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Efficacy of Seed Treatment by Fungicides and Neem Products for Controlling Seed and Seedling Rot of Blackgram

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ABSTRACT *Fusarium moniliforme*, a potential seed borne pathogen, was isolated from farmer's seed samples of black gram. Infected seeds of var. Sarala were treated with neem leaf extract, neem seed extract, neem oilcake extract, Neemazal (commercial formulation), fungicides; Bavistin and Vitavax, separately. The treated seeds were stored in cloth bags as well as polythene bags. Samples were drawn at bimonthly intervals and observations on germination and seed moisture content were taken. The per cent association of the fungi from the seeds and incidence of seed rot were also studied. All the treatments reduced infection of *Fusarium moniliforme*, improved germination and reduced incidence of seed and seedling rot. Both fungicides were superior to neem products in reduction of seed infection and incidence. Among neem products Neemazal (0.3%) and neem oil (0.3%) were significantly superior. Seed infection and seed moisture content were influenced by atmospheric moisture and temperature. Seeds stored in polythene bags were better than cloth bags.

Key words: *Fusarium moniliforme*, seed quality, neem products, control.

Blackgram (*Vigna mungo* L.) is an important pulse crop of Orissa grown both in *Kharif* and *Rabi* seasons. Out of various fungal pathogens associate with the black gram seeds [1, 2], *Fusarium moniliforme* is important in reducing seed quality, plant stand and crop yield in the field. The pathogen is not only soil borne but also predominantly seed borne, causes rotting of the seeds in storage under high humid conditions as well as rotting and wilting of the seedlings in the field. Though these diseases can be managed by use of fungicides [3], these are discouraged due to adverse effects on environment and human health. Therefore, five different neem products along with two important fungicides were evaluated for their effectiveness in reducing fungal association with seeds in storage and management of their diseases under greenhouse conditions.

MATERIALS AND METHODS

Blackgram seeds were collected from farmers and plated in moist chamber following ISTA [4]. *Fusarium* sp. developing on seeds were isolated and maintained in pure culture. The morphology of the fungus was studied and compared with standard literature which revealed the species to be *Fusarium moniliforme*. Healthy seeds of variety Sarala were collected, surface sterilized and inoculated with spore suspension of the fungus (10^4spore/ml). Inoculated seed lot was dried under shade in the laboratory for 24h [5]. These seeds were treated with five neem products viz., neem leaf extract (0.5%), neem seed extract (0.5%), neem oilcake extract (0.5%), neem oil (0.3%), Neemazal (commercial formulation, 0.3%) and the chemical fungicides, Bavistin (0.15%) and vitavax (0.15%). Untreated seeds inoculated with pathogen served as control.

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Two hundred fifty grams of black gram seeds of each treatment was kept in cloth bags and polythene bags separately and stored in the laboratory conditions. Before storing, the moisture content of the seeds and fungal colonization were recorded [4]. Samples were drawn at bimonthly intervals commencing from February to October consecutively for three years (2004-2007)). The extent of *F. moniliforme* association on seeds and per cent rotting were recorded based on moist blotter method. The samples from each treatment were also sown in pots filled with sterilized soil and observation on seedling rot was recorded up to three weak after sowing [6].

Initial seed moisture content and also at bimonthly intervals was recorded from treated seeds. Data on average relative humidity and average atmospheric temperature during the experimental period were also collected. Experimental trial was conducted in a randomized block design with four replications and the data were statistically analysed.

RESULTS AND DISCUSSION

Observation on black gram seed infection by *Fusarium moniliforme* in response to seed treatments at bimonthly intervals revealed that the pathogen was associated with variable percentages. Earlier studies have also revealed that different species *of Fusarium* are associated with infected urd bean seeds [2]. They are responsible for reduction of seed germination and seed rot. In the present study, association of *F. moniliforme* with the seeds of different treatments varied with the month of storage and type of storage container. The per cent association of test pathogen in untreated seeds stored in cloth bags was 52.3 per cent which gradually increased up to 72.4 per cent in August and then decreased to 47.6 per cent in October (Table 1). Though similar trend was observed in all other treatments, lowest association was in seeds treated with Vitavax (6.4% in February and 12.3% in August). Seed treated with Bavistin was also effective in significantly reducing the fungal colonization. Bavistin and vitavax are the important chemicals used as seed treatment to control various seed borne pathogens. Bavistin was highly effective in controlling web blight disease of urd bean caused by *Rhizoctonia solani* [3]. Neemazal and neem oil were next effective treatments where per cent fungal association varied from 21.4-31.3 per cent to 22.4-26.4 per cent respectively. Seed treatment with neem seed extract resulted in reduced fungal association (43.4% to 60.7%) during the months of observation, but it gave significantly high value in comparison to fungicidal seed treatments. Neem leaf extract and neem oilcake extract were less effective. Qais *et al.* [6] while studying the effect of fungicides and oilcake extracts on mycelial growth and myceliogenic germination of *Sclerotinia sclerotiorum* reported that Bavistin @100 ppm reduced sclerotial viability. The ethanol extract of neem seeds also showed toxic properties against *S. sclerotiorum* [6].

Storing in polythene bags resulted less percentage of fungal association as compared with cloth bags. Per cent association was less in Vitavax seed treatment (5.4 to 6.4%) followed by Bavistin (6.4 to 10.4%). Among the neem products, neem oil was most effective with 14.8 per cent to 18.4 per cent fungal association followed by Neemazal (17.3% to 20.5%). Neem leaf and oilcake extracts were less effective against the test pathogen.

Seed moisture content was 7.8 per cent in cloth bags in February and increased up to 8.8 per cent in July. Subsequently, it got reduced to 8.7 per cent in October along with increase and decrease in atmospheric moisture (Table 2). The trend was almost similar in all the treatments and not much variation was observed. Seed moisture content influenced the percentage of *F. moniliforme* association with the seeds. Per cent fungal association was low in the month of February but increased to maximum in August and then decreased in October. Agrawal [7] reported that among several factors affecting the seeds in storage seed moisture, relative humidity and storage temperature are the most important.

Variation in seed moisture content was much less in polythene bags than in cloth bags. However, the variation in per cent isolation of the fungus from seeds in polythene bags of different treatments increased during April and June and then decreased. This may be due to

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Table 1. Percentage of *Fusarium moniliforme* infection on black gram seeds during the period of storage

Treatment	Cloth bag					Polythene bag				
	Feb	Apr	Jun	Aug	Oct	Feb	Apr	Jun	Aug	Oct
Control	52.3 (46.3)	64.4 (53.4)	65.6 (54.1)	72.4 (58.3)	47.6 (43.6)	53.4 (46.9)	48.4 (44.1)	50.4 (45.2)	52.3 (46.3)	45.4 (42.3)
Neem leaf extract (0.5%)	50.6 (45.4)	62.3 (52.1)	65.4 (53.9)	68.8 (56.0)	53.6 (47.0)	51.4 (45.8)	52.3 (46.3)	51.4 (45.8)	52.4 (46.4)	48.6 (44.2)
Neem seed extract (0.5%)	43.4 (41.2)	53.4 (47.0)	54.6 (47.7)	60.7 (51.2)	41.3 (40.0)	44.4 (41.7)	49.4 (44.6)	42.6 (40.7)	45.4 (42.3)	40.4 (39.4)
Neem oilcake extract (0.5%)	55.4 (48.1)	66.2 (54.5)	66.4 (54.6)	71.3 (57.6)	66.6 (54.7)	43.3 (41.1)	48.3 (44.0)	45.8 (42.6)	48.3 (44.0)	40.4 (39.4)
Neem oil (0.3%)	22.4 (28.2)	26.7 (31.1)	23.4 (28.9)	26.4 (30.9)	20.3 (26.6)	14.8 (22.6)	16.6 (24.0)	17.3 (24.5)	18.4 (25.4)	17.6 (24.8)
Neemazal (0.3%)	21.4 (27.5)	24.8 (29.8)	25.5 (30.3)	31.3 (34..0)	26.3 (30.8)	17.3 (24.6)	18.8 (25.7)	16.2 (23.7)	20.6 (26.9)	18.5 (25.4)
Bavistin (0.2%)	10.5 (18.8)	12.6 (18.8)	10.4 (18.8)	15.6 (23.2)	12.1 (20.2)	6.4 (14.6)	8.4 (16.8)	9.4 (17.8)	9.4 (17.8)	10.4 (18.8)
Vitavax (0.2%)	6.4 (14.6)	8.3 (18.7)	10.4 (18.7)	12.3 (20..5)	10.3 (18.6)	5.4 (13.3)	4.3 (11.9)	5.5 (13.6)	5.1 (12.9)	6.4 (14.6)
SEm (+)	1.26	1.19	1.20	0.93	1.38	0.79	0.69	0.83	0.85	0.73
CD(P=0.05)	(2.62)	(2.45)	(2.48)	(1.91)	(2.85)	(1.64)	(1.43)	(1.71)	(1.75)	(1.51)

Table 2. Moisture content of black gram seeds during storage

Treatment	Cloth bag					Polythene bag				
	Feb	Apr	Jun	Aug	Oct	Feb	Apr	Jun	Aug	Oct
Control	7.8	7.9	8.3	8.8	8.7	7.8	7.8	7.9	8.0	7.9
Neem leaf extract (0.5%)	7.9	8.0	8.3	8.9	8.7	7.9	8.0	8.0	8.1	8.0
Neem seed extract (0.5%)	8.0	8.1	8.5	8.8	8.9	8.0	8.1	8.2	8.2	8.1
Neem oilcake extract (0.5%)	8.0	8.2	8.5	8.9	8.8	7.9	8.0	8.0	8.1	8.1
Neem oil (0.3%)	7.9	7.9	8.3	8.3	8.3	7.9	7.9	8.0	8.0	8.0
Neemazal (0.3%)	7.8	7.9	8.2	8.4	8.3	8.0	8.0	8.0	8.0	8.0
Bavistin (0.2%)	7.9	7.9	8.2	8.3	8.3	7.9	7.9	7.9	8.0	8.0
Vitavax (0.2%)	7.9	8.0	8.3	8.4	8.3	7.9	8.0	8.0	8.0	8.0

Atm. Temperature (°C): Feb-23.5, Apr-30.2, Jun-29.8, Aug-28.3, Oct-31.2; Relative Humidity (%): Feb-60.3, Apr-70.3, Jun-82.5, Aug-75.5, Oct-65.5

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Table 3. Effect of seed treatments on black gram seed rotting

Treatment	Cloth bag					Polythene bag				
	Feb	Apr	Jun	Aug	Oct	Feb	Apr	Jun	Aug	Oct
Control	31.8 (34.4)	30.6 (33.6)	35.0 (36.3)	40.1 (39.3)	30.6 (33.5)	30.3 (33.4)	26.8 (31.2)	35.6 (36.6)	24.6 (29.7)	27.6 (31.7)
Neem leaf extract (0.5%)	28.3 (32.0)	25.5 (30.3)	30.3 (33.3)	33.1 (35.1)	30.1 (33.3)	22.4 (28.2)	21.4 (27.5)	25.5 (30.3)	29.8 (33.1)	19.9 (26.5)
Neem seed extract (0.5%)	28.1 (32.0)	25.4 (30.2)	30.4 (33.4)	35.6 (36.6)	28.3 (32.1)	24.4 (29.6)	20.6 (26.9)	25.3 (30.2)	25.3 (30.2)	20.5 (26.8)
Neem oilcake extract (0.5%)	35.3 (36.4)	38.4 (38.3)	35.7 (36.7)	40.9 (39.7)	35.4 (36.5)	28.6 (32.4)	25.4 (30.2)	28.4 (32.2)	30.4 (33.4)	25.1 (30.1)
Neem oil (0.3%)	15.3 (23.0)	14.3 (22.2)	17.4 (24.6)	15.8 (23.4)	18.9 (25.7)	12.4 (20.6)	10.3 (18.5)	12.3 (20.4)	13.4 (21.4)	14.3 (22.2)
Neemazal (0.3%)	14.6 (22.4)	15.4 (23.1)	19.9 (26.4)	16.4 (23.8)	17.8 (24.9)	11.1 (19.4)	10.3 (18.6)	11.4 19.7	12.5 20.6	9.8 18.3
Bavistin (0.2%)	5.5 (13.5)	6.6 (14.8)	7.3 (15.3)	8.4 (16.8)	6.6 (14.8)	4.3 (11.9)	3.4 (10.5)	4.7 12.4	5.6 13.6	3.3 10.2
Vitavax (0.2%)	2.6 (7.6)	2.3 (8.4)	3.8 (11.2)	4.3 (11.4)	3.6 (10.8)	1.3 (4.4)	1.8 (6.5)	1.3 (6.1)	2.4 (8.5)	1.6 (6.5)
Sem ±	2.13	0.95	1.51	1.39	0.97	1.63	1.63	0.95	1.36	1.39
CD(P=0.05)	4.41	1.96	3.11	2.86	2.00	3.36	3.37	1.95	2.81	2.87

increase in atmospheric temperature during these months.

Effect of seed association of the pathogen was more pronounced in expression of disease symptoms in pot culture. Seed rot was 31.9 per cent and 30.3 per cent, respectively in cloth bag and polythene bag during February in untreated control (Table 3). It increased up to 40.1 per cent in August and 35.6 per cent in June in cloth bags and polythene bags, respectively. Incidence of seed rot was minimum in Vitavax seed treatment where it was 2.3 per cent and 1.3 per cent in cloth bags and polythene bags, respectively during February and it rose to the maximum (11.4% and 3.5%, respectively) during August. Bavistin closely followed Vitavax in reducing the disease incidence. Among the neem products, minimum seed rot was in Neemazal seed treatment with 14.6 per cent and 11.1 per cent seed rot in February which increased to 17.8 per cent and 12.5 per cent in October in both the containers. Neem oil was next best neem product in reducing seed rot. Other neem products were less effective.

Seed infection resulted in 74.4 per cent to 80.4 per cent and 68.3 per cent to 78.4 per cent seedling rot in untreated control seeds stored in cloth and polythene bag, respectively (Table 4). Vitavax was effective in reducing seedling rot to 1.3-1.5 per cent in cloth bag and 0-1.4 per cent in polythene bag. Bavistin was significantly superior to neem products in reducing the disease incidence. All the neem products significantly reduced seedling rot incidence. Neemazal was the best neem product with 21.4 per cent and 20.4 per cent seedling rot incidence in cloth bag and polythene bag, respectively followed by neem oil. Other neem products were comparately less effective.

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Table 4. Percentage of black gram seedling rot of the treated seeds in pot culture

Treatment	Cloth bag					Polythene bag				
	Feb	Apr	Jun	Aug	Oct	Feb	Apr	Jun	Aug	Oct
Control	74.4 (59.6)	75.4 (58.3)	75.4 (60.3)	80.4 (63.7)	75.6 (60.4)	68.4 (55.8)	70.4 (57.0)	70.3 (57.0)	78.4 (62.3)	68.5 (55.9)
Neem leaf extract (0.5%)	65.9 (54.2)	68.4 (55.8)	70.3 (60.0)	72.4 (58.3)	68.4 (56.1)	55.5 (48.2)	58.3 (49.8)	60.2 (50.9)	63.3 (52.7)	60.2 (50.9)
Neem seed extract (0.5%)	55.6 (48.2)	54.4 (47.5)	58.4 (49.8)	55.7 (48.3)	50.3 (43.3)	50.8 (45.5)	52.4 (46.4)	57.4 (49.2)	54.3 (47.4)	48.4 (44.1)
Neem oilcake extract (0.5%)	50.1 (45.0)	51.4 (45.8)	55.3 (48.0)	58.7 (50.0)	55.7 (48.3)	46.3 (42.9)	48.3 (44.0)	54.4 (47.5)	58.7 (49.9)	53.2 (46.8)
Neem oil (0.3%)	25.3 (30.2)	30.4 (33.4)	28.4 (32.2)	35.4 (36.4)	30.7 (33.6)	24.4 (29.6)	25.3 (30.2)	25.4 (30.2)	30.4 (33.4)	28.7 (32.4)
Neemazal (0.3%)	24.4 (29.6)	25.4 (30.2)	26.4 (30.9)	21.4 (27.5)	25.6 (30.4)	20.4 (26.8)	22.6 (28.3)	22.7 (28.3)	28.7 (32.3)	25.4 (30.2)
Bavistin (0.2%)	8.3 (16.7)	7.4 (15.7)	6.6 (14.6)	7.4 (15.7)	6.7 (14.9)	6.4 (14.6)	5.3 (13.2)	6.4 (14.5)	6.3 (14.4)	6.0 (14.2)
Vitavax (0.2%)	2.4 (8.0)	1.3 (6.4)	1.5 (6.6)	1.5 (6.6)	1.3 (6.4)	1.4 (6.5)	0.3 (2.8)	1.3 (6.2)	1.4 (6.4)	0.0 (0.0)
Sem ±	1.62	0.95	1.29	1.76	6.15	1.08	1.41	1.15	1.22	1.07
CD(0.05)	3.36	1.95	2.67	3.63	12.71	2.23	2.91	2.38	2.51	2.21

From the experiment it is concluded that neem oil and Neemazal are effective as seed treatments in reducing the seed borne inoculum of *Fusarium moniliforme*. But their efficacy should be evaluated under field condition before recommendation.

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