Comparative Study on Efficacy of Fungicide and Extracts of *Lantana camara*, *Allium cepa* and *Calotropis* sp. against Seed borne Mycoflora of Sorghum

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ABSTRACT

Sorghum is being attacked by several fungal pathogens; many of them are seed-borne. These fungi were detected from the seeds by moistened blotter paper and potato dextrose agar media methods as recommended by the ISTA. Fungi such as *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Colletotrichum graminicola*, *Fusarium moniliforme, Helminthosporium sorghicola, Mucor* spp., *Penicillium oxalicum* and *Rhizopus* spp. were isolated from the sorghum variety HACNL. Studies on different seed treatments of fungicide and botanicals were carried out with an objective to find out effective treatment to control of seed borne fungi. Effect of ridomil and botanicals on the incidence of seed-borne fungi and their effect on seed germination were evaluated. The seed treatment by the fungicide showed that ridomil increased the germination percentage while seed mycoflora were reduced and also noticed the inclined intensity of covered smut. Efficacy of *Lantana camara* and *Calatropis* sp. were found good in controlling the seed-borne fungi associated with sorghum seeds *in vitro*. In the field, Ridomil was more effective to control the covered smut as compared to the other treatments. Overall minimum disease incidence and severity found in using ridomil *i.e* 4.90 and 7.01 respectively, while maximum in the fields of farmers' *i.e.* 22.72 and 20.31. In case of botanical fungicides *Calotropis* sp showed more effective followed by the extracts of *Allium cepa* and *Lantana camara*. Thus, the seeds should be treated invariably by less phytotoxic fungicide and botanical fungicide agents to reduce the seed-borne mycoflora of *Sorghum*.

Key words: *Sorghum bicolor*, Seed-borne fungi, Ridomil, *Lantana camara, Allium cepa* and *Calotropis* sp.

INTRODUCTION

Sorghum (*Sorghum bicolor L.*) is the most important crop of African continent being the chief source of staple food for about two third of this continent population. In Eritrea the major food crop is sorghum occupies 45 percent of the total cultivated area divided for food crops. Some of the seed borne fungi cause deterioration of seeds (Bhowmik, 1969). In Anseba region till 2001 the area cultivated under sorghum and groundnut was about 20,178 hectares with a production of about 8442.1 tones (Anon, 2002). Characterization of sorghum seed such as seed germination, moisture content, seed discolouration and prevalence of seed-borne fungi have long been known to be influenced by various factors during storage. It has been estimated that storage fungi cause about 5% annual loss of the total grains stored. However, the losses higher (15-25%) in underdeveloped countries like Eritrea where high humidity, rainfall and temperature coupled with poor storage conditions exists (Salunkhe *et al*. 1985).

Seed is the basic, vital and central input for the successful agriculture, also governs the impact of other closely inputs to a great extent. Most of the food crops are propagated only through seed. Seeds play a vital role in the transmission of plant pathogens causing plant diseases. Seed also is a passive carrier of the pathogen, especially in developing African countries, where effective seed act is not in use.

The great majority of the farmers in Eritrea operate agriculture on a small scale. Despite much local and regional diversity, they share a number of important characteristics. Most small farmers operate either as independent land owners or under rental arrangement allowing them to make production decisions. They depend mainly on family labour supply. Small-scale farmers are less likely to use capital for commercial inputs like fertilizers, pesticides and agricultural equipments and they tend to use credit for consumption needs rather than for purchasing farming inputs (Syed Danish 2013).

Seed quality attains more significant in view of the emerging biotic and abiotic stresses, issues related quality, phytosanitary measures, market demands and emerging food needs. Pathogenic fungi alone cause nearly 20% reductions in the yield of major food and cash crops (Agrios, 2000). One-third global agricultural production is reportedly destroyed each year by different pests and diseases. To avoid the implication of yield losses due to plant diseases, variety of control measure presently are in use.

The uses of agrochemicals to control seed borne pathogen are well documented by several researchers, however, the use of botanicals based formulations are not being studied in Eritrean conditions. Hence, the study was aimed to conduct the efficacy of chemical fungicides and botanical formulations in regard to fungal count populations, seed germination and disease intensity of sorghum *var*. HACNL (Hamelmalo Agricultural College New Line).

MATERIALS AND METHODS

The experiment was conducted in the research laboratory and field of the Department of Plant Protection, Hamelmalo Agricultural College, Eritrea, in 2014 kharif season which is located at 15^0 53'

N and 38^0 $66^{'}$ E at an altitude of 1280 msl and has an average temperature of 26°C. It receives annual average rain fall of 400 mm.

Seed samples were collected from the progressive farmers of the region. Isolations were made from 200 infected seeds of the sorghum *variety* HACNL (Hamelmalo Agricultural College New Line). Qualitative and quantitative estimation of fungi was done under asceptic conditions by employing Moistened Blotter Paper and Potato Dextrose Agar (PDA) methods (ISTA, 1985). Inoculated plates were incubated for 5 days at 22-25^{o}C under alternating cycles of light and darkness. After 5 days of incubation fungal species found growing on the surface of seeds were identified using standard references (Mathur and Kongsdal, 1998) and their percentage frequency (PF) of occurrence of pathogen was calculated by applying the following formula (Syed *et al.* 2012):

PF= [(No. of seeds on which fungus appear / Total No. of seeds) X100]

Relative abundance by Neha Pathak and Razia (2013) of the fungi was calculated by the following formula:

[(Total No. of colonies of a fungus on seed/ Total No. of colonies of all fungus) X100]

Effect of seed dressing fungicide and botanical fungicides on germination of sorghum seeds *variety* HACNL was studied in *in-vitro* conditions. A total of 200 seed taken from sorghum variety and were treated with three botanical pesticides such as leaf extract of *Lantana camara,* cloves extract of *Allium cepa* and latex of *Calatropis* and Ridomil at 2 gm/ml per kg seeds. For control no treatment was applied. The treatment applied on seeds in conical flasks separately. All treated seeds were placed @ 15 seeds / per plate on Whatman No.1 filter papers in 9 cm diameter Petri plates, moistened with distilled water. Plates were incubated for 5 days at 22-25^{o}C under 12 hour alternating cycles of light and darkness. At the end of the incubation period, fungi growing out from seeds were examined, identified and their percentage frequency (PF) and relative abundance were calculated. Similarly the treated and untreated seeds were placed in PDA media and plates were incubated in the same way.

The treated with botanicals and fungicide seeds were sown in the field of Hamelmalo to observe the rate of disease incidence and severity due to pathogens. At maturity stage of sorghum crop diseases were recorded. The major disease covered smut was assessed on 21 randomly selected panicles 2 weeks after physiological maturity (black layer formation) at fifteen days interval. The smut incidence calculated as:

Disease incidence (%) = (Number of infected plants/Total number of plants) x100.

The smut severity was evaluated and determined by a rating scale of 1-9 (Thakur and King, 1988), where 1 = no visible symptoms, 3 = 1-10% of symptoms, 5 =11-35% of symptoms, 7 = 36-50% and 9 = over 50% of grains replaced by smut sori, was used to score percentage of smut-infected florets in a panicle.

Disease severity (%) = {[B/ (Ax9)] x100}

Where, B= total disease rating, A= total number of samples and 9= maximum grading scale.

The attempt to collect and interpret data of research trial under laboratory and field conditions have been done by Web Based Agricultural Statistics Software Package in the present studies, Analysis of variance method was applied for drawing conclusion from the data. The calculated value was compared with tabulated value at 1 and 5% level of probability (Fisher and Yates, 1968).

RESULTS AND DISCUSSION

The pathogen may be externally or internally seed-borne or associated with seeds as contaminant. In Moistened Blotter Paper method the fungi isolated and identified were *Alternaria alternata, Aspergillus flavus, Aspergillus niger, Colletotrichum graminicola, Fusarium moniliforme, Helminthosporium sorghicola, Mucor* sp., *Penicillium oxalicum* and *Rhizopus* spp. Among these, nine were isolated from the untreated seeds; whereas, five fungi were isolated and identified from the Potato Dextrose Agar method.

The highest percentage frequency and of *Alternaria alternata* was found both in Moistened Blotter Paper method (52.00%) and Potato Dextrose Agar media (32.33%). The same trend was observed in PDA method also. *Fusarium moniliforme* showed least frequency and relative abundance in both culturing methods. *Helminthosporium sorghicola, Mucor* spp., *Penicillium oxalicum, Rhizopus* spp. are not grown in both in blotter and PDA media. The percentage of seed germination was higher in PDA 53% than the blotter method 49% (Table-1).

Table 1: Frequency and Relative abundance of seed mycoflora of sorghum variety HACNL on Moistened Blotter Paper and Potato Dextrose Agar media

Isolated Fungi	Moistened Blotter Paper		Potato Dextrose Agar	
	Frequency percentage*	**Relative abundance***	**Frequency percentage***	**Relative abundance***
Alternaria alternate	52.00	32.00	32.33	19.00
Aspergillus flavus	34.93	14.00	14.33	8.08
Aspergillus niger	41.35	20.66	21.63	14.67
Colletotrichum graminicola	29.75	12.31	31.43	8.09
Fusarium moniliforme	10.50	3.00	9.51	2.43
Helminthosporium sorghicola	51.50	20.46	-	-
Mucor spp.	39.33	10.39	-	-
Penicillium oxalicum	12.85	4.34	-	-
Rhizopus spp.	21.28	5.69	-	-
CD(0.01)	2.43	1.49	1.61	1.48
CD(0.05)	1.77	1.09	1.18	1.09
Percent seed germination	49		53	

*(-) absence of fungus; *: each value is an average of three replications*

The efficacy of different disinfectants has been tested against the sorghum mycoflora @ 2g/kg seeds in laboratory. Ridomil gave the best results and significance increased the seed germination and reduced the mycoflora as compared to other disinfectants and control. *Alternaria alternata, Aspergillus niger, Aspergillus flavus, Fusarium moniliforme, Penicillium oxalicum, Helminthosporium sorghicola, Mucor* sp., *Rhizopus* spp. and *Colletotrichum graminicola* (Plates 1-10) isolated and identified from treated seeds with ridomil, however these fungi showed a very lower frequency values. Seed borne fungi can be controlled by chemical treatment.

Plate 1: *Alternaria alternata*

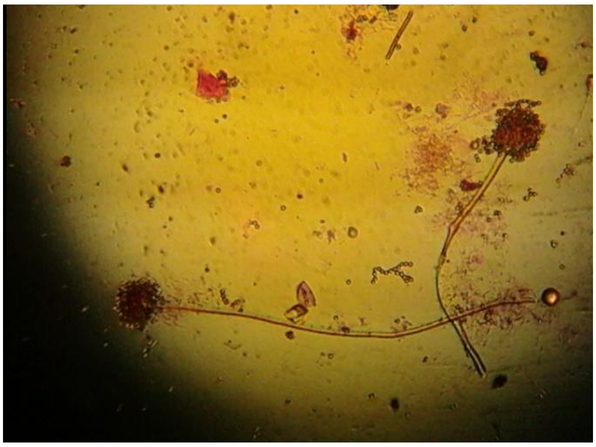

Plate 2: *Aspergillus niger*

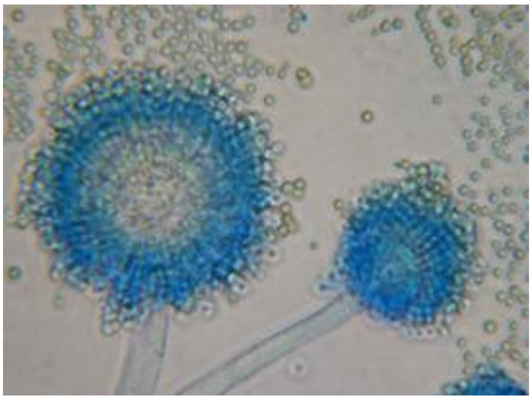

Plate 3: Aspergillus flavus

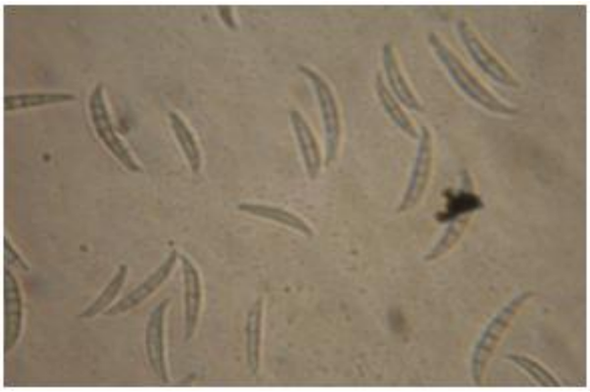

Plate 4: *Fusarium moniliforme*

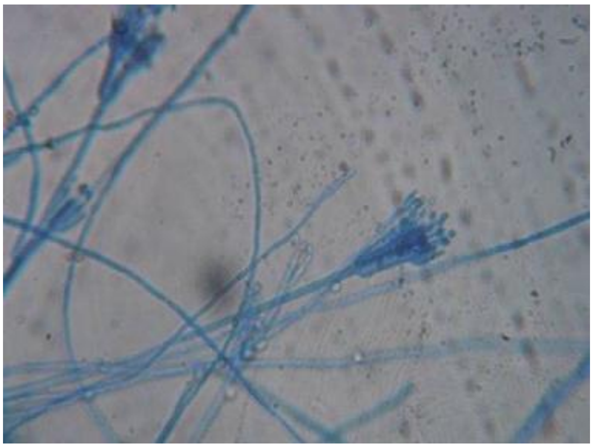

Plate 5: *Penicillium oxalicum*

Plate 6: *Mucor* sp.,

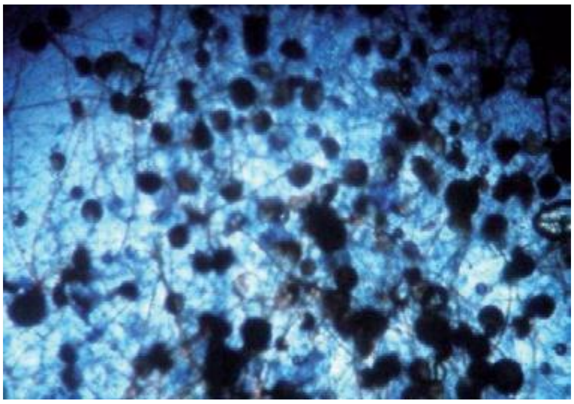
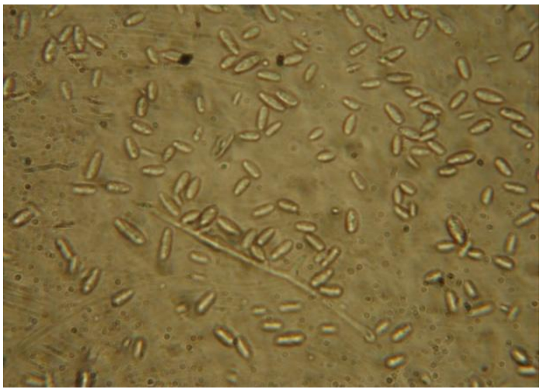

Plate 7: *Rhizopus* spp. Plate 8: *Colletotrichum graminicola*

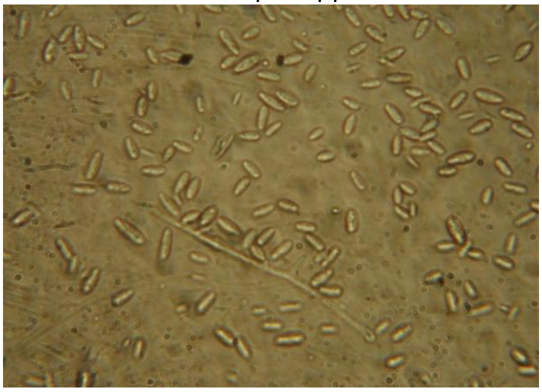
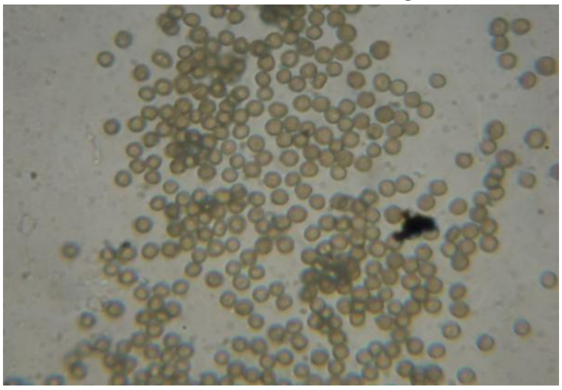

Plate 9: Plate 10: *Sphacelothica sorghi* spores

The effect of fungicide (ridomil) and botanicals on fungal colony formation is shown in fig 1. *Catotropis* sp. had shown more suppressive effect on all fungal flora growth, while the least mycoflora formation recorded for ridomil treatment. Extracts of *Lantana camera* leaves had also shown on the growth of mycoflora on par with *Allium cepa*. Untreated seeds showed highest colony formation units especially it was recorded abundantly in the growth of *A. alternate, Helminthosporium sorghicola, Aspergillus niger and Mucor* spp.

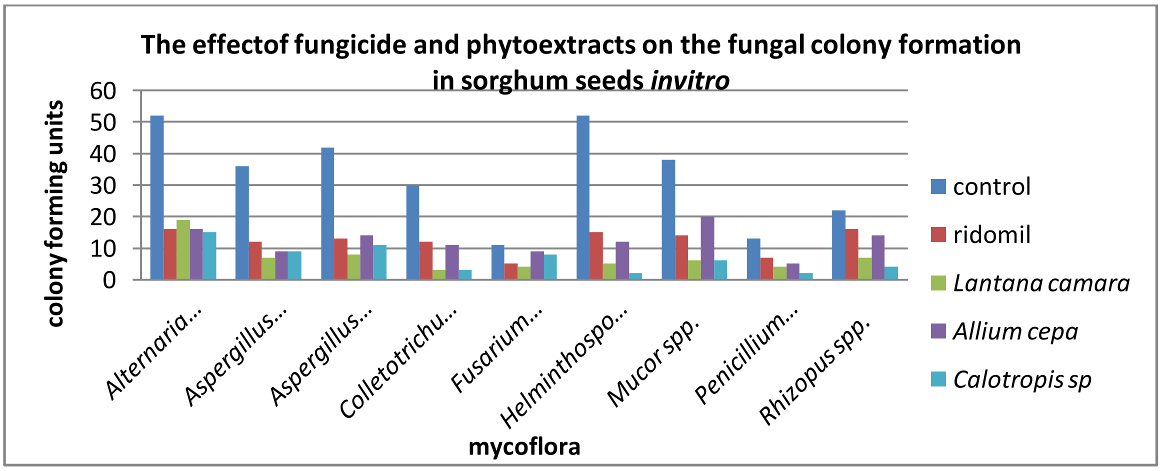

Fig.1 The Effect of Control, Ridomil, *Lantana Camera*, Garlic and *Calotropis* on the Colony Formation of Mycoflora in Sorghum Seeds *in vitro*

It is estimated from the table 2 that the germination of seed had also shown increment in sorghum. Ridomil and *Lantana camara* reduced the percent frequency of seed borne fungi. Seed germination was found 92% in Ridomil treated seeds followed by *Allium cepa* extract (85%), *Lantana camara* (80%) and *Calotropis* sp. (79%)

Table 2: Percentage of Seed Germination in Treated Seeds with Fungicide and Botanicals

Treatments	Percentage of seed germination
Lantana camara	80
Allium cepa	85
Calotropis sp	79
Ridomil	92
Control	71

The results of the incidence and severity of sorghum covered smut are shown in table 3. The results of the different treatments in case of disease incidence at 15 days frequency were observed highest in farmers' field 15.23, 21.69 and 31.23 weekly as compared to control plot i.e 12.34, 18.32 and 24.32. Lowest incidence found in treated with Ridomil @ 2g/kg seed i.e. 4.21, 5.21 and 5.29 at fifteen days interval.

The severity of the disease was 15.45, 21.23and 24.25% in farmers' field of Hamelmalo village being the highest at seven days interval whereas, 12.69, 15.39, 17.23 % in control. Minimum severity recorded in treatment ridomil i.e. 6.45, 6.78, 7.81 at fifteen days interval.

Table 3: Percentage of disease incidence and severity of covered smut on Sorghum at fifteen days interval per 21plants

Treatments	Disease Incidence **(DI)** and Disease Severity **(DS) Days** after					
	15		**30**		**45**	
	D I	**D S**	**D I**	**D S**	**D I**	**D S**
Lantana camara	6.23	11.23	7.69	14.23	8.27	15.21
Allium cepa	6.25	10.24	7.23	11.21	7.98	12.67
Calotropis sp	5.23	9.23	8.32	9.85	8.41	11.23
Ridomil	4.21	6.45	5.21	6.78	5.29	7.81
Control	12.34	12.69	18.32	15.39	24.32	17.23
General field	15.23	15.45	21.69	21.23	31.23	24.25
CD=0.05	1.22	1.23	1.98	1.29	2.05	1.78

The average overall disease incidence was 22.72 and 18.33 in farmers' field and control respectively. The average disease incidence was notices for ridomil 4.90 followed by *Allium cepa* 7.15, *Calotropis* sp. 7.32 and *Lantana camara* 7.40. The overall average disease severity was observed for ridomil treated seeds 7.01 followed by *Calotropis* sp., *Allium cepa* and *Lantana camara* i.e. 10.10, 11.37 and 13.56, respectively. In all treated seeds the disease incidence is lower than the severity except in control and general field conditions. Ridomil treated seeds showed least disease incidence and severity while it was high in both control and general field conditions (Fig.2).

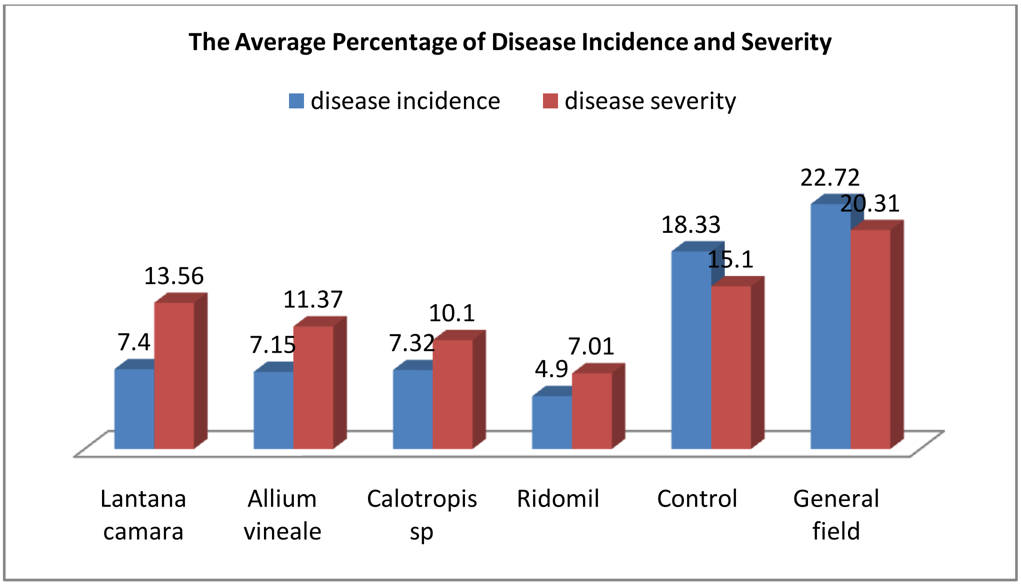

Fig.2 Percentage of disease incidence and severity of covered smut on Sorghum at seven days interval

Panicle/Head diseases were observed throughout the study in all treatments (Plate 11). The prevalence of occurrence of covered smut might be attributed to the biology of the fungal pathogen (*Sphacelothica sorghi*) which transmits covered smut, overwintering primarily as teliospores germinating with the seeds, which is systemic. The results are agreement with those of earlier works by Leather (1958) who reported that smut infection was estimated at 0-60%, with 10% as an average. 'Crop losses' in Nigeria due to covered smut of sorghum is more than seven percent (Dahlburg and Hash, 2000). Ngugi et al. (2002) assessed the disease intensity due to covered smut as more than 30% in 42 to 43% of the fields surveyed. The results are also in accordance with the report of earlier works by Nutsugah et al. (2007) and Kutama et al. (2010).

Plate 11: Filed view of sorghum in HAC research field. (Inset picture: Covered Smut of *Sorghum*)

Extracts of *L. camara, Calotropis*, *A. cepa* have also been quite effective in controlling the seed borne mycoflora of sorghum against control and in general field conditions. The active constituents reported in all parts of these plants but mainly present in leaf of *L. camara,* latex of *Calatropis* and cloves of *A. cepa*. Different parts of these plants are used as green manure and help in controlling pH in soil in addition to antifungal property which reduced the externally seed borne and soil borne fungi.

Plant species such as *Calatropis, L.camara* and *A. cepa* potent in cereal crop seed borne fungi, are available in all over tropical zone. The cost of intensive use of their essential parts of plants should be available and affordable to poor communities in many sorghum producing areas for the controlling of seed borne fungi.

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