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EFFECT OF ORGANIC AND INORGANIC SEED PRIMING ON SOYBEAN GERMINATION AND YIELD PARAMETERS

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ABSTRACT

Efficiency of different biopriming agents i.e. biocontrol agents (*Trichoderma harzianum* @ 0.6%, *Pseudomonas fluorescens* @ 0.6%, *Bacillus subtilis* @ 0.6%) and fungicides (Tebuconazole 5% EC @ 0.2%, Captan 70% WP + Hexaconazole 5% EC @ 0.2%, Carboxin 37.5% + Thiram 37.5% @ 0.2%, Captan @ 0.2%, Carbendazium @ 0.2%, Mancozeb 50% + Carbendazim 25% WS @ 0.2%) in enhancing seed germination, seedling growth and yield were investigated in soybean seeds of JS-335 variety. Primed seeds were subjected for lab and field experiments. Under lab condition, combifungicides carboxin 37.5% + thiram 37.5% @ 0.2% recorded significantly higher seed germination and root length. Whereas, shoot length and seedling length was higher in *Trichoderma harzianum* @ 0.6%. Under field condition the *Bacillus subtilis* @ 0.6% recorded highest seed yield followed by captan 70%WP + hexaconazole 5% EC @ 0.2% and carbendazim 25% + mancozeb 50% WS @ 0.2%. In all other seed priming treatments there was an increase in number of pods per plant, pod weight per plant, seed weight per plant, 100 seed weight and seed yield compared to untreated control.

Key words: Bio priming, Combifungicides, *Trichoderma harzianum*, Biocontrol agents.

INTRODUCTION

Soybean (*Glycine max* (L) Merrill.) belonging to family Papilionaceae, possess a very high nutritional value. It contains about 20 per cent oil and 40 per cent high quality protein, 30 per cent carbohydrates, 4 per cent saponins and 5 per cent fiber. The oil contains about 0.5-1.0 per cent lecithin which is essential for building up of human nerve tissues. Due to high protein content, soybean is known as `poor man's meat'. Soybean protein is rich in valuable amino acid lysine (5%) in which most of the cereals is deficient. In addition, it contains a good amount of minerals, salts and vitamins (thiamine and riboflavin) and its sprouting grains contain a considerable amount of Vitamin A, Vitamin C.

Soybean production and quality of produce in India has to be increased to compete in the world soybean production and its export. The production is primarily constrained by the crop stand, for which one of the reason would be presence of infected seeds in the seed material. Seed treatment with microbial antagonists or fungicides protect the seed from infection by seed borne and soil borne pathogens, enables the seed to germinate and establish as a healthy seedling (Chang and Kommedahl, 1968; Henis and Chet, 1975; Windels, 1981). Seed treatment

is therefore a routine practice to ensure good emergence and better crop stand (Nene and Thapliyal, 1979; Ramos and Ribeiro, 1993). There are diverse opinion whether legume seeds should be treated with fungicides and whether seed dressing materials might adversely affect the *Rhizobium spp*., and hence the nodulation.

A successful antagonist should colonize rhizosphere during seed germination (Weller, 1983). Priming with PGPR increase germination and improve seedling establishment. It initiates the physiological process of germination, but prevents the emergence of plumule and radicle. Initiation of physiological process helps in the establishment and proliferation of PGPR on the spermosphere (Taylor and Harman, 1990). Bio-priming of seeds with bacterial antagonists increase the population load of antagonist to a tune of 10 fold on the seeds thus protected rhizosphere from the ingress of plant pathogens (Callan *et al.*, 1990). There are diverse opinion whether legume seeds should be treated with fungicides and whether seed dressing materials might adversely affect the *Rhizobium spp*., and hence the nodulation. Hence, there is limited information on effect of seed priming treatment the present study was conducted on "Effect of organic and inorganic seed priming on soybean germination and yield parameters".

MATERIALS AND METHODS

The present study was undertaken to know the efficiency of different biopriming agents. Laboratory and field experiments were carried out at Department of Crop Physiology, College of Agriculture, Dharwad. The various methodologies followed during the investigation are summarized here under.

2.1 Seed source and seed priming

Seeds of soybean variety JS-335 were obtained from Seed Unit, Main Agricultural Research Station, Dharwad. Seeds of JS-335 Soybean variety were presoaked for 8 hours in water. Then all seeds were treated with rhizobium culture @10g/kg seeds using natural gum. After that seeds were treated with bioagents or fungicides as per the treatments given below and shade dried overnight by spreading on ground at room temperature (Plate 1.).

Experimental Details

Treatments

T_1 : Tebuconazole 5% EC @ 0.2%
T_2 : Captan 70% WP + Hexaconazole 5% EC @ 0.2%
T_3 : Carboxin 37.5% + Thiram 37.5% @ 0.2%
T_4 : Captan @ 0.2%
T_5 : Carbendazium @ 0.2%
T_6 : Mancozeb 50% + Carbendazim 25% WS @ 0.2%
T_7 : *Trichoderma harzianum* @ 0.6%
T_8 : *Pseudomonas fluorescens* @ 0.6%
T_9 : *Bacillus subtilis* @ 0.6%
T_{10} : Control

Soybean seeds primed with above treatments were subjected to germination studies in laboratory and, seed germination and seedling growth parameters were recorded.

Lab Experiment

Germination (%)

Germination test was conducted in two replications of 50 seeds each by adopting paper towel method as described by ISTA procedures (Anon, 1999). Seeds were incubated at slanting position in Walk-in germinator room in growth cabinets. The temperature of 25 ± 1^0C and RH of 95 per cent was maintained during the germination test. Daily germination count were performed until no further germination occurred for eight consecutive days, then germination percentage was calculated.

Germination (%) = Number of seeds germinated/ Total number of seeds kept for germination× 100

Root length (cm)

Ten normal seedlings were selected randomly in each treatment from all the replications on eighth day from germination test. The root length was measured from the tip of the primary root to base of hypocotyls with the help of a scale and mean root length was expressed in centimetres.

Shoot length (cm)
The ten normal seedlings used for root length measurement were also used for the measurement of shoot length. The shoot length was measured form the tip of the primary leaf to the base of the hypocotyls and mean shoot length was expressed in centimetre.

Seedling length (cm)
Using the same ten normal seedlings, seedling length measurement was also recorded. Seedling length was measured from tip of the primary leaf to the tip of primary root with the help of a scale and mean root length was expressed in centimeters.

Seedling fresh weight (mg)
The ten normal seedlings used for root and shoot length measurements were weighed. The mean fresh weight of the seedlings was recorded and expressed in milligrams.

Seedling dry weight (mg)
The ten normal seedlings used for root and shoot length measurements were put in butter paper pocket and kept in hot air oven at 70 ± 10^0C for 24 hours. The mean dry weight of the seedling was recorded and expressed in milligrams.

Field Experiment

The crop was harvested when plants started drying to pale color, leaflets started shedding and pods turned to pale color. The border row plants were first uprooted manually from all sides of each plot and then the net plots were harvested excluding five plants randomly selected and tagged for recording the observations. The harvested plants were dried in shade for seven days. The seeds were separated manually by gently beating the dried plants with a wooden stick. The seeds were cleaned and dried in the shade, the seed yield (g plant^{-1}) was recorded for each treatment and then seed yield per hectare was computed and expressed as kg ha^{-1}.

No. of pods/plant
The number of pods harvested from five randomly selected and tagged plants in each treatment was counted and average was worked out and expressed as number of pods per plant.
2.4.2 100 seed weight (g)

Hundred seed in each treatment was counted manually and the weight was recorded as per the procedure given by ISTA rules (Anon., 1999). The average hundred seed weight was recorded in grams.

Seed weight per plant (g)
The five plants were uprooted at harvest physiological maturity and processed for seed yield, from which the average yield was calculated and expressed as seed weight per plant.

Seed yield (kgha^{-1})
The matured pods harvested from the net plot in each treatment were sun dried and the seeds were separated. The weight of the seeds from net plot area was recorded and the seed yield obtained from five randomly selected and tagged plants were added to the seed yield of the net plot area for calculation of seed yield per plot (Kg). Then, the seed yield (kg ha^{-1}) was calculated.

Harvest Index (%)
Harvest Index (HI) was calculated by using the formula of Donald (1968) and expressed as percent.

$$HI\ (\%) = \frac{\text{Economic yield (g)}}{\text{Biological yield (g)}} \times 100$$

RESULTS AND DISCUSSIONS

In addition to identification of a tolerant variety, control of disease would further enhance the productivity and quality of produce. In this direction, seed priming could be one of the technique for management of PSS (Satyaprashant, 2004). Significant differences in seed germination and seedling growth parameters were observed due to seed priming in soybean (Table 1.) Significantly higher seed germination (99%) was recorded with carboxin 37.5% + thiram 37.5% @ 0.2%, carbendazim @ 0.2% (94%), tebuconazole 5% EC @ 0.2%

(93%) and *Trichoderma harzianum* @ 0.6% (93%) over control (89%) and other treatments. However seedling length was significantly highest with *Trichoderma harzianum* @ 0.6%, *Pseudomonas fluorescens* @ 0.6%, and carbendazim@ 0.2%, compared to control (Plate 2.). The variation in seed germination percentage and seedling length may be attributed to plant growth promotional effect of seed primers especially bioagents that may produce growth regulatory substances (hormones) upon seed imbibition. These findings are in agreement with the findings of Bapurayagouda (2010), Jin and Tylkowska (2005). Whereas lower seed germination was recorded in untreated control.

The decline in germination percentage may be attributed to ageing effect leading to depletion of food reserves and decline in synthetic activity of embryo apart from death of seed because of fungal invasion, insect damage, fluctuating temperature, relative humidity. Similar results were reported by Vidhyasekaran *et al.* (1980) in sorghum and millet, Ashokan *et al.* (1981) in finger millet and Hooda and Singh (1993) in wheat.

The chemical treatments keep the seed intact, as its acts as binding material; it covers the minor cracks and aberration as the seed coat thus blocking the fungal invasion. It may also acts as a physical barrier, which reduces leaching of inhibitors from seed covering and restricts oxygen movement and thus reducing the respiration of embryo thereby reducing the ageing effect on seed (Vanangamudi et al. 2003). In the present study also combifungicides besides being toxic to fungus might have acted

Table 1. Seed germination and seedling growth parameters as influenced by seed primers in soybean

Treatments	Germination %	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Fresh weight (mg seedling $^{-1}$)	Dry weight (mg seedling $^{-1}$)
T_1 : Tebuconazole 5% EC @ 0.2%	93.00	20.43	5.97	26.40	810	130
T_2 : Captan 70%WP + Hexaconazole 5% EC @ 0.2%	90.00	15.20	2.14	17.34	690	130
T_3 : Carboxin 37.5% + Thiram 37.5% @ 0.2%	99.00	19.87	9.94	29.80	890	120
T_4 : Captan @ 0.2%	91.00	18.14	11.09	29.23	940	120
T_5 : Carbendazim @ 0.2%	94.00	18.18	13.10	31.28	820	120
T_6 : Carbendazim 25% + Mancozeb 50% WS @ 0.2%	91.00	16.40	11.53	27.93	900	130
T_7 : *Trichoderma harzianum* @ 0.6%	93.00	16.73	14.85	31.58	920	120
T_8 : *Pseudomonas fluorescens* @ 0.6%	89.00	18.78	12.58	31.35	910	120
T_9 : *Bacillus subtilis* @ 0.6%	90.00	15.28	13.80	29.08	890	120
T_{10} : Control	89.00	17.35	11.65	29.00	750	110
Mean	**91.9**	**17.63**	**10.66**	**27.74**	**852**	**122**
S.Em±	1.29	0.82	0.59	1.52	3.00	3.32

Table 2. Influence of seed primers on yield parameters in soybean

Treatments	50% flowering (days)	No. of pods/plant	Pod wt./plant (g)	Seed wt./plant (g)	100 seed wt.	% increase in 100 seed wt. over control	Seed yield (kg ha^{-1})	% increase in seed yield over control	Harvest index (%)
T_1:Tebuconazole 5%EC @ 0.2%	44.00	53.33	24.53	18.40	12.22	9.89	2750	1.75	0.56
T_2:Captan 70%WP + Hexaconazole 5%EC @ 0.2%	49.67	76.67	35.20	25.93	13.31	19.69	3116	15.29	0.61
T_3:Carboxin 37.5% + Thiram 37.5% @ 0.2%	41.33	52.40	20.00	18.40	11.96	7.55	2870	6.17	0.54
T_4 :Captan @ 0.2%	41.00	57.40	23.73	16.73	12.43	11.78	2863	5.92	0.53
T_5:Carbendazim @ 0.2%	41.00	49.13	20.87	13.20	11.68	5.04	2746	1.60	0.51
T_6:Carbendazim 25% + Mancozeb 50% WS @ 0.2%	41.00	59.60	32.52	24.80	12.35	11.06	3083	14.06	0.62
T_7:*Trichoder maharzianum* @ 0.6%	39.33	52.53	20.93	15.67	11.58	4.14	2780	2.84	0.55
T_8:*Pseudomonas fluorescens* @ 0.6%	41.33	60.67	22.93	19.00	12.29	10.52	2935	8.57	0.59
T_9:*Bacillus subtilis* @ 0.6%	39.00	70.27	36.50	31.20	12.45	11.96	3221	19.17	0.66
T_{10}:Control	39.00	58.27	23.27	21.20	11.12	-	2703	-	0.47
Mean	**41.67**	**60.43**	**26.00**	**20.20**	**12.14**		**2907**		**0.56**
S.EM±	1.22	4.18	1.75	1.39	0.31		158.26		0.04
CD at 5%	3.63	12.43	5.19	4.13	0.92		470.21		0.11

as seed coat barriers inhibiting seed respiration resulting in delayed aging and improving germination per cent.

Effect of seed treatment with fungicides on seedling length differed significantly in all the treatments. The seeds treated with *Trichoderma harzianum* @ 0.6% recorded significantly highest seedling length followed by *Pseudomonas fluorescens*@ 0.6%. The lowest seedling length was recorded in captan 70% WP + hexaconazole 5% EC @ 0.2% (14.53 cm) treated seeds.Seedling length was significantly reduced with Captan 70% WP + Hexaconazole

5% EC @ 0.2% (17.34 cm) treatment followed by tebuconazole 5% EC @ 0.2% (26.40 cm) and other treatments were on par with control (29.00 cm). The reduction in seedling length with captan 70% WP + hexaconazole 5% EC @ 0.2% was because of significant reduction in both shoot length and root length hypertrophy resulting in stout seedlings (Plate 2.) compared to other treatments. These chemicals must have inhibited apical dominance or cell division at root and shoot apex. The promotion of cambial cell division might have caused thick shoot and root resulting in stout seedlings. Similar results were reported by Poonam Singh *et al.* (2004) in rice. Seedling dry weight differed significantly in all treatments. tebuconazole 5% EC @ 0.2%, captan 70% WP + hexaconazole 5% EC @ 0.2% and carbendazim 25% + mancozeb 50% WS @ 0.2% recorded highest seedling dry weight among the treatments and was on par with most of the priming treatments, and lowest seedling dry weight was recorded in untreated control.

Seed yield differed significantly with priming treatments over control (Table 2.). The yield was significantly higher in *Bacillus subtilis* @ 0.6% (31.20g/plant) followed by carbendazim 25% + mancozeb 50% WS @ 0.2% (24.80g/plant) compared to control (21.20 g/plant) and other treatments. It was indicated that seed priming has differential influence on the allocation of assimilates between vegetative and reproductive organs. In general, crop yield depends on the accumulation of photo-

Table 3. Effect of seed priming on economics in soybean

Treatments	Seed yield (Kg ha^{-1})	Seed yield (q ha^{-1})	Gross returns (Rs ha^{-1})	COC (Rs ha^{-1})	Net returns (Rs ha^{-1})	B:C
T_1:Tebuconazole 5% EC @ 0.2%	2750.67	27.51	55013	16277	38735	2.38
T_2:Captan 70%WP + Hexaconazole 5% EC @ 0.2%	3116.67	31.17	62333	16150	46183	2.86
T_3:Carboxin 37.5% + Thiram 37.5% @ 0.2%	2870.00	28.70	57400	16211	41188	2.54
T_4:Captan @ 0.2%	2863.33	28.63	57266	16082	41184	2.56
T_5:Carbendazim @ 0.2%	2746.67	27.47	54933	16091	38841	2.41
T_6:Carbendazim 25% + Mancozeb 50% WS @ 0.2%	3083.33	30.83	61666	16082	45584	2.86
T_7:*Trichoderma harzianum* @ 0.6%	2780.12	27.80	55602	16054	39548	2.46
T_8:*Pseudomonas fluorescens* @ 0.6%	2935.00	29.35	58700	16054	42646	2.66
T_9:*Bacillus subtilis* @ 0.6%	3221.67	32.22	64433	16054	48379	3.01
T_{10}:Control	2703.33	27.03	54066	16000	38066	2.38

1. Basic cost of cultivation : Rs. 16000.00 ha^{-1}
2. Price of soybean seeds : Rs. 2000.00 q^{-1}
3. Cost of treatments
   - Tebuconazole 5% EC @ 0.2% : 1850kg^{-1}
   - Captan 70% WP + Hexaconazole 5% EC: 1000kg^{-1}
   - Carboxin 37.5% + Thiram 37.5% : 1410kg^{-1}
   - Captan : 550kg^{-1}
   - Carbendazium : 610kg^{-1}
   - Carbendazim 25% + Mancozeb 50% WS: 550kg^{-1}
   - *Trichoderma harzianum* : 120kg^{-1}
   - *Pseudomonas fluorescens* : 120kg^{-1}
   - *Bacillus subtilis* : 120kg^{-1}

assimilates during the growing period and the way they are partitioned between desired storage organs of plant. In the present study, it was revealed that the seed priming with *Bacillus subtilis* @ 0.6%, captan 70% WP + hexaconazole 5% EC @ 0.2% and carbendazim 25% + mancozeb 50% WS @ 0.2% resulted in significantly increased the number of seeds, number of pods, 100 seed weight which have contributed for higher seed yield per plant compared to control and other treatments. Sushma (2003), Pederson and Lauer (2004a) and Gawade *et al* (2009) have also reported similar effect in different crops.

Chaudhary (1981) and Anand et al (2013) opined that HI is the only physiological parameter which is enough to account for variation in yield potential in soybean. Harvest index reveals the efficiency of translocation of assimilates to economic parts. It differed significantly due to priming in the present study and high yielding treatments *viz.*, *Bacillus subtilis* @ 0.6%, *Pseudomonas fluorescens* @ 0.6% and carbendazim 25% + mancozeb 50% WS @ 0.2% showed higher HI indicating that these treatments enhanced translocation efficiency of assimilates to economic parts.

Economics of seed production

The maximum net returns of Rs. 48379 per hectare were recorded in priming treatment with *Bacillus subtilis* @ 0.6% just before sowing with benefit-cost ratio of 1:3.01 which was followed by captan 70% WP + hexaconazole 5% EC @ 0.2%, Rs. 46183 per hectare with benefit-cost ratio of 2.86 and carbendazim 25% + mancozeb 50% WS @ 0.2%, Rs. 45584.10/ha with benefit-cost ratio of 2.83. Whereas, minimum net returns (Rs. 38066/ha) and benefit-cost ratio (2.38) was recorded in untreated control (Table 3.). This could be due to full yield potential of soybean indicating that it is essential to go for seed treatment that enhance crop growth and seed yield by minimizing the pest and disease incidence rather than adopting only normal sowing (without seed treatment).

CONCLUSIONS

Recently many types of seed treatments such hydration pre-sowing, seed priming, seed coating with bio control agents and bio-priming seed treatments have been considerable and environmentally acceptable alternatives to the existing fungicide seed treatment. Thus many different primers have been used for treatment of diseases and yield improvement. It is necessary to study the mechanism of seed primers by which it alters the plant metabolism leading to higher productivity in soybean. And the response of different genotypes of soybean to various seed primers needs to be studied for further to know the influence fungicide and other biocontrol agents to get higher yields.

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