International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Identification and Control of Seed Born Fungal Parhogens of Maize Seed (*Zea mays*) Using Plant Extracts

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Abstract: ***Seed-borne fungal pathogenic infections of maize (Zea mays L.) were studied using seed samples collected from four States in North-east Nigeria. The deep-freeze blotter method was used to detect fungal pathogens on the seeds. Five fungal pathogens associated with the rot identified based on their colonial and morphological characteristics and their severity of occurrence were Aspergillus ustus (83.3%), Fusarium solani (75%), Aspergillus flavus (83.3%), Rhizopus stolonifer (66.7%) and Botrytis cinerea (100%). Aqueous extracts of Cymbopogoncitratus (lemongrass), Vernomiaamygdalina (bitter leaf) and Azadirachtaindica (neem) seeds were tested for their inhibitory activity against seed-borne fungal pathogens in the infected seeds using 20%, 40% and 60% (w/v) concentrations which suppressed their mycelial growth. Statistically, the result showed it was significant with analysis of variance (P= 0.0001).The effect of concentration was proportional to inhibition as the highest inhibition is at 80%. Neem seed extract exhibited the best control, reducing infection of F. solani.***

Keywords: Isolation, Identification, Fungi, Control and Plant Extract

1. Introduction

Maize (Zea mays L.) belongs to the tribe Maydae, family Poaceae and was originated in Mexico and Central America. It possesses somatic chromosome number of 20, a genome size of 2.3 gigabase and more than 32,000 genes (Schnable et al. 2009). Maize grows well in various agroecologies and is unparalleled to any other crop due to its ability to adapt in diverse environments. It has emerged as a crop of global importance owing to its multiple end uses as a human food and livestock feed and serves as an important component for varied industrial products. Besides, maize serves as a model organism for biological research worldwide. Globally, about 1016.73 million metric tonnes of maize is produced every year the highest among major staple cereals (FAOSTAT 2015). A major portion of maize produced worldwide is used for animal consumption as it serves as a vital source of proteins and calories to billions of people in developing countries, particularly in Africa, Mesoamerica and Asia (Shiferaw et al. 2011). Further, it is a source of important vitamins and minerals to the human body. Along with rice and wheat, maize provides at least 30 % of the food calories to more than 4.5 billion people in 94 developing countries. Maize provides over 20 % of total calories in human diets in 21 countries and over 30 % in 12 countries that are home to a total of more than 310 million people (Shiferaw et al. 2011). At present, the developed world uses more maize than the developing world, but forecasts indicate that by the year 2050, the demand for maize in the developing countries will double owing to the rapid growth in poultry industry, the biggest driver of growth in maize production (Rosegrant et al. 2009 ;Prasanna 2014). Improved maize hybrids with substantial increase in production per unit area are required to feed the ever growing population. Further, with changing climatic conditions, several new biotic stresses have emerged and minor disease and insect pests have become more prevalent and started inflicting more damages. Among abiotic stresses drought, heat and water logging are the major one and their simultaneous occurrence are now more frequent than ever. Malnutrition caused by deficiency of minerals and vitamins, especially iron, zinc and vitamin A, has been identified as one of the most important problems that require urgent attention worldwide (Bouis et al. 2011 ; Gupta et al. 2015). Germplasm including wild relatives and landraces possess enormous potential as genetic resource for harbouring important and novel alleles/genes. These valuable germplasm can be systematically and effectively utilized in the crop improvement programmes worldwide, to develop high yielding and nutritious maize with resilience to biotic and abiotic stresses. Farmer-saved seed planted by most farmers is infected with seed-borne fungal pathogens (Tagne*et al*., 2008). Efforts have been employed to reduce crop losses due to diseases in different parts of the world to ensure higher yield which include breeding for disease resistant varieties, the use of chemicals in reducing seed-borne pathogens and the use of biological control agents to reduce plant pathogens (Abdulsalam, 2011). Chemical methods involving seed treatment with fungicides have been employed to improve germination, vigor, crop establishment, crop stands, and yield (Chapman and Harris, 1981). However, indiscriminate use of chemicals for controlling plant diseases has resulted in environmental pollution and health hazards (Chapman and Harris, 1981). Moreover, haphazard use of chemicals breaks down the natural ecological balance by killing beneficial soil microbes, in some cases farmers in developing countries cannot afford high cost of chemical pesticides as a result of these problems there is an increasing attention towards the development of safer methods of disease control (Neergaard, 1988). This has resulted in trials to botanical fungicides for the control of seed-borne pathogens of food crops that are effective and have little or no adverse effect on the environment (Abdulsalam, 2011). In this work, therefore, an attempt will be made to evaluate botanical products for the

control of seed-borne fungal pathogens of maize seeds of farmers in the north eastern states. Certain botanicals with reported fungicidal properties will be investigated to determine their efficacy in reducing seed-borne fungal pathogens of farmer-saved maize seeds.

Pests and diseases are also some of the problems of maize production in the world. According to Neergaard (1988), *Drechsleraturcica*, northern leaf blight, caused considerable losses in 1970 in maize amounting to approximately one billion dollars in maize-growing areas of United States. In Africa *Diplodia*spp and *Fusarium* spp caused losses of more than 10 per cent in Nigeria and Rhodesia, and less than 10 per cent in Tanzania (Neergaard, 1988). In developing countries with poor farming practices seed-borne pathogens are among the major constraints as two-thirds of total losses of maize due to diseases are caused by seed-borne fungi, and of these (*Diplodia*and *Fusarium graminearum)* account for about 25 per cent and *Drechslera*spp for almost 20 percent (Neergaard 1988).To set up a healthy field that will give a good yield, healthy seeds are needed because seed-borne pathogens bring about poor germination, poor vigour, poor crop establishment and crop stands, non-healthy plants, lodging and poor yield (Wiese, 1984). Although a lot of studies have been conducted on pest and disease control of maize using chemical and biological methods, there is still the need to develop a method that is environmentally friendly. It is therefore important to investigate into fungal seed-borne pathogens of farmer-saved seed maize (*zea mays* L.) collected from north east region and efficacy of plant extracts in controlling the pathogens.

Neem seed oil protected the seeds of chickpeas against the serious fungal pathogens *Rhizoctonia solani*, *Sclerotiumrolfsii* and *Sclerotiniasclerotior um*it also slowed the growth of *Fusarium oxysporum*but did not kill it (NRC, 1992)..

Aqueous extract of lemongrass is effective against seed-borne fungal pathogens of melon (Bankole and Adebanjo, 1995).

Aqueous extract of bitter leaf was also effective against seed-borne fungal pathogens of melon (Bankole and Adebanjo, 1995).

The aim of this study was to determine the efficacy of the extracts of *Azadirachtaindica*, *Cymbopogoncitratus* and *Vernoniaamygdalina* in *in-vitro* control of fungal isolates from maize rot. The specific objectives of this research were:

1) To investigate the incidence and level of pathogen rot on seeds in the study area.
2) To determine the efficacy of three plant extracts: neem (*Azadirachtaindica*), lemongrass (*Cymbopogoncitratus*) and bitter leaf (*Vernoniaamygdalina*. *Del*) for reduction or control of seed-borne fungal pathogens of seed maize.

Seed-borne diseases are controlled by seed treatment practices; seed treatment is the oldest practice in plant protection (Nelson *et al*., 1993). The origins of seed treatment can be traced to the 18th century with use of brine for the control of cereal smuts (Neergaard, 1988). The modern era of seed treatments began with the introduction of organo-mercury fungicides in which were widely used for several decades (McGee, 1995).

Seed treatments can be classified as physical, biological, chemical and botanical, regardless of type, successful seed treatment practices must satisfy the following biological requirements (McGee, 1995).

1) Consistently effective.
2) Safe to operators during handling and planting.
3) Safe to wildlife.
4) Compatible with other materials used on seeds.
5) Should not produce harmful residues on plant or soil.
6) Chemical or biological methods should have desirable qualities with respect to application and retention on the seeds.

2. Materials and Methods

The seed samples were collected from four states of the North-east which comprises of Adamawa, Borno, Gombe and Taraba respectively.

Two hundred (200) seeds each were collected from the four states. A survey was conducted and farmers who produce maize were identified. From each state two locations (town/village) popularly known for production of maize crops was covered, and from each location two farmers were identified for the seed collection. The seed samples were collected after harvested and stored. The seed samples were labeled and put in plastic bags and tightly sealed and stored. The seed samples were taken to plant science laboratory for analysis as was done using the method of Mashi and Chimbekujwo, (1999). Incidence of rot was determined using simple percentage as was done by Joseph (2011).

$$\text{Percent Frequency} = \frac{Total number of diseased \quad maize \ seeds}{Total number \quad of \ maize \ seeds} \times 100$$

Seed health analysis determined fungal pathogens on the seed samples. The seed samples wereanalysed using the Deep-freeze blotter method (Mathurand Kongsdal, 2003) as described in sections below. The design for the analysis was Completely Randomized Design (CRD).

On a clean and disinfected working table the required number of sterilized 90mm diameter Petri dishes per sample were poured on PDA.

After incubation for 7 days, the dishes were removed and arranged serially. Moving from one Petri dish to the other, each seed was examined under a stereomicroscope. Habit characters of each fungus was observed and used in identifying the fungi that grow on seeds with the help of identification scheme of Snowdon (1990). Slide preparations of fruiting structures and spores of certain fungi were examined under compound microscope to further confirm their identities by consulting mycological literature and experienced seed health analysts adopting the method of Mashiand Chimbekujwo (1999).

The seeds were surface sterilized with 0.2 % sodium hypochlorite solution for 3-5 minutes and rinsed in three

changes of sterile distilled water, Petri dishes used were sterilized in an oven at 160^{o} for 1 hour, the inoculating needles was sterilized by flaming over a Bunsen burner and cooled by dipping them into ethanol, the media was autoclaved in an autoclave for 15 minutes at 101 Lbs pressure at 121^{o}C and allowed to cool. The incubation of the organism was done in a sterile environment in the inoculation chamber, the table in the inoculation chamber was wiped with 95% ethanol and then ultra violet (UV) light was switched on for 30 minutes before carrying out the inoculation

Determination of rot severity

Observation for level of fungal growth and seed rot was made on daily for 7 days and results were recorded, percentage rot was also determined using modified Echemede, (1985) visual scale of 1-5.

Isolation of fungal pathogen

The main fungal pathogens that were identified during the examinations were sub-cultured on to potato dextrose agar (PDA) as done by Smith & Onion (1983) into pure cultures for preservation for further studies. Sub-culturing was repeated until pure isolates of single species was obtained on PDA.

Pathogenicity test

To ascertain the pathogenicity of the various fungi that were isolated, the approach of Mashi and Chimbekujwo (1999) was employed.

Medium for isolation and identification

The medium that was used for this study was potato dextrose agar (PDA) and was prepared adopting the method of Smith and Onion (1983) below.

Preparation of potato dextrose agar

In preparing the media, thirty nine grams (39g) of potato dextrose agar (PDA) powder was placed in five liters conical flask, 100mls of distilled water was added, mixed and completely dissolved the powder, the supernatant was carefully poured into sterile conical flask which the mouth was covered with sterile cotton wool and then wrapped with aluminum foil before autoclaving at 120^{o} C for 15 minutes at 101 lbs pressure (Smith and Onion, 1983).

Preparation of Plant Extracts

Fresh leaves of lemongrass (*Cymbopogoncitratus D.C*) and bitter leaf *Vernoniaamygdalina Del*) andneem (*Azadirachtaindica. A*) Seeds were collected as treatment materials for the experiment. Aqueous extracts of each of the plant materials were prepared. One gram of a stock solution was dissolve in 20,40 and 60 mls of distilled water respectively and obtained the required concentrations.

3. Results and Discussion

The aim of this study was to investigate the incidence of seed-borne fungal pathogens in seed maize collected from the four states of the north east which comprises of Adamawa, Borno, Gombe, and Taraba respectively and to determine the efficacy of plant extracts in controlling seed-borne fungal pathogens in maize. The occurrence of the various fungal pathogens varied in the four states. The frequency of pathogens showed *Aspergillus ustus*had the highest frequency of 43.27% in all the states visited, followed by *Fusarium solani* with 26.21%, *Aspergillus flavus* had 21.67%, *Botrytis cinerea* had 6.32%, while *Rhizopus stolonifer* had the least frequency of 2.54%. The frequency of fungi isolated from Adamawa state showed *Aspergillus ustus*and *Aspergillus flavus* had the highest frequency of 31.58%, followed by *Fusarium solani* with 26.32%, while *Rhizopus stolonifer* and *Botrytis cinerea* had the least frequency of 5.26%. Borno state showed *Aspergillus ustus*with the highest frequency of 46.34%, followed by *Fusarium solani* and *Aspergillus flavus* with 24.39%, *Rhizopus stolonifer* with 4.88%, while there was no presence of *Botrytis cinerea*. In Gombe state, *Aspergillus ustus*had the highest frequency of 40.00%, followed by *Fusarium solani* with 30.00%, *Botrytis cinerea*with 20.00%,*Aspergillus flavus* had 10.00%, while there is no presence of *Rhizopus stolonifer*. *Aspergillus ustus*had the highest frequency of 55.17% in Taraba state, followed by *Fusarium solani* with 24.14%, *Aspergillus flavus* with 20.69%, while there was no presence of *Rhizopus stolonifer* and *Botrytis cinerea.* Treatment with neem seed extract reduced the incidence of the fungal pathogens significantly at all soaking periods. It also had increased effect on germination. Moreover, all the three plant extracts had increasing effect on germination except for 24 hours of treatment.

Table 1: Frequency (%) of Fungal Pathogens of Maize Seed Rot in North-east Nigeria

Number of isolates per states (%)					
Fungi isolated	ADM	BOR	GOM	TAR	Average
Aspergillus ustus	31.58	46.3	40	55.17	43.27
Fusarium solani	26.32	24.4	30	24.14	26.21
Aspergillus flavus	31.58	24.4	10	20.69	21.67
Rhizopus stolonifer	5.26	4.88	-	-	2.54
Botrytis cinerea	5.26	-	20	-	6.32
Total	100	100	100	100	100

Key:
ADM: Adamawa
BOR :Borno
GOM: Gombe
TAR: Taraba
- : Nil

Interaction of inhibition effect of plant extracts and pathogens

The interaction effect of the inhibition of neem seed oil on the pathogens showed that the effectiveness was in this order *B. cinerea*(0.70) being the highest followed by *A. ustus*(0.59), *R. stolonifer* (0.56), *A. flavus* (0.44) and the least is on *F. solani* (0.33). The interaction effect of the inhibition of bitter leaf on the pathogens showed that the effectiveness was in this order *R. stolonifer* (1.12) being the highest, followed by *B. cinerea*(1.00) *A. flavus* (0.67), *A. ustus*(0.46), and the least is on *F. solani* (0.45). The interaction effect of the inhibition of lemon grass on the pathogens showed that the effectiveness was in this order *B. cinerea*(1.22) being the highest, followed by *A. ustus*(0.74) *R. stolonifer* (0.70), *A. flavus* (0.49), and the least is on *F. solani* (0.46) (Table 2).

Table 2: Interaction of Inhibition Effects of Plant Extracts and the Pathogens

Source	A. ustus	F. solani	A.flavus	R.stolonifer	B.cinerea
Neem	0.59	0.33	0.44	0.56	0.7
Bitter Leaf	0.46	0.45	0.67	1.12	1
Lemon Grass	0.74	0.46	0.49	0.7	1.22

4. Conclusion and Recommendations

In conclusion, this study had shown that neem seed extract have the potential in the protection of seed maize rot by fungi. Therefore due to the chemical control of diseases is environmentally harzadous and very expensive, this inexpensive, non-harzadous and biodegradable plant material could be used as an alternative way of reducing and controlling rot diseases by farmers to increase maize production.

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