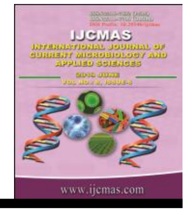
Int.J.Curr.Microbiol.App.Sci (2019) *8*(6): 715-721

EXCELLENT PUBLISHERS

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 06 (2019)**
Journal homepage: http://www.ijcmas.com



Original Research Article https://doi.org/10.20546/ijcmas.2019.806.084

Isolation and Evaluation of Potent Bio-Control Agent against *Fusarium oxysporum* f.sp. *lentis, Sclorotium rolfsii* and *Sclorotinia sclerotiorum* causing Soil Borne Disease in Lentil (*Lens culinaris* Medik)

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Keywords

Fusarium, Sclerotium, Sclerotinia, Lentil, Biocontrol, *Trichoderma* and *Pseudomonas fluorescens*

Article Info

Accepted:
07 May 2019
Available Online:
10 June 2019

A B S T R A C T

Lentil (*Lens culinaris* Medik) is an important crop, the major states producing lentil are Madhya Pradesh, Uttar Pradesh, Bihar and Punjab. Lentil is an important component of farming systems in our country. It is one of the important and most nutritious *rabi* pulses. Vascular wilt causing by *Fusarium oxysporum* f.sp. *lentis, Sclerotium rolfsii Sacc. causing collar rot and Sclerotinia sclerotiorum (Lib.) de Bary) causing stem rot,* is most destructive soil borne diseases of lentil growing area worldwide. Use of chemicals continues to be major strategy to mitigate the menace of crop disease. However, because of the environmental concerns and other hazards associated with use of chemicals, use of biocontol agents is gaining importance. In the present investigation the isolation of potent bio- control and evaluated their antagonistic efficacy against *Fusarium oxysporoum* f.sp. *lentis*, *Sclerotium rolfsii Sacc* and *Sclerotinia sclerotiorum (Lib.) de Bary in vitro.* Effect of selected bio-control agent on percent inhibition of pathogen was recorded. All the isolated *Trichoderma* spp isolate. *Pseudomonas fluorescens* isolate and *Bacillus subtilis* were inhibit the growth of pathogen *in vitro*. It was observed that maximum percent inhibition recorded *Trichoderma spp.* isolates 7 (77.50 %) followed by (75.00%) *Pseudomonas fluorescens* Pf 008 and *Trichoderma spp.* isolate 6 (75.00%) against *Fusarium oxysporoum* f.sp. *lentis*, where, in case of *Sclerotium rolfsii* Sacc, maximum percent inhibition was recorded in *Trichodema* spp. isolate TS001 (74.22%), followed by *Trichodema* spp. isolate 7 (62.22%), while, *Sclerotinia sclerotiorum* (Lib.) de Bary), maximum percent inhibition was recorded in *Pseudomonas fluorescens* Pf 008 (95.50%), followed by *Trichoderma spp.* isolates 7 (75.55 %). This indicates above bio-control have potential and important role in biologically based strategy for management of soil borne diseases in lentil and enhance the plant growth and yield.

Introduction

Lentil (*Lens culinaris* L.) is an important legume crop. It belongs to the family "Leguminoceae" sub family "Papilionaceae". It is one of the most important multipurpose pulse crops. It is native plant of Southwest Asia (Turkey-Cyprus). This crop is a global importance and grown particularly in India, Pakistan, Bangladesh, Nepal, Iran. Lentil is an annual, bushy herb, erect or sub-erect, 15-75 cm tall, highly branched, softly hairy, stems slender, square and ribbed. It is adapted to cool temperate steppe through sub-tropical dry to moist ambience. It does not tolerate water logging. It is able to withstand 4-12 week drought. Lentils are usually sown in areas where temperature ranges 20-30 ^{o}C. The lentil production during year 2016- 17 was 6.71 million tones with 5.45 million hectares of area and productivity was 1105 kg/ha while in India, total area under lentil crop is about 1.276 million hectares, with production 0.976 million tones which contributes 6.18% share of total pulse production and productivity 764.9 kg/ha (Sources: FAOSTATS, 2016-17). Uttar Pradesh accounts for the maximum production in the country contributing to around 45% of the country's production as well as forth maximum area under lentil cultivation. In Uttar Pradesh, total area under lentil crop is about 0.44 mha. With production 0.24 mt. and productivity 537 kg/ha (Sources: Agriculture statistics & amp; crop insurance, 2014 – 2015). The lentil production is greatly affected by many biotic and abiotic factors, among which diseases are the main constraint in the lentil production. The Lentil crop is affected by various disease Among all the fungal diseases e.g. Vascular wilt (*Fusarium oxysporum* f.sp. *lentis*) Sclerotinia stem rot (*Sclerotinia sclerotiorum* (Lib.) de Bary) collar rot (*Sclerotium rolfsii* Sacc.), is most destructive soil borne diseases of lentil growing area of worldwide.

Vascular wilt (*Fusarium oxysporum* f.sp. *lentis*: Fol) plays a major role in reducing lentil yield (Pouralibaba *et al.,* 2015), and causes severe damage to leaves, stems, roots and pods (Singh, 2015). This pathogen can cause infection at all stages of plant growth with more incidences at flowering and podding stages than early vegetative stage (Chavdarov, 2006). Fusarium wilt, which is a vascular fungal disease, is the most devastating of all lentil diseases worldwide that can cause extensive yield losses reaching up to 100% in prolonged favorable environments (Kumar *et al.,* 2010). Collar rot disease caused by *Sclerotium rolfsii* on lentil crop is a non specialized soil borne fungal pathogen of worldwide importance and has a host range of over 500 species (Punja and Grogan, 1988). The pathogenic fungus is soil-borne in nature and produces sclerotia, which can survive in the soil for many years. Infected young seedlings show damping-off symptoms. Plants infected at an advanced stage gradually turn pale, droop and dry (Njambere and Chen, 2011). *Sclerotinia sclerotiorum* (Lib.) de Bary is the causal agent of sclerotinia stem rot in lentil, leading to serious losses in yield due to lodging and premature shattering of seedpods (Gugel and Morrall, 1986). It results in damage of the plant tissue, followed by cell death and soft rot or white mould of the crop. The stem rot fungus overwinters as sclerotia in the soil, in stubble at the soil surface and mixed with seed. Sclerotia can remain viable in the field for five years or more. Use of chemicals continues to be major strategy to mitigate the menace of crop disease. However, because of the environmental concerns and other hazards associated with use of chemicals, use of biocontol agents is gaining importance. So, the present study was undertaken to find out the potential biocontrol agent for the management of soil borne diseases.

Materials and Methods

An experiment was conducted to evaluate different isolated bio-control agent percent inhibition of *Fusarium oxysporoum* f.sp. *lentis, Sclerotium rolfsii* Sacc and *Sclerotinia sclerotiorum* (Lib.) de Bary) ***in vitro.*** The experiment was conducted at Centre of Excellence for Sanitary and Phytosanitary

(SPS), Department of Plant Pathology of Sardar Vallabhbhai Patel University of Agriculture and Technology Modipuram, Meerut, U.P.

Collection of samples

Soil samples were collected from different locations of university as well as KVKs, research centers and University jurisdiction including Hastinapur sanctuary, Meerut. Several samples randomly were taken from the localities using an opened soil borer (20 cm in depth, 2.5 cm in diameter) as described by Lee and Hwang. Samples were air-dried at room temperature for 7-10 days and then were passed through a 0.8 mm mesh sieve and preserved in polyethylene bags at room temperature before use.

Isolation, purification and identification of antagonistic microorganisms

Isolation of microorganisms having bio control potential was done from serial dilutions technique. 10 gm of this soil was dissolved in 100 ml of sterile distilled water to get 10^1 dilution. From this 1 ml of soil suspension was taken and added to 9 ml of sterile distilled water to get 10^2 dilution. This is repeated until a final dilution of 10^7 was obtained. Antagonistic microorganisms were isolated on Potato Dextrose Agar medium and Nutrient Agar medium by using a dilution of 10^2 to10^4 for fungal and 10^5 to10^7 for bacterial. Then 1 ml of each soil suspension was taken and poured in sterilized petriplates, containing medium. Plates were rotated gently to get uniform distribution of soil suspension into the medium. Then the plates were incubated at 24±2°C and observed at frequent intervals for the development of colonies. 3-day-old colonies of mycoflora were picked up and purified by single hyphal tip method and streaking for bacterial colonies. Mycoflora were identified based on mycological keys described by Barnett and Hunter. Identification of bacterial microorganisms was identified based on Bergey's manual.

Isolation and purification of the pathogen

The diseased plant showing the symptoms were washed thoroughly with tap water, small pieces from infected parts 1–2 mm dimension from the advancing margin of the spot, adjacent to healthy portions were cut with the help of sterilized blade. These pieces were surface sterilized with 1 percent sodium hypochlorite solution for 30 seconds and finally washed well in three changes of sterilized distilled water to remove trace of sodium hypochlorite.

The pieces were then transferred aseptically to Petri plates containing Potato Dextrose Agar. Inoculated Petri plates were incubated at 26 ±1^{o}C for three to five days and examined at frequently intervals to see the growth of the fungus developing from different pieces. After fragments of hyphal growth from the growing tips were transferred to fresh PDA slants. Pure culture was made, following repeated hyphal tip transfer.

Antagonistic activities of bio control agent were tested against soil borne plant pathogen *F. oxysporum* f.sp. *lentis, Sclorotium rolfsii* and *Sclorotinia sclerotiorum* by employing dual culture techniques of Morton and Stroube (1955) on PDA.

Dual culture technique

A mycelial disc (5 mm.), obtained from the peripheral region of 5-7 day old culture of pathogen on PDA, was placed on fresh PDA plate (3 cm from centre) then a 5 mm mycelial disc, obtained from the periphery of a 5-7 day old culture of fungal bio agents were placed 3 cm away from the inoculum of the pathogen, for bacterial bio agents were streaked 3 cm away from the inoculum of the pathogen. Three replication of each treatment were maintained with one control set without inoculating the bio inoculants.

Then the plates were incubated at 26±1^{0}C, the measurements were taken after 7 days. At the end of incubation period, radial growth was measured. Radial growth reduction was calculated in relation to growth of the control as follows:

$$\% \text{ inhibition of mycelial growth} = \frac{C - T}{C} \times 100$$

Where C is the radial growth measurement of the pathogen in control and T is radial growth of the pathogen in presence of the bio-agent.

Results and Discussion

In the present investigation evaluated antagonistic effect of isolated potent biocontrol agent against soil borne plant pathogen *F. oxysporum* f.sp. *lentis, Sclorotium rolfsii* and *Sclorotinia sclerotiorum* by employing dual culture techniques *in vitro.*

Antagonistic effect of biocontrol agents against *F. oxysporum* f.sp. *lentis*

The results from the (Table 1) indicated that, all tested bioagents inhibited the growth of *F. oxysporum* f.sp. *lentis.*

Among the maximum inhibition per cent (77.50%) of *F. oxysporum* f.sp. *lentis* was recorded with *Trichoderm*a spp. isolate TS007 after 144 hours, which is superior from all the tested isolates followed by (75.00%) *Pseudomonas fluorescence* Pf008 and *Trichoderm*a spp. isolate TS006 both, (74.25%) *Trichoderm*a spp. isolate TS004. While least mycelial growth inhibition was recorded with *Bacillus spp.* B005 (56.00%).

Antagonistic effect of biocontrol agents against *Sclorotium rolfsii*

The observations recorded (Table 1) revealed that all tested bioagents isolates inhibited the growth of *Sclorotium rolfsii*. *Trichoderm*a spp. isolate TS007 showed maximum inhibition (74.22%) followed by *Trichoderm*a spp. isolate TS007 (62.22%) whereas, *Pseudomonas fluorescence* Pf008 (60.68%) While least mycelial growth inhibition was recorded *Trichoderm*a spp. isolate TS004 (28.22%).

Antagonistic effect of biocontrol agents against *Sclorotinia sclerotiorum*

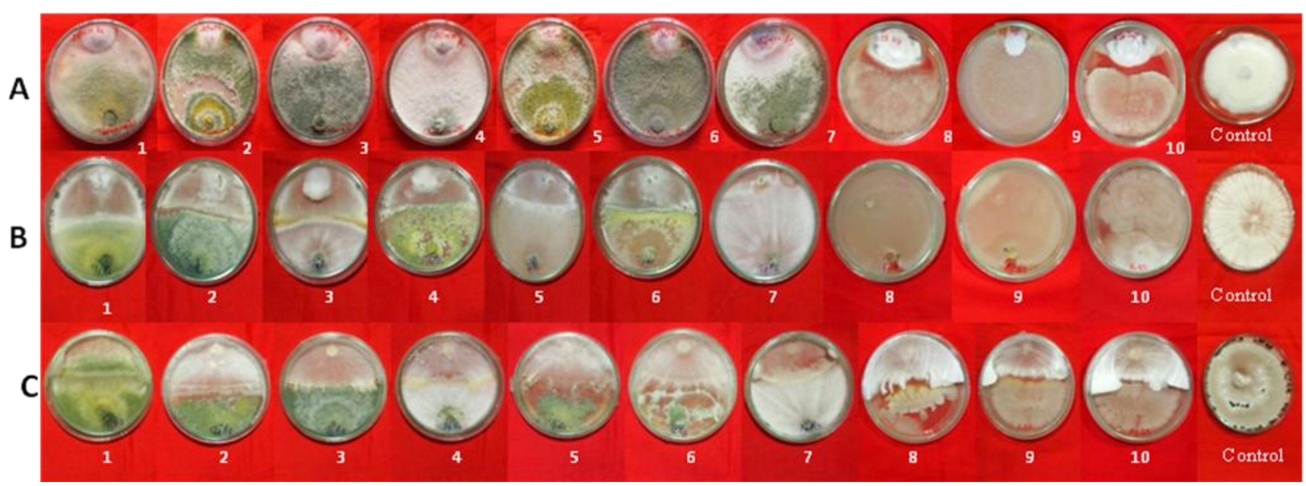
The results from the (Table 1) reveled that, all tested bioagents inhibited the growth of *Sclorotinia sclerotiorum*. The maximum percent inhibition was observed in *Pseudomonas fluorescence* Pf008 (95.55%) followed by *Pseudomonas fluorescence* Pf024 (88.88%), whereas, in case of *Trichoderm*a spp. isolate TS007 inhibited the mycelia growth (75.55%). While least mycelial growth inhibition was recorded *Trichoderm*a spp. isolate TS001 (52.00%).

Biological control is the best alternative, especially against soil borne pathogens such as *Fusarium* sp. *Sclorotium rolfsii* and *Sclorotinia sclerotiorum.* The limitations to biocontrol use are scarce knowledge on the ecology of rhizosphere and use of *in vitro* antagonism for selection of biocontrol agents. However, the advantages of using biocontrol include environmental friendly, cost and extent of protection (Gohel *et al.*, 2006). *Trichoderma* spp. that are common saprophytic fungi found in almost any soil and rhizosphere micro flora, have been investigated as potential biocontrol agents because of their ability to reduce the incidence of disease caused by plant pathogenic fungi, particularly many common soil borne pathogens (Papavizas, 1985; Sivan and Chet, 1986; Calvet *et al.*, 1990; Elad *et al.*, 1993; Spiegel and Chet, 1998; Freeman *et al.*, 2004; Ashrafizadeh *et al.*, 2005; Dubey *et al.*, 2007), although some have been occasionally recorded as plant pathogens (Menzies, 1993). Similar observations on inhibition of soil borne fungal pathogens by *T. harzianum. T. viride* and *T. virens* were made by Mukherjee and Tripathi (2000), Mathew and Gupta (1998). Upadhyay and Mukhopadhyay (1987) and Mukhopadhyay (1986) (Fig. 1).

Table.1 Efficacy of different locally bio inoculants against *F. oxysporum* f.sp. *lentis, Sclorotium rolfsii* and *Sclorotinia sclerotiorum*

Bio- Inoculants	***Fusarium oxysporum* f.sp. *lentis***		***Sclorotium rolfsii***		***Sclorotinia sclerotiorum***	
	Radial growth of pathogen (mm)	% inhibition	Radial growth of pathogen (mm)	% inhibition	Radial growth of pathogen (mm)	% inhibition
***Trichodema* spp. isolate TS001**	25.20	68.50	23.20	74.22	42.20	52.00
***Trichodema* spp. isolate TS002**	22.00	72.50	62.00	31.11	39.20	56.44
***Trichodema* spp. isolate TS003**	22.60	71.75	46.60	48.22	32.00	64.44
***Trichodema* spp. isolate TS004**	20.60	74.25	42.60	52.66	40.60	54.88
***Trichodema* spp. isolate TS005**	21.20	73.50	64.60	28.22	41.20	54.22
***Trichodema* spp. isolate TS006**	20.00	75.00	63.20	29.77	27.20	69.77
***Trichodema* spp. isolate TS007**	18.00	77.50	34.00	62.22	22.00	75.55
***Pseudomonas fluorescens* Pf 024**	26.60	66.75	51.20	43.11	10.00	88.88
***Pseudomonas fluorescens* Pf 008**	20.00	75.00	35.20	60.68	4.00	95.55
***Bacillus spp.* B005**	35.20	56.00	36.60	59.33	26.00	71.11
Control	80.00	-	90.00	-	90.00	-
C.D. $_{(0.05)}$	1.35	-	1.55	-	1.12	-

Fig.1 Row A- Antagonistic effect of isolated bioagents against *Fusarium oxysporum f.sp. lentis*, Row B- Antagonistic effect of isolated bioagents against *Sclorotium rolfsii*, Row C- Antagonistic effect of isolated bioagents against *Sclorotinia sclerotiorum*



The inhibitory effect of these biological control agents against soil borne fungal pathogens was probably due to competition, antibiosis and mycoparasitism (Papavizas, 1980; Cook and Baker. 1983).

In conclusion, in this work, the results of dual culture revealed the rapid colonization of the medium by *Trichoderma* isolates and antibiosis by *Pseudomonas fluorescence* and *Bacillus subtilis*. All *Trichoderma, Pseudomonas fluorescence* isolates and *Bacillus subtilis* evaluated were effective in controlling colony growth of the *F. oxysporum* f.sp. *lentis, Sclorotium rolfsii* and *Sclorotinia sclerotiorum*. The results reported here suggest that from the isolates of *Trichoderma, Pseudomonas fluorescence* and *Bacillus subtilis* used in this study were more capable of influencing the growth of all tested pathogens in dual culture under controlled condition, and may be used as broad spectrum biological control agents under field condition.

Acknowledgement

The authors are greatly acknowledged to the Council of Science and Technology, Uttar Pradesh (CST, U.P) Lucknow, for providing financial assistance to conduct this Research. The authors are also thankful to Chief Secretary and Director of Agriculture, Govt. of U.P. for providing financial assistance to establish research infrastructure under RKVY scheme in the Departments of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, U.P.

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How to cite this article:

Ramesh Singh Yadav and Amit Kumar Yadav. 2019. Isolation and Evaluation of Potent Bio-Control Agent against *Fusarium oxysporum* f.sp. *lentis*, *Sclorotium rolfsii* and *Sclorotinia sclerotiorum* causing Soil Borne Disease in Lentil (*Lens culinaris* Medik). *Int.J.Curr.Microbiol.App.Sci.* 8(06): 715-721. doi: https://doi.org/10.20546/ijcmas.2019.806.084