopus stolonifer* did not show any significant difference (Table 4.5). Prevalence of *Alternaria* spp. and *Curvularia* spp. were reduced from 16.4% and 16.8% respectively in the untreated seeds to 4.1% each. Similarly, *Macrophomina phaseolina* and *Aspergillus flavus* were reduced to 5.3% each, while the untreated seeds were increased to 14.8% and 15.3% respectively (Table 4.5).

Table 4. 3 : Prevalence of Fungal Pathogens after Treatment and Storage for 30 days

	Seed Treatments				
Fungal Isolates (%)	Monceren GT 390 FS	Insector T 45	Neem Seed Extract	Garlic Extract	Untreated Seeds
Alternaria spp.	6.6	9.9	13.5	14.7	15.2
Aspergillus flavus	8.6	5.3	7.8	10.7	13.4
Aspergillus niger	7.8	7.4	10.0	11.5	14.1
Cercospora spp.	6.6	7.8	9.9	10.7	12.3
Curvularia spp.	7.8	6.6	6.6	10.8	13.3
Fusarium spp.	7.4	7.8	9.9	11.5	12.9
Macrophomina phaseolina	8.6	8.6	10.7	12.1	13.3
Penicillium spp.	8.6	9.1	11.5	9.4	14.7
Rhizopus stolonifer	13.5	12.9	14.7	17.4	19.3
LSD	4.4	3.7	3.51	3.8	3.5

Table 4. 4 : Prevalence of Fungal Pathogens after Treatment and Storage for 60 days

Fungal Isolates (%)	Seed Treatments				
	Monceren GT 390 FS	Insector T 45	Neem Seed Extract	Garlic Extract	Untreated Seeds
Alternaria spp.	4.1	7.4	9.9	10.7	16.4
Aspergillus flavus	5.3	5.3	6.6	9.1	15.3
Aspergillus niger	6.6	5.3	7.8	9.9	14.8
Cercospora spp.	5.3	7.4	7.8	7.8	12.9
Curvularia spp.	4.1	5.3	5.3	8.2	16.8
Fusarium spp.	6.6	5.3	7.4	9.9	14.1
Macrophomina phaseolina	5.3	6.6	8.6	7.8	14.8
Penicillium spp.	6.6	6.6	9.1	7.4	14.8
Rhizopus stolonifer	14.1	11.3	13.5	14.1	20.6
LSD	3.4	4.39	3.7	3.8	2.3

Table 4. 5 : Prevalence of Fungal Pathogens after Treatment and Storage for 90 days

Fungal Isolates (%)	Seed Treatments				
	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	1.5	2.1	3.1	3.8	2.4

4.3.2 Insector T 45 Seed Treatment

There was a significant difference ($P<0.05$) among fungal prevalence when seeds were treated and stored for 30 days. However, there were no differences among prevalence of *Aletrnaria* spp., *Aspergillus niger*, *Cercospora* spp., *Curvularia* spp., *Fusarium* spp. and *Macrophomina phaseolina* (Table 4.4). The lowest fungal prevalence was revealed on *Aspergillus flavus* 5.3%, while the highest was revealed on *Rhizopus stolonifer* 12.9% (Table 4.4).

There was a significant ($P<0.001$) reduction on fugal prevalence when seeds were treated and stored for a period of 90 days. Prevalence of *Aspergillus flavus*, *Aspergillus niger*, *Curvularia* spp.

Fusarium spp. and *Macrophomina phaseolina* were reduced to 4.1% each compared with the untreated seeds (Figure 4.8), while prevalence of *Rhizopus stolonifer* was reduced from 21.5% in untreated seed to 9.1%.

Though there was a significant (P<0.05) difference among fungal prevalence 60 days after treatment, with the exception of *Rhizopus stolonifer* all other fungi did not show any differences (Table 4.6). Prevalence of *Alternaria* spp., *Penicilluim* spp. and *Cercospora* spp. were reduced form 19.4%, 16.9% and 16.4% respectively in untreated seed to 5.3% each in treated seeds (Figure 4.6).

However, no significant (P<0.05) difference was revealed among fungi prevalence when seeds were treated and stored for a period of 60 days (Table 4.5). *Fusarium* spp., *Aspergillus flavus, Curvularia* spp. and *Aspergillus niger* observed the least prevalence of 5.3% each compared with untreated seeds which observed 14.1%, 15.3%, 16.8% and 14.8% respectively (Table 4.5).

4.3.3 Neem Seed Extract Seed Treatment

The results shows a significant (P<0.05) reduction on fungal prevalence when seeds were treated and stored for a period of 30 and 60 days. There was no difference among prevalence of *Cercospora* spp. and *Fusarium* spp. (Table 4.4). Prevalence of *Cercospora* spp. and *Fusarium* spp. were reduced to 9.9% each compared with the untreated seeds which observed an increase of 12.9% and 14.1% respectively. The lowest fungi prevalence was revealed of *Curvularia* spp. with 6.6%, while the untreated seeds obtained 13.3% (4.4%)

Among the fungal pathogens, *Aspergillus flavus*, *Aspergillus niger*, *Cercospora* spp., *Fusarium* spp. and *Macrophomina phaseolina* did not revealed any differences (Table 4.5). Prevalence of

Curvularia spp. was decreased to 5.3% compared with the untreated seeds which increased to 16.8% (Table 4.5).

Although, there was no significant (P<0.05) difference among fungal prevalence when seeds were treated for a period of 90 days, prevalence of *Aspergillus niger*, *Penicillium* spp. and *Curvularia* spp. were reduced to 4.1% each compared with the untreated seeds which observed increases to 16.4%, 16.9% and 15.4% respectively (Figure 4.8). 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 6.6% (Figure 4.8).

4.3.4 Garlic Extract Seed Treatment

From the results, there were significant (P<0.05) difference among fungal prevalence when seeds were treated for a period of 30 and 60 days. However, prevalence of *Aspergillus flavus*, *Cercospora* spp., *Curvularia* spp. and *Penicillium* spp. did not show any differences. Similarly, no differences were revealed on *Aspergillus niger*, *Fusarium* spp. and *Macrophomina phaseolina* (Table 4.4). Prevalence of *Aspergillus flavus* and *Cercospora* spp. were reduced from 13.4% and 12.3% to 10.7% each respectively (Table 4.4).

There was a significant (P<0.05) difference among fungi prevalence when seeds were treated and stored for a period of 60 days. However, with the exception of *Alternaria* spp. and *Rhizopus stolonifer*, all other fungi did not show any differences (Table 4.5). Prevalence of *Aspergillus niger*, *Macrophomina phaseolina* and *Penicillium* spp. were reduced from 14.8% each in the untreated seeds to 9.9%, 7.8% and 7.4% respectively (Table 4.5). *Rhizopus stolonifer* (14.1%) and *Alternaria* spp. (10.7%) revealed the highest prevalence in treated seeds, while untreated seeds revealed 20.6% and 16.4%respectively (Table 4.5).

Result obtained when seeds were treated and stored for a period of 90 days revealed no significant ($P<0.05$) difference among fungi prevalence (Figure 4.6). Both *Macrophomina phaseolina* and *Penicillium* spp. revealed the least (5.3%) prevalence, while the untreated seeds revealed 18.4% and 16.9% respectively (Figure 4.6).

4.4 Germination and Seedling Vigour Test of Farmer-saved Soybean Seed Samples before Fungicide Seed Treatment

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 revealed on seed samples obtained from Nalong, Tindando and Yankazia did not show significant differences (Table 4.7). The highest germination was revealed on seed samples obtained from Garimata (79.8%) and Gbadaagba (78%), while seed sample obtained from Nalong and Yankazia revealed the least with 76.8% each (Table 4.6).

Similarly, there were differences among the seed samples obtained Yendi district (Table 4.7). Seed sample obtained from Zangban observed the highest germination (78%), while the lowest was observed on seed sample obtained from Zang with 71.3%. Meanwhile, seed samples obtained from Kanishiegu, Sunsong-Gbung and Zangban obtained a germination of 75%, 74.3% and 73.3% respectively

In the Saboba district, there were differences among seedling vigour in all the seed samples (Table 4.7). However, seed sample obtained from Tindando was observed to have the highest (1114.3) seedling vigour, while seed samples obtained from Garimata, Gbadagbam, Nalong, and Yankazia also obtained 1098.6, 1092.1, 1047.7, and 1034.2 respectively (Table 4.7).

Similarly, there were difference among the seed samples (Table 4.7) obtained from the Yendi district. However, the highest seedling vigour was revealed on seed sample obtained from Zangbang with 1092.0, while the lowest was revealed on seed sample from Zang (892.6). Meanwhile, seed samples obtained from Gumbaliga, Kanishiegu and Sunsong-Gbung also revealed seedling vigour values of 1062.2, 1020.0 and 1007.9 respectively (Table 4.7).

Table 4. 6: Germination (%) and Seedling Vigour of Farmer-saved Soybean Seed Samples from 11 communities before Fungicide Seed Treatment

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	1.4	45.7

4.5 Germination and Seedling Vigour of Farmer-saved Soybean Seeds Treated with Fungicide and Plant Extracts and Stored over a period of 90 days.

4.5.1 Monceren GT 390 FS Seed Treatment

There was a significant (P<0.05) increase in germination and seedling vigour when seeds were treated and stored for a period of 90 days.

Monceren GT 390 FS seed treatment increased seed germination from 60.8% in untreated seed to 85% (Figure 4.3). However, germination was increased to 81.8% and 83% when seeds were treated and stored for a period of 30 and 60 days respectively, while the untreated seeds was reduced to 66.8% and 63.3% respectively (Figure 4.3).

When seeds were treated and stored for 30 days, seedling vigour observed an increase to 1307.9, while untreated seeds were decreased to 805.6. Similarly, seedling vigour was observed to have increased to 1323.8 and 1360.1 when seeds were treated for a period of 60 and 90 days respectively, while the untreated seeds were decreased to 761.9 and 731.9 respectively (Figure 4.4).

4.5.2 Insector T 45 on Seed Treatment

Seed treatment with Insector T 45 for a period of 30 and 60 days revealed an increase in germination from 66.6% and 63.3% in untreated seeds to 80% and 81.5% respectively. However, when seeds were treated for a period of 90 days, it was observed that, germination was increased to 83%, while untreated seeds was decreased to 60.8% (Figure 4.3).

Insector T 45 seed treatment was able to increase seedling vigour from 732.2 in untreated seeds to 1360.1. Also, untreated seeds observed a decrease in seedling vigour (804.6 and 761.9) when seeds were stored for a period of 30 and 60 days respectively, while treated seeds were both increased to 1247.9 (Figure 4.4).

4.5.3 Neem Seed Extract Seed Treatment

Neem seed extract revealed a significant ($P<0.05$) increase in germination and seedling vigour. Germination was increased from 60.8% in untreated seed to 78% when seeds were treated and stored for 30 days. Also, when seed were treated and stored for a period of 60 and 90 days, 73% and 75% germination was observed respectively compared with the untreated seeds (Figure 4.3).

While treated seeds observed an increase in seedling vigour; 1036.7 (30 days), 1065 (60 days) and 1107.5 (90 days), untreated seeds were decreased to 804.6 (30 days), 761.9 (30 days) and 732.2 (90 days) (Figure 4.4).

4.5.4 Garlic Extract Treatment

It was observed from the results that, seed treated with garlic extract and stored for a period of 90 days obtained a high germination of 75.5%. However, when seeds were treated for a period of 30 and 60 days obtained a germination of 72% and 73.35%, while the untreated seeds obtained 63.3% and 60.6% respectively (Figure 4.3).

The results revealed a high increase (1070.2) in seedling vigour when seeds were treated and stored for a period of 90 days, while the untreated seeds obtained 732.2. Similarly, seedling vigour of 1020.6 and 1017.1 was revealed when seeds were treated and stored for 30 and 60 days (Figure 4.4).

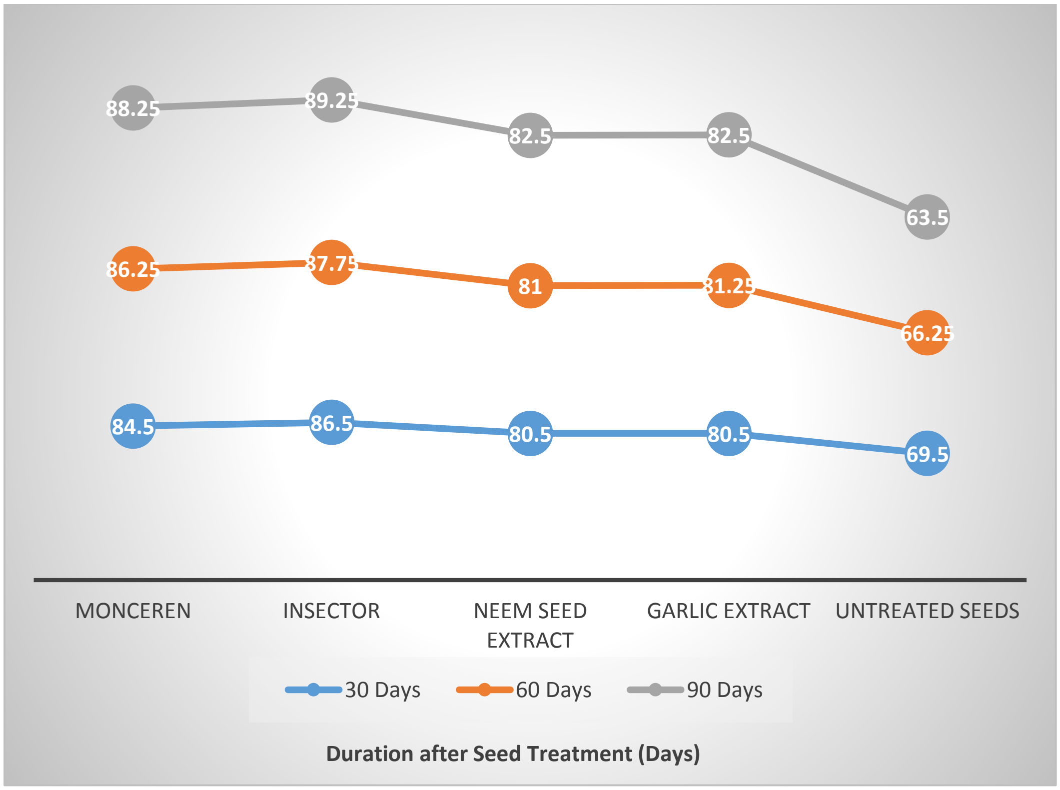

Figure 3: Germination (%) of Farmer-saved Soybean seeds treated and stored for 90 Days

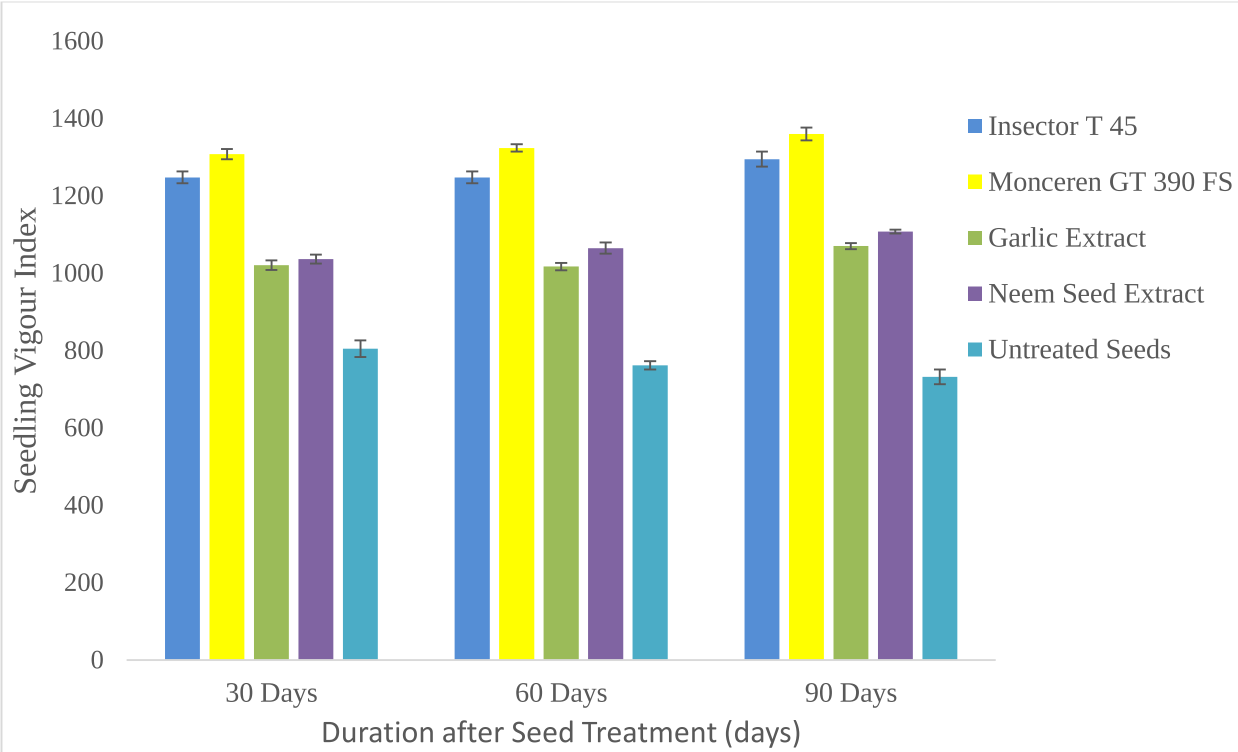

Figure 4 : Seedling Vigour of Farmer-saved Soybean seeds treated and stored for 90

4.6 Pathogenicity of Four Fungal Isolates on Soybean Plants

From the pathogenicity test conducted, it was observed that all plant samples inoculated with fungal isolates developed disease symptoms **(**Figure 5**).** The fungal isolates inoculated include, *Microphomina phaseolina*, *Cercospora* spp., *Alternaria* spp., *Fusarium* spp. Plants developed symptoms a week after inoculation. Re-isolation tests conducted revealed that, fungal isolates inoculated were the cause organism for disease symptoms that were observed on the plants. It was observed that, all plants samples had a degree of chlorosis, lesions (spot) and wilt. From the experiment, soybean plants inoculated with the isolate of *Macrophomina phaseolina* developed symptoms such as leaf spots, wilting and defoliation. Lower stems of infected plants also showed brown lesions, with black strips when they were cut open.

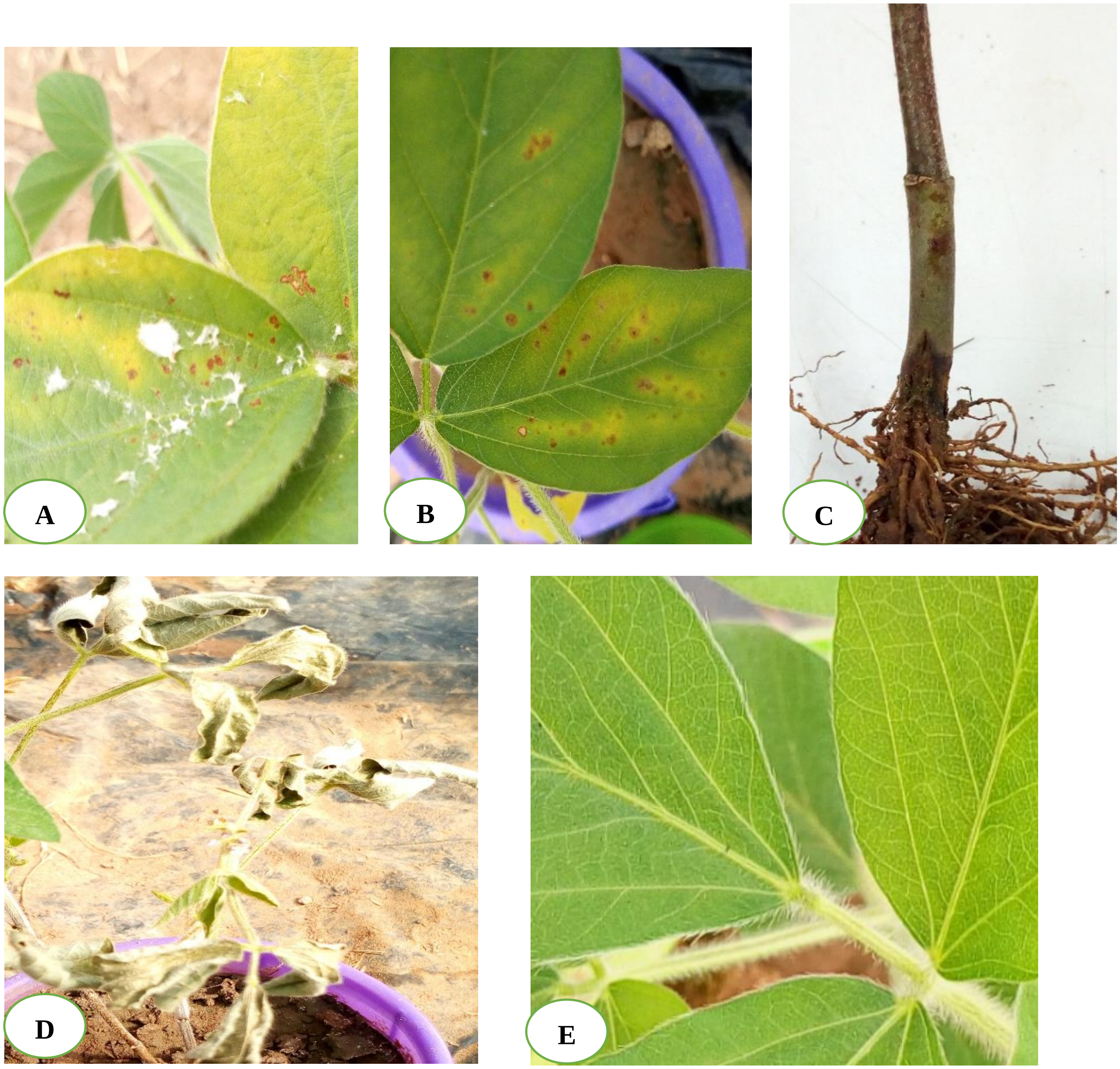

Figure 5 : Soybean seedlings showing symptoms of: A. Leaf spot (*Cercospora* spp.) B. Leaf spot (*Alternaria* spp.), C. rot (*Macrophomina phaseolina)*, D. Post-emergence death (*Fusarium* spp.), E) Control

CHAPTER FIVE

5.0 DISCUSSION

5.1 Appearance Quality of Farmer-saved Soybean Seeds from different Locations

All seed samples obtained from the different location were found to be discolored, shrilled, broken and cracked. Discolored, shriveled and cracked seeds could be attributed to pathogen infection. Basant, (2014) reported high prevalence of pathogens associated with discolored seed samples. Soybean seeds with pale purple to dark purple seed colour has been reported to be a sign of *Cercospora kikuchii* infection (Schuh, 1999; Cai *et al*., 2009).

Similarly, a study has revealed that shriveling of seeds, seed coat cracking and white mold appearance on seed surface is as a result *Phomopsis longicolla* infection (Kmetz *et al*., 1974; Kmetz *et al*., 1978; Kulik and Schoen, 1981; Shortt *et al*., 1981; McGee *et al*., 1980). Bisht and Sinclair, (1985) reported that seed infection by *Cercospora. sojina* results in light to dark gray or brown color.

Discolored, shrilled, broken and cracked seed may result in poor seed quality. Discoloration of seeds by pathogen infection may reduce the quality of the seed (Roy and Baruah, 1972; Awoderu, 1974; Danquah *et al.*, 1976). Most fungal pathogens have been reported to cause seed discoloration (Ou, 1985; Arshad *et al*., 2009) which result in seed deterioration. Vachspati *et al*., (2000) also observed high seedling mortality on discolored seed samples. This could be a reason why seed sample from Zang recorded high prevalence of fungal pathogens as well as low germination and seedling vigour.

5.2. Seed-borne Fungal Pathogens on Farmer-saved Soybean Seed Samples obtained from CSIR-SARI, Saboba and Yendi District of Ghana

5.2.1 Seed Health of Seed Samples before Fungicide and Plant Extracts Seed Treatment

All eleven samples tested 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 stolonifer, *Fusarium* spp. and *Aspergillus niger* fungal pathogens were found on all the seed samples obtained from the different locations. Prevalence of *Rhizopus stolonifer*, *Penicillium* spp., *Aspergillus flavus*, *Aspergillus niger* and *Curvularia* spp. could be attributed to poor handling and storage practices by the farmers. These pathogens have been reported to be associated with stored soybean seeds (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 (Shovan *et al.*, 2008, Ibrahim, 2015; Venugopal *et al.*, 2015). Similar records have been revealed by other authors (Moss and Smith, 2006; Ramesh *et al.*, 2013).

Prevalence of *Rhizopus stolonifer* was high in seed samples from all three locations. In Saboba district, the highest fungal prevalence were revealed on *Rhizopus stolonife*, *Fusarium* spp. and *Macrophomina phaseolina*, In the Yendi district, *Penicillium* spp. and *Alternaria* spp. fungal revealed the highest average prevalence with *Curvularia spp*. being the least. Sample from CSIR-SARI did not reveal *Curvularia* spp. and *Penicillium* spp. fungal infection.

Prevalence of fungal pathogen varied across location, this variation could be as a result of different farming practices which contributes to increase in pathogens (Pickett and Pruitt, 2010). Poor cropping system, post-harvest handling and storage practices carried out at these locations could also be a factor of seed infection by these fungal pathogen. High prevalence of *Fusarium moniliforme* have been reported to be a result of continuous cropping (Pickett and Pruitt, 2010).

5.2.2 Pathogenicity of Four Fungal Isolates on Soybean Plants

All the four fungal isolates tested on the soybean plants proved to be pathogenic as a results of the symptoms developed. Similar records have been reported, when pathogenicity test was conducted on adzuki bean with isolate of *Macrophomina phaseolina*. The adzuki bean showed symptoms of leaf chlorosis, wilting, stunted growth, withering, dried leaves as well as dark microsclerotia on the stem (Sun *et al*., 2015). Other authors has also reported similar results (Gupta and Chauhan, 2005; Mishra, 2017).

Plants inoculated with isolate of *Alternaria* spp. displayed symptoms of chlorosis, dark brown lesions on the leaves and eventually infected leaves withered and dropped. Similar result was revealed 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 dropping of leaves. 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).

Plants inoculated with inoculum of *Cercospora* spp. showed symptoms of leaf chlorosis and lesions on the leave surfaces. Infected plants resulted in wilting and defoliation. This study is in agreement with reports by Poornima, (2010), who observed symptoms of brown to dark brown spots on the upper leaves of *Beta vulgaris* during a pathogenicity test conducted using isolates of *Cercospora beticola*. Lartey *et al*., (2005) also 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. From observation, plants inoculated with *Fusarium* spp. exhibited foliar symptoms such as chlorosis, wrinkling, defoliation. Some of the infected roots had rot and eventually there was complete wilting and drying up of the plants.

5.2.3 Effect Fungicide and Plant Extracts Seed Treatment on Seed-borne Fungal Pathogen, Germination and Seedling Vigour of Soybean Seeds

This study was conducted to develop a measure in controlling seed-borne pathogens that are associated with soybean seeds. All seed treatments suppress the prevalence of the pathogens that were isolated. 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).

With the exception of the untreated seed, all seed treatment improved germination percentage and seedling vigour. Improvement in germination and seedling vigour could be attributed to the effectiveness of the active ingredients in the treatments (Mancini and Romanazzi, 2014). Higher germination recoded could be an indication that the treatments protected the seedling against adverse conditions such as phytopathogens (Taye *et al*., 2013). Increased vigour index in treated seeds of tomato, rice, castor and chickpea seeds has been reported (Jamadar and Chandrashekar, 2015; Patil *et al*., 2015).

5.2.3.1 Monceren GT 390 FS Seed Treatment

Monceren GT 390 FS seed treatment was effective in suppressing fungal pathogens that were associated with the seed samples. Treatment of seed samples with Monceren GT 390 for a period of 90 days was very effective in reducing *Alternaria* spp., *Aspergillus flavus*, *Aspergillus niger*, *Cercospora* spp. *Curvularia* spp., *Macrophomina phaseolina* and *Penicillium* spp. to a lower prevalence. This could be as a result of the active ingredient (Imidacloprid and Pencycuron) inhibiting fungal inoculum.

Germination percentage and seedling vigor of seeds treated with Monceren GT 390 FS increased compared with the untreated at the end of the 90 days. The increment observed in the treated seeds could be attributed to the reduction in fungal prevalence by the seed treatment. This is in agreement with reports by Patil *et al*., (2015) and Sivparsad *et al*., (2014), who observed that germination percentage of sesame and chickpea seeds were increased as a result of seed treatments. Seeds infected with pathogens may result in poor seed germination and seedling vigour. Untreated seeds are mostly infected by pathogens which result in poor seed germination and seedling vigour. 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).

5.2.3.2 Insector T 45 Seed Treatment

All fungal pathogens were reduced to a lower prevalence when seeds were treated with Insector T 45. This could be attributed to the effect of the active ingredient (Imidacloprid and Thiram) reducing fungal inoculum. Thiram has been reported be to effective in suppressing pre- and post–emergence damping-off of cultivars artificially inoculated with *Fusarium graminearum* Group 1 (Lamprecht *et al*., 1990). Similar result has been reported by Solanke *et al*., (1997) who detected that pre- and post-emergence mortality caused by *Aspergillus* spp., *F. moniliforme, Curvularia lunata, A. alternate* and *Penicillium* spp. were controlled by the use of thiram. The finding agrees with Song *et al*., (2004) who discovered that combination of thiram and procloraz application suppressed mycelial growth of some *Fusarium* species. Southwell *et al*., (2003), also revealed that, combination of thiram and carboxim effectively reduced *Gaeumannomyces zeae* in the seed.

Seed treated with Insector T 45 resulted in significant increase in germination and seedling vigour over the untreated seeds. Seed treatment with thiram has proved to be effective in improving maize viability and emergence (Pinto, 1997). Similar result was obtained when pea seeds were treated with thiram, resulting in increased germination and emergence by 33% and 29% (Xue, 2003). High germination percentage and emergence were recorded on maize seeds treated with thiram and carboxim (Southwell *et al*., 2003).

5.2.3.3 Neem Seed Extract Seed Treatment

Neem seed extract was effective in managing the fungi that were isolated. The reduction of these pathogens could probable be the azadirachtin property in the neem seed extract. Neem seed extract has been shown 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). From a study, neem extract has been revealed to suppress seed-borne fungal diseases (Howlader, 2003). This is in partial agreement with the findings of Mondall *et al*., (2009), who reported that seed treatment with garlic extract, neem, gagra, vatpata, Bishkatali leaf extracts reduced seed-borne prevalence and increased germination percentage of wheat seeds.

5.2.3.4 Garlic Extract Seed Treatment

From the study, it was revealed that garlic extract seed treatment was effective in controlling seed-borne fungal pathogens. Garlic contains an active ingredient called allicin, which in turn produces other sulphur compounds including ajoene, allyl sulfides, and vinyldithiins (Koscielny *et al*., 1999). Garlic has been scientifically confirmed to be a natural antibiotic, antiviral and antifungal agent (Michelle, 2003). Mansur *et al*., (2013), has confirmed that allicin in garlic extract was able to control rice seed-borne pathogens. A study was conducted and it was revealed that, application of garlic extract reduced the infection of *Bipolaris sorokiniana* and *Drechslera tritici-repentis* in two cultivars of wheat (Perelló *et al*., 2013).

Seed treatment with garlic extract improved germination from 60.65% in untreated to 75.5% in treated seeds as well as seedling vigour. This is in agreement with a result obtained when rice seeds were treated with garlic extract which increased germination from 67.68% in untreated to 91.67% in treated seeds (Mansur *et al*., 2013). Low germination percentage recorded on untreated seeds could be as a result of the high fungal infection (Islam and Monjil, 2016a).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

- All seed samples obtained from the different locations were found to be discolored, shriveled, broken and cracked during visual extermination of dry seed samples.
- Different seed-borne fungal pathogens were found to be associated with the seed samples. Four pathogenic fungal species: *Cercospora* spp., *Alternaria* spp., *Fusarium* spp., *Macrophomina phaseolina* and five saprophytic species: *Aspergillus flavus*, *Aspergillus niger, Penicillium* spp., *Curvularia* spp. and *Rhizopus stolonifer* were identified. Fungal prevalence varied across location, with the highest and lowest prevalence observed on seed samples obtained from Yendi district and CSIR-SARI respectively. *Aspergillus niger*, *Fusarium* spp. and *Rhizopus stolonifer* fungal pathogens were revealed on all the eleven seed samples. *Microphomina phaseolina*, *Cercospora* spp., *Aternaria* spp., *Fusarium* spp. proved to be pathogenic.
- The use of Monceren GT 390 FS, Insector T 45, Garlic extract and Neem seed extract as seed treatments over a period of 90 days, led to a decrease in fungal prevalence. Prevalence of fungal pathogens increased in untreated seed samples.
- Germination percentage increased to 85% when seeds were treated with Monceren GT 390 FS. Similarly, Insector T 45, Neem seed extract and Garlic extract improved germination percentage of 83%, 78% and 75.5% respectively, while untreated seeds recorded 60.6%.
- Seed treatment with Monceren GT 390 FS, Insector T 45, Garlic extract and Neem seed extract improved seedling vigour.

6.2 RECOMMENDATIONS

- Farmers should be educated on seed treatment measures before storage to prevent the build-up of seed-borne fungi which results in higher disease prevalence and poor seed germination.
- Further studies should be conducted to determine bio-efficacy of the neem seed and garlic extract which have proved to have some degree of antifungal effect in the study.

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APPENDICES

Appendix 1: Analysis of variance table for appearance quality of farmer-saved soybean seeds

Discolored seeds

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	10	54.409	5.441	1.67	0.130
Residual	33	107.500	3.258		
Total	43	161.909			

Shriveled seeds

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	10	79.136	7.914	6.29	<.001
Residual	33	41.500	1.258		
Total	43	120.636			

Broken seeds

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	10	171.636	17.164	9.72	<.001
Residual	33	58.250	1.765		
Total	43	229.886			

Undamaged seeds

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample	10	401.682	40.168	6.17	<.001
Residual	33	214.750	6.508		
Total					

Appendix 2: ANOVA Table of prevalence of fungi on seed sample obtained from the 11 locations

Nalong

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogens	7	518.78	74.11	6.97	<.001

Residual	28	297.67	10.63		
Total	35	816.45			

Tindando

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogens	7	757.956	108.279	12.74	<.001
Residual	28	237.952	8.498		
Total	3	995.907			

Gbadagba

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogens	7	888.53	126.93	8.40	<.001
Residual	28	422.87	15.10		
Total	3	1311.40			

Garimata

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogens	7	1114.39	159.20	10.06	<.001
Residual	28	442.90	15.82		
Total	35	1557.29			

Yankazia

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogens	7	1533.13	219.02	11.58	<.001
Residual	28	529.40	18.91		
Total	35	2062.53			

CSIR-SARI

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogens	7	797.927	113.990	11.49	<.001
Residual	28	277.724	9.919		
Total	35	1075.651			

Zang

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogens	7	202.871	28.982	6.61	<.001
Residual	28	122.783	4.385		
Total	35	325.654			

Kanisheigu

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogens	7	262.92	37.56	2.48	0.041
Residual	28	424.43	15.16		
Total	35	687.34			

Zangban

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogens	7	1046.21	149.46	9.84	<.001
Residual	28	425.15	15.18		
Total	35	1471.36			

Gumbaliga

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogens	7	1309.533	187.076	24.99	<.001
Residual	28	209.647	7.487		
Total	35	1519.180			

Sunsong-Gbung

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogens	7	1187.73	169.68	16.21	<.001
Residual	28	293.15	10.47		
Total	3	1480.88			

Appendix 3: ANOVA Table for farmer-saved seeds treated with fungicide and plant extracts

Monceren GT 390 FS seed treatment for 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogen	8	138.457	17.307	1.89	0.104
Residual	27	247.482	9.166		
Total	35	385.939			

Monceren GT 390 FS seed treatment for 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogen	8	292.795	36.599	6.64	<.001
Residual	27	148.930	5.516		
Total	35	441.725			

Monceren GT 390 FS seed treatment for 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogen	8	153.941	19.243	17.92	<.001
Residual	27	28.993	1.074		
Total	35	182.934			

Insector T 45 seed treatment for 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogen	8	149.365	18.671	2.83	0.020
Residual	27	178.066	6.595		
Total	35	327.430			

Insector T 45 seed treatment for 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogen	8	119.066	14.883	1.62	0.164
Residual	27	247.292	9.159		
Total	3	366.358			

Insector T 45 seed treatment for 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogen	8	86.173	10.772	5.08	<.001
Residual	27	57.213	2.119		
Total	35	143.386			

Garlic extract seed treatment for 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogen	8	193.324	24.165	3.55	0.006
Residual	27	183.991	6.814		
Total	35	377.315			

Garlic extract seed treatment for 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogen	8	140.932	17.617	2.59	0.031
Residual	27	183.593	6.800		
Total	35	324.525			

Garlic extract seed treatment for 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogen	8	118.910	14.864	2.19	0.061
Residual	27	183.335	6.790		
Total	35	302.245			

Neem seed extract seed treatment for 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogen	8	203.892	25.487	4.35	0.002
Residual	27	158.165	5.858		
Total	35	362.057			

Neem seed extract seed treatment for 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Pathogen	8	173.484	21.686	3.26	0.010
Residual	27	179.657	6.654		
Total	35	353.142			

Neem seed extract seed treatment for 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogen	8	56.507	7.063	1.50	0.204
Residual	27	127.141	4.709		
Total	35	183.647			

Appendix 4: ANOVA Table for untreated seeds

Untreated seeds stored for of 30 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogen	8	141.549	17.694	3.05	0.014
Residual	27	156.395	5.792		
Total	35	297.944			

Untreated seeds stored for of 60 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogen	8	157.019	19.627	7.59	<.001
Residual	27	69.845	2.587		
Total	35	226.864			

Untreated seeds stored for of 90 days

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Pathogen	8	98.193	12.274	4.40	0.002
Residual	27	75.381	2.792		
Total	35	173.574			

Appendix 5: ANOVA Table for germination percentage

Germination percentage before seed treatment

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Location	10	262.7273	26.2727	28.90	<.001
Residual	33	30.0000	0.9091		
Total	43	292.7273			

Germination percentage 30 days after seed treatment

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	604.700	151.175	88.93	<.001
Residual	15	25.500	1.700		
Total	19	630.200			

Germination percentage 60 days after seed treatment

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	973.300	243.325	144.55	<.001
Residual	15	25.250	1.683		
Total	19	998.550			

Germination percentage 90days after seed treatment

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	1463.200	365.800	277.82	<.001
Residual	15	19.750	1.317		
Total	19	1482.950			

Appendix 6: ANOVA Table for Seedling vigour

Seedling vigour before seed treatments

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Location	10	187117	18712.	18.56	<.001
Residual	33	33275	1008.		
Total	43	220393			

Seedling vigour 30 days after seed treatment

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

Treatment	4	645341.6	161335.4	174.71	<.001
Residual	15	13852.0	923.5		
Total	19	659193.7			

Seedling vigour 60 days after seed treatment

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	771915.5	192978.9	328.61	<.001
Residual	15	8809.0	587.3		
Total	19	780724.5			

Seedling vigour 90 days after seed treatment

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	4	964033.5	241008.4	271.26	<.001
Residual	15	13327.4	888.5		
Total	19	977360.9			