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Identification of potential isolates of *trichoderma* antagonist to *sclerotium rolfsii* in the garden soil of litchi and mango orchard.

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Abstract
The fungal population in soils of mango and litchi orchards was studied on Peptone dextrose rose bengal agar medium using dilution plate technique. The population of total fungi in different soil samples of mango and litchi orchrd of Pusa and Birauli ranged from 9.00 $X10^3$ cfu/g of soil to 9.60 $X10^3$ cfu/g of soil. The population of total fungi in different soil samples did not differ significantly. In soil samples of KVK, Birauli, population of *Trichodrma* (ranged from 2.50-2.60 cfu/g soil) while in the the soil samples of RAU, Pusa it ranged from 3.3- 3.6 cfu/g soil. The result indicated that population of *Trichodrma* is available in all soil samples with varying population. Five isolates of *T. harzianum* such as Th1, Th2, Th3, Th4 and Th5 were compared for their capability of antagonism towards *S. rolfsii* in dual culture. *T. harzianum* isolate 4 proved best in dual culture and parasitized the colony of *S. rolfsii* earliest. *T.hazianum* 4 also showed maximum inhibition in formation of sclerotia of *S. rolfsii* (73.30%) in dual culture. Viability studies on mycelium and sclerotia of *S. rolfsii* in dual culture with *T. harzianum* in antagonized plate revealed lysis of both mycelium and sclerotia.

Keywords: *Sclerotium rolfsii,* viability, sclerotia, *Trichoderma* sp.

Introduction
Trichoderma are free-living soilborne fungi which are highly interactive in the rhizosphere and foliar environments. *Trichoderma* are known as imperfect fungi but now their perfect stage (Hypocrea) is known, are fast growing in culture and produce numerous green spores and chlamydospores. *Trichoderma* have created ecofriendly, safe and non-chemical disease management system which have great importance in organic agriculture. *Trichoderma*, a soilborne mycoparasitic fungus has been shown effective against many soil borne phytopathogens (Rajkonda *et al*., 2011 and Dolatabadi *et al*., 2012) [4, 14]. Biological control of soil borne phytopathogens has been the subject of extensive research in the last few decades. However, with the increasing interest in biological control, owing to environmental and economic concerns, thousands of research experiments are going on for searching novel, potential, safe and have ability to inhibit wide range of soilborne phytopathogens. *Trichoderma* spp. is well documented as effective biological control agents of soilborne diseases which inhibit the pathogens by direct antagonism or by secreting several cell wall degrading enzymes, antibiotics (Smith *et al.,* 1991) [2]. Biological control using species of *Trichoderma* has been reported as effective in reducing sclerotial viability and controlling damping-off of beans caused by *S. rolfsii* in the greenhouse (Henis, 1984) [6]. However, the importance of the antagonistic effect of *Trichoderma* depends on several factors, such as the physicochemical properties and microflora composition of the soil, and the competitive ability of the antagonists. Many reports indicated that the application of *T. viride* and *T. harzianum* Rafai were found to be highly antagonistic to *S. rolfsii* and successful management of diseases in vegetables and legumes (Kamala *et al*., 2012) [12]. Due to the knowledge of their potentials, several *Trichoderma* based several commercial products are manufactured and marketed in Asia, Europe and USA worldwide for the management of plant diseases (Herman *et al*., 2006) [5]. Biological control of soilborne plant pathogens can be achieved successfully by seed coating, furrow application and root dip of seedlings with Antagonists. Many researchers have demonstrated that the potential of *Trichoderma* sp. in controlling wilt and damping-off diseases of crop plants caused by *Fusarium* sp. and *Rhizoctonia solani* (Kamala *et al*., 2012) [9].

Sclerotium rolfsii Sacc. and *Rhizoctonia solani* are important soilborne phytopathogens which are very common in tropical, subtropical and temperate regions of the world. Both the phytopathogens are survives in the form of vegetative mycelium and/or sclerotia and causes several diseases in crop plants and infected more than 500 species of cultivated and wild plants (Maurya *et al.,* 2008 and Yaqub *et al.,* 2011) [18]. Keeping these views in mind, the experiments were designed to find out the potential isolates of *Trichoderma harzianum* for the management of potent sclerotial fungi viz., *S. rolfsii* in the garden soil of litchi and mango orchard.

Materials and Methods

Isolation of the pathogen

Collar rot infected plants of gram (*Cicer arietinum* L.) were collected from research plots of Tirhut college of Agriculture Dholi, Muzaffarpur in poly bags and brought to laboratory. Affected tissues from the collar region of plants were cut into small bits and washed thoroughly in sterilized water to remove dirts. These were then dipped in 0.1% mercuric chloride solution for 30 seconds, washed in two changes of sterile water, transferred on Potato Dextrose Agar (PDA) medium for hyphal growth in culture tube. After 48 hours of incubation in a B.O.D. incubator, tips of the hyphal growth was taken with inoculating needle and transferred to fresh PDA slants. Pure culture of the fungus was obtained by several transfers of tips of hyphal growth.

Collection of Soil Sample

Soil samples were collected from 4 locations i.e. garden soil of litchi and mango orchard of Krishi Vigyan Kendra Birauli, Samastipur and RAU, Pusa (Samsatipur) to find out the total microbial population of soil. Separate soil samples were taken from each plot.

Soil Analysis

Soil analysis was done in the Laboratory of Department of Soil Science, Rajendra Agricultural University, Pusa, Samastipur. The soil samples were analysed for texture and organic carbon by Rapid Feel Method (Prasad *et al.* 2006) [13] and Walkley's and Black's method (1934) [17].

Isolation of Soil Micro Flora

The soil samples collected from specified areas were subjected to serial dilution for isolation of total fungi. Ten grams of soil from each sample was transferred aseptically to 250 ml conical flask containing 90 ml sterile distilled water separately and mixed thoroughly by shaking horizontally. One ml sample was drawn from the suspension and transferred to 9 ml of distilled water to obtain 10^{-2} dilution. Again the process was repeated for making 10^{-3} dilution. One ml of suspension from 10^{-3} dilution of each soil sample was poured through pipette aseptically into sterile Petri plate. Twenty ml of molten peptone dextrose rose Bengal agar medium cooled to <45°C was poured in each Petri plate and gently rotated horizontally to get uniform mixing of soil suspension. The plates were then incubated in a BOD incubator at 28 ± 1°C. Fungal counts were assessed on 3rd day and converted as colony forming units (cfu) per gram of soil.

Quantitative Estimation of Fungal Population

Number of colonies that appeared on plate were counted and multiplied by dilution factor to determine fungal population as colony forming unit (cfu/g of soil).

Identification of soil micro flora

Most of the fungi that appeared on medium were identified based on morphological and cultural characters. Two methods were adopted. i) Visual observations for colony characters on medium and ii) by preparing slides using lactophenol or cotton blue as a mounting medium and observation under compound Olympous microscope. Identification was done based on morphological characters of vegetative and asexual reproductive structures of fungus reported in literature.

Percent distribution of dominating fungi and *Trichoderma* isolates

The number of colonies per plate that appeared from 10^3 dilution of soil was calculated. The percentage contribution of each isolate was done by using following formula (Mahalingam *et al.,* 2012) [11].

$$\text{Percent distribution} = \frac{\text{Colony of particular fungus (CFU) in a sample}}{\text{Total fungal colonies in a sample}} \times 100$$

Antagonism

The antagonistic activity of *Trichoderma harzianum* against *Sclerotium rolfsii*, was studied by dual culture technique (Morton and Stroube, 1955) [12]. The fungus was cultured on PDA plates for 72 hours. A 6 mm disc of *Trichoderma* was inoculated on the PDA medium on one side of Petri plate aseptically and 6 mm disc of *S. rolfsii* was inoculated at the opposite side in the same plate in such a way that the discs were 4 cm apart from each other. The plates were then incubated at 28 °C for 4 days. Antagonism was apparent when sporulating *Trichoderma* started over growing on the pathogen colony which was noted at 24 hours interval. Based on observation, percentage inhibition was calculated by the following formula.

$$\text{Percentage Inhibition (PI)} = \frac{C-T}{C} \times 100$$

C = Radial growth of pathogen in control plate.
T = Radial growth of pathogen in dual culture.

Viability studies of mycelium and sclerotia of *Sclerotium rolfsii*

To study the viability of test fungus, 6 mm of mycelium disc cut by the cork borer from the zone where the test fungus was already overgrown or lysed by the antagonist was transformed centrally into sterilized petri plates containing PDA. For viability study of sclerotia, the sclerotia formed in antagonized plates were picked up with sterilized forcep, washed in distilled water in several changes of water and then transferred to fresh PDA plates. Sclerotia produced in pure culture of *S. rolfsii* were used as control.

To study the growth of individual organism, the test organism and antagonist were inoculated centrally in sterilized plates containing plates containing PDA. These plates were incubated in B.O.D. incubator at 28 $\pm$ 1^0c. Colony diameter was measured at every 24 hours interval till the fungus occupied full space of plate. The isolate found superior to others in respect of growth and antagonism was selected for further detailed investigation.

Screening of the antagonists

A dual culture technique developed by Morton and Stroube (1955) [12] was used for screening of antagonists against

Sclerotium rolfsii. Twenty ml of sterilized and melted PDA medium was poured into 90 mm sterilized petri plates, allowed to solidify and then inoculated with 6 mm disc of *S. rolfsii* (test fungus) and the antagonist taken from the margin of 4 days old colony and placed towards the edge of the plate approximately 4 cm away from each other and incubated at 28 $\pm$ 1^0c in a B.O.D. incubator. Three replicates were maintained for each treatment. Observation on radial growth was recorded after every 24 hours till the colony of test fungus was completely overgrown by the antagonist.

Mycoparasitism

Mycoparasitism between *Trichoderma harzianum* (the most effective antagonist selected after screening) and the *S. rolfsii* (test pathogen) was studied on PDA. Twenty ml of sterilized melted PDA was poured into 90 mm sterilized petri plate. Six millimeter discs of pathogen and the antagonist cut with the help of sterilized cork borer from 4 days old colonies were placed on solidified PDA approximately 4 cm apart from each other towards the edge of plates. All the petri plates were incubated at 28 $\pm$ 1^0C in a B.O.D. incubator. Observation on radial growth was recorded at every 24 hours interval. From the area of interaction of the two fungi *S. rolfsii* and *T. harzianum* in dual culture, small mycelia fragments of hyphae were taken out on the glass slides with the help of sterilized needle at every 24 hr interval till 96 hr. The hyphal fragments were then stained with cotton blue and mounted in lactophenol. The slides were then examined in Olympus microscope at a magnification of x 400 for hyphal interaction between two organisms.

Results and Discussion

The soil samples collected from mango and litchi orchards of RAU Pusa and KVK, Birauli were analyzed for texture, pH and organic carbon content. The texture of soil samples collected from RAU, Pusa and KVK Birauli was sandy loam except the mango orchard of KVK, Birauli which was silt loam. The pH value of the soils of litchi orchards was 6.4 and 6.6 while the soli of mango orchards were 7.0 and 7.1 at Pusa and Birauli respectively. The soil pH of litchi orchard was in general lower than the soil pH of mango orchard. The organic carbon content in different soil samples ranged from 0.54 to 0.86 which may be considered ideal (Table 1).

Table 1: Soil texture, pH and organic carbon content in soil samples of mango and litchi orchards of RAU, Pusa and KVK Birauli, Samastipur

Location	Soil sample	Soil texture	pH	Organic carbon (%)
RAU, Pusa	Mango orchard	Sandy loam	7.0	0.86
	Litchi orchard	Silt loam	6.4	0.58
KVK, Birauli	Mango orchard	Sandy loam	7.1	0.85
	Litchi orchard	Sandy loam	6.6	0.54

Enumeration of total fungal population in soil

Enumeration of fungal population in soils of mango and litchi orchard was studied on Peptone dextrose rose bengal agar medium using dilution plate technique. The population of total fungi in different soil samples of mango and litchi orchard of Pusa and Birauli ranged from 9.00 $X10^3$cfu/g of soil to 9.66 $X10^3$cfu/g of soil. The population of total fungi in different soil samples did not differ significantly. In soil samples of mango orchard of KVK, Birauli, population of *Trichodrma* (2.60 cfu/g soil) was lower than *Fusarium* species (3.0 cfu/g soil) was observed as dominant fungus. On the other hand the soil samples of mango orchard of RAU, Pusa showed equal population *i.e* 3.6 cfu/g soil of *Trichodrma* and other fungi. In orchard of litchi of RAU, Pusa, *Aspergillus niger* was dominant fungus (3.00 cfu/g soil) but sufficient population of *Trichodrma* (3.00 cfu/g soil) was also observed. The results indicated that *Trichodrma* was available in all soils of mango and litchi both the orchards at both the locations with varying population. The population of *Trichoderma* dominated in soil of mango orchard of RAU, Pusa (Table 2).

Table 2: Enumeration of fungal population in different soil samples

Location	Soil sample	Population of soil micro flora $X10^3$cfu/g of soil*			Distribution (%)		Dominant fungal genera
		Total fungi	Dominating fungus	*Trichoderma* species	Dominating fungus	*Trichoderma* species	
KVK, Birauli	Mango orchard	9.60	3.00	2.60	31.00 (33.25)	27.40* (31.51)**	*Fusarium* sp.
	Litchi orchard	9.00	3.00	2.50	42.96 (40.93)	31.11 (33.88)	*Fusarium* sp.
RAU, Pusa	Mango orchard	9.00	3.60	3.60	40.73 (39.60)	40.73 (39.60)	*Trichoderma* sp.
	Litchi orchard	9.60	3.30	3.00	34.44 (35.89)	31.11 (33.88)	*Aspergillus niger*
	CD at 5%	0.94	0.70	0.76	4.90	4.60	-
	CV	5.30	11.60	17.4	6.90	6.93	-

*Mean of 03 replications.

**Values given in parentheses are Arcsin $\sqrt{}$ transformaion

Microorganism in soil are critical to the maintenance of soil function in both natural and managed agricultural soil because of their involvement in such a process like soil structure, formation, decomposition of organic matter, cycling of carbon, nitrogen, phosphorous and sulphur. In the present study fungal flora were isolated from soil samples of mango and litchi orchard of KVK Birauli and RAU Pusa. Population and distribution of soil were studied with special focus on bioagent, *Trichoderma* species. The soil texture, pH and organic carbon content was also determined. In another experiment the total population of soil micro flora ($9x10^3$ cfu/g of soil) was recorded in litchi orchard (KVK Birauli) and Mango orchard (RAU Pusa, Samastipur). During investigation, it was observed that *Trichoderma* sp. was present in four soil samples taken from mango and litchi orchard of KVK Birauli and RAU Pusa, Samastipur. Regarding distribution of *Trichoderma* maximum population was recorded at low pH. Soil acidity had been reported as a

factor affecting the presence and density of *Trichoderma* species. Soil texture of mango orchard was sandy loam which may be one of the factors that is responsible for *Trichoderma* distribution. Several researchers had worked on the soil texture and found that clay loam soil favoured more fungal occurrence than other soil. Lowler *et al.* (2012) [10] reported that agricultural management practices can affect soil chemical properties, number and activities of numerous microorganisms. Higher population of *Trichoderma* was recorded from the soil of sugarcane plot B2 which had slightly acidic pH. *Trichoderma* has been found to adapt an acidic habitat (Rahman *et al*., 2011).

Identification of *Trichoderma*

Five isolates of *Trichoderma* were observed in isolation of total fungi from soils of mango and litchi orchards of RAU, Pusa and KVK, Birauli. These were purified on PDA. The microscopic slides using small bit of fungal culture in cotton blue was prepared and observed in compound microscope at ×400 for studying the morphological characters. The observations given in table 3 with the cultural and morphological characters match with of *Trichoderma harzianum* reported in the literature. Hence, all the 5 cultures were identified as isolates of *Trichoderma harzianum.*

Table 3: Screening of *Trichoderma harzianum* isolates for antagonism against *S. rolfsii*

***Trichoderma* isolates.**	**Radial growth of *S. rolfsii* (mm)**	**Inhibition of growth of *S. rolfsii* (%)**
	96hr	**96hr**
*T. harzianum*1	26.9* (15.44) **	70.11* (44.50) **
T. harzianum 2	27.23 (15.93)	75.41 (48.92)
T. harzianum 3	30.17 (17.55)	66.48 (41.65)
T. harzianum 4	22.13 (12.78)	79.74 (52.86)
T. harzianum 5	22.50 (13.00)	75.0 (48.57)
Control (*S. rolfsii*)	90.00 (64.13)	0.00
SE(m)	0.31	0.32
C.D at 5%	1.12	1.01
C.V	1.96	2.12

*Mean of 03 replications.

**Values given in parentheses are Arcsin √¯transformaion

Screening of *T. harzianum* isolates against *S. rolfsii*

Five isolates of T*richoderma harzianum* (Th1, Th2, Th3, Th4 and Th5) were screened for their antagonistic potential against *S. rolfsii* by dual culture technique on PDA. The colony of the antagonist and the pathogen grew on PDA until they met each other. Thereafter, the growth of *S. rolfsii* ceased and the colony of *T. harzianum* continued to caused lysis of growth of *S. rolfsii.* earlier advance around *S. rolfsii. T. harzianum* 4 occupied the vacant surface of PDA first and then gradually started spreading around the host fungus and *T. harzianum* isolate 4 proved superior in antagonism over other antagonist isolates as it occupied more space around colony of *S. rolfsii* than rest of the isolates (Table 3). It is evident from the table 4 that *T. harzianum* isolate 4 caused maximum inhibition of 79.74 per cent growth of *S. rolfsii* at 96 hours of incubation in dual culture followed by *T. harzianum* 2 and *T. harzianum* 5 which caused 75.41 and 75.0 per cent inhibition of radial growth of *S. rolfsii* respectively and were at par from each other. Minimum inhibition of growth of *S. rolfsii* (66.48%) was noted in *T. harzianum* 3(Table 3). Thus, the study indicated the superiority of *T. harzianum* isolate 4 in mycoparasitism over *T. harzianum* 1, *T. harzianum* 2, *T. harzianum* 3 and *T. harzianum* 5 isolates. Hence, the *T. harzianum* isolate 4 was selected for further studies.

Viability test of mycelium of *S. rolfsii* in dual culture

Five isolates of *T. harzianum* were used to test the viability of mycelium of *S. rolfsii* in dual culture plate. A disc of 6mm of *S. rolfsii* from each isolate of *T. harzianum* was taken out from the completely lysed area of dual culture plate and kept on PDA in sterilized plates and allowed for the growth of *S. rolfsii*. There was no growth of *S. rolfsii* in any of the plate, instead all the plates showed full growth of different isolates of *T. harzianum in vitro*. This study indicated that mycelium of *S. rolfsii* lysed after mycoparasitism by *T. harzianum* (Table 4).

Formation of sclerotia by *S. rolfsii* in dual culture

Formation of sclerotia by *S. rolfsii* in dual culture with different isolates of *T. harzianum* was studied. Final observation on sclerotia formation was recorded on 15 days old culture plates. After 15 days of inoculation, number of sclerotia formed by *S. rolfsii* was counted in dual culture of *T. harzianum* as well as monoculture of *S. rolfsii*. The results indicated over all significant reduction in formation of sclerotia by *S. rolfsii* in dual culture when compared with control (Table 4).The observation revealed that only 58.67 sclerotia were formed in dual culture with *T. harzianum* isolate 4 as compared to 220 sclerotia in control which in terms of inhibition of sclerotia formation comes to 73.70 cent followed by *T. harzianum* isolate 3 in which sclerotia formation was restricted to 60.15 per cent (Table 4). Minimum number of sclerotia was formed in *S. rolfsii* grown in dual culture with *T. harzianum* isolate 1.

Table 4: Viability of mycelium, formation and viability of sclerotia of *S rolfsii* in dual culture with *T. harzianum* isolates

	Viability of mycelium		**Formation of sclerotia**		**Viability of sclerotia**	
***T. harzianum* isolates**	**Growth of *S. rolfsii* (mm)**	**Growth of *T. harzianum* (mm)**	**No. of sclerotia formed in 15 days.**		**Growth of *S. rolfsii* (mm) for sclerotium.**	**Growth of *T. harzianum* (mm)**
	96 hr	**96 hr**	**Total sclerotia produced**	**Inhibition in formation of sclerotia with *T. harzianum* in dual culture (%)**	**96 hr**	**96 hr**
T. harzianum 1	0	87.90* (61.50)**	121.00	44.55* (26.44)**	0	87.9* (61.50)**
T. harzianum 2	0	90.00 (64.13)	92.00	58.18 (35.56)	0	90 (64.13)
T. harzianum 3	0	90.00 (64.13)	87.67	60.15 (36.96)	0	90 (64.13)
T. harzianum 4	0	90.00 (64.13)	58.67	73.30 (47.12)	0	90 (64.13)
T. harzianum 5	0	90.00 (64.13)	118.00	46.21 (27.51)	0	90 (64.13)
Control.	90 (64.13)	90.00 (64.13)	220.00	0.00	79.74 (52.86)	90 (64.13)
SME	0.12	0.45		0.12	0.12	0.45
C.D at 5%	0.19	1.40		0.40	0.19	1.40
C.V	1.49	1.22		0.77	1.49	1.22

*Mean of 03 replications.

**Values given in parentheses are Arcsin √¯transformaion

Viability test of sclerotia of *S. rolfsii* in dual culture

Viability of sclerotia of *S. rolfsii* formed in dual culture plate with all the five isolates of *T. harzianum* was studied. The sclerotia formed in dual culture of each isolate of *T. harzianum* was picked up with sterilized forcep and placed centrally on PDA and observations were recorded after 96 hours of inoculation. Neither germination of sclerotia nor growth from sclerotium into mycelium was observed. Instead, the growth of *T. harzianum* was noted in all plates (Table 4). This indicated that sclerotia were not viable in antagonized plates.

Mycoparasitism of *Trichoderma harzianum* 4 on *S. rolfsii*

Critical microscopic examination of slides prepared from the zone of interaction between *Trichoderma harzianum* and *S. rolfsii* revealed that the principal mechanism of mycoparasitism was coilling of antagonist hyphae around the hyphae of *S. rolfsii*, disorganization of protoplasmic content upon coiling and finally lysis of host hyphae. In the present investigation *T. harzianum* in dual culture with *S. rolfsii* ceased growth of host fungus upon contact on PDA. Microscopic examination of slides prepared from the zone of interaction of two fungi revealed that hyphae of *T. harzianum* coiled around the hyphae of *S. rolfsii* and disorganized/ digested the protoplasmic contents. Similar observations have been reported for *Trichoderma harzianum* and *Sclerotiniia sclerotiorum* interaction by Inbar *et al.* (1995) [8]. The phenomenon of physical contact followed by the disorganization of the host cells and concomitant changes in the cells of the parasite have been unequivocally established for *Gliocladium* (Huang, 1987) [7] and *Trichoderma* (Chet, 1987) [1]. In Present study there was reduction in formation of sclerotia of *S. rolfsii* in dual culture as compared with control and maximum per cent inhibition of sclerotia formation was recorded in *T. harzianum* isolate 4. Tiramsetti and Adiver (2012) [15] reported maximum inhibition of sclerotial germination with *Trichoderma viride* (63.33%) compared to *T. harzianum* (55.65%). Similarly *Pseudomonas flourescens* inhibited maximum germination (90%) than *Bacillus subtilis* (81.11%) different ages of sclerotia.

Screening of antagonists

Before proceeding for biological control of plant pathogen (s), the first step is to choose a potential antagonist against the target pathogen(s). If there is availability of more than one antagonist, it is always desired to screen them against the test pathogen (s) and select the most potential one. Screening can be done both *in vitro* and *in vitro*. The important characters for which the antagonists should be screened are its high antagonistic potential, radial growth *in vitro* high survival ability, insensitivity to commonly used fungicides and of course, high disease control potential. During the present investigation, three important characters were choosen to screen the antagonists (i) antagonistic potential in dual culture (ii) radial growth in monoculture and (iii) disease control potential. During the present investigation five isolates of *T. harzianum* viz., Th1, Th2, Th3, Th4 and Th5 were evaluated for the above mentioned characters. *T. harzianum* isolate 4 showed best performance in dual culture and mycoparasitized the colony of *S. rolfsii* earliest i.e. in 120 hours. Other isolates of *T. harzianum* could partially check the test pathogen in the above span of time. Hence *T. harzianum* isolate 4 was selected for further studies. On the other hand Virupaksha Prabhu *et al*. (1997) [16] while working with collar rot of cotton caused by *S. rolfsii* reported maximum inhibition zone with *T. harzianum* and *T. viride*. This might be due to the production of antibiotics, which diffused air filled pores and become detrimental to the growth of *S. rolfsii* (Dange, 2006) [3].

Conclusion

The soil samples for isolation of *Trichoderma* were collected from mango and litchi orchards of RAU, Pusa and KVK, Birauli. The soil textures were sandy loam and silty loam. pH of soil samples ranged from 6.4 to 7.1 The organic contents of litchi soils were lower than mango orchards. Isolation of micro flora from the soil of litchi and mango orchard revealed the presence of sufficient population of *Trichoderma* in orchard soils of mango and litchi of RAU, Pusa and KVK, Birauli both the locations. During the isolation of total fungal flora of soil, 5 isolates of *Trichoderma* were brought in pure culture and they were identified as *Trichoderma harzianum* based on cultural characters and microscopic observation on Conidiophore, Phialides and Phialospores. In dual culture of *T. harzianum* and *S. rolfsii*, the principal mechanism of antagonism observed was coiling of antagonist hyphae around the hyphae of *S. rolfsii*, disorganization of protoplasmic content of hyphae and its lysis due to enzymatic action or toxin production by *T. harzianum.* Formation of sclerotia by *S. rolfsii* was also restricted due to presence of *T. harzianum in vitro.* Viability studies on mycelium and sclerotia taken from the zone where *S. rolfsii* was completely antagonized by *T. harzianum* by transferring on fresh PDA revealed no mycelial growth or sclerotial germination. This suggested the loss in viability of *S. rolfsii* due to action of *T. harzianum in vitro.* These five isolates of *Trichoderma harzianum* were screened against *S. rolfsii* for their antagonistic potential against *S. rolfsii* on PDA by dual culture technique. *T. harzianum* isolate 4 proved superior over other 04 isolates of *T. harzianum* in respect of growth rate and antagonism.

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