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CHEMICAL CONTROL OF SEED BORNE MYCOFLORA OF *VIGNA SINENSIS*

Dr. Deepti Sharma

Department of Botany, N.A.S (P.G) College, Meerut (U.P.) India.

Abstract

All the five fungicides namely benomyl,bavistin,captan,thiram and thiobendazole inhibited the growth of all the pathogenic seed tested (*Alternaria alternate*, *Aspergillus flavus*, *Colletotrichum lindemuthianum*, *Fusarium oxysporum* , *F. Semitectum* and *Macrophomina phaseoli*) at different concentration(1000ppm,100ppm,10ppm,1ppm).When they were incorporated in the media, thiram was most effect and bavistin considerably reduced the growth of most fungi . *Alternaria alternate, Aspergillus flavus, Colletotrichum lindemuthianum, Fusarium oxysporum, F. semitectum* were eliminated from the seed treated with thiram. In the present study thiram was most effective fungicides followed by bavistin,captan, benomyl and thiobendazole.

Key Words : *Vigna sinensis*, Seed-borne fungi, Fungicides, Chemical Controls.

Introduction

The present investigation hsas been undertaken to study of the control measures for seed disorders of *Vigna sinensis* (Linn.) belonging to family fabaceae. For the protection of seed against pathogen a range of chemical procedure have been adopted. Seed coat mycoflora is responsible directly for the decay of the seeds or indirectly for the weakening of the seedling.

Chemical control of seed –borne mycoflora has been studied by many worker in our country.(Chohan and Kaur,1975; Rai and Singh, 1976; Shrotri *et al.*, 1985; Narayanappa and Sohi, 1995; Kumar and Singh, 1996; Solanke *et al.*, 1997). They observed that treated seed retained sufficiently higher viability without any phytotoxic effect than untreated seeds. Saharan and Gupta (1972) stated that pathogens carried internally as well externally and can be successfully controlled by seed treatment. Diamond and Horsfall (1955) stated that chemical method of control of seed- borne disease has many advantages. The important advantages of such seed treatment is that while every seed has some chemical on it ,less chemical is used per acre than would be needed for a conventional spray dust treatment (Potter *et al.*,1956). Fungicidal application prior to storage of seeds has been shown to improve germination and reduced seed infestation (Nene and Agarwal,1969).

Material and Methods

During the course of study the seed sample were collected from the farmers from different places in meerut Namely- (1) Abdulapur (2) Datawali (3) Dorli (4) Gesupur (5) Kaili (6) Mawana (7) Rasana.

Datawali sample of the seed were used in the trial, due to higher incidence of the pathogens.

Chemotherapy

A) Effect of fungicides on the growth of Fungi *in vrtro*

Considerable work has been undertaken to control various plant diseases by using fungicides and is considered to be the best popular method.With this view,most popular and easily available fungicides, *viz.* Benomyl (Benlate), Bavistin (Carbendazim), Captan(N-trichloromethly thio-cyclohexene, 1,2 dicarboximide), Thiram (Tetramethyl thiram disulphide) and Thiobendazole (Tecto) were selected for the present study. The efficacy of these fungicides on the growth of each fungus was tested under laboratory condition, with different concentrations, *viz.* 1000ppm, 100ppm, 10ppm, 1ppm. Required amount of different fungicides in 20% ethanol was added separately to the Czapek's agar medium. The medium was poured into sterilized Petri dishes. Each Petri dish was inoculated by mycelia

Table 1: Effect of different concentrations of fungicides on the radial growth(cm) of some seed-borne fungi.

		Fungi														
		Alternaria alternata					*Aspergillus flavus*					*Colletotrichum lindemuthianum*				
Days>>>>		**3rd**	**4th**	**5th**	**6th**	**7th**	**3rd**	**4th**	**5th**	**6th**	**7th**	**3rd**	**4th**	**5th**	**6th**	**7th**
Control>>>>		**2.0**	**3.0**	**4.0**	**4.0**	**5.0**	**2.5**	**3.0**	**4.5**	**5.5**	**6.5**	**1.9**	**3.9**	**4.8**	**5.1**	**5.3**
Benomyl	1000 ppm	-	-	-	-	-	-	-	-	-	-	0.2	0.2	-	-	-
	100 ppm	-	-	0.1	0.2	0.2	-	-	-	0.2	0.4	0.6	1.0	1.2	1.2	1.8
	10 ppm	0.5	0.8	1.0	1.4	1.6	0.5	0.8	1.0	1.5	2.0	0.8	1.3	1.5	1.8	2.1
	1 ppm	0.8	1.0	1.5	1.8	2.0	0.8	1.0	1.5	2.0	3.0	1.0	1.8	2.1	2.3	2.9
Bavistin (Carbendazim)	1000 ppm	-	-	-	0.5	0.8	-	-	-	0.2	0.2	-	-	-	0.2	0.2
	100 ppm	0.5	1.0	1.5	2.0	3.0	0.8	1.0	1.0	1.1	1.1	0.3	0.5	0.6	0.9	1.2
	10 ppm	0.8	1.0	1.5	2.0	3.0	1.0	1.5	2.0	2.2	3.0	0.9	1.2	1.5	1.8	2.0
	1 ppm	1.2	1.5	2.2	2.6	3.0	1.2	2.0	2.5	3.0	4.0	0.9	1.8	2.0	2.1	2.5
Captan	1000 ppm	-	-	-	-	-	-	-	-	-	-	0.1	0.2	0.2	0.8	1.0
	100 ppm	-	0.2	0.5	0.8	1.0	-	-	-	-	1.0	-	-	0.2	1.2	1.3
	10 ppm	0.5	0.8	1.0	1.5	1.8	1.0	1.5	1.8	2.0	2.5	0.5	0.9	1.1	1.3	1.5
	1 ppm	1.0	1.2	2.0	2.2	3.0	1.22	1.8	2.0	2.5	3.5	0.6	1.0	1.2	1.5	1.9
Thiobendazole (As Tecto)	1000 ppm	-	-	-	-	-	-	-	-	0.5	0.8	-	-	-	-	-
	100 ppm	-	-	0.5	1.0	1.5	0.2	0.5	1.0	1.5	2.0	0.3	-	-	-	-
	10 ppm	0.5	1.0	1.2	1.5	2.0	0.5	1.0	1.5	2.0	2.5	0.7	1.2	1.8	2.0	2.8
	1 ppm	0.8	1.2	2.0	2.0	2.5	1.0	1.5	2.0	2.5	3.5	1.0	1.6	2.0	2.5	3.2
Thiram (Thyride)	1000 ppm	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.3
	100 ppm	-	-	0.2	0.5	1.0	-	0.4	0.5	0.8	1.0	0.1	0.1	0.2	0.2	0.8
	10 ppm	0.2	0.5	0.8	1.0	1.2	0.5	0.5	0.8	1.0	1.1	0.3	0.3	0.5	0.8	1.0
	1 ppm	0.3	0.8	1.2	1.5	1.6	0.7	1.0	1.5	2.0	2.5	0.3	0.5	0.8	1.0	1.2

Table 2: Effect of different concentrations of fungicides on the radial growth(cm) of some seed-borne fungi.

Concen-trations		*Macrophomina phaseoli*					*Fusarium semitectum*					*Fusarium oxysporum*				
	Days>>>>	**3rd**	**4th**	**5th**	**6th**	**7th**	**3rd**	**4th**	**5th**	**6th**	**7th**	**3rd**	**4th**	**5th**	**6th**	**7th**
	Control>>>>	**1.5**	**2.3**	**3.5**	**4.6**	**5.3**	**0.5**	**2.5**	**3.5**	**4.5**	**5.5**	**2.0**	**3.5**	**4.5**	**5.5**	**5.5**
Benomyl	1000 ppm	-	-	-	-	-	0.3	0.2	0.6	0.8	0.8	0.5	0.6	0.8	0.8	0.9
	100 ppm	0.3	0.3	0.6	0.8	1.0	0.5	0.5	1.6	1.8	1.8	0.5	0.6	1.4	1.8	1.8
	10 ppm	0.5	0.7	0.9	1.2	1.9	1.3	0.5	2.0	2.0	2.5	1.0	1.1	1.5	2.0	2.5
	1 ppm	0.9	1.3	2.5	2.6	2.9	0.5	1.0	1.9	2.6	3.5	1.5	2.0	2.5	3.0	3.4
Bavistin (Carbendazim)	1000 ppm	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	100 ppm						0.5	1.0	1.5	1.8	1.8	-	-	-	-	-
	10 ppm	-	-	-	0.2	0.2	0.8	1.0	1.6	1.8	2.0	-	-	-	-	-
	1 ppm	0.1	0.2	0.2	0.3	0.5	1.0	1.5	2.2	2.8	3.5	-	-	-	0.1	0.5
Captan	1000 ppm	-	-	-	0.2	0.5	-	-	-	-	-	-	-	-	-	-
	100 ppm	0.2	0.5	0.9	1.1	1.5	0.2	0.5	0.8	1.0	1.2	0.5	0.6	0.7	1.0	1.5
	10 ppm	0.6	0.9	1.1	1.4	2.1	0.5	0.8	1.0	1.5	2.0	0.9	1.1	1.6	2.0	2.5
	1 ppm	0.9	1.6	2.0	2.3	2.6	0.8	1.5	2.0	2.3	2.5	1.8	2.0	2.5	2.5	3.0
Thiobendazole (As Tecto)	1000 ppm	0.2	0.2	0.4	0.5	0.8	-	-	-	-	-	0.2	0.3	0.3	0.3	0.4
	100 ppm	0.2	0.5	0.5	0.6	0.8	0.3	0.5	0.6	0.8	0.8	0.2	0.5	0.8	0.8	1.0
	10 ppm	0.3	0.7	0.9	1.8	1.8	0.5	0.7	0.9	1.0	1.5	1.0	1.2	1.5	1.8	2.0
	1 ppm	0.7	1.1	1.8	2.4	3.2	1.0	1.0	1.5	1.8	2.0	1.8	2.0	2.0	2.5	3.0
Thiram (Thyride)	1000 ppm	0.4	0.5	1.1	1.1	1.2	-	-	-	-	-	-	-	-	-	-
	100 ppm	0.4	0.6	1.1	1.2	1.3	-	-	0.2	0.3	0.5	-	0.3	0.8	1.0	1.5
	10 ppm	0.5	0.9	1.7	1.9	2.7	0.2	0.6	0.8	1.0	1.0	0.8	1.0	1.2	1.5	2.0
	1 ppm	0.5	1.2	1.8	2.2	3.0	0.5	0.8	1.2	1.5	2.5	1.5	1.8	2.0	2.5	2.8

Table 3 : Number of fungal colonies developed from seeds of *Vigna sinensis* treated with different fungicides.

	Storage Months																																	
	Control						Benomyl						Bavistin						Captan						Thiobendazole						Thiram					
Mycoflora	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
Alternaria alternate	4	4.5	3	4	3	2.6	3	1	-	-	-	-	1.5	2	1.5	1.5	0.5	0.5	3	2	1	1	0.5	-	2	2	1	1	-	1	-	-	-	-	-	-
Aspergillus floyus	2	3.2	3	5.5	6.5	6.5	4	4	4	-	-	-	4	5	4.5	5	4	4	2	1.5	1.5	0.5	0.5	0.5	-	-	-	-	-	-	2.5	1.5	1	0.5	0.6	0.5
A. niger	2	3.5	3.5	3.5	4.5	5.5	3	3.5	3	2.3	2.5	1.5	2	3.5	2.5	3	2.5	2.5	2	1	1.5	1.5	1	1	3	2.5	3	2	1.5	1.5	2	2	1.5	1.5	0.5	-
A. sydowi	-	-	1.5	3	2	1.5	-	-	-	2	-	2	-	-	-	-	1.3	-	-	-	-	-	1	-	-	-	-	2	1.5	0.5	-	-	-	0.5	-	-
A.terreus	-	2.1	2	1.5	3	3	-	-	1	-	-	-	1	1	1.5	1	0.5	-	-	-	-	1	1	1	1.4	1.4	1.5	1	0.5	0.5	-	-	1	0.5	-	-
Cladosporum herbarum	-	-	-	1	1.5	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.2	1	-	1	-	-	-	-	-	-
Curyularia lunata	2.5	2	1.5	1	1	0.5	1	0.5	-	-	-	-	2	1.5	0.5	-	-	-	2.1	1	-	-	-	-	1.5	1	0.5	-	-	-	0.5	-	-	-	-	-
Drechslera australiensis	2	1	1	-	0.5	0.5	1	0.3	-	-	-	-	2	1	0.5	-	-	-	2	-	-	-	-	-	1	1	-	-	-	-	0.5	-	-	-	-	-
D.tetramera	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fusarium oxysporum	2	1.5	1.5	1.5	1	1	2	1	1	0.5	1	-	-	-	-	-	-	-	1	1	1	0.5	0.5	0.3	2	2	1	0.5	0.2	0.2	1	0.5	-	-	-	-
F. sernitectum	1	1	-	1.5	1.3	1	-	-	1	1	1	1	1	1.3	-	-	-	-	1	-	-	-	-	-	-	-	1	1	0.2	0.2	-	-	1.2	-	-	-
Macrophomina phoseoli	0.5	1	1.5	1	2	2.5	0.5	1	0.5	0.3	0.2	0.2	-	-	-	-	-	-	-	1	1	0.5	-	-	1	1	0.5	-	-	-	-	0.5	0.5	-	-	-
Penicillum sp.	-	-	1	1.5	1	1	0.5	1	0.5	-	-	-	-	-	1	1	-	-	-	0.5	0.5	-	-	-	1.3	1	1	1	1	0.3	5.3	1	0.5	0.5	-	-
Rhizopus arrhizus	-	-	-	0.5	1.5	2	-	-	1	1	1.5	2.5	-	-	-	-	-	-	1	0.5	0.5	9.5	-	-	0.3	0.5	1	-	-	-	1	0.5	-	-	-	-
Total fungal species	8	9	9	13	13	14	8	8	8	6	5	5	7	7	7	5	5	4	8	8	8	7	5	4	9	9	10	8	7	7	8	6	6	5	2	2
Total incidence of fungi	16	19.8	18.5	26.5	28.8	30.6	15	12.3	12	7.1	6.2	7.2	13.5	15.3	12	12	8.8	8	14.4	8.5	8	14.5	4	2.8	139	12.4	11.7	9.5	5.4	4.2	14	6	5.7	3.5	11	2
Percentage germination of seeds	79	79	78.5	78	76.1	75	77	77	76.3	76.1	75	76.3	77	76.9	76.5	70	75.8	75.9	75.8	76	70	74.5	74.2	74.2	77.1	76.9	76.3	75.8	76.5	76	77	77	78.6	76.8	76.1	76

1,2,3,4,5 and6 denotes the months of samples.

disc(5mm disc) separately with 7 days old cultures of *Alternaria alternata, Aspergillus flavus, Colletotrichum lindemuthianum, Fusarium oxysporum, F. semitectum and Macrophomina phaseoli*. The Petri dish were kept in an inverted position so the fungus was in direct contact with poisoned medium. The medium with 20% ethanol without the fungicides was also inoculated with the above mentioned culture which served as control. Diametre of the colony was measured up to 7th standing from the 3rd day of the inoculation(Nene, 1971).

B) Seed treatment by the Fungicides

In order to study the efficacy of the fungicides to eliminate seed-borne fungi of *Vigna sinensis* seed were treated with the above fungicides separately. Each lot was treated with the fungicides (0.2% bavistin, 0.2% benomyl, 2% captan, 0.2% thiram and 0. 2% thiobendazole) separately by shaking the seeds with fungicides in a conical flask on a rotary shaker for 20 minutes. The sixth lot was not treated with fungicides served as control. All the sample were stored in wide glass jars, plugged with cotton wool to exclude the entry of insects and to prevent the free movement of air and stored for 6 months at room temperature(25 to 30). Samples were drawn at monthly intervals to study the microfungi associated with the seed and the germination percentage of seeds.

Results

A) Tables 1and 2 shows that all the 5 fungicides inhibited the growth of these taken fungi when they were incorporated in the medium at different concentration. Even at lowest concentration (*i.e.* 1 ppm) of fungicides, Significant inhibition of growth of fungi as compared to the control was observed. Thiram and bavistin were most effective to control the fungal growth. The growth of Alternaria alternate was completely checked by benomyl, captan and thiram at 1000 ppm concentration. Growth of *Aspergillus flavus* was stopped by thiobendazole . Inhibitory effect of *Macrophomina phaseoli* was noticed by benomyl and captan. Most effective fungicides against *M. phaseoli* was bavistin. At 10 ppm concentration of bavistin completely checked the growth of *Fusarium oxysporum*.

B) Table 3 revealed that when seed were kept after treating with fungicides, the mycoflora on the seed was reduced. In control set, it was observed that there was increase in total percentage of incidence of fungal organism with advance of storage period up to 6 month, but mycoflora gradually declined on fungicides- treated seeds storage with advance of time and after 4 months of treatment of seeds, the mycoflora declined sharply on the seeds. Thiram proved to be most efficient fungicide to reduce the seed-borne fungi on seeds. Besides this fungicide, bavistin and benomyl were effective on seed-borne fungi. All the fungicides were effective in preserving the viability of seeds. Thiram maintained the maximum percentage of seed germination level.

Discussion

It was noticed that (table 1, 2) Thiram and Bavistin considerably reduced the growth of most of fungi.The efficacy of thiram in inhibiting the growth of *A. flavus* in groundnut seed has been reported by Chohan and Gupta (1970). Similar effect of thiram was reported on *Alternaria sp*. and *Rhizoctonia sp.* on seed of *Phaseolus aureus, Lens esculentus and Vigna variegate* by Suhag and Suryanarayana (1976). Agarwal and Singh (1974b) have reported that 0.2% thiram completely inhibited the growth of *Alternaria tenuis, Curvularia lunata, Cephalosporium sp., Helmintho sporium tetramera, Macrophomina sp*. In jute seeds. Sharma and Basu Chaudhary (1975) stated that thiram at 20% ppm completely in inhibited the growth of *of Alternaria alternate, C. lunata, Acremonium sp., Drechslera rostrata, F. equiseti* in pearl millet seed. In Invitro investigation growth of *F. oxysporium* was eliminated completely by captan, but on treated seed with the fungicides growth of the fungus was only reduced (table3). However, these were eliminated completely by bavistin and thiram.

Seed deterioration due to various seed-borne mycoflora is a common feature under faulty condition of storage leading to loss of seed viability. The effect of seed treatment prior to storage appeared to offer a certain amount of protection during storage.

In the present work, the fungicide thiram afford effective protection to the seeds. The viability of the fungicides treated seeds were found to be higher than the untreated seeds. The reduction in germination in the control indicated that fungicides used as seed treated have no phytotoxic effect leading to the loss of viability (table 3).

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