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Efficacy of bioagents and fungicides against web blight of mung bean caused by *Rhizoctonia solani* (Kuhn)

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Abstract
Efficacy of antagonists and fungicide were evaluated under *in vitro* conditions for the mycelial growth inhibition. It was observed that all the biocontrol agents were significantly superior in inhibiting the mycelial growth of test fungus over control. Mungbean (*Vigna radiate* L.) is an important pulse crop in Indian continent. Mungbean is infected by several fungal, bacterial and viral diseases but foliar blight caused by *Rhizoctonia solani* considered as one of the most devastating diseases in the mungbean. The main aim of this study was to find out the effective fungicides and bioagents for the timely management of *R.solani*. Efficacy of Fungicides with Concentrations Vitavax (0.03,0.05 and 0.1 %), Carbendazim (0.03,0.05 and 0.1 %), Mancozeb (0.03,0.05 and 0.2 %), Thiram (0.03,0.05 and 0.1 %), Antracol (0.03,0.05 and 0.1 %), Nativo (0.03,0.05 and 0.1 %), Azoxystrobin (0.03,0.05 and 0.1 %), Prism (0.03,0.05 and 0.1 %), Melody (0.03,0.05 and 0.1 %), and Conrol. Bioagents efficacy *Trichoderma harzianum* (Th_1), *Trichoderma harzianum* (Th_2), *Trichoderma harzianum* (Th_3), *Trichoderma harzianum* (Th_4), *Trichoderma harzianum* (Th_5), *Trichoderma harzianum* (Th_6), *Trichoderma viride* (Tv_1), *Trichoderma viride* (Tv_2), *Trichoderma viride* (Tv_3), *Trichoderma viride* (Tv_4), *Trichoderma viride* (Tv_5), *Trichoderma viride* (Tv_6) and Control.

Keywords: Bioagents, fungicides, web blight and mung bean

Introduction
Mungbean/green gram [*Vigna radiata* (L.) Wilczek] is one of the most important pulse crops. It is grown in almost all parts of the country and belongs to family Leguminosae. Mung bean is an excellent source of high quality protein. It is consumed in different ways as dal, halwa, snack and so many other preparations. Ascorbic acid (Vitamin-C) is synthesized in sprouted seeds of mung bean. The leguminous crops have the capacity to fix-atmospheric nitrogen through symbiotic nitrogen fixation. It is also used as green manure crop. It is grown in summer and *Kharif* season in northern India and in southern India. In India, it is the third important pulse crop after chickpea and pigeonpea. Mungbean also known as greengram, green bean, mash bean, golden gram and green soy is an important source of dietary protein across Asia. It is a short duration grain legume crop with wide adaptability, widely grown in tropical and sub-tropical regions as a monoculture and as a component in cropping systems, with low input requirement Mungbean is under cultivation since prehistoric times in India. Mungbean is thought to be of Indian origin as evidenced by occurrence at archaeological sites in the Indian Subcontinent. In India mungbean is cultivated in three different seasons, viz., *Kharif*, rabi and summer. It is grown under rainfed conditions during *Kharif* and on residual moisture during Rabi in eastern and southern parts of the country. However maximum area of its cultivation is under *Kharif*, season crop during *Kharif*, mungbean is generally grown with maize, sorghum, pearlmillet, pigeonpea and cotton as intercrop. In U.P., Bihar, Maharashtra, Gujarat and Tamilnadu, urdbean mungbean grown in 1:1 ratio with pigeonpea. During spring, mungbean or urdbean is grown in 2:1 ratio with sugarcane in U.P and northern Bihar as a tradition. The nutritive value of mungbean lies in its high and easily digestible protein, and Amino acid analysis indicates that the concentration of sulphur containing amino acids, namely methionine and cystine are low. Methionine concentration is larger in urdbean than in mungbean, lysine values are comparatively large and that is why, the protein of mungbean is an excellent complement to rice in terms of balanced human nutrition. The Mungbean is cultivated in different kind of soils, which include the light sandy soil of Rajasthan to heavy black loam soil of (cotton soil) and red lateritic soil of Southern India. However, best soil for its cultivation is loam soil with good drainage. The crop should not be raised on alkaline, saline or waterlogged soils. The mung bean plant is 18 to 36 inches tall and produces a cluster of 2 to 8 slender, black pods 3 to 4 inches long.

The slightly fuzzy pods contain very small seeds, which are green in the commercial varieties. Each pod may contain as many as 15 small oval seeds, depending on cultural conditions. The mung bean is an ancient crop of Asia, where the seeds are available in many sizes, shapes, and colors. Domestic mung bean sprouts are produced from green-seeded cultivars. The mung bean's ancestors are annual plants with both short and long-day cultivars. Most domestic cultivars are sensitive to temperature rather than day length. Web blight of mungbean caused by *Rhizoctonia solani* Kuhn is in important disease and causes severe losses in soybean production cob web like mycelium and sclerotia developed over infected leaves were described by Atkins and Lewis (1954) [1]. The *R. solani* produces sclerotia as survival structure, which is brown to black composed of clusters of melanin encrusted, thick walled cells, formed by repeated branching from short, thick, lateral hyphae, when produced on plant parts, it is difficult to separate the sclerotia from their surrounding embedded sclerotia. Temperature is more considerable parameter for their growth and development along with sclerotia production. Under certain conditions young plants were found heavily affected with the disease leading to premature death of plants and substantial losses in yield. Due to this disease, the vigorousness of the affected plants is reduced there by production decreased. The disease attacks all the aerial part of the plants *i.e.* leaves, stem, petioles and pods, when the disease occurred in severe form. The leaves and host plant has given blighted appearance. The mycelium has found web like appearance on the leaves so that disease has been named as web blight of Soybean. It also inhibited root elongation causing seedling root rot, yellowing and shredding of cotyledons and leaves in soybean.

Materials and Methods

Collection of diseased specimens lentil plants showing the typical symptoms of collar rot were collected from Crop Research Centre of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut and from farmers' field in the vicinity of Meerut and Budaun, during the crop season of 2013-14, for collar rot disease of Lentil caused by *Sclerotium rolfsii* Sacc. Diseased plants of lentil showing characteristic disease symptoms were collected. The specimens were brought to the laboratory and critically examined and studied for the symptoms of the disease and isolation of the pathogen. Some specimens were dried, pressed and maintained in herbarium file for further studies. For all the laboratory experimental studies, the standard Potato Dextrose Agar (PDA) medium was used for culturing the *Sclerotium rolfsii*. The composition of PDA used is given below.

Peeled potato	-	200 gms
Dextrose (C6H12O6)	-	20 gms
Agar agar	-	20 gms
Distilled water	-	1000 ml

Potatoes were cleaned, washed, peeled and chopped into slices. There 200 gms of these pieces were boiled in 1000 ml of distilled water and the extract was collected by filtering through clean muslin cloth. 20 gms of Dextrose, and 20 gms of agar-agar were dissolved in the potato extract and the volume was made up to 1000 ml by adding distilled water. Known quantity of medium was dispensed into number of conical flask and plugged with non absorbent cotton and finally wrapped with brown paper. The flasks containing PDA medium were sterilized at 121.6^{0}C temperature (1.1 kg/cm^2 pressure) for 15 minutes.

In vitro evaluation of bio-agents against *S. rolfsii*

Experiments were conducted to find out the efficacy of the antagonists viz. *Trichoderma sp.* and *Pseudomonas fluorescence* against the pathogen *in vitro* by adopting the dual culture technique (Morton and Strouvle, 1955) [4]. Fungal antagonists were collected and evaluated by inoculating the pathogen at one side of the petriplates and antagonist at exactly opposite side of the same plate by leaving about 4 cm gap. For this, actively growing cultures were used. In case of bacterial antagonist, the bacterial culture was streaked at the periphery of the petriplate with the help of a inoculation loop. Three replications were maintained for each treatment. After 96 hours of incubation, when the growth in control plate recorded 90 mm in diameter, the radial growth of the pathogen was measured. The per cent inhibition of the growth over control was calculated by following the equation given by Vincent (1927) [8].

$$\mathrm{I} = \frac{C - T}{C} X100$$

The *Trichoderma* and *Pseudomonas* were isolated from the soil near the rhizosphere of different crops. The soil samples were taken from different crops and different locations. The *Trichoderma* sp. and *Pseudomonas* sp. were isolated by using serial dilution method.

In vitro evaluation of chemical fungicides against *S. rolfsii*

The effect of eight fungicides belonging to different groups (Table-3) was tested *in vitro* for their efficacy to inhibit the growth of the pathogen to a maximum extent. Effect on the growth of *S. rolfsii* was studied using poisoned food technique (Nene and Thapliyal, 1982) [5]. Different concentrations viz., 0.03, 0.05 and 0.1 per cent for systematic fungicide and 0.05, 0.1 and 0.2 per cent for non-systemic fungicides were used. Potato dextrose agar (PDA) was prepared and 100 ml of the medium was taken in 250 ml flasks and sterilized. To the molten cooled sterile medium requisite quantity of the fungicides were added separately and thoroughly mixed so as to get the required concentrations for each of the fungicide. Twenty ml of poisoned medium was poured in to each of the 90 mm sterilized petriplates. Each plate was inoculated with five mm disc of mycelium at the center and incubated at 27± 1^{0}C. Three replications were maintained for each treatment. Potato dextrose agar medium without any of the fungicide served as control. And plates were incubated at 27± 1^{0}C till the growth of the colony touched the periphery in control plate. The per cent inhibition of the growth over control was calculated by following the equation given by Vincent (1927) [8].

$$\mathrm{I} = \frac{C - T}{C} X100$$

Results and Discussion

Identification of the fungus obtained from the affected plant tissue was compared with the type species originally described and were found to resemble *Rhizoctonia solanii*. In all morphological characters.

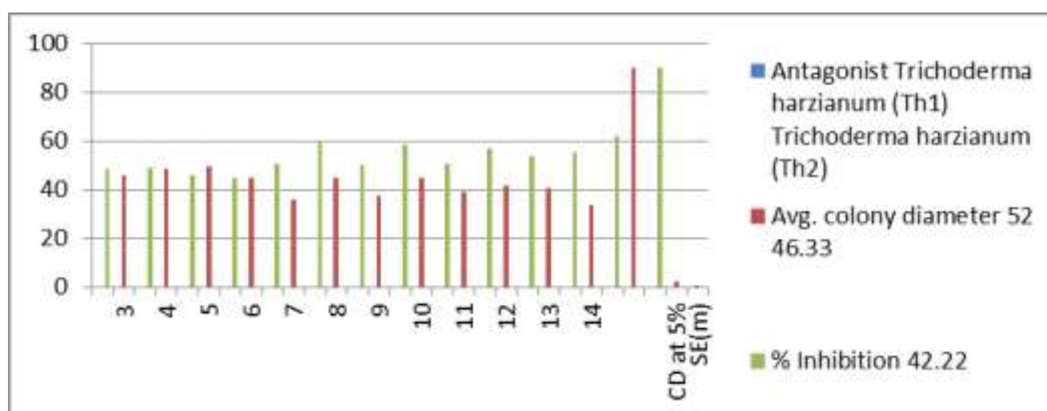
Evaluation of bio-agents against *Rhizoctonia solanii in vitro*

Antagonistic activities of thirteen isolates of *Trichoderma* sp. and one strain of *Chaetomium* sp. were evaluated against *Rhizoctonia solani* in *vitro.* The results from the table revealed that, significant difference in per cent inhibition of mycelial growth of *Rhizoctonia solani* by all the tested bioagents. Among the maximum inhibition per cent (70.7%) of *Sclerotium rolfsii* was recorded in *Trichoderma* isolate T16, which is significantly superior from all the tested isolates followed by T14 (70.0%), T2 (69.3%), T11 (68.5%), T8 (68.1%), and other isolates T1, T4, T7, T3, T5, were found 67.7% mycelial inhibition, whereas T10, T18, T15, T20, T17, T12, T16, T19 and T13 inhibited the mycelial growth of the pathogen between the range of 66.6% to 61.1%, inhibit the growth was significant as compared to control. However, the other bioagent *Pseudomonas fluorescence* inhibit 52.5 % mycelial growth of the pathogen. The minimum inhibition per cent of mycelial growth was recorded in *Trichoderma* isolates T19 (47.0%).

All the tested bioagents showed statistically significant but among the isolates T1, T4, T7, T3, T5 and T6, T12, T17, T20, T15 were non-significant among each other but significant over control. Table No.1.

Table 1: Evaluation of bio-agents against *Rhizoctonia solanii in vitro*

S. No.	Antagonist	Avg. colony diameter	% Inhibition
1	*Trichoderma harzianum* (Th_1)	52.00	42.22
2	*Trichoderma harzianum* (Th_2)	46.33	48.52
3	*Trichoderma harzianum* (Th_3)	46.00	48.88
4	*Trichoderma harzianum* (Th_4)	48.67	45.92
5	*Trichoderma harzianum* (Th_5)	49.67	44.81
6	*Trichoderma harzianum* (Th_6)	44.67	50.36
7	*Trichoderma viridae* (Tv_1)	36.00	60.00
8	*Trichoderma viridae* (Tv_2)	45.00	50.00
9	*Trichoderma viridae* (Tv_3)	37.33	58.52
10	*Trichoderma viridae* (Tv_4)	44.67	50.36
11	*Trichoderma viridae* (Th_1)	39.00	56.66
12	*Trichoderma viridae* (Tv_5)	41.67	53.70
13	*Trichoderma viridae* (Tv_6)	40.33	55.18
14	Chaetomium globosum (Cg_1)	34.00	62.22
	Control	90.00	90.00
CD at 5%		1.983	
SE(m)		0.683	



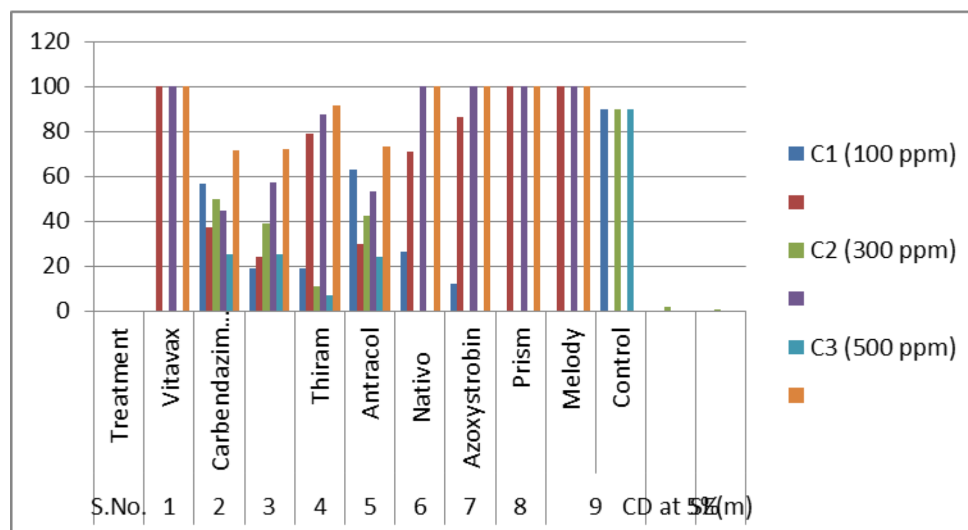
***In vitro* evaluation of different fungicides against pathogen**

Efficacy of five systemic and four non systemic fungicides were tested at different concentrations by Poisoned Food Technique. The results thus obtained have been presented in Table-No 2, Fig-2. The results from table – revealed that there is significant difference in per cent inhibition of mycelial growth of *Sclerotium rolfsii* with the fungicides which were tested. The per cent inhibition of mycelial growth of *Sclerotium rolfsii* was found highest (100%) in the treatment with Propiconazole, Tabuconazole, Hexaconazole and Ridomil at all three concentrations (0.03%, 0.05% and0.1%) and Carbendazim+ Mancozeb at all three concentrations (0.05%, 0.1%, and 0.2%). Other fungicides also inhibited the growth of the pathogen sigficantly. Captan (0.2%) and Mancozeb (0.2%) showed 88.5 per cent and 48.9 per cent inhibitions respectively and were found significantly superior over remaining treatments tested. No inhibition was recorded in the treatment with copper oxy chloride at all concentrations. Table No. 2.

Table 2: Evaluation of different fungicides against pathogen *in vitro*

S. No.	Treatment	C_1 (100 ppm)		C_2 (300 ppm)		C_3 (500 ppm)	
		Colony diameter (mm)	Percent inhibition	Colony diameter (mm)	Percent inhibition	Colony diameter (mm)	Percent inhibition
1	Vitavax	0.0	100	0.0	100	0.0	100
2	Carbendazim (Bavistin)	56.70	37.00	49.70	44.77	25.07	71.44
3	Mancozeb (Dithane M-45)	18.70	24.07	38.70	57.00	25.00	72.22

4	Thiram	18.70	79.22	11.00	87.77	7.07	91.44
5	Antracol	63.00	30.00	42.33	52.96	24.33	73.00
6	Nativo	26.33	70.74	0.0	100	0.0	100
7	Azoxystrobin	12.33	86.30	0.0	100	0.0	100
8	Prism	0.0	100	0.0	100	0.0	100
9	Melody	0.0	100	0.0	100	0.0	100
	Control	90	0.0	90	0.0	90	0.0
CD at 5%				2.101			
SE(m)				0.707			



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