SEED HEALTH TESTING OF RICE AND THE COMPARISON OF FIELD INCIDENCE AND LABORATORY COUNTS OF *Drechslera oryzae* (Bipolaris *oryzae*) and *Pyricularia oryzae* IN GHANA

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BY

OSUMAN ALIMATU SADIA

B.Sc. (Hons) Agric-Tech (Agronomy)

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DECLARATION

I hereby declare that this work being submitted is my own original research work and that it has neither in part nor in whole been used for any degree elsewhere. All other works cited is duly acknowledged.

Osuman Alimatu Sadia	…………………….	……………………
(Student)	Signature	(Date)

Certified by:

Dr. Robert Asuboah	…………………..	……………………
(Supervisor)	Signature	(Date)
Prof (Mrs.) Nana Sakyiwa Olympio	……………………	……………………
(Supervisor)	Signature	(Date)
Dr. Ben K. Banful	……………….…….	……………………
(Head of Department)	Signature	(Date)

DEDICATION

This piece of work is dedicated to my late grandmother Salamatu Sumaila (may her soul rest in peace), my dear mum Aisha Ahmed, My Siblings Nasirudeen, Umar Faruk, Ahmed Tawfiq and Salma for your encouragement, understanding support, Sacrifice and care during this programme.

ABSTRACT

A study was set up to find out the prevalence and occurrence of two fungi pathogens, *Bipolaris oryzae* and *Pyricularia oryzae* on rice (*oryzae sativa* L) seeds. Thirty- three (33) seed samples were collected from rice growers in seven communities in the four agro-ecological zones of Ghana (Sudan Savannah, Guinea Savannah, Forest Zone and Coastal Savannah). Laboratory seed health test was conducted on all the samples by blotter method, and four varieties were selected, one each from the ecological zones and a comparable count of different fungi was obtained under near ultraviolet light before sowing and after sowing. Visual symptom of blast and brown spot were also observed on the rice plants during the field experiments. *Pyricularia oryzae* was not observed on any of the seed samples collected when the blotter method was used for the health test. Sudan Savannah recorded the highest infection of *Bipolaris oryzae* with nine numbers of infections recorded out of nine samples used with infection rate ranging from 1.5% to 29.5%, whilst Guinea Savannah recorded the least infection rate, ranging from 0 to 0.5% when one sample was used. Nerica 14 was used for Guinea Savannah zone and before sowing it recorded 0.5% of *Bipolaris oryzae* but recorded 0% at harvest. Jet 3 variety from the Coastal Savannah recorded 0.5% infection before sowing and recorded 9% infection level at harvest. Also Nerica 2 from the forest zone recorded 0% infection level before sowing but a recorded high infection level of 5% at harvest. *Curvularia pallescens* was not recorded in the forest zone (nerica 2 variety) and in the Guinea savannah (Nerica 14 variety) before sowing but recorded significantly higher percentages at harvest at 86% and 46.5% respectively.Also Sudan Savannah (Jasmine 85 variety) and Coastal Savannah recorded 8.5% and 4.5% infection of *Curvularia pallescens* before sowing

respectively but recorded significantly higher percentages of 60% and 61% respectively.

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ACRONYMS

°C	Degrees Celsius
%	Percentage
AGRA	Alliance for A green revolution in Africa
B	Bipolaris
CRI	Crops Research Institute
CSIR	Council for Scientific and Industrial Research
DTMA	Drought Tolerant Maize for Africa
FAO	Food and Agricultural Organization
IITA	International Institute for Tropical Agriculture
ISTA	International Seed Testing Association
KNUST	Kwame Nkrumah University of Science and Technology
MoFA	Ministry of Food and Agriculture
Msc	Master of Science
NERICA	New Rice for Africa
NPK	Nitrogen Phosphorus Potassium
O	Oryzae
P	Pyricularia

RCBD	Randomized Complete Block Design
RSHT	Routine Seed Health Test
Spp	Species
USD	United State Dollar
USDA	United State Department of Agriculture
UV	Ultra Violet

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CHAPTER ONE

1.0 INTRODUCTION

Rice as food provides a major source of calories for a large percentage of the world's population, particularly in Asia, where more than 90% of all rice is grown and consumed by about 60% of the world's people (Screenivasaprasad *et al*., 2003). It is also a staple food in the Latin Americas, part of the Middle East, and Africa. Today, rice is produced on about 10% of all cropland (144 million in hectares harvested). Again, as a food crop rice provides more calories per hectare for human consumption than wheat (DeDatta, 1981). Rice accounts for 20% to 50% of total caloric consumption of many countries in the world (Nutsugah *et al*., 2004). Rice constitutes the diet of half of the world's population and its production is expanding even in areas which are not traditional producers of the crop.

Africa produces 2.7% of the world's rice and it is the second largest rice producing continent in the world. (6.5 million Mt in 2003) (FAO, 2003). Still rice production does not meet the required quantity to feed the growing population. The deficit is met through importation (FAO, 2003). Rice is one of the main staple cereal food crops in most part of Africa (Traoré *et al*., 2006). Optimum rice production in Africa is constrained by various biotic and abiotic factors. Jones *et al*., (1996), pointed out that grain yield are constrained by unfavorable weather, water and soil conditions, diseases and insect pest outbreak.

Generally, fungi are ubiquitous and play significant roles in our lives. They attack and destroy a variety of organic materials; cause fermentation; are used in the production of organic acids, vitamins, and medicines; and cause diseases. As saprophytes, they help recycle a variety of complex organic materials. As pathogens, they cause a number of destructive diseases. Fungi are known to cause 55 diseases in rice, 43 of which are seedborne or seed-transmittable (Ou, 1985; Richardson 1979, 1981). The three most important pathogens in rice are *Pyricularia oryzae,* which causes blast, *Drechslera oryzae*, which causes brown spot and *Xanthomonas oryzae*, which causes bacteria blight. In the USA the first two account for 30% of losses (USDA, 1965). The most notorious case of brown spot was the outbreak in Bengal, India, in the 1942-1943 when between 50 and 90 % of the rice crops were destroyed thus contributing to a major famine in which two million people died of starvation (Ghose, Ghatge and Subrahmanyan. 1956: Padmanabhan, 1973).

In the USA, the average loss caused by *Drechslera oryzae* was only approximately 0.5 per cent (Cramer, 1967): In Zanzibar according to the survey made by Padwick (1956), it was about 5 per cent, whereas in Nigeria losses ranged from 12- 40 per cent (Awoderu and Onuarah, 1974). However, North America contributes less 2% to the world's production. In the major rice producing countries such as India, the situation is different. In India about 20 percent of world production and other Asian countries, blast and brown spot are common and devastating. *Pyricularia oryzae* often kills seedlings or plants at the tillering stage and may largely destroy the panicles. In Japan, blast has been regarded as by far the most serious biological menace to food production and was responsible for famine in several districts in the 1930's and 1940's (Padwick, 1950). *Drechslera oryzae* and *Pyricularia oryzae* are two most important rice pathogens in the world. Of all the seed borne rice disease probably brown spot caused by *Drechslera oryzae* (*Cochliobolus Miyabeanus*), and blast (*Pyricularia oryzae*) are the two most important. It has been found to induce loses up to 30% in weight of rice grain (Bedi and Gill, 1960).

Rice blast disease, caused by *Magnaporthe oryzae* B.Couch, anamorphe *Pyricularia oryzae* Cav., is a major constraint to rice production and is known to occur in most rice-producing areas of the world (Ou, 1985). The disease causes yield loss as high as 70–80% when predisposition factors (high mean temperature, relative humidity higher than 85–89%, presence of dew, drought stress and excessive nitrogen fertilization) favour epidemic development (Piotti *et al*., 2005). Losses of almost 80% were reported in the 1970s in West Africa (Delassus, 1973). Particularly dangerous in upland rice, it also causes serious damage in rain fed lowland and irrigated systems, mainly when farmers seek to intensify production by the use of improved varieties and fertilizers. Therefore, blast constitutes one of the main constraints to intensification for increasing rice production in Africa.

Notwithstanding, little documentation has been carried out on their prevalence and distribution in Ghana as well as their possible effect on rice in Ghana, therefore there is the need to find out the degree at which the diseases can cause serious economic losses. While all health testing methods for rice have been studied intensively during recent years in order to select suitable procedures, little attempt has been made to correlate occurrence of pathogens in the seed crops with the amount of inoculums recorded on the seed in laboratory tests, or to correlate laboratory records with disease intensity in the following crops developed from tested seed. The main objective of this investigation is to study the prevalence / occurrence of the two pathogens on the seeds collected from the different ecological zones of Ghana.

The specific objectives are:

- To find the possible pathogens of rice across the country.
- To compare initial rate of infection of the two pathogens of the rice seeds to the final infection of the seed after harvest.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ORIGIN OF RICE

The origin of rice was long shrouded by disparate postulates because of the pantropical but distunct distribution of the 20 wild species across four continents, the variations in characterizing and naming plant specimens, and the traditional feud concerning the relative antiquity of rice in India versus China. Among the botanists, Roschevicz (1931) first postulated that the center of origin of this rice, *Sativa* Roschev., to which *O. glaberrima* and *O. sativa* belong, was in Africa and that *O. sativa* had originated from multiple species. A divergent array of wild species was proposed by different workers as the putative ancestor of *O. sativa* (Chang, 1976b).

Several workers considered "*O. perennis* Moench" (an ambiguous designation of varying applications) as the common progenitor of both cultigens (Chang 1976b). A large number of scholars had argued that Asian rice originated in the Indian subcontinent (South Asia), although de Candolle (1884), while conceding that India was more likely the original home, considered China to have had an earlier history of rice cultivation

On the basis of historical records and the existence of wild rice's in China, Chinese scholars maintained that rice cultivation was practiced in north China during the mythological Sheng Nung period (c. 2700 B.C.) and that *O. sativa* of China evolved from wild rices (Ting 1961). The finding of rice glume imprints at Yang-shao site in north China (c. 3200— 2500 B.C.) during the 1920s reinforced the popular belief that China was one of the centers of its origin (Chinese Academy of Agricultural Sciences, 1986).

Since the 1950s, however, rice researchers have generally agreed that each of the two cultigens originated from a single wild species. But disputes concerning the immediate ancestor of *O. sativa* persist to this day (Chang 1976b, 1985; Oka, 1988). A multidisciplinary analysis of the geographic distribution of the wild species and their genomic composition in relation to the "Glossopterid Line" (northern boundary) of the Gondwanaland fragments (Melville, 1966) strongly indicated the Gondwanaland origin of the genus *Oryza* (Chang 1976a, 1976b, 1985). This postulate of rice having a common progenitor in the humid zones of the supercontinent Pangaea before it fractured and drifted apart can also explain the parallel evolutionary pattern of the two cultigens in Africa and Asia respectively. It also reconciles the presence of closely related wild species having the same genome in Australia and in Central and South America. Thus, the antiquity of the genus dates back to the early Cretaceous period of more than 130 million years ago (www.cambridge.org/us/book/kiple/rice.htm).

2.2 EVOLUTION OF RICE

The parallel evolutionary pathway of *O. glaberrima* in Africa and of *O. sativa* in Asia was from perennial wild — Æ annual wild — Æ annual cultigen, a pattern common to other grasses and many crop plants. The parallel pathways are:

Africa: *O. longistaminata* — Æ *O. barthii* — Æ *O. glaberrima.*

Asia: *O. rufipogon* — Æ *O. nivara* — Æ *O. sativa*.

This scheme could to a large extent resolve the past disputes that have characterized the putative ancestors of the two cultigens. Wild perennial and annual forms having the same A genome are present in Australia and in Central and South America, but the lack of incipient agriculture in Australia and of wetland agronomy in tropical America in prehistoric times disrupted the final step in producing an annual cultigens (www.cambridge.org/us/book/kiple/rice.htm). It needs to be pointed out that the putative ancestors, especially those in tropical Asia, are conceptually wild forms of the distant past, because centuries of habitat disturbance, natural hybridization, and dispersal by humans have altered the genetic structure of the truly wild ancestors (www.cambridge.org/us/book/kiple/rice.htm). Most of the wild rices found in nature today are hybrid derivatives of various kinds (Chang, 1976b; 1985). The continuous arrays of variants in natural populations have impaired definitive studies on the wild progenies (Chang, 1976b).

The differentiation and diversification of annual wild forms into the early prototypes of cultigen in South and mainland Southeast Asia were accelerated by marked climatic changes during the Neothermal age of about 10,000 to 15,000 years ago. Initial selection and cultivation could have occurred independently and nearly concurrently at numerous sites within or bordering a broad belt of primary genetic diversity that extends from the Ganges plains below the eastern foothills of Himalaya, through upper Burma, northern Thailand, Laos, and northern Vietnam, to southwest and southern China (www.cambridge.org/us/book/kiple/rice.htm).

From this belt, geographic dispersal by various agents, particularly water currents and humans, lent impetus to ecogenetic differentiation and diversification under human cultivation. In areas inside China where winter temperatures fell below freezing, the cultivated forms (cultivars) became true domesticates, depending entirely on human care for their perpetuation and propagation. In a parallel manner, the water buffalo was brought from the swamps of the south into the northern areas and coevolved as another domesticate (Chang, 1976a).

In West Africa, *O. glaberrima* was domesticated from the wild annual *O. barthii* (Chevalier 1932); the latter was adapted primarily to water holes in the savannah and secondly to the forest zone (Harlan, 1973). The cultigen has its most important center of diversity in the central Niger delta. Two secondary centers existed near the Guinean coast (Porteres, 1956).

Cultivation of the wild prototypes preceded domestication. Rice grains were initially gathered and consumed by prehistoric people of the humid regions where the perennial plants grew on poorly drained sites. These people also hunted, fished, and gathered other edible plant parts as food. Eventually, however, they developed a liking for the easily cooked and tasty rice and searched for plants that bore larger panicles and heavier grains (www.cambridge.org/us/book/kiple/rice.htm).

The gathering-and-selection process was more imperative for peoples who lived in areas where seasonal variations in temperature and rainfall were more marked. The earlier maturing rice which also tended to be drought escaping would have been selected to suit the increasingly arid weather of the belt of primary diversity during the Neothermal period. By contrast, the more primitive rice of longer maturation, and those, more adapted to vegetative propagation, would have survived better in the humid regions to the south (Chang, 1976b; 1985). In some areas of tropical Asia, such as the Jeypore tract of Orissa State (India), the Batticoloa district (Sri Lanka), and the forested areas of north Thailand, the gathering of free-shattering grains from wild rice can still be witnessed today (Chang, 1976b; Higham, 1989).

2.3 Nutritional Considerations

Rice is unquestionably a superior source of energy among the cereals. The protein quality of rice (66 percent) ranks only below that of oats (68 percent) and surpasses that of whole wheat (53 percent) and of corn (49 percent). Milling of brown rice into white rice results in a nearly 50 percent loss of the vitamin B complex and iron and washing milled rice prior to cooking further reduces the water-soluble vitamin content. However, the amino acids, especially lysine, are less affected by the milling process (Kik, 1957; Mickus and Luh 1980; Juliano, 1985a; Juliano and Bechtel, 1985). Rice, which is low in sodium and fat and is free of cholesterol, serves as an aid in treating hypertension. It is also free from allergens and now widely used in baby foods (James and McCaskill, 1983). Rice starch can also serve as a substitute for glucose in oral rehydration solution for infants suffering from diarrhoea (Juliano, 1985b).

The development of beriberi by people whose diets have centered too closely on rice led to efforts in the 1950s to enrich polished rice with physiologically active and rinse-free vitamin derivatives. However, widespread application was hampered by increased cost and yellowing of the kernels upon cooking (Mickus and Luh 1980). Certain states in the United States required milled rice to be sold in an enriched form, but the campaign did not gain acceptance in the developing countries. After the 1950s, nutritional intakes of the masses in Asia generally improved and, with dietary diversification, beriberi receded as a serious threat (www.cambridge.org/us/book/kiple/rice.htm). Another factor in keeping beriberi at bay has been the technique of parboiling rough rice. This permits the water-soluble vitamins and mineral salts to spread through the endosperm and the proteinaceous material to sink into the compact mass of gelatinized starch. The result is a smaller loss of vitamins, minerals, and amino acids during the milling of parboiled grains (Mickus and Luh 1980), although the mechanism has not been fully understood. Parboiled rice is popular among the low-income people of Bangladesh, India, Nepal, Pakistan, Sri Lanka, and parts of West Africa and amounts to nearly one-fifth of the worlds rice consumed (Bhattacharya 1985).

During the 1970s, several institutions attempted to improve brown rice protein content by breeding. Unfortunately, such efforts were not rewarding because the protein content of a variety is highly variable and markedly affected by environment and fertilizers, and protein levels are inversely related to levels of grain yield (Juliano and Bechtel 1985).

2.4 RICE PRODUCTION IN AFRICA

In Africa, rice is the staple food in Gambia, Guinea Bissau, Guinea, Côte d'Ivoire, Liberia, Senegal and Sierra Leone and now Ghana (Séré. *et al*., 2004). In other countries, rice is replacing traditional grain crops (sorghum, millet, maize) (Séré. *et al*., 2004). Rice (*oryza sp*) is after wheat, the most widely cultivated cereal in the world and it is the most important food crop for almost half of the world's population (IRRI, 2009). It is estimated that rice sustains the livelihood for 100 million people and its production has employed more than 20 million farmers in Africa (WARDA, 2005).

In 2008, sub-Saharan Africa (SSA) imported far more than USD 3.6 billion of rice, mainly from Asia, to fill the gap between production and consumption. However, Africa cannot continue to rely on imports, as Asia may soon not be able to export rice and may even become a net importer (Seck *et al*., 2010). The recent dramatic decrease in world rice availability and increase in prices indicate that importing rice is no longer a sustainable strategy. Therefore, African countries are developing their largely unexploited rice potential to boost domestic production. However, both the development of new areas and the intensification of rice cultivation will bring new problems, among which rice diseases are the most important.

2.5 Rice as source of livelihood

The demand for rice in Sub-Saharan Africa is double the rate of population growth and rice consumption is growing faster than that of any other major staple food. (Séré *et al.*,2004). Rice is grown in all but the desert zones and is the main staple in eight West Africa countries. (Séré *et al.*,2004). Rice account for 20-50% of the total calorific consumption and its availability and price impact directly on the poor and major food security issues in the region (Screenivasprasad *et al*., 2003).

The estimated burden of malnutrition is slightly greater for females than for males as anemia affects mostly women in the reproductive stage. Malnutrition raises the risk of death and may reduce the physical and mental capacity of children. So, increasing rice production is essential since the poorest urban household in many African countries obtain a larger share of their cereal-based calories from rice than do higher income households, and rice purchases represent a greater share of their total cash expenditure (Séré. *et al.*, 2004). However, the average yield of 1.7 tha^{-1} in the region is the lowest in the world and FAO estimates nearly 4 million tonnes of annual rice imports into West Africa worth more than US$1 billion per annum. Thus there is a clear gap between consumption and local production owing to a number of biophysical constraints. Blast caused by *Pyricularia grisea* (Cke) Sacc. (Teleomorphe; *Magnaphorthe grisea* (Hebert) Barr) is one of the major constraints to increased rice production. Rice blast is more important in upland and rainfed lowland ecosystems than in other agro ecologies. It causes significant and unpredictable losses in rice fields mainly when farmers try to improve their traditional and poor – yielding system by using new varieties and fertilizer, consequently this disease is one of the major constraints to intensification of rice production (Séré. *et al*.,2004).

2.6 Rice Production in Ghana

Rice is becoming increasingly an important staple food throughout Ghana. The per capita consumption has risen from 13.9kg / year in 1995 to 14.5 in 2000 (MOFA, 2002). Ghana imports 564,000 mt paddy annually compared with domestic production of 180.000 mt. Imported milled rice alone was worth US $95 million in 1999 and it is likely to be slightly higher at present. Rice production has expanded in recent times and it is being given the needed attention to achieve national food security, alleviate rural poverty and contribute to the overall economy through import substitution and foreign exchange conservation (MOFA, 2001).

Rice production in Ghana is constrained by a number of biotic factors including diseases, pests and weeds. Blast disease caused by the fungus *Pyricularia grisea /oryzae* (Rossman *et al.*, 1990) (tele-magnaporthe greisea) (Webster, 1980) remains a threat to rice production in both temperate and tropical regions despite the increased research effort directed towards the control of the disease (Teng, 1994). The ability of the pathogen to infect rice at different stages of growth and its adaptation to both upland and lowland rice ecosystems are indication of plasticity of *P. grisea* to changing environment (Bonman *et al.*, 1992; Teng, 1994). In the West African sub-region, blast is recognized as the primary constraint to rice production causing 3.2-77.0% yield losses (Fomba and Taylo,r 1994). Rice blast was first recorded in Ghana by Bunting and Dade (1925) and then Piening (1962). It was also listed as an important disease by Clerk (1974) and Oduro (2000). Devastation of some rice cultivars by blast was observed in Northern Ghana in 1969. Since then various reports by Twumasi (1996, 1998), Twumasi and Adu-Tutu (1995), Nutsugah (1997a, 1997b) and Nutsugah and Twumasi (2001) have identified the disease as a serious threat to rice production in Ghana. Together with brown spot (*Bipolaris oryzae*), blast disease has been recently listed as one of the serious constraints to rice production in the country (Gerken *et al*., 2001).

2.7 Symptoms of rice blast (*Pyricularia oryzae*) disease

Blast symptoms develop on all the aerial organs of the rice plant, mainly on the coleoptiles, leaf sheaths and leaf blades as indicated in (Fig.1), neck of panicles (Fig. 2), stem nodes (Fig. 3) and spikelet. The foliar lesions reduce the leaf area available for photosynthesis and, when they are severe and occur in the early development stages, they are likely to destroy the whole tiller. Neck blast and node blast are characterized by a brown rot that disorganizes the tissues and prevents the migration of the nutrients that should ensure grain filling. This leads to early maturity of the panicles, causing indirect and seldom quantified yield losses through grain shedding. In farmers' fields, neck blast is considered more destructive than leaf blast, because it is more closely tied to yield losses (Zhu *et al*., 2005). Moreover, the production of spores is abundant (Fig. 4) and contributes to the disease spread, mainly in African irrigated systems where there is no synchronization of planting.

Fig. 1 symptoms on aerial leaf sheath and leaf blade

Source (Bagre (Burkina Faso) in 2008

Fig 2. Blast symptoms on neck of panicles

Source: Banicoara (Benin) in 2009

Fig. 3 blast symptoms on stem nodes and spikelet at

Source: Farako-Bâ (Burkina Faso) in 1984

Fig 4. Blast spore on leaf

Source: JIRCAS Working Report No.70

2.7 Research on rice blast in Africa

2.7.1 Importance of the disease

Rice blast was reported for the first time in Africa in 1930 (Feakin, 1974). Today, it has become the most widespread rice disease in Sub-Saharan Africa (SSA). Despite the fact that high relative humidity and the presence of dew are important for pathogen infection and development, the disease is found in Sudano-savanna and even in the sub-savanna zone in places like Bagre in Burkina Faso, a country where neck blast was found more destructive than leaf blast (Séré, 1999).

In rain fed lowlands of the province of Comoe, Burkina Faso, yield losses between 17 and 599 kg/ha (1–22% of production) were recorded (Séré. *et al*., 2004). In the same province but in the irrigated perimeter of Karfiguela, yield losses were greater: between 134 and 867 kg/ha, representing 4.2–45.3% of the production. A 5-year survey at the irrigated perimeter of Vallee du Kou (15 km from Bobo-Dioulasso) indicated yield loss up to 1965 kg/ha, representing 44% of production. Therefore, it seems that intensifying rice cultivation at Karfiguela and Vallee du Kou, where farmers used modern varieties and fertilizers, led to increased yield losses due to blast (Séré. *et al*., 2004). This was confirmed by an evaluation of demonstration plots in farmers' lowland fields. The incidence of neck blast in plots without fertilizer was one-seventh to one-half of that in plots that received fertilizers. Thus, blast constitutes a serious constraint to intensification of production as a great part of the benefit due to fertilizer application is lost by the increase of the damages due to the disease (Séré, 1999).

In Ghana, surveys conducted on more than 264 farmers' fields in all the major rice-growing areas across the country indicated that the incidence of blast varied considerably across sites, which have been grouped into low, moderate and high blast areas, with incidence levels of 1–10%, >10–50% and >50–100%, respectively(Nutsugah *et al.*, 2008). Heavy yield losses (up to 100%) largely due to blast infection were reported by the farmers. The survey results suggest that Datano, Hohoe and Nyankpala are blast hot spots and key sites for resistance screening, which correlates with the diversity of the blast pathogen populations in Ghana (Nutsugah *et al*., 2008).

Rice blast is also a major disease in the upland and lowland ecologies of Gambia. Crop losses attributed to rice blast vary according to localities and varieties. In Gambia, although the economic importance of rice blast has not been documented, yield losses of up to 100% have been observed in some locations, especially under stress conditions (Jobe *et al.*, 2002).

In Sierra Leone, losses ranged from 3.2% to 14.5% in several popular upland and lowland rice cultivars, and losses in excess of 80% were not uncommon in less adapted cultivars and accessions in experimental plots (Fomba, 1984). In mangroves, losses due to neck blast ranged from 16.0% to 30.9% (Fomba, 1986)

2.7.2 Economic importance of *Pyricularia oryzae*

Pyricularia oryzae cavara [synonym *Pricularia grisea* (Cooke) Sacc, anamorph of *Magnaporthe grisea* (T.T.Hebert) Yaegashi and Udagawa]. The cause of rice blast is one of the most important fungal pathogens of rice (*Oryzae sativa* .L.) because of its widespread occurrence and destructive nature (Ou, 1985). The fungus can attack any aerial part of the rice plant, including seeds, in which the fungus may over winter for several years (Ito, 1932; Marandhar, 1996). Recent investigation (Marandhar, 1996) suggested that systemic transmission of P. oryzae was first reported from Japan (Kurlbayashi, 1928; Marandhar, 1996) and later from other parts of the world (Chung, and Lee, 1983; Lamey, 1970; Mayee, 1975; Reddy and Bastawsi, 1989). However, most of the earlier studies were based on the growth of *P. oryzae* on seeds or seedlings incubated under laboratory condition (Chung, and Lee, 1983; Kurlbayashi, 1928; Lamey, 1970; Suzuki, 1930) or were based on brief observation with few details given (Mayee, 1975; Reddy, and Bastawsi, 1989). In the tropics where airborne inoculum is present throughout the year, overwintering of *P. oryzae* is not important in the disease cycle (Ou, 1985). But in other regions, where overwintering is necessary for the fungus, infected seeds may not play an important role in the disease cycle (Lee, 1994). Infected seeds can also be the primary source of inoculum when seeds are densely sown in seedling boxes for mechanical transplanting as has been the practice in Japan (Honda and Goto, 1963)

2.8 *Bipolaris oryzae* in Africa and other parts of the world

Bipolaris oryzae causes seedling blight and damages the foliage and panicles of rice, particularly when rice is grown in nutritionally deficient or otherwise unfavorable soils (Marchetti and Peterson, 1984). In Egypt, brown spot disease comes in the second rank after blast disease (El-Wahsh, 1997). At first the causal agent of brown spot disease was named by Breda de Haanas *Helminthosporium oryzae* (Gangopadhyay & Padmanabhan, 1987). At present, the graminicolous *Helminthosporium* species are divided into three genera based on colony, conidiophore and conidial morphology, type of conidial germination and the type of hilum structure: *Bipolaris, Drechslera and Exserohilum*. Their telemorphs were from ascomycetes and consist of: *Cochliobolus, Pyrenophora and Setosphaeria*, respectively (Sivanesan, 1987). Brown spot is one of the most important diseases of rice in Guilan province in the north of Iran (Safari Motlagh *et al*., 2006). Brown spot of rice, a fungal disease is caused by *Bipolaris oryzae* Breda de Haan that is Prevalent in all the rice growing countries of the world and most of the cultivars grown in the world are susceptible to this pathogen (Huynh and Ashok, 2004). *Bipolaris oryzae* causes seedling blight and damages the foliage and panicles of rice, particularly when rice is grown in nutritionally deficient or otherwise un-favorable soils (Marchetti & Peterson, 1984). It can be a serious disease causing a considerable yield loss. Where, it affects the quality and the number of grains per panicle and reduces the kernel weight

(Myers *et al.*, 2000). Although it is considered as a minor disease, it is known to cause considerable economic losses during normal years such as during the great Bengal famine of 1942 (Padmanabhan, *et al.*, 1948). The pathogen is known to cause damage at different stages during storage, seed germination and seedling establishment, vegetable growth and reproductive phase. The nature of damage caused by the pathogen differs at different stages. During storage it affects seed quality parameters such as germination, viability, and vigor. During seed germination, it attacks both the root and shoots systems and affects the survival of seedlings by causing seedling blight (Huynh and Ashok, 2004). Due to formation of brown spots and blight symptoms on leaves, the total photosynthetic area is reduced during the vegetative phase. The damage is in the form of grain discoloration, poor grain filling and reduced yield during the reproductive phase (Huynh and Ashok, 2004).

Fig 5 conidium on B. oryzae X 460

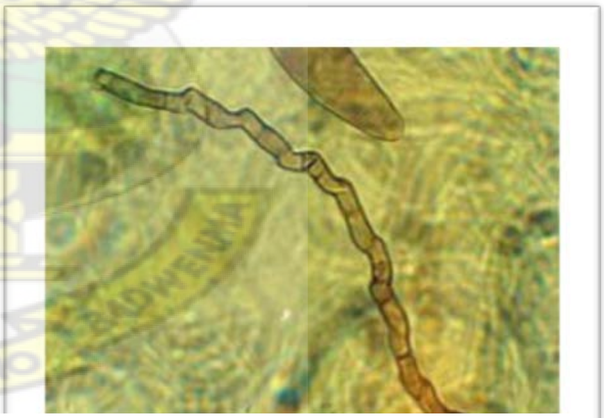

Fig 6. Conidiophore of B. oryzae X 460

(Source: MOTLAGH AND KAVIANI / Int. J. Agri. Biol., Vol. 10, No. 6, 2008)

2.9 Symptoms of Bipolaris oryzae

Bipolaris oryzae is classified in the subdivision Deuteromycotina (imperfect fungi), class Deuteromycetes, order Moniliales and family Dematiaceae and is the causal agent of brown spot disease of rice (Shabana, 2008). Brown spot is one of the important rice diseases in the world. Conspicuous brown spots appear on the leaves, spots measures 2-1 x 0.5cm, are oval and evenly distributed. They are brown with gray or whitish centers on maturity (Misra *et al*., 1990). In severe infections, a spot fuse and leaves wither. Spots also develop on glumes. When conditions favor fugal development, a velvety growth can be seen over the seed and fungus may enter the glumes and leave blackish spots on the endosperm. Brown spot symptoms may appear on the leaf coleoptiles, Leaf sheaths and panicle branches. Blackish lesions may be seen on young roots (Misra *et al*., 1989). Both upland and lowland ecosystems support brown spot development. Brown spot is seed borne and. seedling infection (seedling blight) results from infected seeds. Secondary infection which appears at the post tillering stage occurs through windborne spores (conidia) (Misra *et al.*, 1989).

CHAPTER THREE

3.0 Materials and Methods

3.1 Study Approaches

The study approaches comprised (i) sample collection and (ii) laboratory and field experiments. The field survey was conducted in 2011/2012 cropping season to find out the prevalence or incidence of Pyricularia oryzae and Bipolaris oryzae in Ghana and the distribution of fungi micro flora of rice in Ghana. The laboratory and field experiments were carried out to assess the health status of the seeds from the farmers' fields.

3.2 Field survey

A field survey was conducted from 7th September to 1st October, 2011 in rice-growing areas where rice seeds where collected from farmers' field and research fields and from farmers saved seeds. In all 7 communities, covering four agro-ecological zones comprised the study areas. These were Nobewam and Ejura for the Forest zone; Afife, Asutuare and Ashiaman in the Coastal savannah zone; Nyankpala (Tamale) in the Guinea savannah zone; Navrongo in the Sudan Savannah.

3.3 Outline of Research

A laboratory experiment was conducted at the Pathology laboratory of CSIR- Crops Research Institute, Kumasi, Ghana. In the second part of the experiment, one sample each from the ecological zones was taken and planted in pots arranged in a Randomized Complete Block Design (RCBD) behind the insectary of the Faculty of Agriculture, Kwame Nkrumah University of Science and Technology (KNUST) Kumasi.

3.4 Collection of Samples

Samples of rice cultivar were collected from across the country from selected ecological zone (Forest Zone, Sudan Savannah, Guinea Savannah and the Coastal Zone).

3.5 Seed health

Seed samples were tested for the presence or absence of *Pyricularia oryzae* and *Drechslera oryzae* by the standard blotter method modified from Mathur and Kongsdal (2003). Four hundred seeds were plated in replicates of 25 seeds per 9-cm-diameter Pyrex petri dish (Sterlin, Bibby Ltd. Stone, Staffordshire, U.K) lined with three layers of water soaked blotter papers. The dishes was incubated

12h alternation cycles of light and darkness (Leach, 1962). After incubation, fungi developed on each seeds were examined under different magnifications of a stereomicroscope and identified. The identification of the fungi was based on the way they grow on seeds “habit characters’ and on the morphological characters of fruiting bodies, spores/conidia observed under a compound microscope (Mathur and Kongsdal, 2003).

3.7 Pot Experiment and field layout

The second part of the experiment was done at KNUST, on the field of the Department of Crops and Soil Sciences soil science. Soil was sterilized by heating at about 1

Second ammonia application was done on the 3rd of February, 2012, because the plants were showing some deficiency of nitrogen at the same rate.

3.7.3 Haversting

Harvesting was done immediately the seeds matured and were well dried. Harvesting was carried out in batches since the varieties planted were different and had different days of flower initiation.

3.7.4 Disease score

Diseases occurrence was ranked from 1- 5. With 1 as the least infection and 5 as the highest infection.

CHAPTER FOUR

4.0 RESULTS

Table 1. Prevalence / Incidence of Fungi Microflora detected on 33 rice seed samples collected from the Major Ecological Zones of Ghana

pathogens	Sudan Savannah(nine varieties)		Guinea Savannah(one variety)		Forest Zone(ten varieties)		Coastal Savannah(thirteen varieties	
	Number of infections	**Range of % infection**	**Number of infections**	**Range of % infection**	**Number of infections**	**Range of % infection**	**Number of infections**	**Range of % infection**
Alternaria altanata	3/9	0.5 - 1.0	-	-	0/10	-	3/13	0.5-2.5
Alternaria padwickii	2/9	1.0-4.0	1/1	0.0-4.5	0/10	-	2/13	0.0-0.5
Bipolaris oryzae	9/9	1.5 -29.5	1/1	0 - 0.5	7/10	0.5-13.0	5/13	0.5-9.5
Cladosporium spp	4/9	0.5-5.5	1/1	0.0-4.5	6/10	0.5-7.0	8/13	0.5-60.0
Curvularia lunata	2/9	0.5-1.0	-	-	2/10	0.5-2.0	2/13	0.5-1.0
Curvularia spp	9/9	1.0-13.5	1/1	0.0-40.0	9/10	0.5-28.0	10/13	1-18.0
Fusarium monilliforme	8/9	0.5 – 8.0	-	-	8/10	2.0-6.0	10/13	0.5-3.0
Fusarium oxysporium	1/9	0.0-0.5	-	-	1/10	0.0-0.5	-	-
Fusarium pallidoroseum	3/9	0.5-7.5	0/1	-	6/8.5	0.5-8.5	5/13	0.5-2.0
Fusarium solani	4/9	0.5-8.0	-	-	5/10	0.5-12.0	4/13	0.5-1.0
Nigrospora oryzae	1/9	0.0-3.5	1/1	0.0-4.5	3/10	0.5-5.0	8/13	0.5-3.5
Phoma spp	6/9	0.5-4.0	1/1	0.0-29.0	2/10	0.5-1.0	7/13	0.5-3.5
Pyricularia oryzae	-	-	-	-	-	-	-	-
Sarocladium oryzae	4/9	1.0-8.5	0/1	-	4/10	0.5-3.5	8/13	0.5-4.5
Tricoderma spp	0/9	0.0	-	-	1/10	0.0-0.5	-	-

In the Sudan savannah zone nine (9) varieties were sampled out from the farms along the Tono and Vea Dam. Among the samples some were not known to the farmers so it was indicated as unknown. There were Whyter, four Jasmine 85, Two (2) unknown varieties, Tox 3107 and Tox 3377.

In the Sudan Savannah zone, *Bipolaris oryzae* recorded the highest infection level from all the nine varieties recording the pathogen, the infection percentage ranging from 1.5% - 29.5%. *Nigrospora oryzae, Fusarium oxysporium* and *Curvularia lunata* recorded the least number of occurrences and percentage infection of 1/9 and ranges from 0.0 to 0.5, 1/9 and ranges from 0.0 to 0.5, 2/9 and ranges from 0.5 to 1.0 respectively.

In the Guinea savannah zone only one sample was taken from the Savannah Agricultural Research Institutes (SARI) field. That is Nerica 14. This Zone recorded the least percentage of pathogens with the exception of *Curvularia spp* and *Phoma spp* which recorded percentage incidence ranging from 0.0 - 40.0% and 0.0 to 29.0 respectively. In the Forest Zone ten (10) varieties of rice were, on these varieties a seed health test was carried out and the following pathogens were observed on them as indicated in the Table 2 with their respective percentage infection. *Curvularia spp* was detected on nine out of the ten samples taken with the infection level ranging from 0.5 to 28.5%. *Bipolaris oryzae* was also detected on seven out of the ten samples with the infection level ranging from0.5 to 13.0%, *Fusarium solani* was also detected from five out of the ten samples with infection percentage ranging from 0.5 to 12.0% and *Fusarium monilliforme* was detected on eight out of ten samples with percentage infection ranging from 2.0 to 6.0%.

In the Coastal zone thirteen (13) varieties were sampled, with Togomashall from Afife, one unknown variety, five (5) Jet3 varieties, Aromatic short A and B and Jocop rice from Asutuare and two (2) Jasmine85 and one (1) unknown from Ashiaman were tested for their seed health. Five out of the thirteen samples from the Coastal zone recorded *Bipolaris oryzae,* with infection level ranging from 0.5 to 9.5 on the seeds. *Cladosporium oryzae* was detected on eight out of thirteen samples with infection percentage ranging from 0.5 to 60.0%. *Curvularia spp* and *Nigrospora oryzae* followed with ten out of thirteen samples with 1 to 18.0 percentage infection and eight out of thirteen samples recording *Nigrospora oyzae* with percentage infection ranging from 0.5 to 3.5% respectively.

Table 2. Results of seed infection levels to major rice seed borne fungi detected from four rice varieties harvested in a field grow-out experiment with seeds with different initial seed infection levels and collected from 4 different agro-ecological zones in Ghana.

Pathogen	% seed infection level Ecological Zones							
	Forest zone (Nerica 2)		**Sudan Savannah (Jasmine 85)**		**Guinea Savannah (Nerica 14)**		**Coastal savannah (Jet 3)**	
	Before sowing	**At harvest**	**Before Sowing**	**At harvest**	**Before Sowing**	**At harvest**	**Before sowing**	**At harverst**
Alternaria altanata	**-**	**-**	**-**	**-**	**0**	**1**	**-**	**-**
Alternaria padwickii	**0.5**	**0**	**0.5**	**8.5**	**29**	**1**	**1.5**	**0.5**
Bipolaris hawainensis	**-**	**-**	**0**	**0.5**	**-**	**-**	**-**	**-**
Bipolaris oryzae	**0**	**5**	**27**	**12**	**0.5**	**0**	**0.5**	**9**
Cercospora janseana	**0**	**0.5**	**0**	**8.5**	**0**	**1.5**	**-**	**-**
Cladosporium spp	**0.5**	**14**	**0.5**	**17**	**-**	**-**	**0.5**	**14**
Colletotrichum gloesporioides	**5**	**6**	**-**	**-**	**4.5**	**9.5**	**0**	**2**
Corynespora cassicola	**-**	**-**	**0**	**0.5**	**-**	**-**	**-**	**-**
Curvularia pallescens	**0**	**86**	**8.5**	**120**	**0**	**46.5**	**4.5**	**61**
Curvularia spp	**0**	**7**	**0**	**17**	**4.5**	**12**	**3.5**	**16.5**
Fusarium monilliforme	**3**	**2**	**4**	**11**	**0**	**8.5**	**1**	**8.5**
Fusarium oxysporium	**1**	**1**	**1**	**0.5**	**0**	**1.5**	**0.5**	**1**
Fusarium pallidoroseum	**0**	**1**	**0**	**8.5**	**4.5**	**4.5**	**0**	**9.5**
Fusarium solani	**-**	**-**	**0**	**3-**	**-**	**-**	**-**	**-**
Nigrospora oryzae	**0**	**12.5**	**-**	**-**	**0**	**17.5**	**0.5**	**7.5**
Pericuna spp	**-**	**-**	**-**	**-**	**-**	**-**	**0**	**0.5**
Phoma sorghina	**-**	**-**	**-**	**-**	**-**	**-**	**0**	**0.5**
Phoma spp	**6**	**0**	**-**	**-**	**-**	**-**	**0**	**0.5**
Pyricularia oryzae	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Sarocladium oryzae	**28**	**0**	**2**	**0**	**40**	**5.5**	**14**	**14**

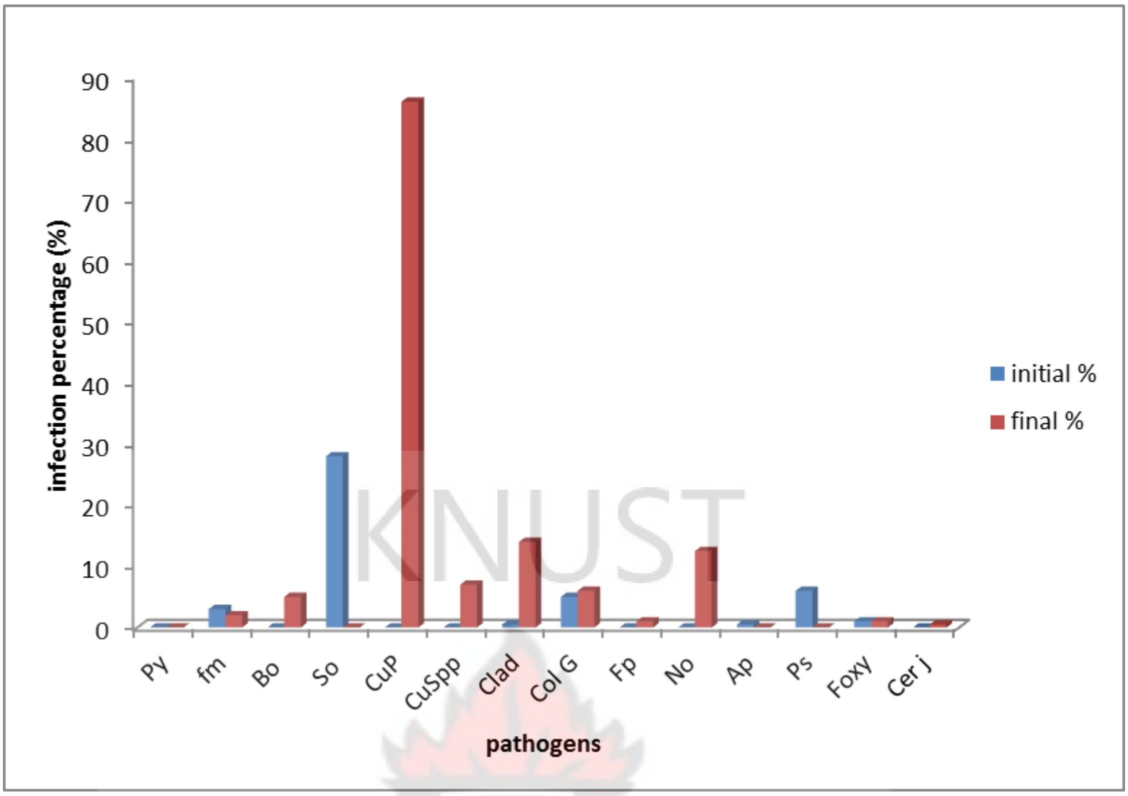

Fig 7: Initial and Final Infection Levels of Pathogens Recorded on Nerica2 from the Forest Zone

Fig 7 above is the result of initial and final seed infection levels of fungal pathogens detected on the variety Nerica 2 obtained from Nobewan within the Forest Zone of Ghana. .

The figure shows that *Pyricularia oryzae,* the causal organism for rice blast, a common disease in Ghana was not recorded both in the initial seed used for the field investigations as well as well as in the final testing; this may not preclude the absence of *Pyricularia oryzae* the blotter method employed in the seed health testing may not have been sensitive enough to detect the fungi. Probably a more powerful method like the agar method should have been also used for the detection.

On the other hand, initial seed infection of the variety Nerica2 obtained from the Forest Zone by Bipolaris *oryzae*, the causal organism of leaf spot in rice was 0% but harvested seeds after field plantings showed a seed infection level of 5% infection level. Also in the same forest zone, *Curvularia pallescens*, the causal organism of curvularia leaf spot was absent but in the harvested seeds from field investigations, a rather very high seed infection level of 86% was recorded. Similar trends of seed infection levels were obtained for the following pathogens, *Curvularia spp, Cladosporium spp, Fusarium pallidoroseum, Nigrospora oryzae, Colletorichum gloesporioides* and *Cercospora janseana*, causal organisms for the rice diseases of pecky rice disease, leaf spot of rice, narrow brown leaf spot all these fungi had fairly low seed infection levels than that obtained in the harvested seeds after the field investigations.

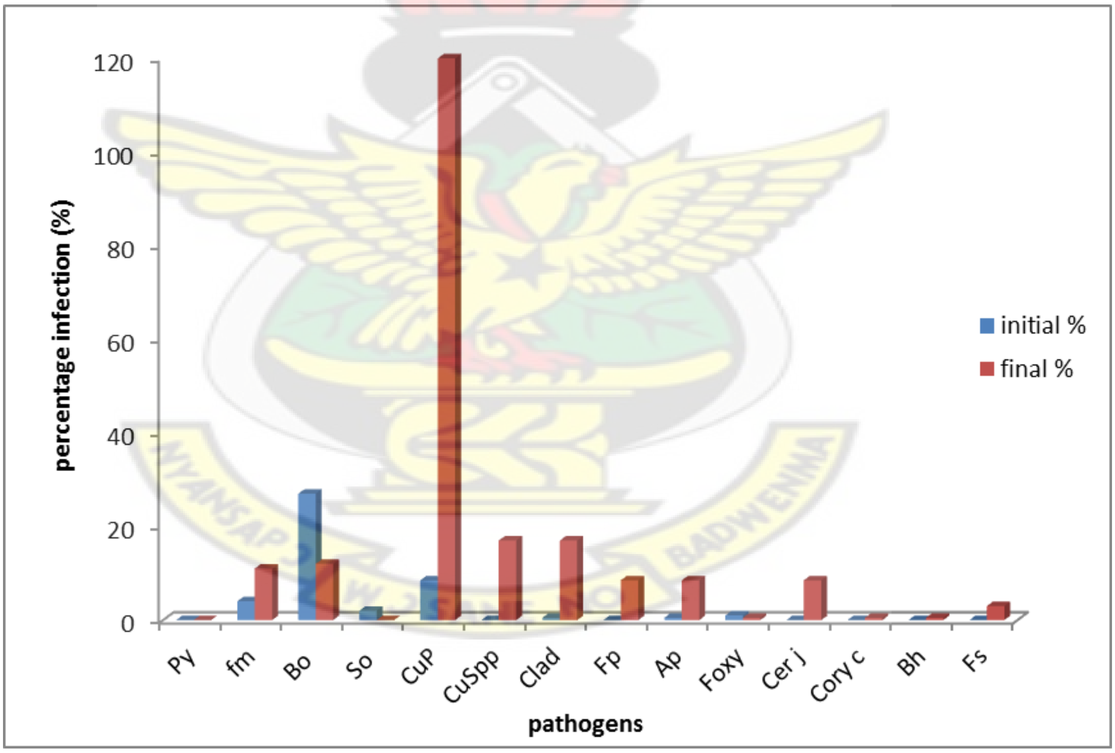

Fig 8: Initial and Final Infection Level of Pathogens Recorded on Jasmine 85 from the Sudan – Savannah

Jasmine 85, a variety obtained from the Sudan Savannah Zone of Ghana with different initial seed infection levels of various fungi was the second variety used in the field investigations. *Pyricularia oryzae* was not detected in both the initial seed planted as well as in the seed harvested after the field investigations.

Fusarium monilliforme which is also very pathogenic in rice and causes seed rot in the crop during germination and also bakanae disease in rice (Javid and Anjum, 2006), had an initial seed infection level of 4% in the seed used for field studies whilst 11% seed infection level was detected in the final seed harvested after the field experiment.. Similar trends of higher seed infection levels for harvested seed in the field experiment compared to initial seed used for field studies were detected for *Curvularia spp, Cladosporium Spp, Fusarium pallidoroseum, Alternaria altanata, Cercospora janseana, Bipolaris hawaeinensis* and *Fusarium solani* which are all pathogenic to rice plant.

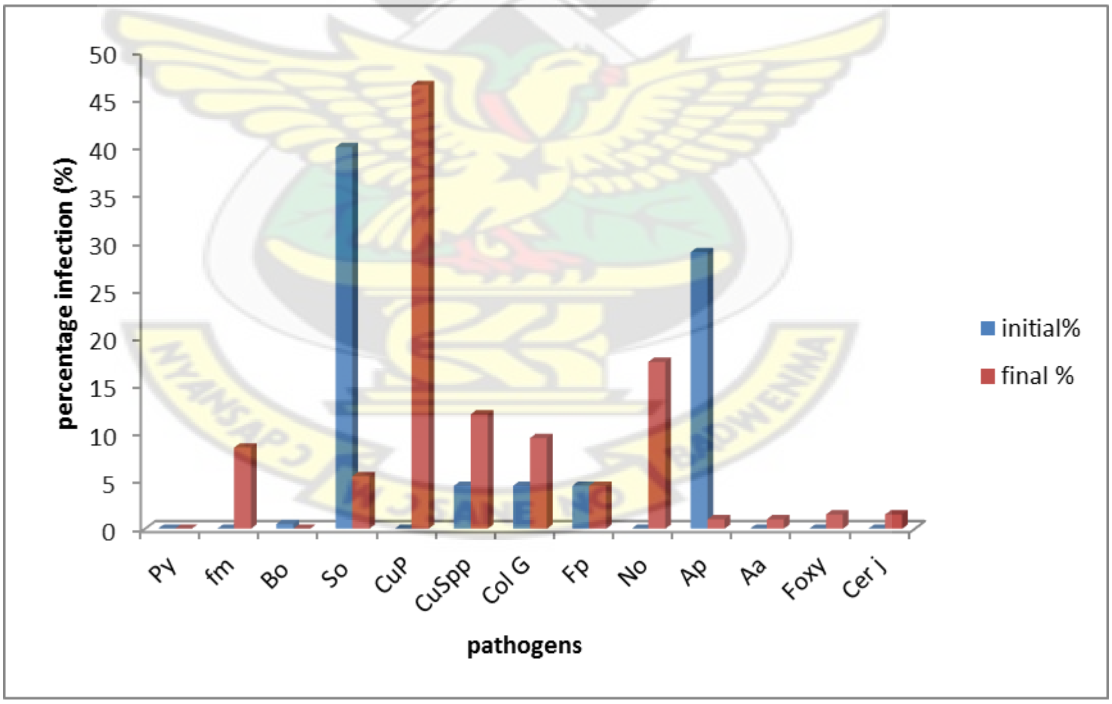

Fig 9: Initial and Final Infection Level of Pathogens Recorded on Nerica14 from the Guinea Savannah

The variety Nerica 14 from the Guinea Savannah Zone was the third variety used for the field investigations.

Fig 9 shows that *Pyricularia oryzae* was not detected (0%) in the initial readings and 0% in the final reading, even though it had a heavy infection (8.0) observation on the field. As shown in table 3, this may be due to the fact the blotter method which the least method is used in detecting pathogens was employed in this current research. *Bipolaris oryzae* had 0.5% infection level in the initial seed used for planting but none was detected from seed harvested from the field experiment (0% seed infection recorded). Rice seed borne and seed transmitted pathogens like *Sarocladium oryzae* and *Alternaria padwickii* which reduce the market value of rice (Neegaard, 1977) were also recorded in higher percentages: (40 %) in the initial seed before planting (5.5 %) and infection levels in the harvested seed of *Sarocladium oryzae*.

Curvularia pallescens, the causal organism of curvularia leaf spot desease of rice was not detected in the initial seed used for sowing but was detected at an infection level of 46.5% in the harvested seed from the field studies. This trend was also the same for *Fusarium monilliforme*, *Curvularia spp*, *Coletotrichum gloesporioides*, *Nigrospora oryzae*, *Alternaria altanata*, *Fusarium oxysporium*, and *Cercospora janseana*.

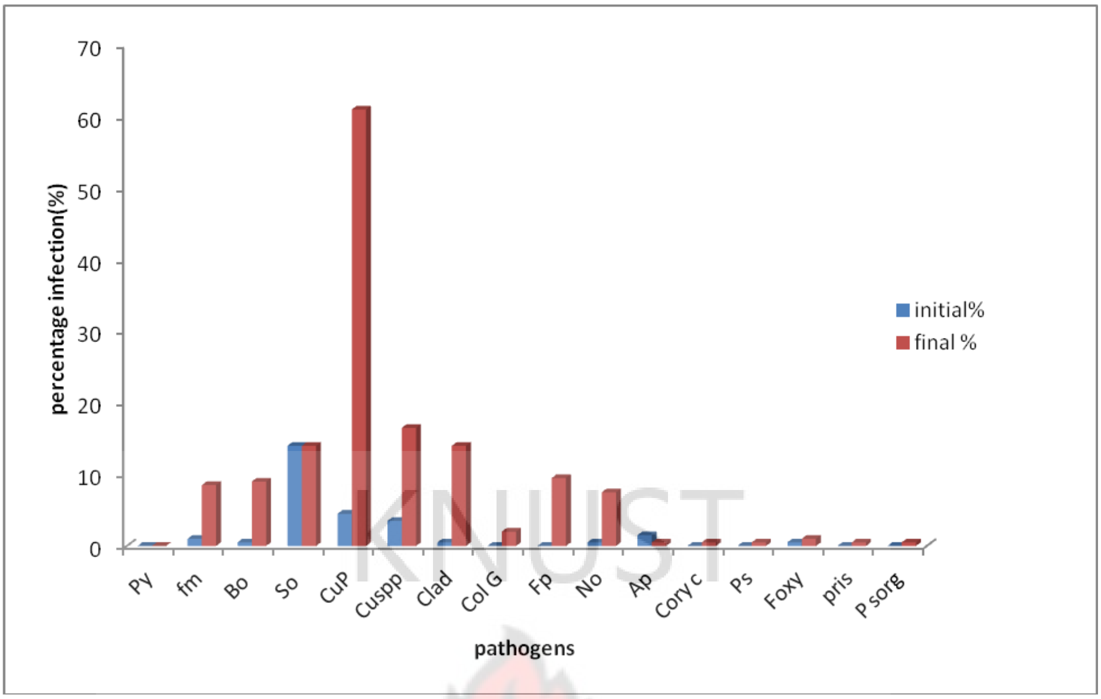

Fig 10: Initial and Final Infection Level of Pathogens Recorded on Jet 3 from the Coastal Zone

The fourth variety used for the field investigations was Jet 3 obtained from the Coastal Zone. There was 0% infection of *Pyricularia oryzae* in the initial seed used for sowing and also seed infection of 0 % was detected in the harvested seed. But from the field observation, there was 8.7 % as indicated in table 5. Similarly, a 0.5% initial seed infection level before sowing was detected for *Bipolaris oryzae* whilst the infection level increases in the harvested seeds with a 9 % detection.

For other fungal pathogens, 14 % seed infection of *Serocladium oryzae* was detected in the pre-planted seed and a resultant seed infection level of 14 % was also detected in the harvested seed from the field investigations. Other pathogens like *Fusarium monilliforme, Curvularia pallescens, Curvularia spp, Cladosporium spp, Collectotrichum gloesporioides, Fusarium pallidoroseum, Nigrospora oryzae, Corynespora cassicola, Phoma spp, Fusarium oxysporium, Pericuna spp, and phoma sorghina* had lower seed infection levels before sowing seeds but rather higher infection levels in the harvested seeds obtained from the field experiment. Other exceptions to this situation were also observed in the investigations; for example Alternaria *padwickii* was detected at a level of 1.5% in the pre-sowing seed but 0.5% seed infection was detected in the harvested seed during the studies.

Table 3: Visual observations on field incidence of rice blast disease (*Pyricularia oryzae*) and brown spot disease (*Bipolaris oryzae*) in the field experiment conducted.

Origin of initial seed	Variety	Incidence of *Pyricularia oryzae* (rice blast disease)	Incidence of *Bipolaris oryazae* (Brown spot disease)
Forest	Nerica 2	8.0	3.3
Sudan savannah	Jasmine 85	11.0	8.0
Guinea savannah	Nerica 14	8.0	0.0
Coastal Savannah	Jet 3	8.7	6.7

Key

0 = No infection

0 – 3 = Trace infection

3 – 6 = Moderate infection

6 – 12 = Heavy infection

In the Forest zone Nerica 2 variety was used in the health test. *Pyricularia oryzae* was not recorded in the initial and the final test. Also in the Forest zone *Bipolaris oryzae* symptoms were observed on the field at 3.3 score, and also recorded 5.5% when seed health test was conducted on it.

In the Sudan savannah zone Jasmine85 variety was used in the health test. It was observed that *Pyricularia oryzae* was not recorded in the initial and also in the final test of the seed test after harvest; nonetheless on the field there was heavy infection on the plant. And also with respect to brown spot disease symptoms, (*Bipolaris oryzae*) the field incidence recorded heavy infection and it reflected with what was recorded on the seeds in the laboratory (27% infection level).

In the Guinea Savannah zone, Nerica 14 was used for the health test. Rice blast disease (*Pyricularia oryzae*) was observed on the field with a heavy infection (8.0). But on the other hand when it was tested in the laboratory for the health aspect it was realized that there was no *Pyricularia oryzae* on the seeds. Also with respect to *Bipolaris oryzae* when the plant was observed on the field there were no symptoms of brown spot disease on the plant, this was confirmed also in the laboratory when a seed health test was carried on them.

In the Coastal zone Jet 3 variety was used for the health testing, it was observed that even though Pyricularia *oryzae* was not seen on the seed during the health test, there was heavy infection (8.7) of the disease symptoms on the plant in the field. With respect to *Bipolaris oryzae*, heavy disease infection was observed on the field and it also confirmed what was seen in the laboratory during the health test.

CHAPTER FIVE

5.0 Discussion

5.1 Occurrence of *Bipolaris oryzae* and *Pyricularia oryzae* from different ecological zones in Ghana

Seed health test was conducted on 33 rice seed from four ecological zones of Ghana, The Sudan Savannah recorded higher percentage of *Bipolaris oryzae* followed by the Forest Zone, Coastal Zone and Guinea Savannah Zone. This result contradicts the normal trend where dryer regions are supposed to record less pathogenic infection as compared to wetter regions. This could be attributed to the fact that the environment in the Sudan Zone where seeds were taken from (Tono and Vea dam) was very conducive for the growth of the fungus due to the availability of water and adequate sunlight.

The Guinea Savannah recorded the least percentage of *Bipolaris oryzae*, and this could be explained that the sample was taken from research fields of SARI where seeds were treated and examined well before planting and also good cultural practices were observed. Nonetheless *Curvularia spp*, *Phoma spp* which were also recorded in higher percentages might not necessarily affect yield but cause discoloration of seeds and affect their market value.

Pyricularia oryzae was absent in all the seed samples tested when the blotter method was used. This could be attributed to the fact that the blotter method was not a powerful method for detecting *Pyricularia oryzae*. But on the contrary during the field experiments visual observations of rice blast caused by *Pyricularia oryzae* and Brown spot caused by *Bipolaris oryzae* were made on the plants except with Nerica 14 variety from the Guinea Savannah zone which recorded no visual symptoms. The presence of *Pyricularia oryzae* on the field and absence during the seed health test further indicates that the blotter method was not a reliable method for health testing. It is also possible that the presence of other organisms could have overcome the presence of the Pyricularia oryzae on the seeds.

5.2 Possible Pathogens of Rice accross the Country

In the study a wide range of Fungi micro flora of rice were detected during the health test conducted on the seeds. The following pathogens were present: *Fusarium monilliforme, Pyricularia oryzae, Bipolaris oryzae, Curvularia spp, Sarocladium oryzae, Cladosporium spp, Fusarium pallidoroseum, Nigrospora oryzae, Alternaria padwickii, Alternaria altanata, Phoma spp, Fusarium oxysporium, Tricoderma spp, Curvularia lunanta, Fusarium solani*. This confirms the works earlier done by Hasany *et al.*, 1968, Kamal and Moghal 1968) on rice and wheat in Pakistan. Also earlier works have reported the above pathogens being isolated from different varieties of rice (Wahid *et al*., 1993, Khan, 1999, 2000; Javid *et al*., 2002)

5.3 Comparison of initial rate of infection of fungi to rice seed and final infection rate at harvest.

Out of the four varieties selected, for the field experiments, most of the pathogens from Sudan Savannah recorded lower initial infection rate but at harvest, pathogens recorded higher infection rate, with the exception of *Bipolaris oryzae*, *Sarocladium oryzae* and *Fusarium oxysporium* which had a relatively higher initial infection rate and finally at harvest recorded a higher infection rate. This could be attributed to the fact that the plant was now grown in a more humid environment (Southern sector) which favors the growth of pathogens. Also in the Sudan zone Pyricularia oryzae was not seen during the health test, but recorded heavy infection during the field experiment?

Also most varieties from all the zones recorded a 0% initial infection rate for most of the fungi seen, but finally at harvest recorded a relatively higher percentage. This trend confirms the postulate that not all healthy looking seeds are free from pathogens further agrees with the earlier work done by Manandhar *et al.*, in 1998. It also confirms the work done by Suzuki in 1934. This finding is very important, especially in seed certification programs where seed lots are certified on the basis of field inspection.

Curvularia pallescens, the causal organism of *Curvularia* leaf spot which was absent in all the ecological zones with the exception of Sudan Savannah zone (Jasmine 85 variety) had 8.5% initial infection rate and recorded higher infection rate at harvest. The probable explanation for such an occurrence could be that seeds used for the field experiment were latently infected with the pathogens, this is in agreement with the work earlier done by Manandhar and others in 1998, where latent infection was evident when growth of *P. oryzae* was observed on 2weeks-old seedlings, but *P. oryzae* was not seen during seed health test. This could also be explained that environment where probably the seed originally came from was not conducive for the growth of the pathogen. This confirms the disease triangle theory postulated by Agrios (2005) and Stevens, (1960).

The detection of higher fungi seed infection levels in the harvested seeds from the field experiment compared to that in the initial seeds used for the field experiment may be due to the fact that the investigations were carried out in Kumasi which falls within the forest zone of Ghana, which is associated with more humidity compared to the more hot dryer savannah in the north from where the seeds for the investigations were obtained from. Such conditions usually reduced seed infection because of the unfavourable, non-conducive environment which is a requirement for seed infections. This explains the phenomena of the disease triangle which says that, for a disease to occur it requires a conducive environment, a susceptible host and a virulent pathogen (Agrios, 2005 and Stevens, 1960). For pathogens like *Sarocladium oryzae* and *Fusarium oxysporium* which gave higher values in the initial seed before planting but ended with lower levels of seed infection in the final harvest from the field investigations, this could be attributed to a non-conducive environmental conditions for the development of those pathogens on the field.

CHAPTER SIX

6.0 Conclusion and Recommendation

6.1 Conclusions

This study showed that *Bipolaris oryzae* and *Pyricularia oryzae* are still existence in the country and that the percentage prevalence is alarming. This study brought to light that the notion that the dryer region (Sudan savannah) has less prevalence of pathogen is a thing of the past due to climatic change and the pathogens adapting to the environment. The Sudan Savannah zone recorded the highest *Bipolaris oryzae* infection percentage of 29.5% followed by Forest zone with 13.0% infection level, Coastal with 9.5% infection level and Guinea savannah recording the least percentage infection level of 0.5%.

Also, in this study *Pyricularia oryzae* was not seen in the health test but was obvious and seen on the field with Jasmine 85 variety from the Sudan savannah recording the highest value of 11.0, followed by Jet 3 variety from the Coastal zone recording 8.7, Nerica 14 from the Guinea savannah zone and Nerica 2 from Forest zone recording the same value of 8.

Moreover, Jasmine 85 from the Sudan savannah recorded the highest value (8.0) of *Bipolaris oryzae*, Jet 3 from Coastal zone and Nerica 2 from the Forest zone also recorded 6.7 and 3.3 of *Bipolaris oryzae* respectively with Nerica 14 from the Guinea Savannah recording a 0% occurrence of *Bipolaris oryzae* on the field.

Furthermore, this study discovered that, some pathogens that initially were not present were now seen in higher percentages after harvest during the seed health test. Examples were *Curvularia pallescens*, *Curvularia spp*, and *Nigrospora oryzae* in the forest zone. Also in the same zone other pathogens that were present in a higher percentage during the initial health test were now not seen or in a lower percentage after harvest. This can clearly explained that some pathogen thrive in the dryer region and some in a relatively moist region.

In the Guinea savannah zone, *Pyricularia oryzae* was not seen both in the initial seed health test and the final test, but recorded a higher value on plants in the field. This indicates that even though the pathogen was not seen on the seed the pathogen could be present and if a much sensitive method like the agar method was used it could be seen.

Finally, this study observed most of the pathogens that are pathogenic to rice but are not considered as economically important to rice, but when nothing is done about them they can become very devastating to rice and will finally affect the yield of farmers and reduce their income as most of these pathogens reduced the appeal of the rice by discoloring them. E.g. of these are: *Curvularia spp, Curvularia pallescens, Fusarium spp* and *Sarocladium oryzae*.

6.2 Recommendation

In connection to the main findings from this study the following are recommended:

- ✓ The breeders should revisit the breeding program for Blast and Brown spot disease resistant variety, so as to clean the seeds in circulation in the country as the disease might have developed a different race.
- ✓ Pathogens like *Curvularia pallescens* should be looked at before they becomes economically important to rice as the pathogen causes discoloration of the seeds to reduces the market value of the grain and the seed.
- ✓ Proper health testing must be done before seeds are certified as this study revealed that initial level of pathogen infection are different from the final

infection when seed are cultivated and also this study made it clear that the fact that symptoms do not show does not necessarily mean they are healthy.

- ✓ This work should be repeated by using a sensitive method like the agar method to authenticate the results of the present work.

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APPENDICES

Appendix i: Weather Data during the Experimental Period

Rainfall (mm)

Months	Rainfall (mm)
November, 2011	0.00
December, 2011	0.00
January, 2012	0.00
February, 2012	0.00
March, 2012	0.11
April, 2012	0.21
May, 2012	0.29
June, 2012	0.35

(SOURCE; CSIR- CROPS RESEARCH METEOROLOGY DEPARTMENT)

Appendix ii Legend of Pathogens

Aa = Alternaria altanata

Ap = Alternaria padwickii

Bo = Bipolaris *oryzae*

Clad = Cladosporium spp

Colg = Colletotrichum gloesporioides

Cory = Corynespora casiicola

CuP = Curvularia pallescens

Cuspp = Curvularia spp

Fm = Fusarium monilliforme

Fp = Fusarium pallidoroseum

No = Nigrospora oryzae

Pris = Pericuna spp

Ps = Phoma spp

Psorg = Phorma sorghina

Py = Pyricularia oryzae

So = Serocladium oryzae

Appendix iii: Ecological Zone, Communities and Varieties of Rice Taken From There.

Ecological zone	Community	Variety
Sudan Savannah	Navrongo (Tono and Vea Dam)	Whiter, four Jasmine 85, Tox3107, Tox 3377, Two unknown.
Guinea Savannah	Tamale (Nyankpala)	Nerica 14
Forest Zone	Nobewam	Nerica 1, Nerica 2 and Two Jasmine 85
	Ejura	Mr. harry, Jasmine 85, Nerica 4, Nerica 9, Nerica 14 and Congo rice.
Coastal Savannah	Afife	Unknown variety
	Asutuare	Unknown, five Jet 3, Aromatic short A and B, Jacoprice
	Ashiaman	Two Jasmine 85 and Unknown

PLATES

Plate 1 POTS ARRANGEMENT IN RCBD IN THREE REPLICATION

Plate 2 FULLY RIPED RICE

Plate 3 BROWNISH COLOURATION ON THE SEEDS

Plate 4: SEED ARRANNGED IN PETRI DISH TO BE INCUBATED

Plate 5 PICTORIAL VEIW OF *Bipolaris oryzae* UNDER THE MICROSCOPE

Plate 6: RSHT AT FUMASUA SEED PATHOLOGY LAB.