



RESEARCH ARTICLE

## Management of lettuce rot caused by *Fusarium oxysporum* f. sp. *lactucae* in hydroponically grown crop using microbial consortia

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**ABSTRACT:** Hydroponics or growing plants on mineral nutrient solutions without soil has many advantages, as the system has higher water and fertilizer use efficiency, and crop encounters very low pest and disease incidence which inevitably check the requirement of plant protection chemicals. However, recently few of the diseases caused by commonly observed pathogens in conventional soil based system have also been detected in hydroponic culture, bringing a serious risk to hydroponic crop production. The present investigation was made to assess the effectiveness of bio-intensive management of *Fusarium* rot of lettuce incited by *Fusarium oxysporum* f. sp. *lactucae* (FoL) under hydroponic culture tank system in Assam during 2014-2016. Three rhizosphere inhabiting microbial agents, viz., *Trichoderma harzianum*, *Bacillus thuringiensis* and *Pseudomonas fluorescens* were tested singly as well as in consortia against the pathogen. The microbial agent, *T. harzianum* exhibited comparatively high antagonistic activity showing highest suppression of FoL *in vitro*. In hydroponic tanks, *T. harzianum* applied as root application, showed lowest root infection (15.39%), leaf infection (18.09%) and overall lower disease incidence (17.29%) with highest crop growth attributes and yield. This was followed by treatment with consortia of *T. harzianum* and *B. thuringiensis*. The highest root infection (85.08%), leaf infection (70.02%), rot incidence (80.97%) and lowest yield (30.00 g/plant) have been recorded in untreated lettuce plants in control tanks.

**Keywords:** Antagonists, *Fusarium oxysporum* f. sp. *lactucae*, hydroponic, lettuce

In conventional agriculture, crops are grown in open soil with or without irrigation, application of concentrated fertilizers and agrochemicals which possess numbers of notable negative impacts. The major downside of conventional crop cultivation includes - large patch of land requirement, inefficient and high use of fresh water, pest and disease pressure, nutrients and toxic pesticides runoff, soil degradation accompanied by erosion and other associated detrimental impacts on the environment (Killebrew and Wolff, 2014). Hydroponics, on the other hand, is growing plants on mineral nutrient solutions without soil is becoming a popular technology for commercial food production. Unlike conventional agriculture, hydroponic agriculture system of food crop production has a better control of pest and disease factors which reduces the requirement of plant protection chemicals and produces higher yield mostly free from chemical residues (Libia *et al.*, 2012). This versatile crop cultivation system can range from rudimentary backyard setups to highly sophisticated commercial enterprises (Barbosa *et al.*, 2015). In a controlled environment, it can support continuous production throughout the year. Other benefits of hydroponic culture are short production period, relatively better product quality and sanitation practices (Khater and Ali, 2015). Presently, hydroponic agriculture system has been successfully employed to

grow various commercial and specialty crops including strawberries, tomatoes, peppers, leafy vegetables, eggplants, cucumbers and much more.

Lettuce (*Lactuca sativa*) the second most important salad vegetable crop after tomato is currently cultivated in the hydroponic system either with or without artificial medium. This leafy vegetable performs best in the nutrient film technique or NFT (Tognoni *et al.*, 2015). In conventional soil based system, lettuce crop is heavily affected by several diseases; among them, *Pythium* wilt and damping, *Fusarium* root rot, *Rhizoctonia* rot, lettuce drop (*Sclerotinia minor*) and bacterial soft rot (*Pectobacterium carotovorum*) are more common. Few of these diseases have also been detected in hydroponic culture systems, bringing a serious risk to hydroponic lettuce production (Jensen and Abad, 2009). The contamination of plant pathogens in hydroponic system can come from growing media, plant materials, insects, and through irrigation waters (Postma *et al.*, 2008). The colonization is often triggered by steady temperatures and moisture regimes and the common fact that root colonizers can easily spread through recirculating water (Rosberg, 2014). In hydroponic systems, fungal development resulted from conidia bound to the root surface, which is the only source of carbon to support the growth of the fungus in the mineral nutrient solution

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(Chantal, 2006). Though these can be prevented by conventional chemical based methods but nonchemical measures should be preferred over chemical methods in hydroponics due to environmental, food safety and health concerns. The biological control involving antagonists and botanicals has been suggested to be ideally suited for the closed structure of soilless systems. Biocontrol agents can easily be added to nutrient solutions in a hydroponic system and be dispersed. The antagonistic ability of *Trichoderma* against different formae speciales of *F. oxysporum* has been well documented. The present study has been made to determine the suitability of bio-intensive approach by use of microbial antagonists for sustainable management of *Fusarium* rot disease in lettuce crop under the hydroponic system.

## MATERIALS AND METHODS

The study was carried out in the laboratory and green shade net house of the Department of Plant Pathology, Assam Agricultural University, Jorhat, Assam, during 2014-2016.

### Hydroponic cultivation

Artificial hydroponic culture tanks (HCT) were made using plastics tubs (30 inch x 18 inch) to grow lettuce plant variety "Linda" following 'Static Solution Technique'. Floating styrofoam boards were used as growing tray to place the seedlings in the upper side of the HCT tank. Disposable plastic glasses (7.5 cm diam.) filled with sterilized coco peat were used as growing media and to keep the lettuce plants upright. Modifications were made in such way that 90 per cent of the disposal glass planted with lettuce plant remained inside the hydroponic solution. Artificial aqua tank air pumps were connected with the HCT tank for continuous aeration. As a source of micro and macro nutrients in required concentrations, artificial nutrient solution (Flora series, General Hydroponics, 32500 Fleurance, France) was used following manufacturer's guidelines. The nutrient rich HCT tank water was stirred at regular interval and the pH was adjusted to neutrality by adding dry pH down powder.

### Pathogen isolation

Infected lettuce plants showing typical symptoms of *Fusarium* rot were collected from the horticultural orchard, AAU, Jorhat for isolation of the pathogen (Cabral *et al.*, 2014). Potato dextrose agar (Himedia, Mumbai) supplemented with 20 mg of tetracycline was used as a basal medium. For purification of the fungus, hyphal tip culture technique was employed. The isolated pure culture of the pathogen was maintained on PDA at 8°C to conduct subsequent experiments. The pathogenicity test of the isolated *Fusarium* culture was done as per the technique described by Zhao *et al.* (2014).

### Microbial antagonists

Widely accepted three antagonists, *viz.*, *Trichoderma harzianum* (MTCC 7477) *Pseudomonas fluorescens*

(MTCC 25036), *Bacillus thuringiensis* (MTCC 25035) were collected from the Biocontrol Laboratory of the Department of Plant Pathology, AAU, Jorhat, Assam. These were transferred to fresh medium and preserved at 4°C for evaluation study of their bioactive potential against *Fusarium* rot pathogen. The cultures of the fungal pathogen as well of all three antagonists *T. harzianum*, *P. fluorescens* and *B. thuringiensis* were maintained throughout the period of study in PDA, Nutrient Agar and King's B (Himedia, Mumbai) media respectively, by routine sub-culturing at regular intervals and stored at 4°C.

### Evaluation of compatibility among different bioagents and development of microbial consortia

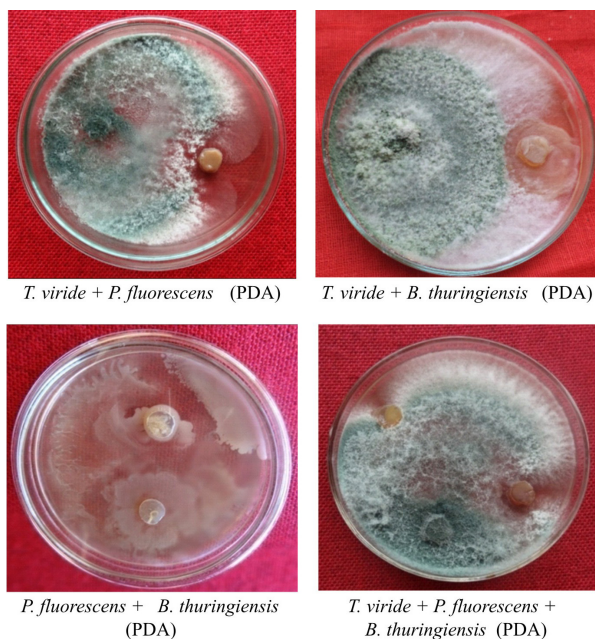
Compatibility among the microbial bioagents was tested *in vitro* adopting dual culture assay plate technique with minor modification (Rahman *et al.*, 2009) using PDA as basal media. The treatment combinations were: Growth of *T. harzianum* alone; *B. thuringiensis* alone; *P. fluorescens* alone; *T. harzianum* + *B. thuringiensis*; *T. harzianum* + *P. fluorescens*; *B. thuringiensis* + *P. fluorescens*; and *T. harzianum* + *B. thuringiensis* + *P. fluorescens*. The radial growths of each bioagent individually and in combinations were recorded upto 120 hrs of incubation.

### *In vitro* evaluation of antagonistic effect of different microbial bioagents against *FoL*

The inhibitory effect of different antagonists and their combinations were tested *in vitro* against *FoL* adopting plate assay technique (Rahman *et al.*, 2009) in pathogen favourable PDA medium. Agar plug of 5 mm diameter was prepared from 48 hr old culture plates of *Fusarium* and *T. harzianum* and placed near the periphery of freshly prepared PDA plates. Similarly, agar plugs containing 48 hr old culture of *P. fluorescens* and *B. thuringiensis* were scooped out from plate cultures and placed near the periphery of respective plates with the growth of *F. oxysporum*. The plates were then incubated at 28±1°C for 72 hrs. Fungal growths as colony diameter were measured and percentage inhibition was calculated following standard methods.

### Preparation of microbe-based bioformulation

For the preparation of microbe-based bioformulation, the fungal and bacterial antagonists were at first multiplied individually in shake flasks using PD broth and nutrient broth respectively as a growth medium. The flasks containing 200 ml of respective medium were inoculated with 48 hrs old growth of *T. harzianum*. The inoculum was prepared by pouring sterile water into slant culture of the fungus and subsequently shaking gently to lodge the vegetative parts into the suspension. A loop of the inoculum suspension (10<sup>8</sup> cfu/ml) was transferred to flasks containing PD broth. Similarly, the bacterial antagonist *B. thuringiensis* and *P. fluorescens* were multiplied in individual flasks. Inoculated flasks were incubated at 28±1°C with orbital shaking at 150 rpm for



**Fig. 1.** Compatibility reaction among different bioagents in PDA as basal media

72 and 48 hrs for fungal and bacterial antagonists respectively. The consortia formulation was prepared by mixing individual broth culture of *T. harzianum*, *B. thuringiensis* and *P. fluorescens* at a ratio of 1:1:1 after adjusting cell population to  $10^8$  cfu/ml for each of the bioagents.

#### **In vivo evaluation of microbe-based bioformulation treatments**

The *in vivo* evaluation of antagonists based bioformulation was conducted in artificially infested hydroponic culture tanks under the net house. The inoculum of *FoL* was prepared by culturing in PD broth in shake culture at 150 rpm, for 10 days at  $28 \pm 2^\circ\text{C}$  (Singh *et al.*, 2013). The microconidia of the fungus were harvested by filtering through 4 layers of muslin cloth, centrifuged at 6000 g for 10 min, adjusted to a spore population of  $2 \times 10^6$  cfu/ml and was used to infest all the hydroponic tanks before planting. The antagonist based formulations were added to the nutrient solution before transplanting of lettuce seedling and again applied as spray after transplanting. After transplanting lettuce seedlings, the tanks were kept in the net house to protect from direct exposure to sunlight. The observations recorded during experimentation were - Disease incidence in roots and leaves (%); dry weight of the roots (g); dry weight of the shoots (g); height of the plant (cm); yield/plant (g).

## **RESULTS AND DISCUSSION**

### **Compatibility among the bioagents**

*In vitro* study revealed that there was compatibility among the three bioagents viz., *T. harzianum*, *P. fluorescens* and *B. thuringiensis* (Fig. 1). All the three bioagents showing

**Table 1.** Results of compatibility test conducted with different bioagents

Bioagents	<i>T. viride</i>	<i>P. fluorescens</i>	<i>B. thuringiensis</i>
<i>T. viride</i>	+ve	+ve	+ve
<i>P. fluorescens</i>	+ve	+ve	+ve
<i>B. thuringiensis</i>	+ve	+ve	+ve

+ve – Positive (Compatible)

compatibility was selected for downstream experimentation (Table 1). Sumana and Devaki (2012) reported positive compatibility amongst antagonists like *T. harzianum*, *B. subtilis* and *P. fluorescens* in dual cultures. Similar compatible observations amongst bioagents like *P. fluorescens*, *T. harzianum*, and *B. bassiana* was made by Bora *et al.* (2013).

### **Antagonistic effect of the bioagents against *FoL***

The three compatible bioagents, viz., *T. harzianum*, *B. thuringiensis*, and *P. fluorescens* were tested against *FoL* singly and in consortia. Highest radial growth inhibition of *FoL* was recorded in case of *T. harzianum* (68.56%) followed by consortia of *T. harzianum* and *B. thuringiensis* (60.90%). This was followed by *B. thuringiensis* where 55.15 per cent growth inhibition recorded (Table 2).

Dagostin *et al.* (2011) reported that the bioagent *Trichoderma* has ability to produce a series of antibiotics and fungal cell wall degrading enzymes. These enzymes play important role in mycoparasitism and mycelia lysis of the target haustoria and hyphae. Ayer and Adam (1981) showed that *T. harzianum* could produce diffusible substances toxic to the pathogens and effectively act as ectoparasite by overcrowding the target organisms. Lee and Wu (1984) mentioned that *T. harzianum* could produce antibiotics and inhibit the mycelial growth of *S. sclerotiorum* completely and induce swelling and plasmolysis of target pathogen cells. *Trichoderma* sp. is known to produce different kinds of enzymes such as cell wall degrading enzyme, biotic and abiotic stress tolerance, mycoparasitism, antagonistic activity against different plant pathogens (Sharma *et al.*, 2014). *B. thuringiensis* is a naturally abundant Gram-positive bacterium and a well-known, effective bio-insecticide. They have the ability to produce antimicrobials such as bacteriocins, which are actually inhibitory peptides or proteins against different pathogens (Mobolaji, 2009). *Bacillus* spp. acts as potential biocontrol agents of plant pathogenic fungi such as *F. oxysporum*, *F. sambucinum*, and *F. graminearum* (Baysal *et al.*, 2013). Barruzi *et al.* (2011) reported a number of antifungal compounds, including polypeptides that interact with the fungal membrane, are produced by *Bacillus* species. *B. thuringiensis* can secrete chitinases and chitin-binding proteins; together these proteins control fungi by binding to cell wall chitin and disrupting the cell polarity, leading to fungal growth inhibition. The suppressive effect of *T. harzianum* and *B. thuringiensis* shown against *FoL* in our study might be due to production of similar type of antimicrobials and toxins.



**Table 2.** Suppressive effect of different antagonists and their consortia against radial growth of *F. oxysporum* f. sp. *Lactucae*

Bioagents	Radial growth (mm)	Inhibition(%)
<i>F. oxysporum</i> f. sp. <i>lactucaae</i> (FoL)	90.00	0.00(0.57)
<i>T. harzianum</i> + FoL	12.25	68.56(55.86)*
<i>P. fluorescens</i> + FoL	40.00	46.91(43.22)
<i>B. thuringiensis</i> + FoL	22.45	55.15(47.93)
<i>T. harzianum</i> + <i>P. fluorescens</i> + FoL	57.0	37.23(37.58)
<i>T. harzianum</i> + <i>B. thuringiensis</i> + FoL	21.25	60.90(51.30)
<i>P. fluorescens</i> + <i>B. thuringiensis</i> + FoL	74.75	23.77(29.13)
<i>T. harzianum</i> + <i>P. fluorescens</i> + <i>B. thuringiensis</i> + FoL	49.50	42.09(40.40)
S.Ed ( $\pm$ )		1.77
CD <sub>0.05</sub>		3.02

\*Data in the parenthesis are angular transformed values

**Table 3.** Effect of different microbe based bioformulations on incidence of root and leaf infections caused by *F. oxysporum* f. sp. *lactucaae* in hydroponically grown lettuce plants

Treatments	Root infection (%)	Reduction of root infection (%)	Leaf infection (%)	Reduction of Leaf infection (%)
T <sub>0</sub> = Control	85.08 (67.21)*	00	70.02 (56.79)*	00
T <sub>1</sub> = <i>T. harzianum</i>	15.39 (23.09)	81.91	18.09 (25.10)	74.16
T <sub>2</sub> = <i>T. harzianum</i> + <i>B. thuringiensis</i>	22.73 (27.83)	73.28	23.64 (29.06)	66.23
T <sub>3</sub> = <i>B. thuringiensis</i>	54.50 (47.58)	35.94	45.23 (42.25)	33.04
T <sub>4</sub> = <i>P. fluorescens</i>	66.74 (54.76)	21.55	55.50 (48.16)	20.73
T <sub>5</sub> = <i>T. harzianum</i> + <i>P. fluorescens</i> + <i>B. thuringiensis</i>	29.58 (32.90)	65.23	29.45 (32.83)	57.94
T <sub>6</sub> = <i>T. harzianum</i> + <i>P. fluorescens</i>	42.18 (40.46)	50.42	36.15 (36.93)	48.37
T <sub>7</sub> = <i>P. fluorescens</i> + <i>B. thuringiensis</i>	75.29 (60.13)	11.50	60.70 (50.77)	13.31
S. Ed ( $\pm$ )	2.49		2.34	
CD <sub>0.05</sub>	4.25		4.00	

\*Data in the parenthesis are angular transformed values

Antagonist mixtures have been thoroughly investigated in soil systems, especially against pathogenic taxa such as *Fusarium* spp. and *Pythium* spp., where enhanced disease control of root infecting pathogens has been attained with multi-strain mixtures (Lemanceau *et al.*, 1992). In the present study combination of *T. harzianum* and *B. thuringiensis* significantly reduced the mycelial growth of FoL. A similar observation was reported by Kamala and Devi (2012).

#### Effect of microbe-based bioformulations on lettuce disease infection in hydroponic tank

Significant reduction of root and leaf infection of lettuce crop has been observed when antagonist based bioformulations were applied into the hydroponic tank. Lowest root infection (15.39%), as well as leaf infection (18.09%), was recorded in the tanks treated with *T. harzianum* ( $1 \times 10^8$  cfu/ml) alone as root application along with the nutrient solution. This treatment was followed by consortia of *T. harzianum* and *B. thuringiensis* where 22.73 per cent and 23.64 per cent root and leaf infection was recorded respectively (Table 3). Reduction in root infection (81.91%) and leaf infection (74.16%) was recorded highest in *T. harzianum* treated tank followed

by *T. harzianum* and *B. thuringiensis* combine treatment where 73.28 per cent and 66.23 per cent reduction in root and leaf infection was recorded respectively. Highest root (85.08%) and leaf infection (70.02%) has been recorded in the untreated control tank.

Application of the bioagents significantly reduced the root and leaf disease incidence in lettuce. The significant decrease of root and leaf infection of lettuce was recorded in two best treatments, *i.e.*, *T. harzianum*, as well as *T. harzianum* and *B. thuringiensis* based bioformulations applied as combinations of root and foliar treatments might have occurred due to direct effects of the bioagents on the suppression of the pathogen population. *Trichoderma* species have the ability to control many soil-borne plant diseases caused by fungi (Elad *et al.*, 1980). Successful reductions of *Fusarium* rot in many crops with the application of different species of *Trichoderma* have been recorded earlier (Ramezani, 2009).

#### Effect of microbe-based bioformulation on disease incidence and yield attributing characters

All the three microbial bioagents *T. harzianum*, *B. thuringiensis* and *P. fluorescens* significantly improved

**Table 4.** Effect of different antagonist based bioformulations on growth parameters of hydroponically grown lettuce plant

Treatments	Growth characteristics							
	Shoot length (cm)	Root length (cm)	No of leaves	Shoot dry weight (g)	Root dry weight (g)	No of roots	Root fresh weight (g)	Yield (g/plant)
T <sub>0</sub> = Control	6.87	2.62	5.00	0.48	0.25	11.00	2.07	30
T <sub>1</sub> = <i>T. harzianum</i>	35.00	28.25	23.00	18.04	5.95	56.00	28.12	251.25
T <sub>2</sub> = <i>T. harzianum</i> + <i>B. thuringiensis</i>	29.25	24.50	19.00	15.00	4.30	53.00	24.37	221.75
T <sub>3</sub> = <i>B. thuringiensis</i>	16.00	12.25	10.00	6.35	0.89	32.00	12.15	80.25
T <sub>4</sub> = <i>P. fluorescens</i>	13.67	8.50	8.00	2.77	0.54	26.00	7.82	63.50
T <sub>5</sub> = <i>T. harzianum</i> + <i>P. fluorescens</i> + <i>B. thuringiensis</i>	24.00	20.50	15.00	12.05	2.82	49.00	20.67	187.75
T <sub>6</sub> = <i>T. harzianum</i> + <i>P. fluorescens</i>	20.25	16.50	12.00	9.41	1.40	40.00	16.80	140.00
T <sub>7</sub> = <i>P. fluorescens</i> + <i>B. thuringiensis</i>	9.75	6.875	6.00	0.90	0.36	19.00	4.97	44.50
S. Ed (±)	1.32	1.13	1.09	0.54	0.29	2.04	0.91	6.57
CD <sub>0.05</sub>	2.25	1.93	1.86	0.92	0.49	3.48	1.55	11.20

the growth parameters as compared to the untreated control. The highest growth attribute in terms of shoot length (35.00 cm), root length (28.25 cm), no. of leaves (23.00), shoot dry weight (18.04 g/plant), root dry weight (5.95 g/plant), root fresh weight (28.12 g/plant) and total biomass (251.25 g/plant) of lettuce plants was recorded with application of bioformulation of *T. harzianum* alone. This was followed by application of consortia formulation of *T. harzianum* and *B. thuringiensis* (Table 4).

Similarly, *Trichoderma* spp. have been reported to enhance plant height, leaf number, tiller number, root length and root fresh weight of rice (Doni *et al.*, 2014). The yield of lettuce treated with consortia of different bioagents followed similar trend as recorded for root and leaf infection (Table 3). Highest yield (251.25 g/plant) with lowest overall disease incidence (17.29%) was recorded in the tanks treated with bioformulation containing *T. harzianum* alone followed by treatment with consortia of *T. harzianum* and *B. thuringiensis* where 26.13% rot incidence and 221.75 g/plant healthy harvest has been observed. The highest fungal rot incidence (80.97%) and lowest yield (30.00 g/plant) of lettuce were recorded in the control treatment where no bioformulation was applied. The increase in yield and decrease in disease incidence might be due to suppression of the pathogen due to the application of bioagents. This finding is in agreement with Bustamante *et al.* (1989). In our study, the plant height and yield increased in *T. harzianum* treated plants. Similar results of increased plant growth due to the application of *Trichoderma gamsii* in cereals and legume crops have been reported earlier (Rinu *et al.*, 2013). The increase of plant growth might be associated with secretion of auxins, gibberellins and cytokinins. Moreover, Alippi and Monaco (1994) reported that *B. subtilis* can secrete several antifungal metabolites such as subtilin, bacitracin, bacillin and bacillomycin which have inhibitory effects on fungal pathogens. Yang *et al.* (2014) reported that *B. subtilis* exerted a heat stable antibiotic interfering with spore germination, or early germ tube development of stone fruit brown rot pathogen. The

present finding suggests that suppression *FoL* by *Bacillus* sp. could be due to similar type of mechanisms. Manikandan and Raguchander (2015) reported that liquid formulation of strain Pf1 has six month shelf life and enhanced disease resistance in tomato plants against *Fusarium* wilt disease. The present finding suggests that suppression of *FoL* could be due to similar type of resistance. Van Peer and Schippers (1989) reported that bacterization of hydroponic nutrient solution with plant-growth-promoting *Pseudomonas* strains increases root and shoot mass of tomato, cucumber, lettuce, and potato most likely by suppressing deleterious rhizosphere microflora.

The application of microbe-based bioformulation of *T. harzianum* or consortia formulation of *T. harzianum* and *B. thuringiensis* as root treatment and foliar spray application can be effective biocontrol option for management of fungal rot of hydroponically grown lettuce plant. Increasing population and declination of arable land due to poor land management, people may adopt new technologies like the soil-less culture of crop production with bio-intensive management practices.

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