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Estimation of Yield Loss in Soybean Due to Collar Rot Disease in Mid Hills of Meghalaya and Its Management

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Abstract Collar rot disease incited by *Sclerotium rolfsii* is one of the most destructive soil borne disease of soybean that causes 30—40% yield losses in India. The disease has also been found to occur frequently in the soybean field of north eastern hill (NEH) states and major losses incurred because of pre and post-emergence seedling mortality, wilting and weakening of older plants. The present investigation focused on assessment of yield losses and *in-vitro* efficacy of plant extracts, bio-control agents (BCAs) and chemical fungicides against *S. rolfsii*. Twenty genotypes were screened, of which, the genotype NRC-80 showed the least (0%) per cent disease index (PDI) and minimum yield loss (1.02%), whereas the genotype MACS-1059 showed the highest PDI (46.15%) and maximum yield loss (46.17%). In the *in- vitro* management study, it was found that garlic extract was more effective followed by neem extract in reducing the radial growth of the fungus, whereas sclerotial germination was inhibited only by garlic extract (18.34%). Dual culture assays carried out to test five antagonists revealed that *Pseudomonas putida* was highly effective in reducing the radial growth as well as sclerotial germination of *S. rolfsii*. Also, seven fungicides were also evaluated for inhibition of radial growth and sclerotial germination of *S. rolfsii*. Folicur was highly effective in inhibition of radial growth and sclerotial germination followed by Benofit and Contaf. The best performed plant extracts, BCAs and fungicides may be recommended as important component of integrated disease management, which needs further *in-vivo* investigations against collar rot of soybean under mid hill conditions of Meghalaya.

Keywords Genotype, Plant extract, BCAs, Fungicides.

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Introduction

Soybean (*Glycine max* L.) is a potential oil seed and pulse crop in India which contains about 20% and 40% oil and high quality protein respectively. Soy-

bean is not very much popular in NEH states as an oilseed crop. But it is grown as a pulse crop for direct food and for making various fermented and non-fermented food stuffs by the people of this region. The NEH states occupy only 17.5 thousand hectares and produces 17.5 thousand tonnes of soybean annually with a productivity of 1000 kg/ha [1]. In Meghalaya, the area under soybean cultivation is about 1055 ha with production of 1163 mt and productivity of 1102 kg/ha [2].

Thirty five major diseases reported to inflict soybean crop in [3]. Among the diseases, that affect soybean production, collar rot caused by *Sclerotium rolfsii* is considered as one of the important disease. The disease is soil borne, fast spreading and destructive which results in 30—40% yield losses in India. The limited availability of information on yield loss and management of collar rot of soybean in NEH region prompted to carry out the present investigations under mid hill conditions of Meghalaya.

Materials and Methods

Isolation and identification of *S. rolfsii*

Fungus causing collar rot of soybean was isolated from naturally infected soybean plants collected from the experimental farm of Plant Pathology Division, ICAR–RC for NEH region, Umiam, Meghalaya, India. Infected plant materials with sclerotia around the stem were collected and were used for isolation. Pure culture of the fungus was then transferred to potato dextrose agar (PDA) slants and maintained in the refrigerator at 4°C.

Estimation of yield losses in soybean genotypes due to collar rot

Estimation of losses in soybean due to collar rot disease was carried out in pot culture experiment with 20 soybean genotypes. One month old pathogenic *S. rolfsii* inoculum was mixed thoroughly with top soil in 50:50 w/w proportions and applied sclerotial bodies @ 200 g/pot around the base of the soybean seedlings, 30 days after sowing. Un-inoculated pot served as control. The crop was regularly monitored for the development of disease and final disease intensity was recorded at maturity (R_7) stage. The mean disease index was converted to percent disease index (PDI). The crop was harvested separately, seeds were sun dried for one week and yield was calculated. Yield losses in each treatment were calculated by subtracting their yields from the protected control.

Management of collar rot of soybean under *in-vitro* conditions

Sets of laboratory experiments were conducted to evaluate the efficacy of different available plant extracts, chemical fungicides and biocontrol agents (BCAs) for *in-vitro* management of *S. rolfsii*.

In-vitro evaluation of plant extracts against mycelial growth of *S. rolfsii*

Seven plant species viz. Neem (*Azadirachta indica*), Datura (*Datura stramonium*), Tulsi (*Ocimum sanctum*), Lantana (*Lantana camara*), Marigold (*Tagetes erecta*), Onion (*Allium cepa*) and Garlic (*Allium sativum*) were evaluated at the concentration (conc.) of 5, 10 and 20% against *S. rolfsii* by poisoned food technique. Three replications were maintained for each treatment including control.

In- vitro evaluation of BCAs against mycelial growth of *S. rolfsii*

Actively growing cultures of two fungal viz. *T. harzianum* and *T. viride* and three bacterial BCAs viz. *P. fluorescens*, *P. putida* and *B. subtilis* were used for evaluating their efficacy *in-vitro* by dual culture technique. Three replications were maintained for each treatment including control. Percent inhibition of *S. rolfsii* over control was worked out [4].

In-vitro evaluation of fungicides against mycelial growth of *S. rolfsii*

The efficacy of four systemic fungicides viz. Benofit (Benomyl 50% WP), Bavistin (Carbendazim 50% WP), Contaf (Hexaconazole 5% EC), Folicur (Tebuconazole 25.9% EC) and three non-systemic fungicides viz. Jatayu (Chlorothalonil 75% WP), Antracol (Propineb 70% WP), Bilzeb (Mancozeb 75% WP) at the concentrations of 0.001, 0.01, 0.1, 0.3 and 0.5% were assayed

Table 1. Assessment of yield losses in soybean genotypes due to collar rot disease. Seed yield recorded after harvest and converted to q/ha. *Means of three replications. '*F*'- test at 5% level.

Genotypes	PDI* Inoculated	Yield (q/ha)* Control	Inoculated	Yield loss* (q/ha)	Yield loss (%)
NRC80	0	24.28	24.03	0.25	1.02
MACS1059	46.15	21.98	11.83	10.15	46.17
MACS1140	35.71	26.44	18.13	8.31	31.42
MACS1184	28.57	27.79	21.12	6.67	24.01
MACS1188	3.34	33.15	31.71	1.44	4.34
MACS1254	24.0	32.17	24.86	7.31	22.72
MACS1259	20.0	17.85	13.93	3.92	21.96
MACS1281	21.73	21.46	16.59	4.87	22.69
RKS52	4.01	27.31	25.47	1.84	6.73
RKS54	11.12	28.79	25.22	3.57	12.41
JS335	33.34	25.05	17.57	7.48	29.86
DSB12	33.34	19.91	13.78	6.13	30.78
DSB16	30.0	27.93	20.78	7.15	25.59
DS2614	16.67	21.05	17.02	4.03	19.14
SL794	26.61	22.41	16.62	5.79	25.83
SL799	30.43	31.77	23.21	8.56	26.94
VLS74	31.57	29.12	20.75	8.37	28.74
VLS75	33.34	33.68	23.64	10.04	29.81
PS1466	12.51	30.79	26.67	4.12	13.38
AMS1	4.01	33.47	31.64	1.83	5.46
SEm±		0.619	0.229	0.174	
CD (p = 0.05)		1.239	0.458	0.349	

under *in-vitro* conditions against *S. rolfsii* by poisoned food technique. Three replications were maintained for each treatment including control without fungicide.

In-vitro evaluation of plant extract and fungicides for inhibition on sclerotial germination

The poisoned agar medium (containing plant extract and fungicides) with desired concentrations was poured into petridishes and then solidified. Twenty sclerotia were placed in each petridish. Three replications were maintained for each treatment along with control and were incubated at 27±1°C. Sclerotial germination was observed at 24 h intervals for seven consecutive days after incubation and un-germinated sclerotia were removed, washed in sterile distilled water for three times (5 min each), blotted dry and

Table 2. *In-vitro* efficacy of plant extracts at different conc on radial growth and percent sclerotial germination of *S. rolfsii*. Figures in parentheses indicate inverse sine transformed values. Means having common letter are not significantly different. '*F*'-test at 5% level.

Sl. No.	Plant extracts	Radial growth (cm) 5%	10%	20%	Percent sclerotial germination 5%	10%	20%
1.	Neem	1.78 (7.66)^{d}	1.51 (7.05)^{c}	0	100(90)^{a}	100(90)^{a}	100 (00)^{a}
2.	Datura	9.0 (17.45)^{a}	9.0 (17.45)^{a}	9.0 (17.45)^{a}	100(90)^{a}	100(90)^{a}	100 (90)^{a}
3.	Tulsi	3.63 (10.98)^{C}	2.7 (9.45)^{b}	1.67 (7.42)^{c}	100(90)^{a}	100(90)^{a}	100 (90)^{a}
4.	Lantana	9.0 (17.45)^{a}	9.0 (17.45)^{a}	9.0 (17.45)^{a}	100(90)^{a}	100(90)^{a}	100 (90)^{a}
5.	Marigold	5.54 (13.61)^{b}	3.01 (9.99)^{b}	6.74 (15.04)^{b}	100(90)^{a}	100(90)^{a}	100 (90)^{a}
6.	Onion	9.0 (17.45)^{a}	9.0 (17.45)^{a}	9.0 (17.45)^{a}	100(90)^{a}	100(90)^{a}	100 (90)^{a}
7.	Garlic	5.8 (13.93)^{b}	0	0	100(90)^{a}	100(90)^{a}	81.66 (64.64)^{b}
8.	Control	9.0 (17.45)^{a}	9.0 (17.45)^{a}	9.0 (17.45)^{a}	100(90)^{a}	100(90)^{a}	100 (90)^{a}
	SEm±						
	CD (p = 0.05)	1.69 5.12	1.59 4.83	1.72 5.22	– NS	– NS	2.33 7.07

Table 3. *In-vitro* antag.nistic effect of various BCAs on radial growth and percent sclerotial germination of *S. rolfsii*. Figures in parentheses indicate inverse sine transformed values. Mean having common letter are not significantly different. '*F*'-test at 5% level.

Sl. No.	BCAs	Radial growth (cm)		Percent sclerotial germination	
1.	*Trichoderma harzianum*	2.18	(8.49)^{c}	100	(90)^{c}
2.	*Trichoderma viride*	2.91	(9.82)^{b}	100	(90)^{c}
3.	*Bacillus subtilis*	2.16	(8.45)^{c}	80	(63.43)^{c}
4.	*Pseudomonas putida*	0		68.3	(55.73)^{b}
5.	*Pseudomonas fluorescens*	2.92	(9.83)^{b}	100	(90)^{c}
6.	Control	9.0	(17.45)^{a}	100	(90)^{c}
SEm±		1.11		2.01	
CD (p = 0.05)		3.37		6.11	

plated onto PDA medium to check the viability of sclerotia. Germination was recorded after three days of incubation at 27±1°C. Percent inhibition of sclerotial germination was calculated by counting the number of sclerotia germinated and un-germinated.

In-vitro evaluation of BCAs for inhibition on sclerotial germination

Sixty numbers of dried sclerotia were immersed in each of the suspension of *T. harzianum*, *T. viride*, *P. putida*, *P. fluorescens* and *B. subtilis* and they were plated at the rate of 20 per plate equidistantly with three replications. For control, twenty sclerotia were immersed in sterile distilled water. Both were incubated at 27±1°C. Percent inhibition of sclerotial germination was calculated.

Experimental design and data analysis

All the above *in-vitro* experiments in the present investigation were arranged as a randomized block design (RBD) with three replications and data obtained were statistically analyzed by one-way analysis of

Table 4. *In-vitro* efficacy of fungicides at different conc on radial growth and percent sclerotial germination of *S. rolfsii*. Figures in parentheses indicate inverse sine transformed values. Means having common letter are not significantly different. '*F*'- test at 5% level.

Sl. No.	Fungicides	Radial growth (cm) (Concentration percentage) 0.001	0.01	0.1	0.3	0.5	Percent sclerotial germination (Concentration percentage) 0.001	0.01	0.1	0.3	0.5
1.	Benofit	9.0 (17.45)^{a}	7.01 (15.35)^{b}	0	0	0	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}	0	0
2.	Bavistin	9.0 (17.45)^{a}	9.0 (17.45)^{a}	9.0 (17.45)^{a}	9.0 (17.45)^{a}	9.0 (17.45)^{a}	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}
3.	Contaf	9.0 (17.45)^{a}	3.98 (11.51)^{c}	1.68 (7.44)^{cd}	1.57 (7.19)^{cd}	0.81 (5.16)^{cd}	100 (90)^{a}	100 (90)^{a}	86.71 (68.61)^{b}	85.01 (67.22)^{c}	73.35 (58.91)^{d}
4.	Folicur	0	0	0	0	0	0	0	0	0	0
5.		6.52 (14.79)^{b}	1.82 (7.75)^{cd}	1.19 (6.26)^{cd}	1.41 (6.81)^{cd}	1.51 (7.05)^{cd}	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}
6.	Antracol	4.25 (11.89)^{c}	3.12 (10.17)^{bc}	1.44 (6.89)^{cd}	0.99 (5.71)^{cd}	0	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}
7.	Bilzeb	9.0 (17.45)^{a}	8.31 (16.75)^{ab}	3.07 (10.09)^{bc}	0	0	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}	83.35 (65.91)^{c}
8.	Control	9.0 (17.45)^{a}	9.0 (17.45)^{a}	9.0 (17.45)^{a}	9.0 (17.45)^{a}	9.0 (17.45)^{a}	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}	100 (90)^{a}
SEm±		2.82	1.67	1.26	1.38	1.45	–	–	2.51	4.85	5.05
CD (p = 0.05)		8.56	5.08	3.84	4.19	4.41	NS	NS	7.63	14.71	15.31

variance (ANOVA). The significant difference, if any, among the treatment means were compared using critical difference (CD) at $p = 0.05$ according to the Fisher's least significant test. Whenever necessary the data were transformed into inverse sine values before statistical analysis.

Results and Discussion

Estimation of yield loss of soybean due to collar rot

The yield of genotypes in un-inoculated pots was higher than that of inoculated pots which showed wide variation in yield (q/ha) and percent yield loss (Table 1). Under inoculated conditions there was significant reduction in yield and the lowest yield was recorded from genotype MACS1059 (11.83 q/ha) and minimum yield loss was recorded for genotype NRC80 (0.25 q/ha), while maximum yield was recorded from MACS1188 (31.71 q/ha) and maximum yield loss was recorded for genotype MACS1059 (10.15 q/ha). In the present findings yield loss was estimated and ranged from 1.02 to 46.17% under natural condition in pots. Heavy yield loss i.e. 46.17% might be due to the inoculation of the pathogenic *S. rolfsii* into the respective pots. Earlier reports also showed yield loss of 30—40% in different soybean cultivars due to collar rot in India. Four genotypes were found to be resistant and could give good yield even under inoculated conditions. These genotypes could be managed to compensate the disease infection by the pathogen. This might be due to genetic make-up of the crops that restrict the pathogen to further multiplication after infection or might be due to genetic variability within a pathogen population or due to presence or appearance of different physiological races of the pathogen.

In-vitro efficacy of plant extracts

The results (Table 2) showed significant difference in percent inhibition of mycelial growth of *S. rolfsii* with the plants extracts tested. Garlic clove extract @ 10 and 20% conc was found most effective in controlling the pathogen by inhibiting maximum radial growth followed by Neem @ 20% conc. Neem contains various chemical constituents such as nimolicinol, isolimolicinolide, azadirachtin, azadirachtol, nimbocinol, nimocin [5]. Moreover, Tulsi @ 5, 10 and 20% conc were also found effective in inhibiting the radial growth *in-vitro*. It was also observed that fungitoxicity of the extracts was higher at increased concentrations.

Among the plant extracts studied for inhibition of sclerotial germination, only garlic extract at 20% conc was found effective (18.34%) in inhibiting sclerotial germination (Table 2). This result shows that garlic may have some antifungal compound that could inhibit both the mycelial as well as sclerotia development [6].

In-vitro efficacy of BCAs

In the present study (Table 3), it was found that the bacterial antagonist *P. putida* (zero radial growth) was superior over all bio-agents, whereas *B. subtilis* inhibited the radial growth by76.12%. It might be because of *Pseudomonas* spp. produces extracellular protease enzyme which suppressed the mycelia growth and also produces antagonistic secondary metabolites such as siderophores and antibiotics like phenozines, pyroluterin and pyrrolnitrin that inhibit pathogens [7]. The broad suppressive properties of *B. subtilis* might be due to their ability to produce volatile and non-volatile substances, abundance of antibiotics [8]. Antagonist *T. harzianum* inhibited 75.89% mycelial growth of *S. rolfsii* during present investigation. Similar findings on the antagonistic parasitism of *T. harzianum* on *S. rolfsii* have also been reported [9, 10]. Since the cell walls of *S. rolfsii* are composed of β-1, 3-glucan and chitin, it was assumed that endochitinase enzyme produced by *T. harzianum* played an important role in the growth of the pathogen.

As for the sclerotial germination assay, BCAs viz. *P. putida* and *B. subtilis* were found comparatively effective in inhibiting *S. rolfsii* (Table 3). The diffusible antibiotics, volatile metabolites and hydrogen cyanide produced by *Pseudomonas* sp. might have degraded the cell wall of sclerotia, made up of melanin and thus inhibited the sclerotial germination.

In-vitro efficacy of fungicides

Among the seven fungicides (Table 4), Folicur (Tebuconazole) was most effective in controlling the pathogen (complete inhibition) tested even @ 0.001% conc followed by Benofit (Benomyl) @ 0.1% conc, Bilzeb (Mancozeb) @ 0.3% conc and Antracol (Propineb) @ 0.5% conc respectively. The results are in accordance with other workers who found that the active ingredient interfered with the growth of the pathogen [11]. However, the most common fungicide Bavistin was least effective on the mycelial growth of *S. rolfsii* at any given concentrations [12, 13].

Among the fungicides used, Folicur was superior over all fungicides tested which completely inhibited (100%) sclerotia germination even at 0.01% conc, whereas Benofit could inhibit (100%) sclerotia germination at 0.3% and 0.5% conc (Table 4). Treatments with Bavistin, Contaf, Jatayu, Antracol and Bilzeb had no significant effect on the inhibition of sclerotial germination.

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