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EFFICACY OF BIOAGENTS AND FUNGICIDES AGAINST SEED BORNE FUNGI OF SOYBEAN

KIRTI PAWAR, S.P. MISHRA AND R.K. SINGH

Department of Plant Pathology, College of Agriculture, RVSKVV, Indore 452001 Madhya Pradesh, India
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ABSTRACT

An experiment was conducted to assess the reduction in association of seed borne fungi and enhancement of germination of soybean (Glycine max (L.)Merill) using four varieties JS 335,JS 9305, JS 9560 and NRC 12 with two storage categories (seeds stored in bins and seeds stored in bags) by treatment with fungicides Thiram(0.25%), Captan (0.25%), Carbendazim (0.1%), Thiram + Carbendazim(1:1) and Chlorothalonil(0.2%) and bioagents T. harzianum and T. viride to evaluate their effect on seed borne mycoflora and germination by two different methods of incubation i.e. Standard blotter and Agar plate methods. In all fourteen fungi including ten pathogens were recorded. Out of five fungicides and two bioagents, Thiram and Thiram + Carbendazim and T. viride showed least association of mycoflora and enhanced per cent germination i.e. Thiram (77.5%), Thiram + Carbendazim (76.4 %) and T. viride (68.3%) with seeds stored in bags of all the four varieties employing standard blotter method.JS 9305 and NRC 12 were found tolerant to expression of seed borne mycoflora along with better germinability viz. (77.5% for JS 9305) and (76.4 % for NRC 12). Among two storage categories, seeds stored in bags showed higher germination and lesser percentage of mycoflora associated with seeds as compared to seeds stored in bins. Overall, two varieties JS 9305 and NRC 12 could be stored in bags and by treating with Thiram (0.25%).

Keywords: Bioagents, seed categories, fungicides, germination, management, mycoflora,

INTRODUCTION

Soybean, (*Glycine max* (L) Merill) is a host of several seed borne fungal pathogens. A large number of pathogens are transmitted through seeds which reduce seed germination, seedling vigour and also cause several diseases like anthracnose,(*Colletotrichum truncatum*), rhizoctonia root rot (*Rhizoctonia solani*), phomopsis seed decay *(Phomopsis sojae*), charcoal rot (*Macrophomina phaseolina*), myrothecium leaf spot (*Myrothecium roridum*), alternaria leaf spot(*Alternaria alternata*), purple stain (*Cercospora kikuchii*) and helminthosporium leaf spot (*Helminthosporium* sp). Mycoflora associated with seed results in greater loss in seed quality and also in yield. Seed treatment is one of the best methods to manage seed borne diseases. Several fungicides have been employed to control of fungal diseases of the crop. Agrochemicals like Thiram, Captan and copper oxychloride were used to control many diseases caused by fungal pathogens in soybean**.** Ibiam *et al* (2000) and (2006) observed that seed dressing fungicides- Benlate, Apron plus 50 DS Fernasan- D, Dithane M-45 and Bavistin controlling seed bore fungi of rice. Mane *et al.* (2010) evaluated efficacy of various fungicidal seed treatment on seed mycoflora and seed germination during storage of sorghum. Even though effective and efficient control of seed borne fungi can be achieved by the use of fungicides, the same cannot be applied to grains for reasons of pesticide toxicity (Harris *et al*. 2001). The continuous and indiscriminate use of chemicals to control diseases results in accumulation of harmful residues of chemicals in the soil, water and seed. In recent years, considerable success has been achieved by introducing antagonists to control seed borne fungal pathogens. A remarkable work has been done for management of seedling diseases of many crops caused by *Rhizoctonia solani* and *Sclerotium rolfsii* both *in vitro* and in pot culture experiments by using *Trichoderma* (Akhter 1999, Pradeep *et al*. 2000, Raihan *et al.* 2003, Haider 2005).The present study has, therefore, been undertaken to evaluate the effect of seed treatment with chemicals and/ or bio-agents in reducing the associated seed-borne fungi and in turn enhancing germination of seeds.

MATERIALS AND METHODS

A study was conducted to assess the association of seed borne mycoflora of soybean using four varieties JS 335,JS 9305,JS 9560 and NRC 12 with two different storage categories i.e seeds stored in bins and seeds stored in bags by using two different methods, namely ,Standard blotter paper method and Agar plate method. Seeds were treated with fungicides (Thiram (0.3%) Captan (0.3%), Carbendazim (0.1%), Thiram + Carbendazim (1:1) and Chlorothalonil (0.3%) and talc formulations of bioagents *Trichoderma viride* and *T. harzianum* containing

Corresponding author e-mail: kirtibhavya@gmail.com

10^7cfu/g by two incubation methods (i) Standard blotter method (ISTA, 1978) (ii) Agar plate method (Modified Ulster method, Muskett and Malone, 1948).

Standard blotter method: A set of 400 seeds treated with fungicides/ bioagents were incubated for seven days under alternating cycles of 12 hours NUV (near ultra violet) light and 12 hours of darkness at a temperature of $27^oC \pm 1$.

Agar plate method: In this method, potato dextrose agar medium (PDA) was poured aseptically in the sterilized glass petri dishes of 10 cm diameter @ 15 to 20 ml per petri dish and keeping the seeds treated with fungicides/ bioagents on sterilized blotting paper in aseptic conditions. These petri dishes were further incubated at 27 ± 1^oC for seven days under 12 hours alternating cycles of NUV light and darkness. These seeds were examined for germination and fungal growth after five and eight days of incubation. The seeds were treated with the talc formulations of bio control agents (BCA). Bio-control agents have been used as seed dressers to see their effect on seed borne mycoflora viz., *Trichoderma viride*, *T. harzianum* @ 5 g/kg along with fungicides carbendazim, thiram, chlorothalonil, captan and thiram+ carbendazim were evaluated by Standard blotter method and Agar plate methods. Untreated seeds served as control.Fungi were identified by preparing temporary slides and examining under the compound microscope. In fewer cases the fungi from the incubated seeds were transferred to PDA in petridishes aseptically and incubated at 28±1˚C for 3-10 days and then examined under compound microscope. Identification of fungi was confirmed observing their growth characters on the slides and by examining the cultures under the microscope through the characters of mycelium, spores and fruiting bodies. The total number of fungal species isolated by two incubation methods was calculated on percent basis to find out the arising fungi and the difference between two incubation methods.The data on the percentage germination and association of fungi in different categories, under the influence of fungicide and management of myco flora by bio control agents and/or fungicide were analysed statistically.The values were transformed by $\sqrt{X} + 0.5$ and angular transformation wherever necessary.

RESULTS AND DISCUSSION

The study using seven seed treatments including five fungicides, namely ,Thiram, Captan, Carbendazim, Chlorothalonil and Thiram + Carbendazim and two bioagents *T. harzianum* and *T. viride* revealed association of fourteen seed borne fungi on four varieties of soybean stored in two conditions. The fungi included *A. flavus, A. imperfectii, A. alternata, C. kikuchii, C. truncatum, D. phaseolorum, Helminthosporium* sp.*, R. stolonifer, F. oxysporum* and *M. phaseolina, M. roridum, R. bataticola, S. rolfsii* and *P. sojae*. Least association of mycoflora was recorded under Thiram and Thiram + Carbendazim followed by Carbendazim by both incubation methods.The averages of all seven treatments have been presented in Table 1.Seed treatment with Thiram and Thiram + Carbendazim effectively reduced all the fungi except *R. bataticola* and *S. rolfsii*. Out of four varieties employed JS 335 and JS 9560 harbored higher number of fungi on seeds in comparison to JS 9305 and NRC 12, consistently in both the Standard blotter and Agar plate methods.

Table 1: Fungi (%) associated with soybean varieties after treatment with fungicides and bioagents by Standard Blotter and Agar Plate methods

Response of fungicides and bioagents on varieties by ↓	***Fungi associated under two storage conditions (%) →***	***Aspergillus flavus***		***Alternaria alternata***		***Aschochyta imperfetii***		***Colletotrichum truncatum***		***Cercospora kikuchii***		***Diaporthe phaseolorum***		***Fusarium oxysporum***		***Helminthosporium sp.***		***Macrophomina phaseolina***		***Myrothecium roridum***		***Phomopsis sojae***		***Rhizopus stolonifer***		***Rhizoctonia bataticola***		***Sclerotium rolfsi***	
Standard blotter method		c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2	c_1	c_2
	JS 335 *	2	1	3	2	3	2	3	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	-	-	-	-
	JS 9305	3	2	3	2	-	-	3	2	-	-	-	-	3	1	3	2	-	-	-	-	2	2	2	1	3	1	-	-
	JS 9560	3	2	3	2	3	2	2	1	3	1	3	1	2	1	3	2	3	2	3	1	2	1	3	1	-	-	2	1
	NRC 12	3	2	3	2	-	-	3	1	-	-	-	-	3	2	3	2	2	1	-	-	-	-	2	1	2	1	-	-
	Control	14	12	12	10	11	10	11	9	13	9	12	10	12	13	13	10	9	8	-	-	-	-	9	8	2	4	-	-
Agar Plate method	JS 335	3	1	3	2	3	1	2	1	2	1	3	1	2	1	2	1	2	1	3	1	2	1	3	1	-	-	-	-
	JS 9305	3	2	3	2	-	-	4	2	-	-	-	-	3	2	3	2	-	-	-	-	3	2	3	2	3	1	-	-
	JS 9560	3	2	3	2	3	2	3	2	3	1	3	2	3	2	3	1	3	2	3	2	3	1	2	1	-	-	2	1
	NRC 12	3	2	3	3	-	-	3	2	-	-	-	-	3	2	3	2	3	2	-	-	-	-	3	2	3	2	-	-
	Control	13	12	13	10	12	10	12	8	13	9	13	9	12	9	12	10	13	12	10	8	12	9	10	8	13	10	9	8

**Average of 7 treatments viz., T1-Captan(0.25%),T2- Thiram (0.25%) ,T3-Carbendazim(0.1%), T_4-Thiram + Carbendazim (0.1%), T_5-Chlorothalonil(0.2%), T_6- T.harzianum, T_7- T.viride , C_1 – stored in bins, C2- stored in bags*

However, fungicides or bioagents applied as seed dressers drastically reduced the intensity of seed mycoflora as compared to control. It was observed that intensity of seed borne mycoflora was higher on seeds stored in bins in comparison to those stored in bags. Thus varieties JS 9305 and NRC 12 showed tolerance to seed borne mycoflora under storage in bags. The data on germination of seeds of four varieties stored in bins and bags as evident by both the test methods, Standard blotter method (Table 2) and Agar Plate method (Table 3) indicated that significantly higher germination was recorded in seeds stored in bags as compared to those stored in bins. Varieties JS 9305 and NRC 12 responded better to seed treatments exhibiting higher germination as compared to JS 335 and JS 9560. Seed treatment with Thiram resulted in maximum percentage germination of seeds stored in bag (77.5%) of all the varieties followed by Thiram + Carbendazim (76.4%) and Carbendazim (73.2%). The corresponding per cent germination in control was (55.5%) through Standard Blotter Method. In case of seeds stored in bin, seed treatment with Thiram (70.2%) and Thiram + Carbendazim (70.06%) showed maximum per cent germination followed by Carbendazim (67.0%) and Captan (66.5%) as compared to 48.55% in control by Standard Blotter Method.By Agar Plate method, Thiram showed maximum seed germination (74.7%) followed by Thiram + Carbendazim(73.2 %) & Carbendazim (70.7%) and (53.7%) in control for seeds stored in bags and for seeds stored in bins (Carbendazim (67.1%) and Thiram (66.7%)showed maximum percent germination followed by Thiram + Carbendazim (64.6%) as compared to (46.2%) in Control (Table 3). Their seed treatment eliminated the seminosphere mycoflora and results in slight improvement in seed germination and seedling vigor.

Table 2: Effect of seed treatment with fungicide and bioagents on germination % of different soybean varieties using Standard Blotter method

Seed category (C) →	Germination(%) of seeds during storage in										Overall mean
	bins					bags					
Seed treatment with ↓	JS 335	*JS 93-05*	JS 95-60	NRC-12	Mean	JS 335	JS 93-05	JS 95-60	NRC 12	Mean	
Captan	60.3	68.4	61	76.2	66.5	70.2	68	66.3	78.4	70.8	68.6
	(50.9)	(55.8)	(51.3)	(60.8)	(54.7)	(56.9)	(55.5)	(54.5)	(62.3)	(57.3)	(56.1)
Thiram	64.4	71.2	65.5	79.6	70.2	75.3	81.2	70.4	83.2	77.5	73.8
	(53.3)	(57.5)	(54)	(63.1)	(57)	(60.2)	(64.3)	(57)	(64.3)	(61.9)	(59.4)
Carbendazim	59.9	65.5	66.7	76.1	67	68.4	72.5	71.1	81.1	73.2	70.1
	(50.6)	(54)	(54.7)	(60.7)	(55.1)	55.9)	(58.3)	(57.4)	(64.1)	(58.9)	(57))
Thiram+	68.3	70.2	63.4	78.2	70.1	73.3	80.7	71.4	80.5	76.4	73.2
Carbendazim	(55.7)	(56.9)	(52.8)	(62.1)	(56.9)	(58.8)	(63.9)	(57.6)	(63.7)	(61.1)	(58.9)
Chlorothalonil	58.2	65.3	60	71.3	63.7	65.3	73.4	68.2	76.3	70.8	67.2
	(49.7)	(53.9)	(50.7)	(57.6)	(53.0)	(53.9)	(58.9)	(55.6)	(60.8)	(57.3)	(55.1)
T. harzianum	58	67.3	62.6	73.2	65.2	60.2	68.3	64.3	76.3	67.2	66.2
	(49.6)	(55.1)	(52.3)	(58.8)	(53.9)	(50.8)	(55.7)	(53.3)	(60.8)	(55.2)	(55.5)
T. viride	61.3	65.2	55.2	74.1	63.9	63.1	69.7	61.2	79.3	68.3	66.1
	(51.5)	(53.8)	(47.9)	(59.4)	(53.1)	(52.5)	(56.6)	(51.4)	(62.9)	(55.9)	(54.5)
Control	42.3	46.4	51	54.4	48.5	49.3	58.8	48.4	65.7	55.5	52.1
	(40.5)	(42.9)	(45.5)	(47.5)	(44.1)	(44.6)	(50.1)	(44.1)	(54.1)	(48.2)	(46.1)
	58.8	64.5	61.2	72.6	64.3	66.9	72.4	65.9	77.5	70.73	67.53
Mean	(50.1)	(53.5)	(51.5)	(58.6)	(53.4)	(55.1)	(58.5)	(54.4)	(61.8)	(57.4)	(55.4)

Comparison of	SEm(±)	CD(P=0.05)	Comparison of	SEm(±)	CD(P=0.05)
Varieties(V)	0.23	0.65	V×T	0.56	1.59
Treatment(T)	0.28	0.79	V×C	0.33	0.92
Category©	0.17	0.46	T×C	0.39	1.12
			V×T×C	0.78	2.25

In the present study also reduced association of the myco flora and improvement in germination was observed with treated seeds and additive effects of fungicides and bio-agents as seed dressers in reducing the internally seed-borne fungi is quite obvious. Hall and Xue (1995) observed Thiram, Carbendazim, benomyl and Captan to be effective in eliminating the pathogens.Maximum per cent germination was recorded by Thiram and Thiram + Carbendazim in seeds stored in bags and by Carbendazim and Thiram in seeds stored in bin using Standard blotter method and Agar Plate method. Rathod and Pawar (2013) isolated seed borne fungi of soybean variety Durga and evaluated the effect of various fungicides on mycoflora and germination and observed that copper oxychloride increased the germination and reduced the mycoflora. In the present study, among the bio-agents *T. viride* was found to be more effective in reducing seed myco flora and enhancing percentage germination (68.3%) followed by *T. harzianum* (67.2%)in seeds stored in bags while in seeds stored in bins, treatment with *T. harzianum* resulted in maximum percentage seed germination (65.2%) followed by *T. viride* (63.9%) by Standard Blotter Method (Table 2) and in Agar Plate Method *T. viride* was observed to show maximum percentage germination (66.1%) on seeds of all the four varieties, followed by *T. harzianum* (65.9%) in seed stored in bag as, while, on seeds stored in bin, treatment with *T. harzianum* resulted in maximum percentage seed germination (64.6%) followed by *T. viride* (62.9%) (Table 3).Girija and Umamaheshwaran (2003) reported the reduction of mycelium growth of *S. rolfsii* in interaction with *Trichoderma* spp .Among the two incubation method, Standard blotter method showed maximum percentage germination as compared to Agar plate method.(Table 2 and 3). Among all the various interactions studied, interactions between varieties with seed category, varieties with treatments, seed category with treatments, varieties and seed category with treatments, all of them were found to be statistically significant. The present observations have shown the value of seed treatments for improving stand and yield of soybean.

Table 3: Effect of seed treatment with fungicide and bioagents on percent germination of different soybean varieties using Agar Plate method

Seed category© →	**Germination (%) of seeds during storage in**										**Overall mean**
	bins					**bags**					
Seed treatment with↓	**JS 335**	**JS 93-05**	**JS 95-60**	**NRC-12**	**Mean**	**JS 335**	**JS 93-05**	**JS 95-60**	**NRC 12**	**Mean**	
Captan	58.0 (49.6)	63.4 (52.7)	55.1 (47.9)	50.6 (45.5)	56.7 (48.9)	68.2 (55.6)	65.3 (53.9)	63.1 (52.5)	77.5 (61.6)	68.5 (55.9)	62.6 (52.4)
Thiram	63.2 (52.6)	66.1 (54.3)	62.3 (52.1)	75.2 (60.1)	66.7 (54.8)	73.2 (58.7)	78.3 (62.2)	66.1 (54.4)	81.3 (64.3)	74.7 (59.9)	70.7 (57.3)
Carbendazim	65.3 (53.9)	69.4 (56.4)	61.5 (51.6)	72.2 (58.1)	67.1 (55.1)	66.2 (54.4)	69.5 (56.4)	68.3 (55.7*)	79.1 (62.7)	70.7 (57.3)	68.9 (56.1)
Thiram+ Carbendazim	61.4 (51.5)	63.2 (52.6)	60.7 (51.1)	73.1 (55.7)	64.6 (53.5)	71.2 (57.4)	77.2 (61.4)	65.4 (53.9)	79.3 (62.9)	73.2 (58.9)	68.9 (56.2)
Chlorothalonil	61.2 (51.4)	65.3 (53.9)	58.1 (50.1)	68.2 (55.6)	63.3 (52.7)	62.1 (52.0)	71.3 (57.6)	67.1 (55.0)	74.3 (59.5)	68.7 (56.0)	66.1 (54.4)
T. harzianum	56.2 (48.5)	65.2 (53.8)	65.3 (53.9)	72.0 (64.6)	64.6 (53.6)	61.3 (51.5)	65.2 (53.8)	62.2 (52.1)	75.1 (60.1)	65.9 (54.3)	65.3 (53.9)
T. viride	61.1 (51.4)	63.7 (52.9)	53.4 (46.9)	73.6 (59.1)	62.9 (52.6)	61.1 (51.4)	66.5 (54.6)	60.0 (50.7)	77.1 (61.4)	66.1 (54.5)	64.5 (53.5)
Control	41.3 (39.1)	42.1 (40.4)	49.2 (44.5)	52.3 (46.3)	46.2 (42.8)	48.5 (44.1)	56.7 (48.8)	46.3 (42.8)	63.3 (52.7)	53.7 (47.1)	49.9 (44.9)
Mean	58.4 (49.8)	61.6 (51.7)	57.9 (49.5)	65.2 (54.2)	60.8 (51.3)	64.9 (53.7)	69.7 (56.7)	62.7 (52.4)	75.8 (60.6)	68.3 (55.8)	64.5 (53.6)

Comparison of	**SEm(±)**	**CD(P=0.05)**	**Comparison of**	**SEm(±)**	**CD(P=0.05)**
Varieties(V)	0.36	1.03	V×T	0.88	2.52
Treatment(T)	0.44	1.26	V×C	0.51	1.46
Category©	0.25	0.73	T×C	0.62	1.78
			V×T×C	1.24	3.57

Seed treatment with Thiram and Thiram + Carbendazim effectively reduced the seed borne mycoflora except *R. bataticola* and *S. rolfsii.* Varieties JS 9305 and NRC 12 were tolerant whereas JS 335 and JS 9560 were more prone to expression of seed borne mycoflora. Varieties JS 9305 and NRC 12 also responded to higher seed germination than the other two varieties in response to seed treatment and storage conditions. Among the two storage categories, seeds stored in bags showed higher germination and lesser percentage of mycoflora associated with seeds as compared to seeds stored in bin for evaluating the effect of fungicides and /or bio-agents on seed borne mycoflora and enhancing the germination.

REFERENCES

Akther, N. (1999) Biological control of seedling mortality of different crops caused by *Sclerotium rolfsii* using antagonistic fungi. MS Thesis, Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur. 76 pp

Girija, V. K. and Umamaheshwaran, K. (2003) Basal rot of balsam and its management through bio agents. , *Plant Disease Research* **18** (1): 52-55.

Haider, M.M. (2005) Biological and chemical control of *Rhizoctonia* dry root rot and foliar blight of soybean(Glycine *max* L. MERR). Department of Plant Pathology, Bangabandhu Sheik Mujibur Rahman Agricultural University, Gazipur. 68 pp

Hall, Robert and Xue, Allen G. (1995) Effectiveness of fungicidal seed treatment applied to smooth or shrivelled soybean seeds contaminated by *Diaporthe phaseolorum. Phytoprotection* **76**: 47-56.

Harris, C.A. Renfrew, M.J. and Woolridge, M.W. (2001) Assessing the risk of pesticide residues to consumers: recent and future developments. *Food Additives and Contamination* **18**: 1124-1129.

Ibiam, O. F. A., Umechuruba, C. I. and Arinze, A. E. (2000) Field Evaluation of seed –dressing

fungicides Bavistin , Benlate Fernasan – D and Apron Plus 50 DS associated with three rice varieties Faro12, Faro 15, Faro 29. *Journal of Health and Visual Science* **2**: 96 – 106.

Ibiam, O. F. A., Umechuruba, C. I. and Arinze, A. E. (2006) Evaulation of the Efficacy of seed Dressing fungicides (Bavistin, Benlate, Fernasna–D, Apron Plus 50 DS, AND Dithane–M45). In the control of Seed – Borne Fungi of Rice (Oryzae sativa L) Variety Faro 15 .*In Vitro Sciencia Africana* **5** (1): 1-10.

ISTA (1978). International Rules for Seed Testing. *Seed Science Technol*ogy **13:** 484-487.

Muskett, A.E. and Malone, J.P.(1948) The Ulster method for the examination of flax seed for the presence of seed-borne parasites. *Annals of Applied Biol*ogy **28**:8-13.

Mane ,P. V., L. R. Rathod G. B. Honna, V. C. Patil & S. M. Muley (2011) Effect of fungicidal seed treatment on seed mycoflora and seed germination during storage of sorghum. *Bioscience Discovery* **02** (2):214-216

Pradeep, K., Anuja, Kumud, K., Kumar, P. and Kumar, K. (2000) Bio control of seed borne fungal pathogens of pigeonpea (*Cajanus cajan* (L.) Millsp.). *Annals of Plant Protection Sciences* **8**:30-32

Raihan, M.G., Bhuiyan, M.K.A. and Sultana, N. (2003) Efficacy of integration of an antagonist, fungicide and garlic extract to suppress seedling mortality of peanut caused by *Rhizoctonia solani* and *Sclerotium rolfsii*, *Bangladesh Journal of Plant Pathology* **19** (1&2): 69-73

Rathod, L.R. and Pawar, N. B. (2013) In Vitro seed treatment of fungicides for the control of seed borne fungi of soybean variety Durga. *Global Research Analysis* **10**(2)**:** 15-16.