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Different techniques of seed treatment in the management of seedling disease of sugerbeet

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The effectiveness of nine combinations (two fungitoxicants in each combination) of five compatible fungitoxicants like PCNB, TMTD, carboxin, carbendazim and captan at the rate of 3.0 g/kg of seed (mixed in equal proportions) applied in two methods as seed dipping and seed coating of sugarbeet seeds against the germination, pre- and post-emergence damping off of seedlings caused by four soil borne plant pathogens viz. *Pythium aphanidermatum, Rhizoctonia solani, Rhizoctonia bataticola* and *Sclerotium rolfsii* was investigatigated. Seed coating of two biological antagonists, viz. *Trichoderma harzianum* and *Gliocladium virens* was also evaluated against these pathogens and compared with fungitoxicant mixtures in reducing the pre- and post-emergence mortality. Seed coating with all combination of fungitoxicants gave better results in reducing the seedling mortality against dipping of seeds in aqueous suspensions of combinations of fungitoxicants. Among the combinations a mixture of PCNB + TMTD gave better results followed by carboxin+PCNB and captan+PCNB. Seed coating of biological antagonist like *Trichoderma harzianum* gave best results in reducing the disease as compared to other treatments.

Key words : Seed treatment technique, seedling blight disease, management, sugarbeet

INTRODUCTION

Sugarbeet (*Beta vulgaris* L.), an important sugar yielding crop in the world is affected by several diseases of fungal and viral origin. Among the fungal diseases the most destructive disease is pre- and post-emergence damping off of sugarbeet seedlings caused by *Pythium aphanidermatum, Rhizoctonia solani, Rhizoctonia bataticola* and *Sclerotium, rolfsii* resulting into gapy stands of the crop in the field (Sen *et al.,* 1974). Being soil borne, these pathogens affect the seedings and are difficult to control because of their multiplication and continual persistence in soil. It has been reported that pelleting of sugarbeet seeds with various seed dressing fungicides provides better protection of seedlings against these pathogens than the conventional seed treatment (Singh *et al.,* 1978; 1982). Singh and Srivastava (1987) reported that mixture of two fungicides showed lesser mortality (pre- and post-emergence mortality) than treatment with a single fungicide. Although fungicides offer certain degree of protection against those pathogens, their adverse effects on other soil microflora and the environment cannot be ignored. Under such conditions the biocontrol agents appear to be promising in disease management. Several organisms such as *Trichoderma* sp. (Harman *et al.,* 1981). *Bacillus* sp. (Capper and Campbel, 1986) and *Pseudomonas* sp. (Vidhyasekaran and Muthamilan, 1995) have been successfully used as biocontrol combinations of fungicides and their comparison in method of application and also difference in mortality among the biocontrol agents. The present study was undertaken under greenhouse condition at the Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya.

MATERIALS AND METHODS

Isolation of pathogen

Sugarbeet seedlings affected by damping off disease collected from sugarbeet field at District seed Farm

(D-Block), Bidhan Chandra Krishi Viswavidyalaya, Kalyani were used for isolation of the pathogen. The pathogen was isolated by tissue segment method (Rangaswami, 1958) on potato dextrose agar medium. The culture was purified by hyphal tip cultural method (Rangaswami, 1958) on plain agar medium. Pathogenicity tests were conducted with these fungi individually as well as in combination, and all were found to be pathogenic under greenhouse conditions. Two biocontrol agents *Trichoderma harzianum* were also isolated from sugarbeet field from the University farm by dilution plate technique on *Trichoderma* specific medium (TSM) (Elad *et al.,* 1980). Seed coating of biological antagonist was done by dipping sugarbeet seed in suspension containing 10^7 spores/ml of *T. harzianum* and *G. virens* separately (Das *et al.,* 2001).

Pot culture experiment

Soil collected from sugarbeet cultivated field was sterilised at 1.4 kg cm^{-2} pressure for 2 h and filled in enamel trays (size 40 cm × 30 cm). The fifteen day old cultures of all the four fungi, *Pythium aphanidermatum, Rhizoctonia solani, Rhizoctonia bataticola* and *Sclerotium rolfsii* multiplied in sand maize meal medium were inoclated in the trays in equal proportions (1:1:1:1).

Seeds of sugarbeet variety LS-6 supplied by Indian Institute of Sugarcane Research, Lucknow, were polished and coated with five compatible fungicides viz., PCNB (penta chloronitrobenzene,), TMTD (thiram), carboxin (vitavax), carbendazim (bavistin) and captan (captaf) in nine combinations (combination of two in equal proportions) at the rate of 3.0 g/kg seed. The seeds were steeped in aqueous suspension of above mentioned fungicides mixture (Table 1). Another lot of seeds were also coated with two biological antagonists following Das *et al.* (2001). Seeds without treatment served as control. Each tray was sown with 75 seeds and irrigated periodically, as and when required. The whole experiment was conducted under glasshouse condition in a completely randomized block design (CRBD) with four replications in consecutive three years (1992, 1993 and 1994). Emergence of seedlings and mortality were recorded up to 50 days after sowing. Disease assessment was done following Das *et al.* (2001).

RESULTS AND DISCUSSION

Seed treatment by seed dipping with fungicides mixture and seed coating with bioatagonists significantly reduced the pre- and post-emergence mortality compared to untreated control. Germination, pre- and post-emergence mortality which fluctuated in 1992, 1993 and 1994, might be due to complex interactions of temperature, moisture and other ecological factors which were known to affect infection and disease development (Garren, 1964).

Highest mean germination of seeds of three years was obtained on vitavax + thiram coated seeds (84.89%) followed by seed sown on auto-claved soil (84.70%). Lowest percentage of germination was obtained in captain + thram mixture in both coated dipped seeds (61.10% and 62.56% respectively). The difference in germination in different treatments were statistically significant, but the type of treatments like seed dipping and seed coating had no significant difference among themselves, except 1993 experiments. The interaction effect of treatments (fungicides mixtures) and type of treatments (seed dipping and seed coating) also showed some significant difference except in 1993 experiments. Similar findings were also reported by many workers in different sugarbeet growing countries (Ferro and Manaresi, 1994; Heubrock and Huubregts, 1995). The mean value of three years data revealed that germinability and plant stand in all the treatments were more as compared to untreated control and Dunkan multiple range test showed that the treaments of fungicides mixture like PCNB + thiram in seed dipping gave similar results like that of seed coating of vitavax + captan. Smilarly seed dipping in vitavax + PCNB was at per with seed coating bavistin + PCNB and coating with biological antagonist like *Gliocladium virens* (Table 1).

With regards to disease managemet of pre-and post-emergence mortality, it was observed that seed coated with all nine fungicide combinations reduced both more significantly than when the seeds were dipped in aqueous suspension of same combinations of fungicides. Similar findings were reported by several other workers in different sugarbeet

growing countries (Veverka, 1976; Osinka and Szymczak-Nowak, 1983; V" rbanov *et al.*, 1984; Singh and Srivastava, 1987). In case of pre-emergence mortality data (three years mean) showed that minimum mortality occurred when the seeds were coated with vitavax + PCNB (13.39%) followed by viavax + thiram (16.28%). Maximum mortality were observed when the seed dipped with bavistin + PCNB (40.10%) followed by bavistin + captan (39.05%). Lowest pre-emergence mortality was observed when the seeds were coated with either of the tested biological antagonists. Of the two antagonists, lower mortality was observed with *T. harzianum* coated seeds (7.43%) in comparison with *G. virens* (11.30%). Biological antagonist proved to be better than either types of seed treatments with fungicides mixtures. The interaction effect of seed dipping and seed coating showed significant difference among themselves but the treatments (fungicides mixture) and their type of applications (seed dipping and seed coating) individually had no significant difference among themselves (Table 1).

In case of post-emergence mortality, the best result was observed when the seeds were coated with

Table 1 : Efficacy of fungicides mixture in a different methods of seed treatment and bioantagonist against germination and pre- and post-emergence mortality of sugarbeet seedlings.

Treatments	Germination (%)				Pre-emergence mortality (%)				Post-emergence mortality (%)			
	1992	1993	1994	Pooled mean	1992	1993	1994	Pooled mean	1992	1993	1994	Pooled mean
Infested Soil												
Seed dipping												
Uncoated seed dipped in water	15.80	16.90	14.10	15.66^{k}	56.55	46.95	51.26	51.59^{A}	87.32	90.50	82.95	86.92^{a}
PCNB + thiram	70.30	78.92	62.85	70.69^{fgh}	23.19	21.05	27.22	23.82^{def}	29.44	12.92	21.00	21.12^{efg}
Captan + thiram	61.50	69.62	56.55	62.56^{ij}	34.08	28.06	32.00	31.38^{bc}	33.56	23.32	28.82	28.57^{cd}
Captan + PCNB	77.25	79.37	63.15	73.26^{defgh}	25.91	20.25	26.70	24.29^{defg}	26.09	14.60	22.40	21.03^{efg}
Bavisitin + thiram	74.87	75.49	73.80	74.72^{cdefg}	34.75	36.53	33.01	34.76^{b}	54.01	56.05	52.83	54.29^{a}
Bavistin + PCNB	69.62	71.80	68.41	69.94^{ghi}	40.22	42.09	38.00	40.10^{a}	49.19	51.26	46.95	49.13^{a}
Bavistin + captan	65.72	67.74	63.69	65.72^{hij}	39.32	41.34	36.49	39.05^{a}	52.38	53.84	50.44	52.22^{a}
Vitavax + thiram	81.70	83.46	79.41	81.52^{abc}	21.86	23.90	19.82	21.86^{fgh}	53.87	55.82	51.86	53.85^{a}
Vitavax + PCNB	71.95	73.95	70.02	71.97^{efgh}	18.78	20.89	17.22	18.96^{hij}	30.21	32.11	31.16	31.16^{c}
Vitavax + captan	68.25	70.09	66.06	68.13^{gij}	27.77	29.71	25.88	27.29^{ced}	30.45	32.52	28.59	30.52^{c}
Seed coating												
PCNB + thiram	82.87	65.87	54.30	67.51^{ghij}	17.62	13.32	21.37	17.44^{ij}	22.75	9.03	17.42	16.40^{gh}
Captan + thiram	73.00	59.70	50.60	61.10^{j}	28.43	22.07	28.02	26.17^{def}	26.75	16.87	24.07	22.56^{ef}
Captan + PCNB	79.35	63.52	52.85	65.24^{hij}	20.68	17.50	24.67	20.95^{ghi}	21.69	9.72	18.17	16.53^{gh}
Bavistin + thiram	77.97	79.97	76.18	78.04^{cdef}	28.22	30.25	26.20	28.22^{cd}	29.59	31.67	27.66	29.64^{cd}
Bavistin + PCNB	72.45	74.56	70.71	72.57^{efgh}	35.93	38.04	33.73	35.90^{ab}	21.25	23.33	19.27	21.28^{ef}
Bavistin + captan	67.52	69.55	65.53	67.53^{ghij}	34.50	36.55	35.96	35.67^{ab}	39.62	28.59	50.42	39.54^{b}
Vitavax + thiram	85.07	86.64	82.95	84.89^{ab}	16.22	18.39	14.22	16.28^{kj}	32.64	34.80	30.56	32.67^{c}
Vitavax + PCNB	75.27	77.00	73.38	75.22^{cdefg}	13.29	15.39	11.49	13.39^{kl}	22.03	24.13	20.16	22.11^{ef}
Vitavax + captan	71.00	72.84	68.92	70.92^{fgh}	22.99	25.21	21.01	23.07^{efgh}	24.12	26.45	22.48	24.35^{de}
T. harzianum coated seeds :	87.32	82.23	65.15	78.23^{bode}	6.98	3.93	11.37	7.43^{m}	15.10	7.35	15.65	12.70^{h}
G. virens coated seeds	80.42	79.83	63.40	74.55^{cdefg}	9.75	7.91	16.25	11.30^{l}	22.98	12.35	20.52	18.62^{fg}
Uninfested soil (Autoclaved soil)												
Coated seeds	90.72	82.60	65.40	79.57^{abcd}	—	—	—	—	—	—	—	—
Uncoated seeds	90.47	90.93	72.70	84.70^{a}	—	—	—	—	—	—	—	—
Sem ±	1.72	1.77	1.46	1.52	1.09	1.03	1.13	0.99	1.39	1.39	1.06	1.16
CDat 5%	4.87	5.01	4.13	4.28	3.11	2.90	3.20	2.82	3.95	3.94	2.99	3.29

	Interaction effect of seed dipping (sd) and seed coating (sc)																	
	Sem ±	CD at 5%	Sem ±	CD at 5%	Sem ±	CD at 5%	Sem ±	CD at 5%	Sem ±	CD at 5%	Sem ±	CD at 5%	Sem ±	CD at 5%	Sem ±	CD at 5%	Sem ±	CD at 5%
Treatments	1.26	3.59	1.29	3.68	1.09	3.13	0.78	2.23	0.75	2.14	0.84	2.38	1.00	2.86	1.02	2.91	0.77	2.21
Types (sd. & sc.)	0.59	1.69	0.61	NS	0.52	NS	0.37	1.05	0.35	1.00	0.39	1.12	0.47	1.35	0.48	1.37	0.36	1.04
Treatments x Types	1.78	NS	1.83	5.21	1.55	4.43	1.11	NS	1.06	NS	1.18	NS	1.42	4.05	1.44	4.12	1.09	3.13

Data transform as angular transformation for calculation

Superscript indicate ranking by Duncan's multiple range test ; Different superscripts indicate signifiant at P<0.05

PCNB + thiram (16.40%) followed by captan + PCNB (16.53%). Here also type of treatments, seed dipping and seed coating, gave significant difference in mortality. Fungicide combinations and their different types of application also showed significant difference in mortality among themselves. (Table 1). Treatments with PCNB + thiram and captan + PCNB seed dipping also showed mininim mortality (21.03% and 21.12% respectively) which were also statistically at par when the seeds were coated with bavistin + PCNB (21.28%) followed by vitavax + PCNB (22.11%) and captan + thiram (22.56%). Here also *T. harzianum* and *G. virens* coated seeds showed minimum mortality (12.70% and 18.62% respectively). Mortality of *G. virens* coated seeds was statistically at par with the seeds coated with PCNB + thiram and captan + PCNB (Table 1).

Singh *et al.* (1982) reported that seed pelleting with fungicides gave minimum pre- and post-emergence mortality in comparison with seed soaking. This could be due to combined effect of fungicides and sufficient availability for a time being at the site when infection occourred. In case of seed soaking the effectiveness of fungitoxicants was lost due to handling and more exposore to soils (Mills, 1972). Sivan *et al.* (1984) reported that seed coating of bean seeds with *T. harzianum* reduced the pre- and post-emergence mortality in comparison with prothiocarb fungicides. Harmen *et al.* (1981) reported that limited inoculum needed to suppress damping off was the major advantage in use of seed treatments with biological antagonists.

It was concluded from the present experiment that management of pre- and post-emergence mortality of sugarbeet seedlings can be done by seed coating with fungicidies mixture like PCNB + thiram, captan + PCNB or vitavax + PCNB in place of soil drenching with fungicides which is not only highly expensive but also damage the soil ecologi-cal environment. Cost of coating of sugarbeet seeds with the above fungicides mixtures were approximately Rs. 5.00/ kg; 7.00/ kg and 9.50/ kg seed respectively and therefore, can be easily adopted by the farmers. Above all seed coating of biological antagonists gave maximum performance in reducing pre- and post-emergence damping off disease and maximum cost involved in coating the seeds was Rs. 6.00 / kg. The cost of sophisticated laboratory required for multiplication of the biological antagonists was, however, not considered. In European countries seed coating or pelleting with fungicides and bioantagonists are being regularly employed for successful control of damping off disease of sugarbeet seedlings (Chavanes, 1995; Rosso *et al.*, 1995).

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