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Integrated management of seedling blight disease of tea caused by *Sclerotium rolfsii*

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Abstract

Tea is one of the important plantation crops in Nepal. One of the important fungal pathogens *Sclerotium rolfsii*, causing seedling blight disease in tea was found to be predominant in the nursery grown plants. The art and science of plant disease control has moved in the direction of biological control of plant pathogen is a distinct possibility for the future and can be successfully exploited in modern agriculture, especially within the framework of integrated disease management systems. Effective integrated management practices against *S. rolfsii* were developed using neem cake, oil cake, aqueous leaf extract of *Azadirachta indica*, bio-control agent like *Trichoderma harzianum* and calixin (0.1%) *in vivo*. Combination with cow dung, neem cake, oil cake, chicken manure and rabbit manure, disease reduction were insignificant. However, combination with neem cake and oil cake showed 66.4% disease incidence, whereas in oil cake, neem cake and *Azadirachta indica* in combination disease incidence were recorded 11.1%. Under pot culture conditions *T. harzianum* alone and in combination with neem cake, oil cake and *Azadirachta indica* provided best effective management practices of seedling blight in all the three modes of application *viz.*, simultaneous, repeated and post infection.

Introduction

Tea is the important cash crop in Nepal. A number of fungal pathogens causes disease in tea plants. One of the important fungal pathogen is *Sclerotium rolfsii* which causes sclerotial blight in tea. A number of fungal pathogens cause diseases of tea which reduces the quality and quantity of tea production. Sclerotial blight caused by *Sclerotium rolfsii* Sacc. (telomorph: *Athelia rolfsii* (Curzi) Tu and Kimbrough = *Corticium rolfsii* Curzi) is one of the fungal diseases which appears in the nursery grown tea seedlings. Effective integrated management practices against *S. rolfsii* were tested *in vivo*. Integrated Disease Management (IDM) as applied to disease means using all the tactics available to the grower (cultural, biological, host plant resistance and chemical) that provides acceptable yield and quality at the least cost and is compatible with tenets of environmental stewardship. The art and science of plant disease control has moved in the direction of biological control of plant pathogens, including use of introduced antagonists. It is now widely recognized that biological control of plant pathogen is a distinct possibility for the future and can be successfully exploited in modern agriculture, especially within the framework of integrated disease management systems. Integrated control is a flexible, multi-dimensional approach to disease control utilizing a range of control components such as biological, cultural and chemical strategies needed to hold diseases below damaging economic threshold without damaging the agro-ecosystem. Effective integrated management practices against *S. rolfsii* were developed using neem cake, oil cake, aqueous leaf extract of *Azadirachta indica*, bio-control agent like *Trichoderma harzianum* and calixin (0.1%) *in vivo*. Combination with cow dung, neem cake, oil cake, chicken manure and rabbit manure, disease reduction were insignificant. However, combination with neem cake and oil cake showed 66.4% disease incidence, whereas in oil cake, neem cake and *Azadirachta indica* in combination disease incidence were recorded 11.1%. Under pot culture conditions *T. harzianum* alone and in combination with neem cake, oil cake and *Azadirachta indica* provided best effective management practices of seedling blight in all the three modes of application *viz.*, simultaneous, repeated and post infection.

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In the present investigation an attempt was made to develop an effective integrated management strategy against seedling blight of disease of tea.

Materials and methods

Fungal cultures

Virulent culture of *Sclerotium rolfsii* Sacc (*Corticium rolfsii* Curzi) was obtained from Immuno-Phytopathology Lab, Department of Botany, North Bengal University. This was originally isolated from Teen Ali-17/1/54 and after completion of Koch's postulate, the organism was identified by the Global Plant Clinic, Diagnostic and Advisory Service, CABI Bioscience UK and designated as Sr-1. Besides, two more isolates (Sr-2 and Sr-3) of *S. rolfsii* which were used in this investigation were isolated from infected tea roots of TV-25 and UP-8 respectively. Cultures of *Trichoderma harzianum* (biocontrol agent) was also obtained from the laboratory, mentioned above.

Inoculation technique

Inoculum preparation

Fungal pathogen

According to Chowdhury and Sinha (1995), sand maize meal medium was prepared in the ratio of 3:1 (sand : maize). In the prepared sand maize meal medium fungal pathogen (*S. rolfsii*) was inoculated and incubated at 28^{0}C for 7 days. The inoculum was mixed with sterile soil at the ratio of 1:8. Fungus soil mixture (100 gm) were mixed with the top soil of earthen pots containing tea seedlings and kept for development of disease reaction.

Biocontrol agents

Trichoderma species prepared in several media *viz.*, wheat bran media (wheat-bran: sand 1:1, and 25 ml of water for 150 g of inoculum in each polythene packet); Saw dust media (saw dust and water), tea waste media (tea waste and water). Media were autoclaved and inoculated as above.

Inoculation of healthy tea seedlings in pot

One year old tea seedlings were planted in earthen pots containing 1 kg soil and allowed to be established. Regular watering was done for two weeks and then 100 g of pathogen inoculum was added carefully in the rhizosphere of each plant. Disease assessment was done after 2-week- intervals and up to 45 days of inoculation.

Inducing agents and their application

In vivo test

Mature leaves (500 g) each of *Azadirachta indica* and *Catharanthus roseus* were harvested, washed thoroughly with running tap water, rinsed with distilled water, air dried and macerated separately homogenized in a electric blender. The leaf extract was filtered through double – layered muslin cloth and centrifuged at 10,000 g for 30 minutes. The supernatant was collected and filtered through Whatman No.1 filter paper. Each filtrate was further filter sterilized and preserved as stock (100%) solution aseptically in bottles at 5^{0}C for further use. Leaf extracts were diluted (1:10) with distilled water, drops of Tween-80 was mixed and sprayed on tea plants with the help of sprayer. The control plants were sprayed with distilled water mixed with Tween-80. Spray was done four times at 7-day intervals. Both treated and untreated plants were inoculated with *S. rolfsii* and disease assessment was made.

Mustard oil cakes and neem cakes were allowed to decompose separately for a week in a clay pot covered with polythene. After decomposition, 100 ml of decomposed oil cake solution was added in each tea seedlings pots. The pots were then inoculated with *S. rolfsii*. Untreated control was kept for comparison. Growth behaviour also observed up to two months. Organic additives (cow dung, rabbit manure and chicken manure), 100 gm of each were taken separately and mixed in 1 kg of soil. These soil mixtures were separately kept in each pot. Tea seedlings were planted in each pot containing different organic components. After one week, 100 gm of pathogen (*S. rolfsii*) inoculum was added in the rhizosphere of each

tea seedling.

Mass cultures of *T. harzianum and T. viride* were prepared on carrier medium comprising of wheat bran and sawdust (WBSD) in 3:1 ratio. Five hundred grams of the contents of carrier medium moistened with 20 percent (w/w) distilled water was filled in each bag. These polythene bags were sterilized at 15 lb pressure for 1 h for 2 consecutive days. Each polythene bag was then inoculated with 4-6 days old bits (0.3 cm) of pure culture either of *T. harzianum* and *T. viride* and incubated at 28±1°C. During incubation, these bags were gently hand shaken to promote uniform sporulation over the carrier medium and to avoid clusters. Addition of biocontrol agents in soil was done 10 days prior to inoculation with *S. rolfsii*. 0.1% of calixin was sprayed with distilled water on tea plants. The control plants were sprayed with distilled water mixed with Tween-80. Spray was done four times at 7-day intervals. Both treated and untreated plants were inoculated with *S. rolfsii* and disease assessment was made.

Result

In vivo evaluation

Growth promotion in tea seedlings

Tea seedlings of two varieties (B-157 and TeenAli-17/1/57) were grown in soil amended with neem cake and oil cake separately. Each treatment consisted of 10 plants, in triplicate and the values are an average of 30 plants. Results were recorded after one-month interval and up to two months following the treatment of neem cake and oil cake and after inoculation with *S. rolfsii*. Results (Table 1) revealed that the growth of tea seedlings had been increased following amendment with neem and oil cakes than those treated plants inoculated with *S. rolfsii* in relation to untreated uninoculated tea seedlings as recorded after two months following treatment.

Similarly seedlings of three tea varieties (UP-3, B-157 and K-1/1) were grown in soil amended separately with cow dung, rabbit manure and chicken manure. Each treatment consisted of 10 plants, in triplicate and the values are an average of 30 plants. Results were recorded after one month interval up to two months following the treatment of organic components and after inoculation with *S. rolfsii*. It has been observed that the growth of tea seedlings had been increased in treated uninoculated than treated inoculated tea seedlings (Table 2). Among the three treatments with organic components, rabbit manure gave very good and healthy growth of tea seedlings than chicken manure and cow dung.

Under pot culture conditions *T. harzianum* alone and in combination with neem cake, oil

Table 1: Growth promotion in tea seedlings following soil amendment with neem cake and oil cake

Tea variety	One month				Two months			
	Healthy		Infected		Healthy		Infected	
	Increase in height (cm)	Increase no. of leaves	Increase in height (cm)	Increase no. of leaves	Increase in height (cm)	Increase no. of leaves	Increase in height cm	Increase no. of leaves
T17/1/54	2±.02	4±.04	0	2±.03	5±.03	6±.02	1±.01	2±.03
Untreated Treated	2±.01	3±.04	1±.01	0	2±.03	8±.04	2±.03	4±.02
Neem cake	1±.02	3±.02	2.5±.1	2±.03	2±.01	4±.03	2±.01	3±.02
Oil cake	1±.01	3±.03	1±.02	0	4±.04	6±.03	1±.01	2±.03
B-157								
Untreated Treated	2±.01	2±.03	1±.02	0	0	1±.01	2±.03	3±.01
Neem cake	1±.04	4±.03	1.5±.1	0	2±.03	0	2±.02	1±.01
Oil cake								

± Stand for standard deviation; Average of three replicates

Table 2: Growth promotion in tea seedlings by different organic components after inoculation with *Sclerotium rolfsii*

Tea variety	One month				Two months			
	Healthy		Infected		Healthy		Infected	
	Increase in				Increase in			
	height (cm)	no. of leaves	height (cm)	no. of leaves	height (cm)	no. of leaves	height (cm)	no. of leaves
UP-3 Untreated	2±.0	0	1±.02	0	3±04	0	1±.01	0
Treated Cow dung	6±.0	1±.02	3±.07	1±.04	4±.03	1±.02	1.5±.02	0
Rabbit manure	9±.0	0	6±.02	0	6±.03	1±.04	4±.02	0
Chicken manure	4±.0	1±.03	2±.01	0	3±.02	1±.06	2±.04	0
B-157 Untreated	1±.0	0	0	0	1±.02	2±.04	1±.02	0
Treated Cow dung	3±.0	1±.03	3±.04	1±.02	4±.05	1±.02	1±.03	1±.04
Rabbit manure	8±.0	1±.06	5±.02	0	5±.03	1±.06	2±.01	1±.03
Chicken manure	4±.0	0	2±.06	1±.03	4±.07	3±.08	2±.07	1±.04
K – 1/1 Untreated	2±.0	0	1±.05	0	2±.02	1±.02	0	0
Treated Cow dung	3±.0	0	1±.03	0	2±.05	0	0	0
Rabbit manure	9±.0	4±.02	7±.04	0	8±.03	0	2±.01	0
Chicken manure	6±.0	2±02	4±.03	0	3±.06	3±.04	2±.03	0

± Stand for standard deviation; Average of three replicates

Table 3: Effect of simultaneous treatments with biocontrol, fungicide, organic amendments and plant extract on development of seedling blight of tea following inoculation with *Sclerotium rolfsii*

Treatment	Disease incidence (%)	Disease control (%)
Trichoderma harzianum	0	100
Oil cake with Neem cake	66.4	33.6
Oil cake, Neem cake and *Azadirachta indica* (aqueous extract)	11.1	88.9
T. harzianum with *Azadirachta indica* (aqueous extract), oil cake and neem cake	0	100
Cow dung, Neem cake and Oil cake	44.6	55.4
Chicken manure, Neem cake and Oil cake	47.5	52.5
Rabbit manure, Neem cake and Oil cake	46.6	53.4
T. harzianum, Calixin (0.1%) and *Azadirachta indica* (aqueous extract)	0	100
Untreated Control	100	0

Table 4: Comparative efficacy of application of organic amendments and formulation against *Sclerotium rolfsii*

Treatment	Disease incidence (%)		
	Simultaneous	Repetitive	Post infection
Trichoderma harzianum	0	0	0
Oil cake, Neem cake and *Azadirachta indica* (aqueous extract)	15.8	0	44.6
T. harzianum, Azadirachta indica (aqueous extract) Oil Cake and Neem cake	0	0	0
Cowdung, Neem cake and Oil cake	40.6	30.5	77.7
Rabbit manure, Neem cake and Oil cake	46.3	33.0	85.8
Chicken manure, Neem cake and Oil cake	47.5	35.5	88.2
T. harzianum, Calixin (0.1%), *Azadirachta indica* (aqueous extract)	0	0	0
Untreated Control	100	100	100

cake and *Azadirachta indica* provided best effective management practices of seedlings blight in all the three modes of application *viz.*, simultaneous, repeated and pot infection. Combination with neem cake and oil cake showed 66.4% disease incidence where as in oil

cake, neem cake and *Azadirachta indica* in combination disease incidence were recorded 11.1%. But in combination with cow dung, neem cake, oil cake, chicken manure and rabbit manure, results were insignificant as shown in (Tables 3 and 4).

Discussion

In vivo trials demonstrated that *Trichoderma harzianum* alone as well as in combination with neem cake, oil cake, aqueous extract of *Azadirachta indica* and calixin (0.1%) provided a total control of sclerotial blight disease. Similar results were obtained by Sonali and Gupta (2004) when *T. viride* alone and in combination with neem oil, neem cake and deodar needles used in radial growth of *S. rolfsii* resulted in a total control of the disease. But repeated application of neem cake, oil cake with various combinations of cow dung, rabbit manure and chicken manure were found to be less significant. Finally it was observed that *T. harzianum* and in combination with neem cake, oil cake, neem extract and calixin (0.1%) were found most effective in reducing disease incidence on tea seedling plants *in vivo*.There are several reports on the management of disease by Integrated Disease Management (IDM). Management of chickpea root rot and collar rot against *S. rolfsii* by integration of biological and chemical seed treatment was reported by Tiwari and Mukhopadhyay (2003). They observed that application of carboxymethyl cellulose (CMC) with *G. virens* powder (10^9 spores per g) in combination with vitavax provided maximum protection (81.9%) to the crop against chickpea root rot and collar rot pathogens in glasshouse. Chickpea seeds treated with GV powder + CMC + vitavax significantly increased seedling emergence (47.9%); final plant stand (85.8%) and grain yield (79.7%) which was statistically at par with the treatment GV powder + vitavax and GV suspension + vitavax in a sick plot. Upamanyu *et al.*, (2002) reported the management of root rot and web blight caused by *Rhizoctonia solani*. They obtained that *T. viride* showed the maximum tolerance to carboxin, tebuconazole and carbendazim followed by *T. virens*, *T. harzianum* and *A. niger* when used in integrated disease management along with fungicides and oil cakes both under glass house and field conditions. Soil amendment (cotton cake) + *T. virens* and carboxin (ST), mustard cake + *T. virens* + tebuconazole and soil amendement (mustard cake) + carbendazim (ST) were found effective in containing the root rot under glass house conditions while soil amendment (mustard cake) + carbendazim (ST) + carbendazim (FS) were found highly effective in reducing pre- and post- emergence root rot and web blight. Severity was best contained by soil amendement (mustard cake) + carbendazim (ST+FS) followed by tebuconazole + *T. virens* (ST) + carbendazim (FS).

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