E-ISSN: 2789-3073
P-ISSN: 2789-3065
IJPPM 2022; 2(2): 19-24
www.plantpathologyjournal.com
Received: 07-05-2022
Accepted: 09-06-2022

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Pathogenic potential of seed borne mycoflora in finger millet (*Eleusine coracana* L. Gaertn)

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Abstract
Finger millet (*Eleusine coracana* L. Gaertn.), commonly known as *Ragi* is an important coarse cereal traditional crop widely grown on marginal soils under poor management practices in semi-arid areas of African and Asian countries. It is the fourth most important millet crop in the world. In the present study, seed infecting fungi were isolated by standard blotter method and agar plate method from four cultivars i.e. GPU 45, GPU 67, REC 69 and Uduru Mallige of finger millet. Eight fungi namely *Pyricularia grisea, Drechslera nodulosa, Curvularia lunata, Alternaria alternata, Fusarium moniliforme, Aspergillus flavus, Aspergillus* sp. and *Penicillium* spp. were found to be associated with the seeds of finger millet. The pathogenic potential of *P. grisea, A. alternata, C. lunata* and *A. flavus* was studied on finger millet cultivar REC 69 by seed coating with fungal spores and culture filtrate of the respective fungi by standard blotter method and paper towel method. All the tested fungi were found to reduce the seed germination, seedling vigour index (SVI) and pre as well as post emergence mortality. In standard blotter method, highest reduction in seed germination (36.1%) and SVI (56.6%) was recorded in the treatment of *P. grisea* closely followed by *A. flavus*. In pot conditions, seed treated with *A. favus* had lowest seed germination (32.5%), highest per-emergence (67.5%) and post emergence mortality (18.2%) followed by *P. grisea, C. lunata, A. alternata* and *F. moniliforme* over control. Culture filtrate of *A. flavus, P. grisea* and *C. lunata* were found inhibitorier as compared to *A. alternata* and *F. moniliforme.*

Keywords: Finger millet, seed born mycoflora, culture filtrate, pathogenic potential, seed and seedling parameters

Introduction
Finger millet commonly known as *Ragi* is the fourth most important millet crop in the world. It can be grown under conditions of very low rainfall. The crop can withstand severe drought and can revive again with a good shower of rain. In India, finger millet is cultivated over an area of 0.988 m ha with a production of 1.587 m. tons and productivity of 1607 kg/ha during 2019-20 (Tonapi, 2020) [19]. In Madhya Pradesh, crop is grown in erratic pockets by tribal farmers. The grains are rich source of protein, fibre, minerals and amino acids, which are crucial to human health and growth. Apart from the major nutrients, the calcium content of finger millet seed is higher than all the cereals and millets. Finger millet seeds are vulnerable to the huge diversity of opportunistic microbe leading to grain mold, seed blackening and discolouration. A number of seed borne fungi were reported to cause severe losses both in fields as well as in storage conditions. They are responsible for both pre and post emergence mortality, affect seedling vigour, development of plant diseases and distribution of pathogen to new areas. Penugonda *et al.* (2010) [12] reported that variety of fungi harboring in finger millet seeds are potentially toxigenic to man and responsible for diseases of poultry and livestock. Saleh *et al.* (2012) [16] isolated fumonicins produced by *Fusarium verticillioides* in finger millet that may cause potential health risk to infants who consume finger millet gruel as a weaning food. Jain (2020) [6] reviewed the work on seed borne mycoflora of finger millet and reported 87 fungal species belonging to 38 genera in different cultivars causing seed rot, seedling blight and leaf spot diseases. Resistance in finger millet cultivars against major diseases was reported by Jain (2021) [7]. In the present study, attempts has been made to isolate and identify the mycoflora associated with the seeds of finger millet cultivars and their effect on seed viability and other seedling parameters which are responsible for higher yield of the crop.

Materials and Methods
Isolation of fungi associated with four finger millet cultivars namely GPU 45, GPU 67, REC

69 and Uduru Mallige (UM) were carried out by taking seeds from the composite samples by standard blotter method and agar plate method as described by Bhale *et al.* (2001) [4]. The seeds were dipped in 1% sodium hypochlorite solution for 1 minute and rinsed three times with sterilized distilled water to remove sodium hypochlorite from the seed surface. Ten sterilized and ten unsterilized seeds were kept in the moist blotter paper using forceps at equal distance (9 in a circle and 1 in centre) in petri plates. In Agar plate method, potato dextrose agar (PDA) medium was prepared and sterilized in autoclave at 121.6 °C temperature and 15 lb pressure for 20 minutes. After semi cooling, 20 ml of PDA was poured in 90 mm sterilized petri plate under aseptic conditions. After solidification of media, petri plates were inverted for 12 h. afterward; ten sterilized and 10 unsterilized finger millet seeds were arranged in the same manner on the media under aseptic conditions. The experiment was conducted in Complete Randomized Design with four replications. These plates were incubated under alternating light and dark period. After 7 days of incubation, seeds were examined one by one and developing fungal growth was recorded. Associated fungi were identified by microscopic observation after purification on the basis of their cultural and morphological characteristics.

The pathogenicity test was carried out for *Pyricularia grisea*, *Alternaria alternata*, *Curvularia lunata*, *Aspergillus flavus* and *Fusarium moniliforme* in apparently healthy seeds of finger millet cultivar REC 69. The seeds of REC 69 were surface sterilized for 1 minute with 1% sodium hypochlorite solution followed by 3 washings in sterile water. The seeds were then soaked in distilled water for 24 hrs and inoculated by rolling on 14 days old culture of each test fungus. Forty inoculated seeds were placed for incubation using *rolled towel paper* method in 4 replications. Control was maintained without inoculation for comparison. After 7 days, rolled towel papers were carefully opened and germinated as well as non-germinated seeds were counted. Shoot and root length (cm) of seedlings were recorded in randomly selected 10 seedlings and seedling vigour index (SVI) were calculated using following formula.

$$\text{Seed germination (\%)} = \frac{\text{Total no. of germinated seeds}}{\text{Total no. of sown seeds}} \times 100$$

SV I = Seed germination (%) x (Mean shoot length + Mean root length)

In another experiment, ten inoculated finger millet seeds were placed in equidistance at 2 cm depth in plastic pots filled with sterilized soil. The experiment was conducted in four replications along with control. The pots were watered everyday with 50 ml. sterilized water. The observations on seed germination were recorded 7 days after sowing and seedling mortality on 14th day after sowing. Pre-emergence and post emergence mortality were calculated using following formula.

$$\text{Pre-emergence mortality (\%)} = \frac{\text{Total no. of non-germinated seeds}}{\text{Total no. of sown seeds}} \times 100$$

$$\text{Post-emergence mortality (\%)} = \frac{\text{Total no. of died seedlings}}{\text{Total no. of germinated seeds}} \times 100$$

The influence of culture filtrate of five fungi was assessed on seed health of finger millet by standard blotter method and paper towel method. Fungi namely *P. grisea*, *A. alternata*, *C. lunata*, *A. flavus and F. moniliforme* were separately cultured on potato dextrose broth medium and incubated at 25±2 °C for 14 days. Liquid medium along with fungal growth of each fungus was filtered through Whatman filter No. 42. Resulting filtrates were used to evaluate their effect on seed germination and seedling growth. Healthy seeds of finger millet cv REC -69 were treated by soaking the seeds for 8 hr into culture filtrate of respective fungus. Seeds soaked in sterilized distilled water served as control. Ten treated seeds of finger millet were kept in the moist blotter paper using forceps at equidistance in petri plates. In another experiment, forty treated seeds from each culture filtrate were kept in rolled paper towel. Observations on seed germination, shoot -root length were recorded after 7 days of incubation at room temperature. Emergence of seedling from the seeds was considered as successful germination of seeds. Percent seed germination, seed vigour index and pre emergence mortality was calculated. Reduction in seed germination and seedling growth parameters were calculated. The data were analysed statistically using completely randomized design. The values expressed in percentage were transformed to angular values before analysis.

Results and Discussion

Association of mycoflora with finger millet seeds

Three fungi namely *Aspergillus flavus, Curvularia lunata and Penicillium* spp. were found associated with the seeds of finger millet in standard blotter method (Table 1). Maximum mean association of *C. lunata* in a range of 6.7 to 16.7% was recorded in sterilized seeds of finger millet with maximum association in REC 69 (16.7%) and minimum in GPU 67 (6.7%). In unsterilized seeds, association of *C. lunata* varied from 10.0 to 23.3% with a mean of 17.5% was maximum in REC 69 and Uduru Mallige (23.3%) and minimum in GPU 67 (10.0%). This fungus was observed in all the four cultivars tested for the fungal association. Association of *A. flavus* was recorded only in sterilized seeds of REC 69 (13.3%) and Uduru Mallige (16.7%) whereas it was recorded in unsterilized seeds of all four cultivars ranging from 3.7% (GPU 67) to 23.7% (REC 69) with a mean of 14.3%. *Penicillium spp.* was observed associated with sterilized seeds of Uduru Mallige (6.7%) and unsterilized seeds of REC -69 (10.0%) and Uduru mallige (13.3%). Average association of *A. flavus* was 14.3% in unsterilized seeds and 7.5% in sterilizes seeds. Whereas, 17.5% association of *C. lunata* was in unsterilized seeds and 11.7% in sterilized seeds. *Penicillium* spp. was associated 3.7% in unsterilized and 1.7% in sterilized seeds.

Eight fungi namely *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*. *Curvularia lunata*, *Drechslera nodulosa*, *Pyricularia grisea*, *Fusarium moniliforme*, and *Penicillium* spp. were found to be associated with the seeds of finger millet in agar plate method (Table 2). All the fungi were found associated with sterilized and unsterilized seeds of REC 69 and Uduru Mallige (UM) except *A. niger* in sterilized seeds of UM. Mean association of all the fungi with four cultivars of finger millet was 2.5 to 20.8% in sterilized seeds and 6.6 to 28.1% in unsterilized seeds. *C. lunata*, *A. flavus* and *P grisea* were predominant fungi associated with finger millet seeds. Maximum mycoflora

were found associated with finger millet cultivar REC 69 and UM whereas minimum association of mycoflora was in GPU 45 and GPU 67. Association and dominance of theses fungi with finger millet seeds were also reported by Ranganathaiah and Mathur (1978), Pall and Lakhani (1991), Adipala (1992), Ghodke *et al.* (2000), Penugonda *et al.* (2007), Ahir (2016) and Shobha Rani and Dorcas (2016) [14, 10, 1, 5, 11, 2,17]. Inter varietal differences among nine varieties of finger millet in the distribution and composition of the grain microflora was also reported by Srinivasa *et al.* (1972) [18].

Pathogenic potential of seed mycoflora on finger millet seeds

Data presented in Table 3 showed the retarding effect of *Pyricularia grisea, Alternaria alternata, Curvularia Lunata, Aspergillus flavus* and *F. moniliforme* on seed germination, shoot length, root length and seedling vigour index (SVI) of finger millet cultivar REC 69. Significant variation in seed germination varied from 56.2 to76.5% was recorded in different treatments as compared to 88.0% in Control. Minimum seed germination was recorded in the treatment of *P. grisea* followed by *A. flavus* (59.5%), *C. lunata* (65.0%), *A. alternata* (68.0%) and *F. moniliforme* (76.5%). Reduction in seed germination due to fungi was 13.1 to 36.1%, maximum in *P. grisea* (36.1%) closely followed by *A. flavus* (32.4%) and minimum in *F. moniliforme (*13.1%) followed by *A. alternata (22.7%) and C. lunata (26.1%).* Shoot length and root length varied from 4.3 to 6.3 cm and 5.6 to 8.4 cm, respectively were recorded. Reduction in shoot length was 9.5 to 31.7% and 11.9 to 31.0% in root length. Seedling vigour index (SVI) ranging from 562.0 to 1293.6 was estimated with 34.3 to 56.6% reduction in SVI due to different treatments. Out of five fungi, maximum reduction in seed germination, shoot length, root length and SVI was recorded in *P. grisea* followed by *A. flavus*. Minimum reduction in seed germination (13.1%), shoot length (9.5%), root length (11.9%) and SVI (22.2%) was recorded in *F. moniliforme.*

Pathogenic potential of *Pyricularia grisea, Alternaria alternata, Curvularia Lunata, Aspergillus flavus* and *F. moniliforme* was assessed in finger millet cultivar REC 69 using plastic pots and data are presented in Table 4. Significant differences in seed germination ranging from 32.5 to 85.0%, pre emergence mortality 15.0 to 67.5% and post emergence mortality 0.0 to 19.0% were recorded in different treatments. Reduction in seed germination was 20.6 to 61.8%. Maximum reduction in seed germination was recorded in *A. flavus*, followed by *P. grisea* (50.0%), *C. lunata* (47.1%) and *A. alternata* (44.0%), while minimum was in *F. moniliforme* (20.6%) compared to control. Pre emergence mortality was maximum in *A. flavus* which was at par with *P. grisea, C. lunata* and *A. alternata*. Similarly, post emergence mortality was at par in *C. lunata, A. flavus* and *P. grisea* with maximum in *C.lunata* followed by *A. flavus* and *P. grisea*. No post emergence mortality was recorded in *F. moniliforme* and Control. Ashokan *et al.* (1979) [3] reported that seed treated with *Aspergillus terreus, A. niger* and *Curvularia* species were most inhibitory on finger millet. Reddy (1983) [15] found significant positive correlation between number of fungi associated with finger millet seeds and loss of viability ranging from 3 to 51% during storage.

Effect of culture filtrates of seed infecting fungi on seed health of finger millet

Effect of culture filtrate obtained from *P. grisea, A. alternata, C. lunata, A. flavus* and *F. moniliforme* was evaluated on seed health of finger millet using standard blotter method and data are presented in Table 5. Seed germination, shoot length, root length and SVI were hampered due to culture filtrate of fungi. Seed germination varied from 48.5 to 65.0% was recorded in the various treatments over control (80.0%). Percent reduction in seed germination was 18.8 to 39.4%. Lowest seed germination and highest inhibition in seed germination was recorded in *P. grisea* followed by *A. flavus* and *C. lunata*. Whereas highest seed germination and lowest inhibition was recorded in F. *moniliforme* followed by *A. alternata*. Shoot length and root length varied from 1.8 to 3.1 cm and 2.3 to 4.3 cm, respectively were recorded. Reduction in shoot length and root length was 9.7 to 41.9% and 14.0 to 46.5%, respectively with maximum reduction in *A. flavus* and minimum in *F. moniliforme*. SVI ranging from 205.0 to 592.0 was lowest in *A. flavus* followed by *P. grisea* (247.3) and (287.6). Reduction in SVI varied 28.6 to 65.4% in different culture filtrates and was maximum in the same treatments. Effect of fungal culture filtrate on pre emergence mortality of finger millet seeds were worked out. Pre emergence mortality ranging from 35.0 to 51.5% was maximum in *C. lunata* followed by *P. grisea* (50.0%) and *A. flavus* (41.3%) as compared to control (20.0%). Minimum pre emergence mortality was recorded in *F. moniliforme* (35.0%) followed by *A. alternata* (38.8%).

Effect of five fungal culture filtrate on seed germination, shoot length, root length, SVI and pre emergence mortality was studied on finger millet cultivar REC 69 using paper towel method and data are presented in table 6. Retarding effect of culture filtrate of all the fungi was recorded in seed germination and seedling growth parameters. Seed germination ranging from 46.0 to 70.0% was recorded in different treatments as compared to control (89.4%). A reduction of 21.7 to 48.5% was maximum in *A. flavus* closely followed by *P. grisea* (47.3%). Least reduction in seed germination was recorded in *F. moniliforme* followed by *C. lunata*. Shoot length, root length and SVI ranging from1.9 to 3.8 cm, 2.4 to 5.2 and 197.8 to 804.6, respectively were recorded in all the treatments. Minimum shoot length, root length and SVI was recorded in *A. flavus* followed by *P. grisea*. Whereas maximum shoot length, root length and SVI was noted in *F. moniliforme* followed by *A. alternate*. Maximum reduction in shoot length (50.0%), root length (53.8%) and SVI (75.4%) was in the treatment of *A. flavus* followed by *P. grisea*. Pre emergence mortality ranging from 10.6 to 54.0% was highest in *A. flavus* followed by *P. grisea* (52.8%). Lowest pre emergence mortality was recorded in *F. moniliforme* (30.0%) and *C. lunata* (32.5%). Previously, Kumar (2010)[9] also reported that culture filtrate of *Aspergillus niger, Penicillium citrinum, Fusarium* species and *Alternaria alternata* significantly affected the seed viability and root-shoot elongation of finger millet. *A. alternata* was highly destructive causing 63.3% inhibition in seed germination and 56.1% inhibition in SVI. Khairnar *et al.* (2011) [8] reported that fungal metabolites of *Aspergillus flavus, Fusarium oxysporum* and *F. moniliforme* were more toxic and cause 100% inhibition in seed germination and root-shoot elongation. Toxicity of culture filtrate of *Fusarium*

spp. in finger millet seed viability and seedling growth was also reported by Penungonda *et al.* (2015) [13].

It may be concluded from the present study that eight fungi namely Alternaria *alternata, Aspergillus flavus, A. niger, Curvularia lunata, Drechslera nodulosa, Pyricularia grisea* and *Penicillium* spp. were found associated with discoloured seeds of finger millet cultivars. Significant reduction in seed germination, seedling growth parameters and increase in pre emergence mortality of seedlings were found due to dominant pathogenic seed borne fungi.

Table 1: Mycoflora associated (%) with finger millet seeds in Standard blotter method

Fungi	Finger millet cultivars								Mean	
	GPU 45		GPU 67		REC 69		Uduru Mallige			
	S	US	S	US	S	US	S	US	S	US
Aspergillus flavus	0.0	6.7	0.0	3.7	13.3	23.7	16.7	23.3	7.5	14.3
Curvularia lunata	10.0	13.3	6.7	10.0	16.7	23.3	13.3	23.3	11.7	17.5
Penicillium spp.	0.0	0.0	0.0	0.0	0.0	10.0	6.7	13.3	1.7	5.7
Mean	3.3	6.7	2.2	4.6	10.0	19.0	12.2	20.0	7.0	12.5

S = Sterilized US = Unsterilized

Table 2: Mycoflora associated (%) with finger millet seeds in Agar plate method

Fungi	Finger millet cultivars								Mean	
	GPU 45		GPU 67		REC 69		Uduru Mallige			
	S	US	S	US	S	US	S	US	S	US
Alternaria alternata	0.0	0.0	0.0	0.0	10.0	16.7	13.3	23.3	5.8	10.0
Aspergillus flavus	10.0	20.0	0.0	10.0	26.7	30.0	23.3	33.3	15.0	23.3
Aspergillus niger	0.0	0.0	0.0	0.0	10.0	16.7	0.0	11.7	2.5	7.1
Curvularia lunata	23.3	33.3	6.7	13.3	31.6	38.3	21.7	27.7	20.8	28.1
Drechslera nodulosa	0.0	0.0	0.0	0.0	6.7	13.3	11.1	13.3	4.4	6.6
Pyricularia grisea	0.0	0.0	3.3	6.7	13.3	16.7	16.7	23.3	8.3	11.7
Fusarium moniliforme	0.0	10.0	0.0	0.0	10.0	16.7	6.7	16.7	5.6	10.8
Penicillium spp.	0.0	23.3	0.0	6.7	16.7	23.3	11.1	16.7	6.9	17.5
Mean	4.2	10.8	1.3	4.6	15.6	21.5	13.0	20.8	8.7	14.4
Association of mycoflora (%)	25.0	50.0	25.0	50.0	100.0	100.0	87.5	100.0	-	-

Table 3: Effect of seed inoculation with different fungi on seed germination, shoot length, root length and seedling vigour index in finger millet (*in vitro*)

S. No.	Fungi	Seed germination		Shoot length	Root length	Seedling vigour index
		Original values	Transformed values			
1	*Pyricularia grisea*	56.2 (36.1)	48.6	4.4 (30.2)	5.6 (33.3)	562.0 (56.6)
2	*Alternaria alternata*	68.0 (22.7)	55.6	5.4 (14.3)	7.1 (15.5)	850.0 (34.3)
3	*Curvularia lunata*	65.0 (26.1)	53.8	4.7 (25.4)	6.3 (25.0)	715.0 (44.7)
4	*Aspergillus flavus*	59.5 (32.4)	50.5	4.3 (31.7)	5.8 (31.0)	601.0 (53.5)
5	*Fusarium moniliforme*	76.5 (13.1)	61.1	5.7 (9.5)	7.4 (11.9)	1002.2 (22.2)
6	Control	88.0 (00.0)	69.9	6.3 (00.0)	8.4 (00.0)	1293. (00.0)
7	SEm ±	-	1.645	0.285	0.269	-
	CD (5%)	-	4.889	0.846	0.798	-

Figures in parentheses are percent reduction, Average of 4 replications

Table 4: Effect of seed inoculation with different fungi on seed germination, shoot length, root length and seedling vigour index in finger millet (*in vivo*)

S. No.	Fungi	Seed germination (%)*	Percent reduction over control	Pre-emergence mortality (%)*	Post-emergence mortality (%)**
1	*Pyricularia grisea*	42.5(40.61)	50.0	57.5(49.39)	17.7(4.26)
2	*Alternaria alternata*	47.5(43.56)	44.1	52.5(40.55)	9.2(2.52)
3	*Curvularia lunata*	45.0(42.05)	47.1	55.0(47.95)	19.0(4.39)
4	*Aspergillus flavus*	32.5(34.56)	61.8	67.5(55.44)	18.2(4.29)
5	*Fusarium moniliforme*	67.5(56.03)	20.6	32.5(33.97)	0.0(0.71)
6	Control	85.0(70.45)	0.0	15.0(19.55)	0.0(0.71)
7	SEm	4.843		4.740	0.459
	CD(5%)	14.390		14.084	1.365

Figures in parentheses are ARC SIN* and Square root** transformation
Average of 4 replications

Table 5: Effect of culture filtrate of major fungi on seed germination, shoot length, root length and seedling vigour index in finger millet (Standard blotter method)

S. No.	Fungi	Seed germination		Shoot length	Root length	Seedling vigour index	Pre-emergence mortality (%)*
		Original values	Transformed values				
1	*Pyricularia grisea*	48.5 (39.4)	44.1	2.0 (35.5)	3.1 (27.9)	247.3 (58.2)	50.0(45.00)
2	*Alternaria alternata*	61.2 (23.5)	51.5	2.5 (19.4)	3.6 (16.3)	373.3 (36.9)	38.8(38.46)
3	*Curvularia lunata*	58.7 (26.6)	45.0	2.1 (32.3)	2.8 (34.9)	287.6 (51.4)	51.5(45.86)
4	*Aspergillus flavus*	50.0 (37.5)	50.0	1.8 (41.9)	2.3 (46.5)	205.0 (65.4)	41.3(39.97)
5	*Fusarium moniliforme*	65.0 (18.8)	53.8	2.8 (9.7)	3.7 (14.0)	422.5 (28.6)	35.0(36.24)
6	Control	80.0 (00.0)	63.6	3.1 (00.0)	4.3 (00.0)	592.0 (00.0)	20.0(26.19)
7	SEm		1.604	0.207	0.191	-	2.085
	CD(5%)		4.767	0.616	0.567	-	6.196

Average of 4 replications
Figures in parentheses are percent reduction
* Figures in parentheses are ARC SIN transformed values

Table 6: Effect of culture filtrate of major fungi on seed germination, shoot length, root length and seedling vigour index in finger millet (Paper towel method)

S. No.	Fungi	Seed germination		Shoot length	Root length	Seedling vigour index	Pre-emergence mortality (%)*
		Original values	Transformed values				
1	*Pyricularia grisea*	47.2 (47.3)	41.8	2.4 (36.8)	3.1 (40.4)	259.6 (67.7)	52.8 (46.61)
2	*Alternaria alternata*	63.1 (29.5)	52.6	2.9 (23.7)	4.1 (21.2)	441.4 (45.1)	36.9 (37.39)
3	*Curvularia lunata*	67.5 (24.5)	55.3	2.3 (39.5)	3.4 (34.6)	384.8 (52.2)	32.5 (34.75)
4	*Aspergillus flavus*	46.0 (48.5)	42.7	1.9 (50.0)	2.4 (53.8)	197.8 (75.4)	54.0 (47.30)
5	*Fusarium moniliforme*	70.0 (21.7)	56.9	3.2 (15.8)	4.1 (21.2)	511.0 (36.5)	30.0 (33.14)
6	Control	89.4 (0.0)		3.8 (0.0)	5.2 (0.0)	804.6 (0.0)	10.6 (18.98)
7	SEm		2.221	0.247	0.245	-	1.308
	CD (5%)		6.601	0.734	0.729	-	3.886

Average of 4 replications
Figures in parentheses are percent reduction
* Figures in parentheses are ARC SIN transformed values

Acknowledgement
Authors are highly indebted to ICAR, Project Coordinator (Small Millets) and authorities of Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.) for providing the financial assistance and all the necessary facilities needed during the present study.

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