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RESEARCH ARTICLE

CAUSES FOR SEED BORNE INFECTION IN FINGER MILLET [*ELUSINE CORACANA* (L.) GAERTN.] AND ITS MANAGEMENT UNDER FIELD CONDITIONS

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ABSTRACT

Finger Millet [*Elusine coracana* (L.) Gaertn.] is generally affected by several seed-borne fungi and causing severe losses both in fields as well as in storage conditions. The fungi associated with seeds at the stage of harvest, transport, processing and under storage bring about several undesirable changes, making them unfit for human consumption and sowing. The total loss of millet grain after harvest is estimated to be as much as 15 per cent in many countries and much higher in developing countries. Studies pertaining to seed borne mycoflora of finger millet are flatter limited. Therefore, in the view of the above facts, the present study is undertaken to find out the mycoflora associated with the seeds of finger millet and its effect on seed and to determine the efficacy of bio-control agents and chemicals for managing seed borne mycoflora under field condition. A total of 27 seed samples were collected in different places of North-eastern districts of Tamil Nadu for the assessment of seed borne infection in finger millet. The mycoflora *viz*., *Alternaria* spp., *Aspergillus flavus*, *Aspergillus niger*, *Curvularia* spp., *Fusarium* spp., *Helminthosporium* spp. and *Pyricularia grisea* were identified as casual organisms in seeds of finger millet. Among them, *Pyricularia grisea* causes blast disease in finger millet under field condition and *Curvularia* spp., *Fusarium* spp., and *Helminthosporium* spp. cause grain mold/seed blackening disease incidence. Based on the results of causal organisms of seed borne infection the field experiments were conducted at Centre of Excellence in Millets, Athiyandal during *kharif*, 2016 and *kharif*, 2017. Seed treatment with pre-mixture of fungicides (Mancozeb 63% + Carbendazim 12%) @ 3g/kg of seed with one spray of pre-mixture of fungicides (Mancozeb 63% + Carbendazim 12%) @ 0.2% at the time of blast incidence recorded less incidence of neck blast, finger blast and seed blackening/grain mold and higher grain yield of 2700 kg/ha and 2492 kg/ha were recorded during *kharif*, 2016 and *kharif*, 2017 respectively on both trials.

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INTRODUCTION

Finger millet is originally native of the Ethiopian highlands and was introduced into India approximately 4000 years ago (Anon, 2012). It is cultivated widely in East Africa and tropical Asia, mainly in the rainy slopes. It is also cultivated in the upland area of the Himalayas at an elevation of 2,300 m. India is the largest cultivator of finger millet, which is primarily grown in the states of Karnataka, Tamil Nadu, Andhra Pradesh, Maharashtra, Uttar Pradesh, Bihar, Orissa and Gujarat. These eight states together account for 95 and 98.13 per cent of the total area and production of finger millet in the country (Sonnad, 2005) respectively. Its grain has long storability even under normal conditions and has made it "famine reserve".

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This aspect is at most important as Indian agriculture suffers from vagaries of monsoon (Michaelraj and Shanmugam, 2013). Finger millet contains 7.7% protein, 1.5% fat, 2.6% ash, 72.6% carbohydrate, 3.6% crude fiber, 350 mg Ca, 3.9 mg Fe, 0.42 mg Thiamin, 0.19 mg Riboflavin, 1.1 mg Niacin, 13.24% moisture and energy 336K (Hulse *et al*.,1980). The seed is cooling, tonic and astringent properties and is a rich source of calcium, iron, phosphorus and essential amino acids like lysine, cystine and triptophane. It is used in the treatment of hepatitis, fever and biliousness. The nutritional quality of finger millet grain makes it an ideal food for expectant women, lactating mothers, children, the sick and diabetics (National Research Council, 1996). Finger millet straw is used as a valuable fodder for both milking and working animals. The grains of the herb are used for making fermented drinks or beer. It has emerged as a food for poor people due to its hardy nature, drought resistant capacity and adequate nutritive value. It can compensate to satisfactory extent the nutritive problem.

Diseases are the major constraints in economic production of finger millet. As many as 25 fungal, 4 viral, 5 bacterial and 6 nematode pathogens have been recorded on this crop (Mundhe, 2005). It is generally affected by several seed-borne fungi and causing severe losses both in fields as well as in storage conditions (Krishna Prasad and Basuchaudhary, 1987). The fungi associated with seeds at the stage of harvest, transport, processing and under storage bring about several undesirable changes, making them unfit for human consumption and sowing (Patil *et al*., 2012). The total loss of millet grain after harvest is estimated to be as much as 15 per cent in many countries and much higher in developing countries (FAO, 1980). The seeds are passive carriers of pathogens that are transmitted when sown seeds germinated under suitable environmental conditions. It held that progressive reduction in the concomitant loss of viability due to seed borne fungal spores. Seed treatment for controlling plant diseases has been termed as the "pain less method" for farmers. In under developing country like India, seed treatment is cheap and effective since we cannot pay the heavy costs of spraying and dusting. Seed treatment with fungicides application can minimize disease and thus increase genetic potential and ultimately yield. Biological agents *viz*; *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas* spp. are managing wide range of seed borne fungi. There is no risk of development of resistance. Studies pertaining to seed borne mycoflora of finger millet are flatter limited. Therefore, in the view of the above facts, the present study is undertaken to find out the mycoflora associated with the seeds of finger millet and its effect on seed and to determine the efficacy of bio-control agents and chemicals for managing seed borne mycoflora under field condition.

MATERIALS AND METHODS

Survey area and collection of seed samples: Finger millet seeds were collected from Northeastern zone of Tamil Nadu (Tiruvannamalai, Tirupathur and Vellore districts) using paper bags and envelopes. The surveyed areas covered the most important finger millet producing regions, the altitude ranging from 171 to 994 msl. The finger millet seed samples were collected from farmers' fields and also purchased from local markets (Table 1). In each location, 250 g of seed samples were collected and stored under laboratory conditions for further studies.

Isolation and identification of the seed borne fungi: Isolation of fungi associated with finger millet seeds were carried out in randomly counted 100 seeds of the composite seed sample by standard blotter method (Bhale *et al*., 2001). Ten seeds per Petri plates, after surface sterilization by 1% Sodium hypochlorite solution for one minute and non-surface sterilized, were placed at equal distance on three layers of properly moistened sterilized blotters. Then the Petri plates were incubated under 12/12 hr alternating light and dark period at 25 ± 2^0C. Developing fungal growth on each of the seed after seven days was observed regularly, identified by microscopic observation and infection levels were recorded as the percentage of infected seeds in a sample. The fungi occurring on each and every seed in the plates were identified preliminary on the basis of sporulation characters like sexual or asexual spores with the help of stereoscopic binocular microscope. The validation and further confirmation of seed-borne fungi were made by preparing slides of the fungal growth and observing them under compound microscope. The identification was made with the help of manuals.

Management studies under field condition: Based on the results of *in vivo* condition, the field experiments were conducted at Centre of Excellence in Millets, Athiyandal during *kharif*, 2016 and *kharif*, 2017 in randomized block design with 3 replications to find out the management of seed borne pathogens infection of finger millet under field condition. Although, fungicides like carbendazim, ediphenphos *etc*., were found to be highly effective against neck and finger blast diseases, the advanced fungicides like pre-mixture fungicide with bio-agents was lacking. Keeping these facts in view, trials were conducted to manage neck and finger blasts of finger millet through fungicides and bioagents during the time of incidence. Initially all the treatments were designed with seed treatment of *Pseudomonas fluorescens* and pre-mixture of fungicides (Mancozeb 63% + Carbendazim 12%). Carbendazim 12% + Mancozeb 63% is a broad spectrum fungicide with protective and curative action with contact and systemic in nature. The leaf blast incidences were recorded more in early stage/tillering stage (30-45 days after sowing). After the occurrence of disease incidence, treatment spray was carried out and observations on leaf blast, neck blast, finger blast and grain mold disease incidence and grain yield were recorded. Using the data statistical analyses were done to draw interferences.

Disease assessment: The observations on leaf blast in individual tillers were recorded by using 1–9 scale (SES); for neck blast and finger blast severity (%), estimated across all the panicles/all tillers in each replication. For neck and finger blast incidences, total number of infected neck and fingers were scored, counted and disease incidence % was calculated using the following formula

$$\text{a) Neck blast (\%)} = \frac{\text{Number of infected panicles}}{\text{Total number of panicles}} \times 100$$

$$\text{b) Finger blast (\%)} = \frac{\text{Number of infected fingers}}{\text{Average no. of fingers per plant} \times \text{Total number of panicles}} \times 100$$

RESULTS

A total of 27 seed samples of finger millet were collected in different places/altitudes of North-eastern districts of Tamil Nadu (Table 1). The seed-borne pathogens were identified through light microscope, based on their morphological and conidial characters as mentioned by earlier workers. The mycoflora *viz*., *Alternaria* spp., *Aspergillus flavus*, *Aspergillus niger*, *Curvularia* spp., *Fusarium* spp., *Helminthosporium* spp. and *Pyricularia grisea* (Fig 1) were identified in seeds of finger millet (Table 2). The data revealed that all the treatments tested were able to reduce the disease significantly over control (Table 3). Among them, seed treatment with pre-mixture of fungicides (Mancozeb 63% + Carbendazim 12%) @ 3g/kg of seed with one spray of pre-mixture of fungicides (Mancozeb 63% + Carbendazim 12%) @ 0.2% at the time of disease incidence (T_3) recorded less incidence of neck blast, finger blast and seed blackening/grain mold and higher grain yield of 2700 kg/ha (Table 3) and 2492 kg/ha (Table 4) were recorded during *kharif*, 2016 and *kharif*, 2017 respectively on both the trials. The next best treatment was seed treatment with *Pseudomonas fluorescens* @ 10 g/kg of seed with one spray of pre-mixture of fungicides (Mancozeb 63% + Carbendazim 12%) @ 0.2% at the time of disease incidence (T_4).

DISCUSSION

The mycoflora *viz*., *Alternaria* spp., *Aspergillus flavus*, *Aspergillus niger*, *Curvularia* spp., *Fusarium* spp., *Helminthosporium* spp. and *Pyricularia grisea* (Fig 1) were identified in seeds of finger millet.

Table 1. Finger millet seed samples collection sites/locations

Sample locality	District	Variety	Latitude (^{0}N)	Longitude (^{0}E)	Altitude (m)
Se.Kudalur -1	Tiruvannamalai	Local	12.0804	78.5943	171
Se.Kudalur -2	Tiruvannamalai	Local	12.0804	78.5943	171
Keelsirupakkam	Tiruvannamalai	Aruvathan kevuru	12.1053	79.0036	201
T.Velur	Tiruvannamalai	Local	12.0726	78.5055	202
Thanipadi-1	Tiruvannamalai	Local	12.0633	78.5006	210
Thanipadi -2	Tiruvannamalai	Local	12.0633	78.5006	210
Thandrampet	Tiruvannamalai	Local	12.0521	78.4844	228
Chinniyanpettai -1	Tiruvannamalai	Local	12.0521	78.4844	228
Chinniyanpettai -2	Tiruvannamalai	Local	12.0521	78.4844	228
Chinniyanpettai -3	Tiruvannamalai	Local	12.0521	78.4844	228
Melnimmiyampattu -1	Tirupathur	Vellaisuruttai	12.3816	78.4104	438
Melnimmiyampattu -2	Tirupathur	Local	12.3816	78.4104	438
Narasingapuram -1	Tirupathur	Local	12.3605	78.4336	479
Narasingapuram -2	Tirupathur	Vellaisuruttai	12.3605	78.4336	479
Narasingapuram -3	Tirupathur	Vellaisuruttai	12.3629	78.4352	485
Narasingapuram -4	Tirupathur	Local	12.3629	78.4352	485
Kalrappatty	Tirupathur	Local	12.3651	78.4536	512
Narasingapuram -5	Tirupathur	Local	12.3719	78.4503	513
RMS Pudur	Tirupathur	Local	12.3538	78.4612	523
Namiyampattu	Tiruvannamalai	Local	12.3937	78.5879	594
Veerakalasai	Tiruvannamalai	Local	12.3516	78.4810	697
Melreddyur	Tiruvannamalai	Local	12.3538	78.4834	701
Arasavalliyur	Tiruvannamalai	Local	12.3850	78.5520	725
Mangalam -1	Vellore	Vellai ragi	12.3450	78.3920	994
Mangalam -2	Vellore	Karunsuruttai	12.3450	78.3920	994
Nilavoor -1	Vellore	Vellai ragi	12.3450	78.3920	994
Nilavoor -2	Vellore	Karunsuruttai	12.3450	78.3920	994

Table 2. Fungal species recorded on finger millet seed samples (values in per cent)

Seed samples from	*Alternaria* sp.	*Aspergillus flavus*	*Aspergillus niger*	*Curvularia* spp.	*Fusarium* spp.	*Helminthosporium* spp.	*Pyricularia grisea*
Arasavalliyur	1.0	2.5	2.0	5.0	-	-	1.0
Chinniyanpettai -1	1.5	3.0	-	-	1.0	1.0	-
Chinniyanpettai -2	1.0	2.0	-	-	1.0	-	-
Chinniyanpettai -3	2.0	1.0	2.5	-	-	-	1.0
Chinniyanpettai 4	1.0	1.5	1.5	-	-	2.5	1.0
Kalrappatty	-	-	-	1.5	2.0	3.0	-
Keelsirupakkam	1.0	-	-	2.0	2.0	5.0	1.5
Mangalam -1	-	1.5	-	-	-	3.5	1.5
Mangalam -2	-	1.5	1.0	1.0	-	-	-
Melnimmiyampattu -1	-	-	1.0	2.5	1.0	1.0	-
Melnimmiyampattu -1	-	1.5	2.5	1.0	3.0	-	1.0
Melreddyur	2.0	-	-	-	1.5	-	1.0
Narasingapuram -1	1.0	3.5	2.0	-	-	-	1.0
Narasingapuram -3	2.5	-	-	-	-	-	-
Narasingapuram -4	1.0	2.0	3.5	-	-	4.0	-
Narasingapuram -5	3.0	2.5	-	-	-	3.0	4.0
Narasingapuram-2	1.0	2.0	-	-	-	-	2.5
Nilavoor -1	1.5	2.0	2.0	1.5	-	-	2.0
Nilavoor -2	1.0	1.0	-	-	-	2.5	1.0
Puthur	-	2.0	2.5	4.0	1.0	2.0	-
RMS Pudur	-	-	1.0	-	3.0	-	1.0
Se.Kudalur – 2	1.0	4.0	2.5	1.0	-	1.0	-
Se.Kudalur -1	2.0	-	1.0	1.0	1.5	-	-
T.Velur	1.5	1.0	1.0	-	-	-	-
Thanipadi -2	-	-	-	2.5	1.0	-	1.0
Thanipadi -1	2.5	1.5	1.0	-	-	-	2.0
Veerakalasai	2.5	-	-	5.0	3.0	-	1.0

Similarly, Kumar (2010) reported four fungi namely *Aspergillus niger, Penicillium citrinum, Fusarium* spp. and *Alternaria alternata* were found to be dominant on seeds of finger millet genotypes. Penugonda *et al.* (2010) reported many fungal species of *Aspergillus, Fusarium* and *Penicillium griseofulvum* associated with the finger millet seed samples. Kumar (2013) also observed several fungi *viz; Aspergillus flavus, A. nidulans, A. terreus, Fusarium spp., Trichothecium roseum, Trichoderma viride* and *Penicillium griseofulvum* associated with the seeds of finger millet. Pande *et al.* (1994) observed gray to black discoloured seeds had higher levels of infection by *Pyricularia grisea* and *Bipolaris nodulosa* than apparently healthy, normal reddish brown finger millet seeds. Plating of seed components showed that both the fungi were present in the pericarp and endosperm but not in the embryo. Seed treatment with pre-mixture of fungicides (Mancozeb 63% + Carbendazim 12%) @ 3g/kg of seed with one spray of pre-mixture of fungicides (Mancozeb 63% + Carbendazim 12%) @ 0.2% at the time of incidence recorded less incidence of neck blast, finger blast and seed blackening/grain mold and higher grain yield of 2700 kg/ha and 2492 kg/ha respectively on both the trials. Nagaraja *et al.* (2007) reported that the fungicides like carbendazim, ediphenphos *etc.*, are found to be highly effective against neck and finger blast diseases in finger

Table 3. Effect of integrated disease management of finger millet blast under field condition during *kharif* 2016

Treatment No.	Leaf blast (G) 30 DAS* (Before treatment)	Leaf blast (G) 51 DAS* (After treatment)	Neck blast* (%)	Finger blast* (%)	Grain mold /Seed blackening* (%)	Yield* (kg/ha)
T_1	1.33	0.67	19.00 (25.84)	21.40 (27.56)	15.36 (23.07)	2157
T_2	1.67	0.67	24.10 (29.40)	25.74 (30.49)	16.50 (23.97)	2175
T_3	1.33	0.33	3.17 (10.25)	2.50 (9.10)	6.25 (14.48)	2700
T_4	1.33	0.33	9.33 (17.79)	7.15 (15.51)	8.53 (16.98)	2610
T_5	1.67	0.67	21.33 (27.51)	20.57 (26.97)	13.25 (21.35)	2395
T_6	1.67	0.67	24.83 (29.89)	23.51 (29.01)	14.37 (22.27)	2345
T_7	1.33	0.67	25.67 (30.44)	26.24 (30.81)	15.04 (22.82)	2298
T_8	1.33	0.67	27.17 (31.41)	28.54 (32.29)	15.79 (23.42)	2256
T_9	2.67	3.67	33.67 (35.47)	40.56 (39.56)	21.85 (27.87)	2086
S.Em ±	0.49	0.48	1.38	1.27	1.25	71.17
CD at (5%)	1.01	0.99	2.86	2.65	2.61	148.00

*Mean of three replications; Figures in the parentheses are arc sine transformed values

Treatment	Details
T_1	Seed treatment with pre-mixture of fungicide (Mancozeb 63% + Carbendazim 12%) 3g/kg of seed
T_2	Seed treatment with *Pseudomonas fluorescens* 10 g/kg of seed
T_3	T_1 + one spray of pre-mixture of fungicide (Mancozeb 63% + Carbendazim 12%) 0.2% at the time of incidence
T_4	T_2 + one spray of pre-mixture of fungicide (Mancozeb 63% + Carbendazim 12%) 0.2% at the time of incidence
T_5	T_1 + one spray of *P. fluorescens* 0.6% at the time of incidence
T_6	T_2 + one spray of *P. fluorescens* 0.6% at the time of time of incidence
T_7	T_1 + one spray of Azadirachtin 0.1% at the time of incidence
T_8	T_2 + one spray of Azadirachtin 0.1% at the time of incidence
T_9	Control

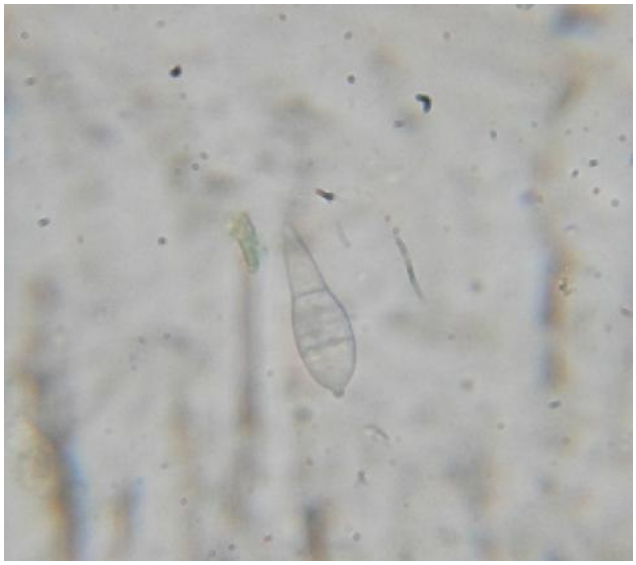
Table 4. Effect of integrated disease management of finger millet blast under field condition during *kharif* 2017

Treatment No.	Leaf blast (G) 30 DAS* (Before treatment)	Leaf blast (G) 51 DAS* (After treatment)	Neck blast* (%)	Finger blast* (%)	Grain mold /Seed blackening* (%)	Yield* (kg/ha)
T_1	1.67	1.00	21.25 (27.45)	23.00 (28.66)	11.36 (19.70)	2060
T_2	1.67	0.67	26.00 (30.66)	27.10 (31.37)	10.50 (18.91)	2117
T_3	1.33	0.33	3.10 (10.14)	4.00 (11.54)	2.50 (9.10)	2492
T_4	1.33	0.33	9.00 (17.46)	10.85 (19.23)	8.00 (16.43)	2410
T_5	1.67	0.67	20.67 (27.04)	20.00 (26.57)	8.50 (16.95)	2339
T_6	1.67	0.67	22.50 (28.32)	25.10 (30.07)	14.37 (22.27)	2300
T_7	1.33	0.67	23.67 (29.11)	28.24 (32.10)	15.75 (23.38)	2310
T_8	1.67	1.00	28.33 (32.16)	28.54 (32.29)	17.50 (24.73)	2210
T_9	3.33	4.33	36.00 (36.87)	38.60 (38.41)	20.50 (26.92)	2025
S. Em ±	0.48	0.52	1.77	1.67	1.53	103.34
CD at (5%)	0.99	1.09	3.69	3.48	3.19	214.90

*Mean of three replications; Figures in the parentheses are arc sine transformed values

Treatment	Details
T_1	Seed treatment with pre-mixture of fungicide (Mancozeb 63% + Carbendazim 12%) 3g/kg of seed
T_2	Seed treatment with *Pseudomonas fluorescens* 10 g/kg of seed
T_3	T_1 + one spray of pre-mixture of fungicide (Mancozeb 63% + Carbendazim 12%) 0.2% at the time of incidence
T_4	T_2 + one spray of pre-mixture of fungicide (Mancozeb 63% + Carbendazim 12%) 0.2% at the time of incidence
T_5	T_1 + one spray of *P. fluorescens* 0.6% at the time of incidence
T_6	T_2 + one spray of *P. fluorescens* 0.6% at the time of time of incidence
T_7	T_1 + one spray of Azadirachtin 0.1% at the time of incidence
T_8	T_2 + one spray of Azadirachtin 0.1% at the time of incidence
T_9	Control

millet. Prajapati *et al.* (2004) reported tricyclazole (beam) (@ 0.045%) was the most effective fungicide for the control of rice blast and increasing yield. Similar results were also reported by Vijaya (2002), Gohel *et al.* (2009), Sood and Kapoor (1997), Sunder *et al*. (1994) and Peterson (1990). Effectiveness of iprobenfos (kitazin) (3.75 kg a.i./ha) in controlling rice blast and increasing grain yield has also been reported by Sharma and Kumar (1992). Lukose *et al*. (2007) reported that carbendazim (0.05%) reduced the blast disease intensity and increased the grain and fodder yield with maximum net return and ICBR. Varma and Santhakumari (2012) have reported that Isoprothiolane (1.5 ml/litre) reduced rice blast incidence with maximum increase in grain and straw yield. Thus, the results of earlier workers are also in line with the results obtained in the present investigations. New generation chemicals like Tricyclazole and Propiconazole can provide effective control against blast disease in rice (Singh *et al*., 2000). Raj and Pannu (2017) reported that Tricyclazole followed by Propiconazole were superior in managing rice blast. Fungicides showed effective control against blast disease in rice ecosystem



(Prajapati *et al.*, 2004; Dutta *et al*., 2012; Sood and Kapoor, 1997). Carbendazim and Tricyclazole showed effective control against pearl millet blast under field conditions (Lukose *et al.*, 2007; Joshi and Gohel, 2015). However, rice blast pathogen isolates showed differential sensitivity to Tricyclazole and Carbendazim (Yuan and Yang, 2003; Mohammad *et al*., 2011). Narayana Swamy *et al*. (2009) and Ganesh Naik *et al.* (2012) reported that Tebuconazole+ Trifloxystrobin have also been reported to be effective against rice blast. Sharma *et al*. (2018) reported that blast disease can be effectively managed with three sprays of Tebuconazole + Trifloxystrobin or Propiconazole in pearl millet. Similarly, Kumar (2013) found that minimum seed blackening (16.05%, 24.51%, 18.84% and 24.44%) was recorded as per the treatment of carbendazim @ 0.1 per cent, *Pseudomonas flurorescens* @ 0.6 per cent, mancozeb @ 0.2 per cent used to control finger millet grain mold or seed blackening.

Conclusion

Good seed is recognized as an important input in any agricultural production system. One of the required characteristic of good seeds other than high germination and purity is the absence of seed-borne pathogens. *Alternaria* spp., *Aspergillus flavus, Aspergillus niger, Curvularia* spp., *Fusarium* spp., *Helminthosporium* spp. and *Pyricularia grisea* were the main fungi causing diseases in finger millet seeds. Out of the fungi *Pyricularia grisea* and *Helminthosporium* spp. were important pathogens whose inoculums present on the surface of seeds, were recorded in the field incidence. The presence of so many pathogenic fungi at high level in seed from various geographical area indicates a clear need for field surveys for these and other pathogens. Based on the present studies, it could be concluded that a clean need to increase public awareness on aspects related to seed health and to develop suitable management practices for control of these seed borne pathogens.

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Glossary of Abbreviations

@ -at the rate of
% - per cent
^{0}C - degree celsius
a.i.-active ingredient
Ca - Calcium
Fe- Iron
g - gram
ha- hectare
hr - hour
K - Boltzmann constant
m - metre
mg - milli gram
ml - milli litre
mm - milli metre
msl- mean sea level
t - tones

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