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RESEARCH ARTICLE

Biological control of cabbage (*Brassica oleracea var. capitata*) head rot disease caused by *Sclerotinia sclerotiorum*

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SUMMARY

Head rot disease of cabbage is caused by *Sclerotinia sclerotiorum*. Efficacy of various biocontrol agents were evaluated for their potential to manage the head rot of cabbage under *invitro* condition. Among the tested isolates, *Trichoderma viride* recorded the maximum (85.71%) inhibition on the mycelial growth of pathogen followed by *Pseudomonas fluorescens* which recorded 79.15 per cent inhibition on the mycelial growth of *Sclerotinia sclerotiorum*. Based on the laboratory analysis, effective biocontrol agents were evaluated under glass house and field condition. Among the treatments tested in field condition, combined application of biocontrol agents (ST+ SA with (Pf + Tv) + Foliar spray with Pf) significantly recorded maximum (75.26) per cent disease reduction. These biocontrol agents were used an alternative to the chemical for controlling the cabbage head rot disease and enhanced the plant growth parameters and there by increased yield in cabbage.

Key Words : Cabbage, Head rot, *Sclerotinia sclerotiorum*, Biocontrol agents, *Trichoderma viride*, *Pseudomonas fluorescens*

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Cabbage is an important cole crops grown in India, accounting for about ten per cent of the total vegetable production of the country. India is the third largest cabbage producer in the world. In India, cabbage is mainly grown in the states like Uttar Pradesh, Orissa, Bihar, Assam, West Bengal, Maharashtra, Tamil Nadu and Karnataka. The major constraints in the production of cabbage are the diseases *viz*., head rot, damping off, club root, black rot, Alternaria leaf blight and downy mildew. Alaganagalingam *et al.* (1978) first reported the cabbage head rot disease in Tamil Nadu. The symptom first 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. Further a white cottony growth appeared on the head of the cabbage later the fungus produced the black coloured hardy resting structures (Purdy,1979). Severe yield losses occured in field and storage due to head rot disease

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(Ramsey, 1925). The disease is caused by the fungus *Sclerotinia sclerotiorum* which is widely distributed in relatively cool and moist areas throughout the world.

The wide spread use of fungicides to control plant diseases has lead to an increase of health hazards due to their phytotoxic residual and pollution effects consequently using some other means of disease control instead of agrochemical. Management of plant diseases through biological method envisage the use of antagonistic organisms like rhizobacteria, bacteriophages and avirulent strains of the pathogen and bacterial metabolites. Among the various antagonists used for the management of plant diseases, plant growth promoting rhizobacteria (PGPR) play a vital role. These bacteria may mediate biocontrol by one or more of the several mechanisms of disease suppression. Because of these reasons, biological control is a good alternative method, as compared to chemical control which destroys a range of micro and macro-organisms and has a limited impact on the environment (Sigee,1993). Plant growth promoting rhizobacteria (PGPR) used for direct plant growth promotion, biological disease management and inducing host resistance (Kloepper and Beauchamp, 1992).

Ecofriendly approach will be always better, it ensures the maximum suppression of the disease without any adverse impact on the ecosystem. All these requirements were considered in the following studies undertaken to develop a suitable ecofriendly management system against head rot of cabbage.

MATERIAL AND METHODS

Survey and disease assessment:

A survey was conducted during 2017 on the incidence head rot disease in different cabbage growing areas of Nilgiris, Tamil Nadu. In each village, four fields were selected and four plots in each field having an average area of ten square meters were marked at random. Head rot affected plants were counted in each plot and expressed as per cent disease incidence.

$$\text{Per cent disease incidence} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

Isolation of pathogen

The pathogen was isolated from the diseased tissues of cabbage by tissue segment method (Rangaswami, 1958). The infected portions of cabbage were cut into small pieces using sterilized scalpel and these were surface sterilized with 0.1 per cent mercuric chloride for one minute and washed in three changes of sterile distilled water and then placed on previously poured and solidified Petridish containing potato dextrose agar (PDA) medium. These plates were incubated at room temperature (28 ± 2°C) for five days and observed for the growth of the fungus. The hyphal tips of fungi grown from the pieces were transferred aseptically to PDA slants for maintenance of the culture.

Screening of biocontrol agents tested against *Sclerotinia sclerotiorum* under *in vitro*:

Biocontrol agents were screened against *Sclerotinia sclerotiorum* by dual culture method (Dennis and Webster, 1971). A nine mm mycelial disc of *Sclerotinia sclerotiorum* and *Trichoderma viride* were placed opposite to each other near the periphery of the Petriplate and incubated at room temperature (28±2^{0}C). After four days of incubation, mycelial growth of the pathogen and inhibition zone was measured in treatment imposed as well as in control plates. Per cent inhibition (PI) of mycelial growth was calculated using the formula suggested by Pandey *et al.* (2000).

$$PI = \frac{Dc - Dt}{Dc} \times 100$$

Dc = Average diameter of fungal growth (cm) in control

Dt = Average diameter of fungal growth (cm) in treatment

The overgrowth of antagonists over the pathogen was measured seven days after incubation. The overgrowth and zone of inhibition was measured and expressed in centi meter and millimeter, respectively.

Efficacy of biocontrol agents against head rot disease of cabbage in pot culture experiment:

A pot culture experiment was conducted to study the efficacy of *Trichoderma viride* and *Pseudomonas fluorescens* and Botanicals against *Sclerotinia sclerotiorum*. The isolates of *Sclerotinia sclerotiorum* were multiplied on sand-maize medium (Riker and Riker, 1936). The medium containing sand and maize powder (19:1) was mixed, moistened with 400 ml of water kg^{-1} and then packed in polypropylene bags. The bags were sterilized at 120^{0}C at 15 psi for 20 min. for two consecutive days and inoculated each bag with two nine mm potato dextrose agar (PDA) culture disc of actively growing *Sclerotinia sclerotiorum*. They were incubated at room temperature (28±2^{0}C) for 15 days and used as inoculum source. Earthern pots (30 cm dia x 60 cm

height) were filled with 5 kg of soil per pot. One cabbage seedling were planted in each pots with seven treatments and three replications. The talc based formulation of the *Pseudomonas* sp. (containing 10^9 cfu ml^{-1}) and *Trichoderma* sp. (10^6 cfu ml^{-1}) were delivered as per the requirement of the treatment. The per cent disease incidence was recorded periodically at 15 days interval upto harvest.

Efficacy of biocontrol agents against head rot disease of cabbage in field trial:

Field experiment was conducted to evaluate the efficacy of biocontrol agents against cabbage head rot in Nanjanad farm, Nilgiris district, Tamil Nadu. Randomized Block Design (RBD) was used in the experiments with plot size 5 x 4 m and spacing 15 x 10 cm. Cabbage seedlings were planted in each plot with seven treatments and three replications. The per cent disease incidence was recorded periodically at 15 days interval upto harvest. All normal agronomical practices were followed at regular intervals.

RESULTS AND DISCUSSION

A survey was conducted to assess the incidence of cabbage head rot disease in different places of Nilgiris district, Tamil Nadu. The incidence of head rot disease ranged between 15.42 to 60.75 per cent. The maximum incidence of 60.75 per cent head rot disease was recorded at Kenthorai, Nilgiris (Table 1). Biocontrol agents and botanicals were tested for their antagonistic activity against head rot pathogen *Sclerotinia sclerotiorum*. Among the tested isolates, *T. viride* recorded the maximum (85.71%) inhibition on the mycelial growth of pathogen with the inhibition zone of 3.15 mm followed by *Pseudomonas fluorescens* which recorded 79.15 per cent inhibition on the mycelial growth of *Sclerotinia sclerotiorum* with the inhibition zone of 2.54 mm (Table 2).

Upadhyay and Mukhopadhyay (2008) reported that *T. harzianum* significantly reduced the incidence of *Sclerotium* root rot and increased the root, green foliage and sucrose yield per ha in sugar beet. The strain T-1-R9 of *T.viride* was effective against the stem canker and black scurf disease of potato caused by *R. Solani* (Beagle Ristanio and Papavizas, 1985). Hydrolytic enzymes produced by *Trichoderma* sp. play an important role in destruction of plant pathogens (Chet *et al*., 1981). The importance of β-1, 3-glucanase and chitinase as key enzymes responsible for fungal cell and sclerotial wall lysis and degradation has been reported (Cook and Baker, 1983). These enzymes have been shown to be produced by several fungi and bacteria and may be an important factor in biological control.

The direct mycoparasitic activity of *Trichoderma* sp. has been proposed as one of the major mechanisms for their antagonistic activity against phytopathogenic fungi (Baker, 1987) *Trichoderma* spp. attach to the host hyphae by coiling, hooks or appressorium like structures

Table 1: Survey on the incidence of cabbage head rot in Nilgiris district of Tamil Nadu during 2017

Sr. No.	Places	Per cent disease incidence
1.	Thambatti	25.43
2 .	Akoni	19.54
3 .	Athiram patti	32.75
4 .	Bettati	48.24
5 .	Kilkunda	52.39
6 .	Selakorai	21.45
7 .	Kilkowhatty	42.74
8 .	Nanjanad	39.55
9 .	Kenthorai	60.75
10.	Thuneri	33.28
11 .	Jeghathala	15.42
12 .	Othanatty	45.58
13 .	Ullathy	54.64
14.	Wellington	37.45
15.	Ellanalli	29.33
	C.D. (P=0.05)	1.45

and penetrate the host cell walls by secreting hydrolytic enzymes such as a basic proteinase (Geremia *et al*., 1993).

In pot culture experiment the efficacy of biocontrol agents against Head rot disease of cabbage was tested, among the seven treatments T_3 (ST+ SA with (Pf + Tv)+ FA with Pf) treatment was significantly recorded maximum (72.47 %) disease reduction of head rot disease followed by T_2 and T_1 treatments were recorded 67.19 and 65.80 per cent reduction of the disease, respectively (Table 3). Biological control assumes special significance in it being an ecology conscious and cost-effective alternative strategy for disease management. Rhizobacteria such as *P. fluorescens* and *Bacillus* strains could provide significant levels of disease suppression and substantially enhance plant growth and grain yield. Antagonistic bacteria are considered as ideal biological control agents owing to their rapid growth, easy handling and aggressive colonization of rhizosphere (Gnanamanickam *et al.*, 2002).

In field condition the efficacy of biocontrol agents against head rot disease was tested, among the seven treatments T_3 (ST+ SA with (Pf + Tv) + FA with Pf) treatment was significantly recorded 73.41 per cent disease reduction of head rot disease with the yield of 75.26 t/ha followed by T_2 and T_1 were statistically on par which accounted 69.62 and 67.45 per cent reduction of the disease (Table 4).

Antibiotics produced by different plant growth promoting rhizobacteria have a broad-spectrum activity.

Table 2: Screening of biocontrol agents and botanicals against mycelial growth of *Sclerotinia sclerotiorum in vitro*

Sr. No.	Treatments	Mycelial growth of *Sclerotinia sclerotiorum* (cm)*	Per cent reduction over control	Inhibition zone (mm)
1.	*Trichoderma viride*	1.28	85.71	3.15
2.	*Pseudomonas fluorescens*	1.87	79.15	2.54
3.	*Bacillus subtilis*	2.48	72.32	1.70
4.	Botanicals (*Adathoda vasica*)	3.28	63.39	0.85
5.	Control	8.96	-	-
	C.D. (P=0.05)	0.40	-	

*Mean of three replications

Table 3: Efficacy of biocontrol agents against head rot disease of cabbage under glass house condition

Sr. No.	Treatments	Per cent disease incidence *	Per cent reduction over control
1.	T_1- ST+ SA with *P. fluorescens*	19.95	65.80
2.	T_2- ST+ SA with *T .viride*	19.13	67.19
3.	T_3 - (ST+ SA with (Pf + Tv) + Foliar spray with Pf)	16.05	72.47
4.	T_4- Foliar spray with botanicals (*Adathoda vasica*) 3 %	37.25	36.12
5.	T_5- Carbendazim (0.1%)	15.42	73.55
6.	T_6- Mancozeb (0.1%)	29.65	49.15
7.	T_7- Untreated control	58.32	-
	C.D. (P=0.05)	1.58	-

*Mean of three replications

Table 4 : Efficacy of biocontrol agents against head rot disease of cabbage under field condition

Sr. No.	Treatments	Per cent disease incidence *	Per cent reduction over control	Yield (t/ha)
1.	T_1- ST+ SA with *P. fluorescens*	16.97	67.45	68.24
2.	T_2- ST+ SA with *T .viride*	15.84	69.62	69.56
3.	T_3 - (ST+ SA with (Pf + Tv) + Foliar spray with Pf)	13.86	73.41	75.26
4.	T_4- Foliar spray of botanicals (*Adathoda vasica*) 3 %	29.87	42.71	36.25
5.	T_5- Carbendazim (0.1%)	12.45	76.12	76.95
6.	T_6- Mancozeb (0.1%)	28.52	45.30	39.65
7.	T_7- Untreated control	52.14	-	28.58
	C.D. (P=0.05)	1.25	-	-

*Mean of three replications
Seed treatment (ST)- 4g/kg of seed (*T.viride*), 10g/kg of seed (*P. fluorescens*) Soil application (SA) - @ 2.5 kg/ha (*T.viride* / *P. fluorescens)*

The antibiotic 2,4 diacetylphloroglucinol (2,4 DAPG) produced by several strains of *P. fluorescens*, not only have activity against a wide range of plant pathogenic fungi but also have antibacterial, antihelminthic and phytotoxic properties (Keel *et al.*, 1992). Kavitha (2004) reported that *P. chlororaphis* isolate PA 24 and *B. subtilis* isolate CBE 4 produced 2,4 DAPG and phenazine which are inhibitory to the growth of the *Pythium aphanidermatum* in turmeric and to the other soil borne pathogens, *viz*., *M. phaseolina*, *F. oxysporum* f.sp. *cubense* and *Sclerotium rolfsii*. Salah Eddin Khabbaz (2006) reported that the antibiotic 2,4 DAPG and phenazine isolated from the cell cultures of *Pseudomonas* isolates Pf 32, Pf 93, and B 49 effectively inhibited the growth of *Macrophomina phaseolina.*

The present study supported that benevolent profuse evidence to prove the field application of bioformulations to manage the head rot disease in cabbage. In conclusion, the potentiality of the biocontrol agents as an alternative to the chemical for controlling the cabbage head rot incidence and enhanced the plant growth parameters and there by increased yield in cabbage.

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