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Management of head rot of Cabbage caused by *Sclerotinia sclerotiorum* through combined application of fungicides and biocontrol *Bacillus amyloliquefaciens*

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Abstract
The pathogen *Sclerotinia sclerotiorum* infects cabbage leading to a diseased condition called head rot. Symptoms of the disease begin with water soaked lesions on the leaves which enlarge over time. In the final stage of the disease, rotting of the entire cabbage head takes place which is followed by formation of cottony white mycelial growth. The mycelial growth becomes dense leading to development of carbon black coloured resting structures called sclerotia. The effect of eight different fungicides *viz*., propineb, carbendazim, tebuconazole, nativo (tebuconazole+trifloxystrobin), fosetyl aluminium, tricyclazole, metalaxyl and kresoxim methyl at concentrations of 25, 50, 100 and 250 ppm were evaluated against the growth of Sclerotinia sclerotiorum under *in vitro*. Results revealed that the fungicide nativo (tebuconazole+trifloxystrobin) was the most effective which inhibited growth of the pathogen at all the four concentrations. This was followed by carbendazim and tebuconazole which exhibited complete inhibition at concentration of 100 and 250 ppm. For field studies the effective fungicides were tested in combination with *B. amyloliquefaciens* isolate B15. Combined application of *B. amyloliquefaciens* isolate B15 and nativo was found to be highly effective with the least disease incidence of 8.67 per cent indicating 78.55 per cent reduction over control followed by nativo alone with disease incidence of 8.74 per cent and 78.37 per cent reduction over control. Both the treatments results were on par with each other.

Keywords: *Bacillus amyloliquefaciens* Fungicides, Nativo, *Sclerotinia sclerotiorum*

1. Introduction
Cabbage (*Brassica oleracea var. capitata*) is a leafy green biennial which is grown for its dense-leaved heads. Cabbage is of Cyprus and Mediterranean origin. It is cultivated extensively in tropical and temperate regions of the world *viz*., China, Germany, India, Indonesia, Japan, Korea, Poland, Russia, Taiwan, Turkey, Ukraine, USA, Uzbekistan and several other countries (Rai and Yadav, 2009) [16]. Head rot disease of cabbage caused by *Sclerotinia sclerotiorum* causes rotting of matured cabbage heads in the field, during post-harvest operations and storage (Hudyncia *et al*., 2000) [8]. The disease causes serious losses in field, storage and under market conditions (Ramsey, 1925) [18]. Initial symptoms on cabbage appear as water soaked spots on lower or upper leaves which enlarge causing the infected tissue to become soft followed by wilting of outer leaves. As the disease progresses a white cottony growth becomes evident on the leaves which finally covers the entire head followed by development of black carbon coloured resting structures called sclerotia (Purdy, 1979) [15]. In India head rot of cabbage was first reported during Febraury 1978 from Kodaikanal area in Dindigul district of Tamil Nadu (Alagianagalingam *et al.,* 1978) [2]. The pathogen is geographically cosmopolitan and has a broad ecological distribution. The pathogen occurs in cool and moist areas of the world (Purdy, 1979) [15].
Different fungicides are used to control *S. sclerotiorum.* Vinclozolin and tebuconazole were the most effective fungicides in suppressing the growth of *S. sclerotiorum* at 10-500 ppm under *in vitro*. In greenhouse soybean plants treated with fungicides survived the inoculation of the pathogen and added new leaves in comparison with the untreated plants which had expanded foliar lesions causing defoliation, fungal colonized stems and dead plants (Mueller *et al.,* 2002) [12].

Iqbal *et al.* (2003) [10] tested nine fungicides against stem rot disease of brinjal caused by *S. sclerotiorum*. Among them benlate, tecto and topsin at 50-100 ppm completely inhibited the mycelial growth of the fungus followed by ridomil gold. Prajapati and Udit (2008) [14] tested ten fungicides against *S. sclerotiorum* causing root rot of dolichos bean and found vitavax, companion and bavistin as most effective in inhibiting the growth of the pathogen completely at the lowest concentration of 100 ppm. Captan was found to be the least effective fungicide. Under field conditions the disease was effectively controlled by the application of vitavax followed by companion. Out of three fungicides tested against *S. sclerotiorum* thiophanate methyl was the most effective at the concentration of 1ppm and considerably affected the mycelial growth of the pathogen at other concentrations tested. Under greenhouse study thiophanate methyl reduced the symptoms caused by the pathogen by more than 30 per cent as compared to control where incidence was 87.25 per cent (Figueiredo *et al.*, 2010) [6].

Out of eight fungicides tested against *S. sclerotiorum* causing whitemold of lima bean phenylthiourea and difenoconazole were found to be the most effective showing an inhibition in the radial growth of the pathogen. In greenhouse experiments phenylthiourea and difenoconazole were found to be the most effective showing 0.14 and 0.22 per cent disease severity respectively as compared to control where it was 18.9 per cent (Ashutosh *et al*., 2012) [4]. Fungicides topsin-M and ridomil gold showed complete inhibition of growth of *S. sclerotiorum* at concentration of 100ppm while the same effect was shown by rizolex-T at 200 and 400 ppm (Abdel-Kader *et al.*, 2012) [1]. Ioannis (2014) [9] tested seven fungicides against *S. sclerotiorum* causing stem rot of lentil plants and found that the pathogen was sensitive to thiophanate methyl and to triazole fungicides group. The pathogen exhibited little sensitivity to strobilurine fungicides.

2 Materials and Methods

2.1 Isolation of Pathogen

Cabbage plants showing symptoms of *Sclerotinia* head rot were collected from Kothagiri area in Nilgiris district of Tamil Nadu. The age of the crop varied from 60 to 75 days. Sclerotia collected from infected cabbage heads were surface sterilized with 0.1 per cent mercuric chloride and rinsed with three changes of sterile water. The surface sterilized sclerotia were plated on Potato Dextrose agar (PDA) in sterile Petri plates and kept in an incubator at 19 ^{0}C for 5 days. The fungus was subcultured and mainatained on PDA. The stock cultures were maintained in PDA slants for long time storage under refrigerated condition at 4 ^{o}C.

2.2 *In vitro* screening of fungicides against *S. sclertiorum*

Inhibitory effects of eight fungicides *viz*., propineb, carbendazim, tebuconazole, native (tebuconazole+trifloxystrobin), fosetyl aluminium, tricyclazole, metalaxyl and kresoxim methyl each at four concentrations (25ppm, 50ppm,100ppm, 250 ppm) were evaluated on the growth of *S. sclerotiorum* by poisoned food technique. Each treatment was replicated three times with proper control. The fungal growth was measured after five days and per cent inhibition and number of sclerotia formed were recorded.

2.3 Selection of Biocontrol agent to be used in combination with effective fungicides in field trials

Biocontrol efficacy of twenty *Bacillus* isolates was screened against *Sclerotinia sclerotiorum* previously. *Bacillus amyloliquefaciens* isolate B15 was the most effective. Therefore this isolate was selected to be used in combination with effective fungicides obtained from *in vitro* studies. Standard isolate of *B. amyloliquefaciens* isolate B15 maintained as glycerol stock was obtained from the Department of Plant pathology, Tamil Nadu Agricultural University, Coimbatore. The isolate was subcultured and maintained on Nutrient agar (NA) medium for further studies.

2.4 Efficacy of fungicides alone and in combination with *B. amyloliquefaciens* isolate B15 under field conditions

To assess efficacy of fungicides and combination of effective fungicides and *B. amyloliquefaciens* isolate B15, a field trial was conducted in Kurkathi village in Kothagiri area of Nilgiris district, Tamil Nadu, during Febraury 2014-March 2014. The trial was laid out in a randomized block design (RBD) with a plot size of 6x2.8m^2 replicated three times. First spraying was given 48 days after planting (DAP) at cupping stage when the cabbage plant had approximately 20-26 leaves and subsequent sprays were given at 7 days interval till head fill stage when the cabbage head measured approximately 3 to 8 inch in diameter.

The treatment details are furnished below

T1- Nativo (Tebuconazole+Trifloxystrobin) @1.5g/L
T2- Carbendazim @ 2g/L.
T3- Metalxyl @ 1g/L.
T4- Fosetyl Al @ 1g/L
T5- Tebuconazole @ 1.5ml/L.
T6- *Bacillus amyloloiquefaciens* (B15) @ 10 ml/L
T7- B15 @ 10ml/L + Nativo @1g/L.
T8- B15 @ 10ml/L + Carbendazim @ 2g/L
T9- Control.

The disease incidence was calculated and results were expressed in terms of per cent disease reduction over control (Vincent, 1927) which was calculated as follows

$$R = \frac{100\ (C-T)}{C}$$

Where, R = Per cent reduction over control, C = Per cent disease incidence in control T = Per cent disease incidence in treatment

2.5 Statistical analysis

The data recorded were analysed statistically using the IRRISTAT version 92 developed by the International Rice Research Institute (IRRI), the Philippines (Gomez and Gomez, 1984). Prior to statistical analysis of variance (ANOVA) the percentage values of the disease index were arcsine transformed. Data were subjected to analysis of variance (ANOVA) at two significant levels ($P < 0.05$ and $P < 0.01$) and means were separated by Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

3.1 Efficacy of fungicides tested *in vitro*

Out of eight fungicides tested against *S. sclerotiorum* combination product nativo (tebuconazole+trifloxystrobin) inhibited the fungal growth completely at all the four concentrations (25, 50, 100, 250 ppm) followed by carbendazim and tebuconazole which exhibited complete inhibition at concentration of 100 and 250 ppm (Table 1). Inhibition due to carbendazim at 25 and 50 ppm was 45.2 and 62.2 per cent respectively (Table 2). Tebuconazole inhibited the fungal growth by 30.4 and 35.5 per cent at 25 and 50 ppm respectively. Tricyclazole was found to be least effective with

3.0 and 26.6 per cent inhibition over control at 25 and 250 ppm respectively.

Asha and Gupta (2001) [3] tested six fungicides against stem rot of mustard caused by *S. sclerotiorum* at concentrtaions of 500, 1000, 1500 and 2000 ppm and found that carbendazim, thiophanate methyl and phenylpyrrole completely inhibited growth of the pathogen at all concentrations tested. Among various fungitoxicants carbendazim was found effective in supressing the growth of *S. sclerotiorum* (Singh *et al*., 2003) [20]. Chand *et al.,* (2009) [5] reported that under *in vitro* carbendazim was the most effective fungicide which completely inhibited growth of *S. sclerotiorum* at 50 and 100 ppm. Zewain *et al.* (2004) [21] evaluated four different fungicides and neem extract against *S. sclerotiorum* and found that carbendazim inhibited growth of the pathogen completely at 50, 100, 500, 1000 and 5000 ppm. Carbendazim completely inhibited radial growth of *S. sclerotiorum* at concentration of 50, 100 and 200 ppm (Monika *et al*., 2013) [11]. Fungicides nativo and metalaxyl inhibited growth of *S. sclerotiorum* completely at 500, 1000 and 1500 ppm (Rajesh, 2013) [17]. Among six fungicides and biopesticides evaluated against stem rot of mustard caused by *S. sclerotiorum*, carbendazim, vitavax and benomyl were the most effective showing complete inhibition to the growth of the pathogen at 1000 ppm (Singh *et al*., 2014) [19].

3.2 Assessing efficacy of fungicides and *B. amyloliquefaciens* isolate B15 under field conditions for the management of *Sclerotinia* head rot of cabbage

Among the nine treatments combined application of *B. amyloliquefaciens* isolate B15 and nativo was found to be highly effective with the least disease incidence of 8.67 per cent indicating 78.55 per cent reduction over control followed by nativo with disease incidence of 8.74 per cent and 78.37 per cent reduction over control (Table 3). Application of fosetyl Al was found to be least effective with 12.71 per cent disease incidence indicating 68.55 per cent reduction over control. Maximum yield per hectare of cabbage was observed in treatment consisting of combined application of B15 and nativo (48.38 ton/ha) followed by nativo (48.21 ton/ha). Least yield per hectare was recorded in control (37.91 ton/ha).

Field spraying of carbendazim and vitavax at 0.1 per cent at 10 days interval minimised stem rot of ajowan (*Trachyspermum ammi*.) caused by *S. sclerotiorum* and increased the yield (Singh *et al.,* 2003) [20]. Combined application of *B. megaterium* with 10 µg mL^{-1} carbendazim showed significant reduction in symptoms of fusarium crown and root rot of tomato by 84 per cent when compared with inoculated control and by 77 per cent when compared with carbendazim treatment alone. Under field conditions carbendazim at 0.1 per cent controlled rot of dolichos bean caused by *S. sclerotiorum* giving mean disease reduction over control of 71.05 per cent over two successive years (Prajapati and Udit, 2008) [14]. Spraying of carbendazim (0.1 per cent) at 10 days interval was effective in controlling stem rot of mustard caused by *S. sclerotiorum* and reduction in disease severity and enhancement of seed yield was observed (Singh *et al*., 2014) [19]. Combined application of *B. subtilis* strain NJ-18 and flutolanil or difenoconazole for controlling sharp eyespot disease of wheat in the field lead to minimised amount of fungicide application in addition to providing effective disease control (Peng *et al*., 2014) [13]

Table 1: Efficacy of fungicides against the growth of *Sclerotinia sclerotiorum* under *in vitro*- Mycelial growth (mm)

Fungicides	Mycelial growth (mm) /reduction over control(%)			
	Concentration(ppm)			
	25	50	100	250
Propineb	78.0^{d} (8.82)	76.0^{d} (8.74)	69.3^{c} (8.31)	66.0^{c} (8.13)
Fosetyl Al	82.0^{d} (9.07)	80.7^{d} (9.00)	67.3^{c} (8.23)	0.0^{a} (0.70)
Carbendazim	49.3^{b} (7.04)	34.0^{b} (5.83)	0.0^{a} (0.70)	0.0^{a} (0.70)
Tricyclazole	87.3^{d} (9.37)	85.3^{d} (9.26)	68.0^{c} (8.26)	66.0^{c} (8.14)
Tebuconazole	62.7^{c} (7.93)	58.0^{c} (7.62)	0.0^{a} (0.70)	0.0^{a} (0.70)
Nativo (tebuconazole+ trifloxystrobin)	0.0^{a} (0.70)	0.0^{a} (0.70)	0.0^{a} (0.70)	0.0^{a} (0.70)
Metalaxyl	78.7^{d} (8.89)	61.3^{c} (7.84)	59.3^{c} (7.73)	0.0^{a} (0.70)
Kresoxim methyl	86.7^{d} (9.33)	85.3^{d} (9.26)	40.7^{b} (6.37)	25.3^{b} (5.07)
Control	90.0^{d} (9.46)	90.0^{d} (9.46)	90.0^{d} (9.46)	90.0^{d} (9.46)

Figures in parantheses are squre root transformed values
In a column means followed by same letter are not significantly different at 5% level of DMRT

Table 2: Efficacy of fungicides against the growth of *Sclerotinia sclerotiorum* under *in vitro*- Inhibition over control (%)

Fungicides	Inhibition over control (%)			
	Concentration(ppm)			
	25	50	100	250
Propineb	13.3	15.5	23	26.6
Fosetyl Al	8.8	10.4	25.2	100.0
Carbendazim	45.2	62.2	100.0	100.0
Tricyclazole	3.0	5.2	24.4	26.6
Tebuconazole	30.4	35.5	100.0	100.0
Nativo (tebuconazole+ trifloxystrobin)	100.0	100.0	100.0	100.0
Metalaxyl	12.6	31.8	34.1	100.0
Kresoxim methyl	3.7	5.2	54.8	71.8
Control	-	-	-	-

Figures in parantheses are squre root transformed values
In a column means followed by same letter are not significantly different at 5% level of DMRT

Table 3: Efficacy of fungicides and *B. amyloliquefaciens* isolate B15 under field conditions for the management of *Sclerotinia* head rot of cabbage

T. No.	Treatments	Disease incidence (%)	Reduction over control (%)	Yield (t/ha)
T1	Nativo (Tebuconazole+Trifloxystrobin)@1.5g/L	8.74a (17.19)	78.37	48.21a
T2	Carbendazim @ 2g/L	10.27c (18.69)	74.59	46.79b
T3	Metalaxyl 1g/L	12.37e (20.59)	69.39	45.24c
T4	Fosetyl Al 1g/L	12.71e (20.88)	68.55	45.90c
T5	Tebuconazole @ 1.5ml/L	11.24d (19.6)	72.19	46.76d
T6	B15 (*Bacillus amyloliquefaciens*) @ 10ml	13.57f (21.61)	66.42	44.72b
T7	B15 @10ml/L+ Nativo@ 1.5g/L	8.67a (17.12)	78.55	48.38a
T8	B15 @ 10ml/L+ Carbendazim @ 2g/L	9.80b (18.24)	75.75	46.73d
T9	Control	40.42g (39.47)	-	37.91e

Figures in parantheses are arc sine transformed values. In a column means followed by same letter are not significantly different at the 5% level of DMRT

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