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BIOCONTROL OF LETTUCE DROP CAUSED BY *SCLEROTINIA SCLEROTIORUM* UNDER PROTECTED CONDITIONS

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ABSTRACT
Lettuce (*Lactuca sativa* L.), belonging to family Asteraceae, is one of the popular green leafy-vegetables. Among various diseases attacking this crop, lettuce drop caused by *Sclerotinia sclerotiorum* is the most destructive disease. Considering the more concern over pesticide residues on this crop, effect of fungal and bacterial biological control agents on incidence of lettuce drop was studied. Out of fungal antagonists, *T. harzianum* was found most effective (65.26%) in inhibiting the test pathogen followed by *T. viride* (60.94%), *T. hamatum* (60.69%) and *T. polysporum* (59.76%), while bacterial antagonists (*Bacillus subtilis* and *Pseudomonas fluorescens*) did not inhibit the pathogen significantly under *in vitro* conditions. *T. harzianum* also resulted in minimum incidence (14.44%) of lettuce drop followed by *T. viride* under *in vivo* conditions. An experiment conducted under protected conditions revealed minimum incidence of lettuce drop by *T. harzianum* giving more than sixty per cent reduction in disease over control and highest yield of lettuce head followed by *T. viride* . Therefore, *T. harzianum* can be used effectively in minimizing the incidence of lettuce drop under protected conditions

INTRODUCTION

Lettuce (*Lactuca sativa* L.), belonging to family Asteraceae, is one of the most widely consumed vegetables around the world for its leaves and most often used as salads. It is also consumed as soups, sandwiches and wraps. Genus *Lactuca* has more than 100 species and six types. Different types of lettuce are Romaine (Cos), crisp head (Ice berg), butter head, stem (asparagus), leaf (cutting) and oil-seed lettuce (Mousavi *et al.,* 2013). It is rich in vitamin A and C and minerals like calcium, iron, magnesium, potassium and sodium. In India, it is gaining popularity with the change in food habit and health consciousness among the people (Kaushal and Kumar, 2010). It is grown especially in temperate and sub-temperate regions in open as well as under protected conditions. Hilly states like Himachal Pradesh provide a congenial climate for its commercial cultivation. During the growing season, lettuce crop is attacked by a number of fungal, bacterial and viral diseases of which, lettuce drop caused by *Sclerotinia sclerotiorum* has been found to be predominant and destructive (Bharat *et al*., 2014). Crop losses may vary from less than 1 per cent to nearly 75 per cent (Purdy, 1979) and in some cases entire field may be lost (Stoneman, 2002).

Presently, there are no commercial lettuce cultivars with resistance to *Sclerotinia sclerotiorum* and it has a broad host range and extended survival in soil in the absence of hosts. Therefore, current management strategies for lettuce drop rely heavily on chemical applications. However, considering the prominence of lettuce in the daily diet and more concern over pesticide residues on lettuce crop support the development of nonchemical approaches like biological control (Matheron and Porchas, 2004). Therefore, present studies were undertaken to study the effect of fungal and bacterial biological control agents on incidence of lettuce drop under protected conditions.

MATERIAL AND METHODS

Isolation of the pathogen

The pathogen *Sclerotinia sclerotiorum* was isolated from naturally infected plants of lettuce using potato dextrose medium and pathogenicity was tested on seedlings of susceptible variety 'Solan Kriti'.

In vitro evaluation of fungal and bacterial antagonists

Six fungal antagonists, namely, *Trichoderma viride, T. harzianum, T. polysporum, T. hamatum, T.* sp. and *T. virens* and two bacterial antagonists, namely, *Bacillus subtilis* and *Pseudomonas fluorescens,* procured from Department of Plant Pathology, UHF, Nauni, were tested for their antagonistic activities against *S. sclerotiorum* under *in vitro* conditions by dual culture technique and streak plate method, respectively. Each treatment was replicated three times.

The inoculated plates were incubated at 25 ± 1°C. Radial growth of fungal antagonist and the test pathogen was recorded for a week. Per cent inhibition over control was worked out according to formula given by Vincent (1947).

Preparation of mass culture of pathogen, fungal antagonists and bacterial antagonists

Mass production of the pathogen inoculum was done on corn meal sand medium whereas mass culture of fungal antagonists was prepared on wheat bran saw dust medium by using standard methods. Bacterial antagonists were multiplied on nutrient broth medium.

***In vivo* evaluation of biocontrol agents against lettuce drop**

Fungal and bacterial antagonists, screened under *in vitro* conditions, were also evaluated to assess their biological potential against *S. sclerotiorum* in trays. Forty days old seedlings of susceptible variety "Solan Kriti" were planted in sterilized soil in trays. Each fungal antagonist containing about 10^6 cfu/g of substrate (@5 g/kg soil) was mixed in the soil and the pathogen inoculum (@5 g/kg soil) was applied as mass culture of *S. sclerotiorum*. Bacterial antagonists were applied as bacterial suspension (100 ml) having a population density of 10^8cfu ml^{-1} and were added simultaneously with the mass culture of pathogen to soil in trays.

Trays inoculated with test pathogen alone were kept as control for comparison. Each treatment was replicated three times. The seedlings were frequently watered so as to maintain uniform moisture in all the trays. Data on disease incidence was recorded one month after inoculation.

Evaluation of biocontrol agents under protected conditions

An experiment was laid out to study the effect of potential biocontrol agents on disease incidence of lettuce drop under protected conditions at the University Research Farm of Department of Vegetable Science, UHF, Nauni. Susceptible lettuce cv. "Solan Kriti" was raised in the nursery and 40 days old seedlings were transplanted in plots (100 x 80 cm). The experiment was laid in completely randomized block design with six biocontrol agents and a check replicated three times. The suspension of bacterial biocontrol agents (@ 10^6-10^8 cfu/ml) as drench and mass culture of fungal antagonists (@ 5 g/kg) by placement method was applied to the soil at the time of transplanting and after 30 days of transplanting. The observations on disease incidence and yield were recorded in the month of February.

RESULTS AND DISCUSSION

***In vitro* evaluation of biocontrol agents on mycelial growth**

Fungal and bacterial antagonists were screened under *in vitro* conditions by dual culture and streak plate method, respectively, and the results have been shown in Fig. 1.

All the fungal and bacterial antagonists inhibited the growth of *S. sclerotiorum* ranging from 27.54 to 65.26 per cent (Fig 1). Among fungal antagonists, *T. harzianum* was found most

Table 1: *In vivo* evaluation of fungal and bacterial antagonists against *S. sclerotiorum*

Antagonist	Disease incidence (%)
Trichoderma viride	18.44(25.40)
T. harzianum	14.44(23.85)
T. polysporum	24.98(29.97)
T. hamatum	20.25(26.62)
T. virens	28.37(32.10)
T. sp.	34.18(35.70)
Pseudomonas fluorescens	32.52(34.78)
Bacillus subtilis	31.94(34.32)
Control	56.35(45.62)
CD$_{(0.05)}$	(3.65)

Figures in the parenthesis are arc sine transformed values

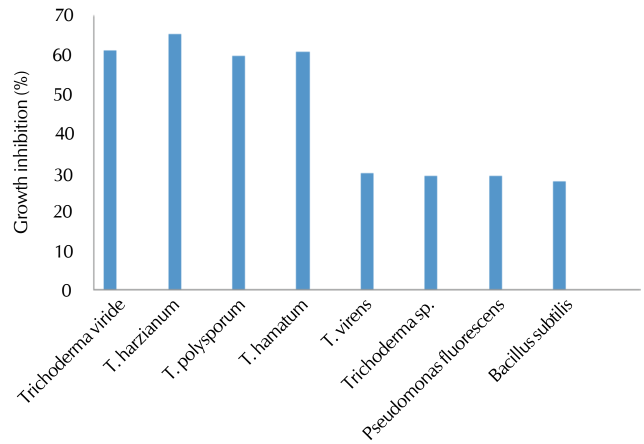

Figure 1: *In vitro* evaluation of fungal and bacterial antagonists against *S. sclerotiorum*

effective (65.26%) in inhibiting the test pathogen followed by *T. viride* (60.94%), *T. hamatum* (60.69%) and *T. polysporum* (59.76%), though statistically at par with each other. *T. virens* was found least effective with 29.84 per cent inhibition of mycelial growth of the pathogen. However, bacterial antagonists, *Bacillus subtilis* and *Pseudomonas fluorescens* did not inhibit the test pathogen significantly and resulted in 27.54 and 28.92 per cent growth inhibition, respectively.

Singh (1998) reported that *T. harzianum* showed strong mycoparasitism and covered 100 per cent colony growth of *S. sclerotiorum*, whereas *T. viride* showed strong antibiosis and formed 2-3 mm zone of inhibition after 6 days of incubation in dual culture. Twenty one isolates of *Trichoderma* spp. were collected by Smith *et al.* (2013), out of which *Trichoderma asperellum, T. atroviride* and *T. harzianum* prevented germination of more than 70 per cent of sclerotia of *S. sclerotiorum* in bioassay tests. Hyphal and sclerotial parasitism by means of coiling, penetration and lytic degradation were the mechanisms by which *T. harzianum* inhibited *S. sclerotiorum* (Mehta *et al.*, 2012).

Phookan and Chaliha (2000) reported suppression of mycelial and sclerotial growth of *S. sclerotiorum* by *Bacillus subtilis* and Aeron *et al.* (2011) found *P. fluorescens* PS1 causing morphological alteration in mycelia of *S. sclerotiorum* by hyphal perforation and fragmented lysis. Shrestha *et al.* (2015) observed that *Bacillus thuringiensis* conferred significant inhibition in mycelial growth, formation and viability of sclerotia of *S. minor in vitro*.

Trichoderma isolates have also been reported effective against different soil borne pathogens by various workers. *Trichoderma harzianum*-55 IIHR was observed to give 70 per cent inhibition of *Sclerium rolfsii* causing collar rot of chickpea followed by *T. harzianum* NBA II giving 63 per cent inhibition (Sab *et al.*, 2014). Similarly, *T. viride* and *T. harzianum* were found effective in inhibiting mycelial growth of *Rhizoctonia solani* causing root rot of chilli under *in vitro* conditions (Patel *et al.*, 2014).

In vivo evaluation of biocontrol agents

Fungal and bacterial antagonists were also evaluated in sick soil by adding the mass culture of these antagonists into the

Table 2: Effect of biocontrol agents on incidence of lettuce drop and yield under protected conditions

Treatments	Disease Incidence (%)	Per cent disease control	Yield (g/plot)
Trichoderma viride	38.41(37.92)	51.35	3,208
T. harzianum	30.50(33.31)	61.37	4,016
T. polysporum	51.95(46.11)	34.20	2,696
T. hamatum	41.97(40.26)	46.84	3,108
T. virens	49.67(45.31)	37.09	2.578
T. sp.	52.78(46.27)	33.15	2,567
Pseudomonas fluorescens	54.54(47.59)	30.92	2,483
Bacillus subtilis	60.89(51.36)	22.88	2,333
Control	78.96(67.21)	-	1,400
$CD_{0.05}$	(11.08)	-	1,101

Figures in the parenthesis are arc sine transformed values

soil and observations on per cent disease incidence after statistical analysis have been presented in Table 1.

All fungal and bacterial antagonists reduced the incidence of lettuce drop significantly; however, *T. harzianum* resulted in minimum incidence (14.44%) of lettuce drop (Table 1). This was followed by *T. viride* (18.44%), *T. hamatum* (20.25 %) and *T. polysporum* (24.98 %), though all were statistically different from each other. Bacterial antagonists did not reduce the disease incidence significantly in comparison to fungal antagonists.

Rabeendran *et al.* (2006) evaluated fungal isolates (*T. hamatum*, *T. virens*, *Coniothyrium minitans*, *Clonostachys rosea* and *T. rossicum*) by incorporating spore suspensions into transplant potting mix and planting lettuce seedlings in them. *T. hamatum*, *T. virens* and *C. minitans* reduced disease by 30-50 per cent at harvest. Villalta *et al.* (2012) found that *T. hamatum,* applied to the potting mix of lettuce transplant and as a combined application (transplant and soil applications at transplanting), significantly reduced disease by 78 per cent and 83 per cent, respectively.

Evaluation of biocontrol agents under protected conditions

Fungal and bacterial antagonists, after initial screening under *in vitro* and *in vivo* conditions, were further evaluated for their efficacy in reducing the incidence of lettuce drop vis-a vis increasing the yield under protected conditions. Mass culture of antagonists was applied in the field and data on per cent disease incidence and yield were recorded and are presented in Table 2.

A significant reduction in the incidence of lettuce drop was achieved by applying all fungal and bacterial antagonists; however, minimum incidence was given by *T. harzianum* (30.50%) followed by *T. viride* (38.41%), though statistically at par with each other (Table 2). Data further showed that more than sixty per cent reduction in disease over control was given by *T. harzianum* (61.37%). This was followed by application of *T. viride* which resulted in 51.35 percent reduction in disease over control. These antagonists also enhanced the yield in addition to disease suppression and similar trend was observed for yield wherein, soil application of *T. harzianum* gave highest yield of lettuce head (4,016 g/plot) followed by *T. viride* (3,208 g/plot), which were statistically different from each other.

Presently, no efficacious biocontrol agent for *S sclerotiorum* is available for commercial application; however, some fungal and bacterial antagonists have been tested by different workers. McLaren *et al.* (1996) reported that incidence of lettuce drop was reduced by treating seedlings with a peat-bran preparation of *T. harzianum* in the green house. Beneden *et al.* (2010) applied *T. harzianum* and *T.* spp. to the soil which inhibited the sclerotia and their carpogenic germination. These were tested in a sequence of celery and two lettuce crops in glass house conditions and showed decreased disease incidence and increased marketable yield. Mehta *et al.* (2012) observed that soil application of *T. harzianum* resulted in less disease intensity as compared to control (54%). Soil incorporation of *T. virens* and *T. hamatum* was shown to have potential to control *S. sclerotiorum* disease in cabbage (Jones *et al.*, 2014). *Trichoderma* exert biocontrol action against soil borne pathogens through different mechanisms such as competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and plant defense mechanisms and antibiosis or directly by mechanisms such as Mycoparasitism (Srivastava *et al.*, 2015).

It can be inferred from this study that soil application of *T. harzianum* can be used for the management of lettuce drop under protected conditions.

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