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Bio-efficacy of different plant leaf extracts and bio-control agents against *Fusarium oxysporum* f. sp. *ciceri*

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Abstract
Chickpea (*Cicer arietinum* L.) is one of the most important legumes in India. The fungal disease *Fusarium oxysporum* f. sp. *ciceri* Butler is dominant and destructive in large areas of chickpea production. Leaf extracts from seven different plant species were evaluated *in vitro* against the pathogen at concentrations of 5% and 10%. Among these leaf extracts, neem was the most effective against *Fusarium oxysporum* f. sp. *ciceri*, followed by garlic, pipal, palmarosa, tulsi, aloe vera, and ashoka, which prevent the growth of fungi. Of the biopesticides, *Trichoderma harzianum* (64.00%) showed the best result in *Fusarium oxysporum* f. sp. *ciceri*, followed by *T. viride* (51.33%), *T. koningii* (44.67%), *T. longibrachiatum* (30.67%), *T. hamatum* (23.33%), and *Chaetomium globosum* (18.67%).

Keywords: Chickpea, *Fusarium oxysporum* f. sp. c*iceri*, plant leaf extract, bio-control agents

Introduction
Chickpea (*Cicer arietinum* L.), commonly known as Gram or Bengal gram or Egyptian pea, belongs to subfamily Papilionaceae (family- Leguminosae) with chromosome number 2n = 2x = 16. It is an important *Rabi* legume crop in India. Legumes are in the diet of most people in India because they provide an excellent combination of biological value as a cereal supplement. It contains 27.42 grams of carbohydrates, 8.86 grams of protein and 2.59 grams of fat per 100 grams of chickpeas. Chickpeas are high in protein, low in fat and sodium, cholesterol-free, and an excellent source of both soluble and insoluble fiber and complex carbohydrates, vitamins and minerals, especially calcium, phosphorus, iron and magnesium (Roy *et al.*, 2010) [11].

The germinated seeds are recommended to be eaten to treat scurvy. Being a legume, it is a good source of protein, making up about 99 percent of the dry weight of the grain, which is very cheap and is therefore called the "poor man's meat" (Muehlbauer and Rajesh, 2008) [10]. Chickpea is third the most important legume in the world after dry beans and peas. It accounts for 20 percent of the world's pulse production. The most important chickpea producers are India, Pakistan and Mexico. India is the largest producer with about 8 million tons, accounting for about 69-71 percent of the world's total production.

The area, production and productivity of major pulses in India, chickpea, is estimated at 9.93 mha; 9.53 mt. and 960 kg/ha. It is grown in Madhya Pradesh, Rajasthan, Uttar Pradesh, Jharkhand, Maharashtra, Bihar, Punjab, Haryana, Andhra Pradesh and Chhattisgarh. However, six major states *viz*. Madhya Pradesh, Rajasthan, Maharashtra, Uttar Pradesh, Karnataka and Andhra Pradesh together contribute 91 percent of the production and 90 percent of the area. Area, production and productivity of chickpea in U.P. is estimated at 6.11 Lakh ha; 6.84 lakh tonnes and 1119.48 kg/ha (Anonymous, 2018) [1].

Despite efforts by various agencies to increase production, the total production and productivity per unit area is very less. Among the various factors affecting the yield, one of the most important factors is chickpea wilt caused by various pathogens. The pathogen is in the soil and can survive in the soil for at least six years in the absence of a host. It is one of the main chickpea diseases nationwide, the yield loss observed was about 60 percent (Singh and Gupta, 2007) [13].

F. oxysporum f. sp. *ciceri* infects chickpea at the seedling, flowering, and pod formation stages, with increased incidence at the flowering and pod formation stages when the crop is

exposed to sudden temperature increases and water stress (Choudhary *et al.,* 2007) [3]. Chickpea losses due to Fusarium wilt have been reported in the range of 10-15% (Jalali and Chand, 1991) [7], but losses of up to 70% have been reported in some years in northern India and Pakistan (Grewal and Pal, 1970) [5]. Four races (1-4) of Fusarium wilt have been identified from India (Haware and Nene, 1982) [6].

Consequently, due to the severity of the disease and the importance of the harvest, the current studies were carried out on the bioefficacy of various plant leaf extracts and biopesticides against *Fusarium oxysporum* f. sp. *ciceri*.

Methods and Materials

Screening of different plant leaf extracts against *Fusarium oxysporum* f. sp. *ciceri* (*in vitro*)

The existence of naturally occurring compounds in plants that have antifungal activities has been identified and tested against the pathogen. In this study, the efficiency of leaf extracts at 5% and 10% concentrations from seven plants was investigated against *Fusarium oxysporum* f. sp. *ciceri*, which causes Fusarium wilt of chickpea.

S. No.	Plant leaf extracts
1.	Palmarosa (*Cymbopogon martinii* var. *motia*)
2.	Pipal (*Ficus* sp.)
3.	Neem (*Azadrachta indica*)
4.	Tulsi (*Ocimum tenuiflorum*)
5.	Garlic (*Allium sativum*)
6.	*Aloe vera* (*Aloe barbadensis*)
7.	Ashoka (S*araca asoca*)
8.	Control (untreated)

Fresh leaves of the plant species were collected, washed with tap water and then with sterilized water, crushed in sterilized distilled water in a ratio of 1:1 (w/v) with a mortar and filtered through 2 layers of muslin cloth to get an extract. 100 ml of sterilized potato dextrose agar medium was mixed with 10 ml of plant leaf extract in a 150 ml conical flask and sterilized in an autoclave at a of 1.1 kg pressure /sq. cm for 5 minutes. The sterilized medium was then poured into three sterilized Petri dishes, and after solidification, a 5 ml disc with a 7-day pathogen culture was placed in the center of the Petri dishes and incubated at 28±1 °C. Unmodified PDA with leaf extract served as a control. Observation of the radial growth of the test fungus was recorded after 7 days of incubation.

Evaluation of bio-control agents against *Fusarium oxysporum* f. sp. *ciceri* (*in vitro*)

For this study, the pathogen was isolated from diseased chickpea plants from the wilt disease area of Legume Research Farm, C.S. Azad University of Agriculture and Technology, Kanpur. One-week-old culture of *Fusarium oxysporum* f. sp. *ciceri* kept in PDA Petri dishes at 28±1 °C. The culture of all bioagents was isolated from the rhizosphere of chickpea plants at Nawabganj Farm, C. S. Azad University of Agriculture and Technology, Kanpur. Antagonistic activity of these bioagents against the test pathogen was determined by the dual-culture method (Dennis and Webster, 1971) [4]. A 5 mm pathogen disk was taken from the actively growing colonies of the test pathogen and the antagonist using a sterilized cork borer. The pathogen disc was aseptically placed on one side of the agar plates and the antagonist disc on the opposite side of the pathogen in the same Petri dish. Each treatment was replicated three times and incubated at 28±10 °C. Antagonists and pathogen growth were recorded after 7 days of incubation. The mechanism of interaction was observed and data were expressed as percentage inhibition according to the following formula (Bliss, 1934) [2].

$$\text{Percentinhibition (P.I.)} = \frac{\text{Growth in control} - \text{Growth in treated plates}}{\text{Growth in control}} \text{ x } 100$$

S. No.	Bio-agents
1.	*Trichoderma harzianum*
2.	*T. viride*
3.	*T. koningii*
4.	*T. longibrachiatum*
5.	*T. hamatum*
6.	*Chaetomium globosum*

Results and Discussion

Screening of plant leaf extracts against the pathogen *in vitro*

Seven plant leaf extracts were evaluated for their inhibitory effect against the pathogen *in vitro* (figure 1). The results of the average of the diameter of fungal colony after 7 days of incubation at 28±1 °C are given in Table 1.

Table 1: Evaluation of plant leaf extracts at 5 percent concentration against the pathogen *in vitro* after 7 days of incubated at 28±1 °C

S. No.	Plant leaf extracts	Dose %	Average diameter of fungal colony (cm)	Percent inhibition
1.	Neem (*Azadrachta indica*)	5	2.75	63.33
2.	Garlic (*Allium sativum*)	5	3.05	59.33
3.	Pipal (*Ficus* sp.)	5	3.45	54.00
4.	Palmarosa (*Cymbopogon martinii* var. *motia*)	5	3.80	49.33
5.	Tulsi (*Ocimum tenuiflorum*)	5	3.95	47.33
6.	Aloe vera (*Aloe barbadensis*)	5	4.30	42.67
7.	Ashoka (*Saraca asoca*)	5	5.65	24.67
8.	Control (untreated)	-	7.50	-
	SEm±		0.062	
	C.D. at 5%		0.189	

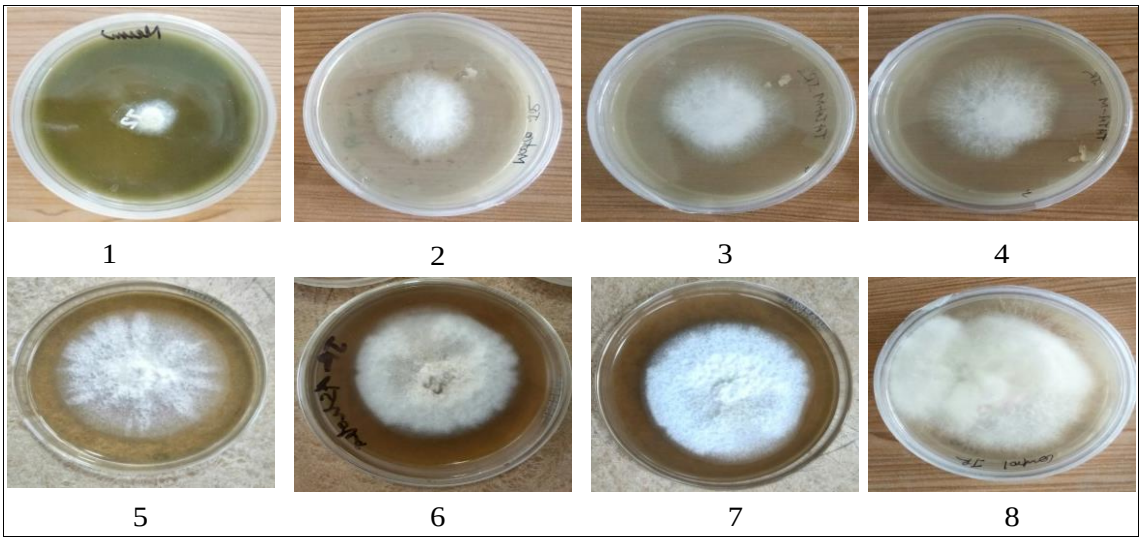
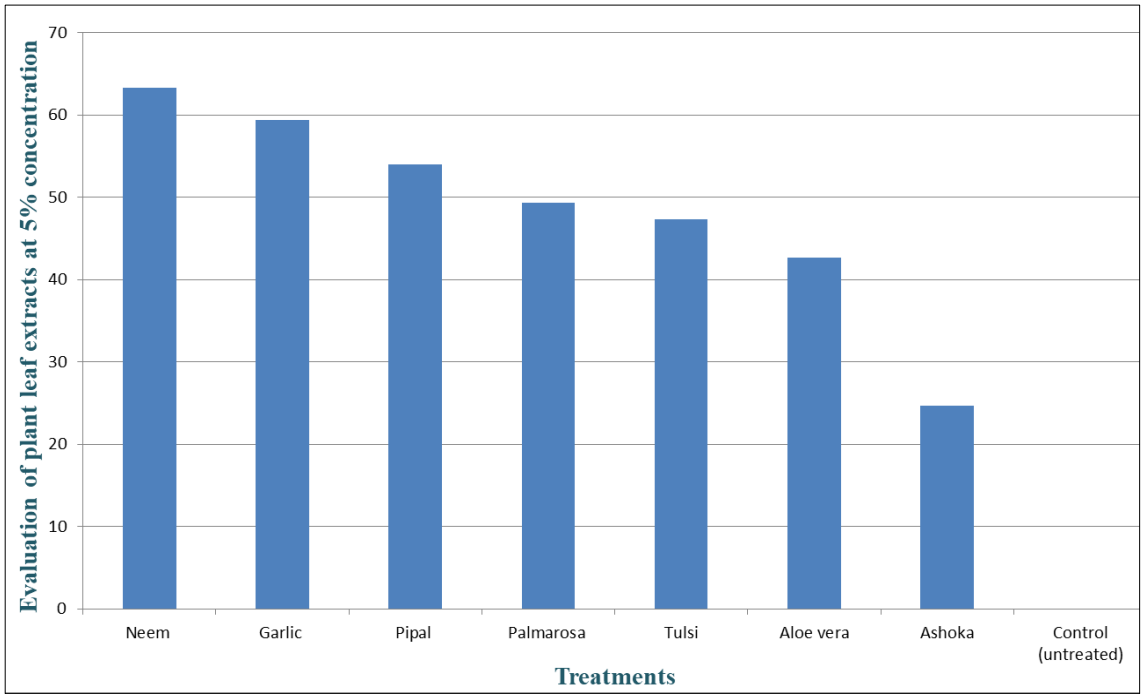


Fig 1: Evaluation of plants leaf extracts 5 perent concentraltion against the pathogen
1. Neem (*Azadrachta indica*), 2. Garlic (*Allium sativum*), 3. Pipal (*Ficus* sp.), 4. Palmarosa (*Cymbopogon martinii* var. *Motia*), 5. Tulsi (*Ocimum tenuiflorum*), 6. *Aloe vera* (*Aloe barbadensis*), 7. Ashoka (*Saraca asoca*) 8. Control



Graph 1: Evaluation of plant leaf extracts at 5% concentration against the pathogen *in vitro* after 7 days of incubated at 28±1 °C

When compared to the control, all of the plant extracts effectively inhibited fungal growth (Figure 1). According to Table 1 and its histogram (Graph 1), out of seven different plant extracts tested in the laboratory, Neem leaf extract (63.33%) was effective against *Fusarium oxysporum* f. sp. *ciceri*, followed by garlic (59.33%), pipal (54.00%), palmarosa (49.33%), tulsi (47.33%), aloe vera (42.67%), and ashoka (24.67%) to inhibit fungal growth in comparison to the control.

Table 2: Evaluation of plant leaf extracts at 10 percent concentration against the pathogen *in vitro* after 7 days of incubated at 28±1 °C

S. No.	Plant leaf extracts	Dose %	Average diameter of fungal colony (cm)	Percent inhibition
1.	Neem (*Azadrachta indica*)	10	2.05	72.67
2.	Garlic (*Allium sativum*)	10	2.35	68.67
3.	Pipal (*Ficus* sp.)	10	3.15	58.00
4.	Palmarosa (*Cymbopogon martinii* var. *motia*)	10	3.20	57.33
5.	Tulsi (*Ocimum tenuiflorum*)	10	3.40	54.67
6.	Aloe vera (*Aloe barbadensis*)	10	3.65	51.33
7.	Ashoka (*Saraca asoca*)	10	4.05	46.00
8.	Control (untreated)	-	7.50	-
	SEm±	-	0.053	-
	C.D at 5%	-	0.160	-

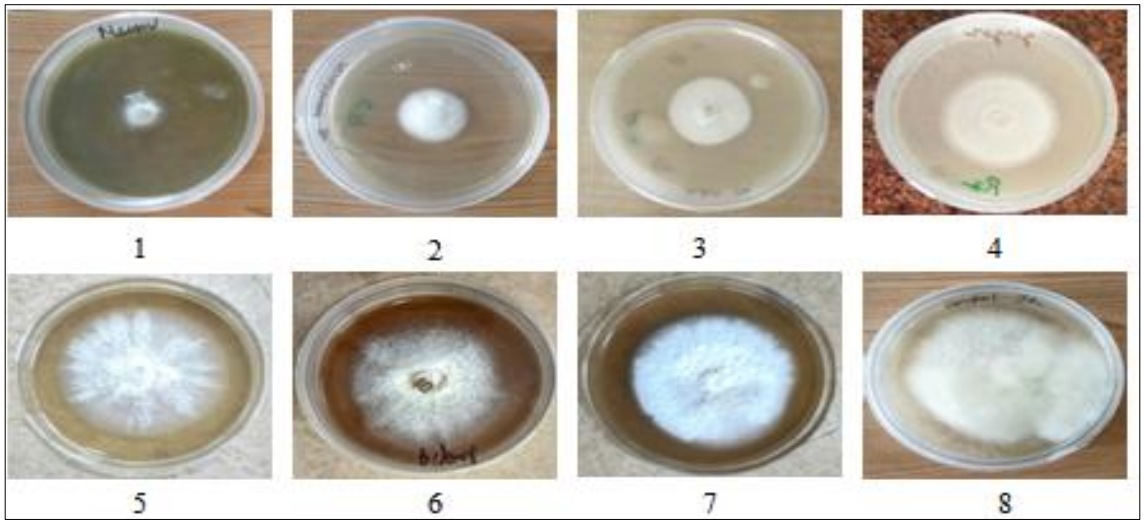
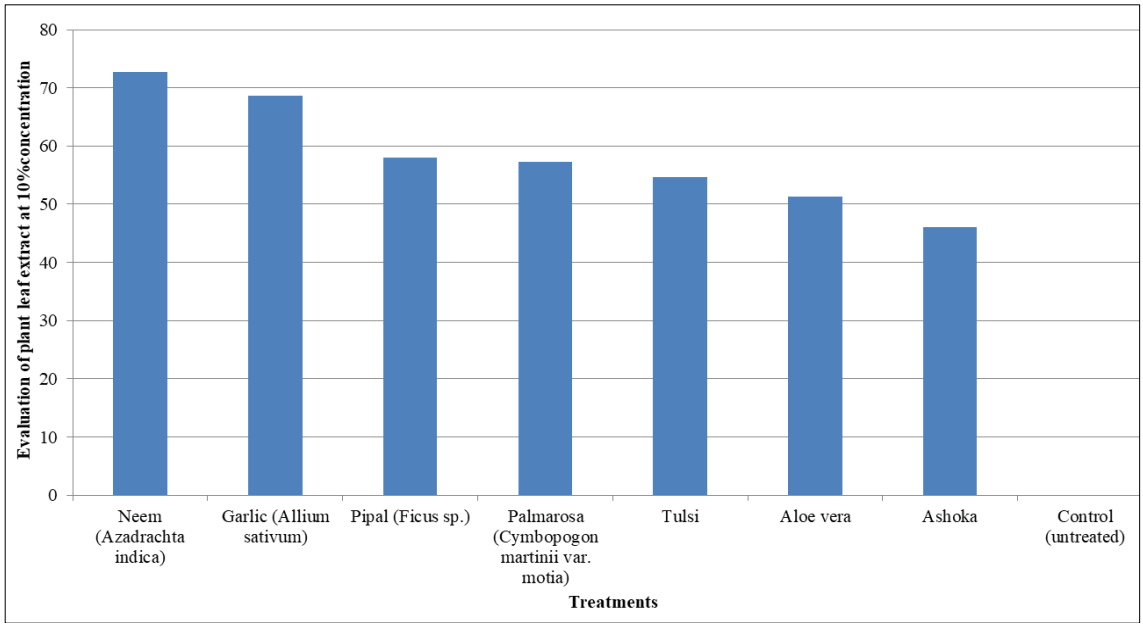


Fig 2: Evaluation of plants leaf extracts 10 perent concentraltion against the pathogen
1. Neem (*Azadrachta indica*), 2. Garlic (*Allium sativum*), 3. Pipal (*Ficus* sp.), 4. Palmarosa (*Cymbopogon martinii* var. *Motia*), 5. Tulsi (*Ocimum tenuiflorum*), 6. *Aloe vera* (*Aloe barbadensis*), 7. Ashoka (*Saraca asoca*) and 8. Control



Graph 2: Evaluation of plant leaf extracts at 10% concentration against the pathogen *in vitro* after 7 days of incubation at 28±1 °C

All plant extracts effectively inhibited the growth of the fungus compared to the control (Figure 2). Table 2 and its histogram (Graph 2) show that among the seven plant extracts tested in laboratories, neem leaf extract (72.67%) was found highly effective against *Fusarium oxysporum* f. sp. *ciceri* followed by Garlic (68.67%), Pipal (58.00%), Palmarosa (57.33%), Tulsi (54.67%), Aloe Vera (51.33%) and Ashoka (46.00%) to inhibit the growth of the fungus compared to the control.

Evaluation of bio-agents against the pathogen *in vitro*:
The inhibitory effect of the bio-agents against the pathogen was evaluated *in vitro* using dual culture methods as described in the Materials and Methods section. The results of the average diameter of the fungal colony incubated at 28±1 °C after 7 days are shown in Table 3 (Figure 3).
The results shown in Table 3 and its histogram (Figure 3) showed that all the bioagents inhibited fungal growth *Fusarium oxysporum* f. sp. *ciceri*.

Table 3: inhibiting effect of different bio-agents on the growth of *Fusarium oxysporum* f. sp. *ciceri in vitro* incubated of 28±1 °C.

S. No.	Bio-agents	Average diameter of fungal colony	Per cent inhibition over control
1.	*Trichoderma viride*	3.65	51.33
2.	*Trichoderma koningii*	4.15	44.67
3.	*Trichoderma harzianum*	2.70	64.00
4.	*Trichoderma longibrachiatum*	5.20	30.67
5.	*Trichoderma hamatum*	5.75	23.33
6.	*Chaetomium globosum*	6.10	18.67
7.	Control	7.50	-
	S.Em±	0.074	
	C.D. at 5%	0.228	

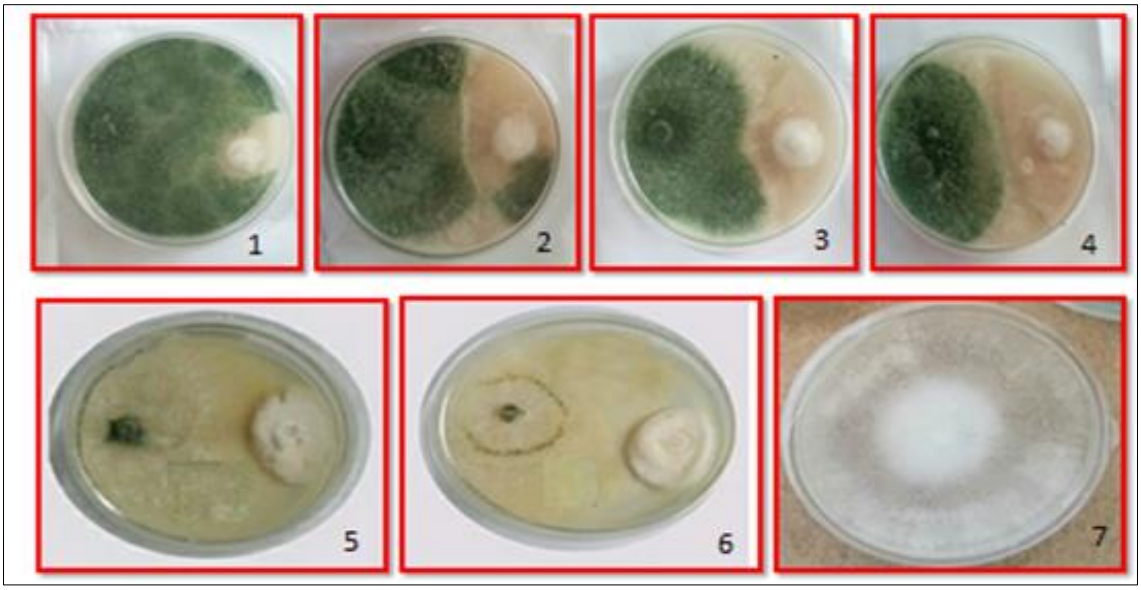
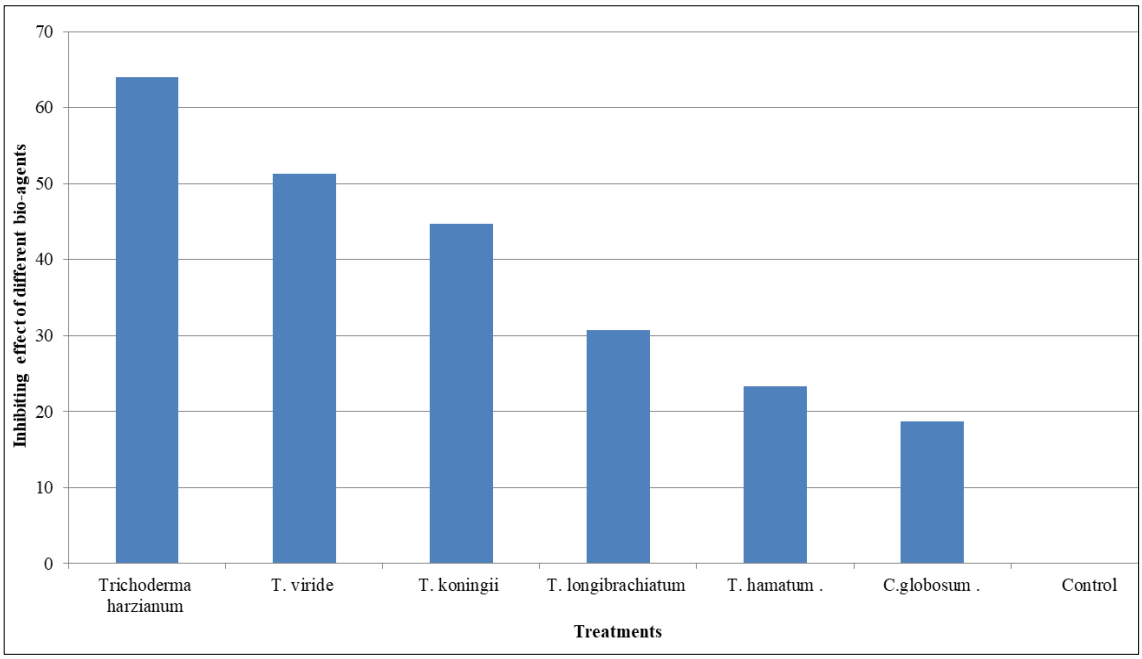


Fig 3: Evaluation of bio-agents against the pathogen *in vitro*
1. *Trichoderma viride*, 2. *Trichoderma koningii*, 3. *Trichoderma harzianum*, 5. *Trichoderma longibrachiatum*, 6. *Trichoderma hamatum*, 7. *Chaetomium globosum*, 8. Control



Graph 3: Inhibiting effect of different bio-agents on the growth of *Fusarium oxysporum* f. sp. *ciceri in vitro* incubated of 28±1^{0}C

Table 3 and its histogram (Fig 3) show that six bioagents tested in the laboratory were effective against *Fusarium oxysporum* f. sp. *ciceri.* Pathogen growth inhibition was highest for *Trichoderma harzianum* (64.00%), followed by *T. viride* (51.33%), *T. koningii* (44.67%), *T. longibrachiatum* (30.67%) and *T. hamatum* (23.33%), which are statistically close to each other. The least effective bioagents was Chaetomium globosum (18.67%).

Discussion

Leaf extract of seven different plant species were also evaluated *in vitro* against the pathogen at 5% and 10% concentration. Among these leaves extract *viz.*, Neem was most effective against *Fusarium oxysporum* f. sp. *ciceri* followed by Garlic, pipal, palmarosa, Tulsi, Aloe Vera and Ashoka inhibiting the fungal growth. These results are similar to those as reported by Minz *et al.* (2012) [9] and Şesan *et al.* (2017) [12].

Six bio-control agents were evaluated in *in vitro* conditions, *Trichoderma harzianum* (64.00%) showed that best performance in checking the growth of *Fusarium oxysporum* f. sp. *ciceri* followed by *t. viride* (51.33%), *T. koningii* (44.67%), *T. longibrachiatum* (30.67%), *T. hamatum* (23.33%) and *Chaetomium globosum* (18.67%). Similar findings were also reported by Yasmin *et al.* (2014) [14] and Jan *et al.* (2015) [8].

Conclusion

Out of seven leaf extracts of different plant species evaluated *in vitro* against the pathogen, Neem leaf extract was found most effective in inhibiting the growth of *Fusarium oxysporum* f. sp. *ciceri*, followed by Garlic and

other leaf extracts. It is safe and ecofriendly method of management of pathogen.

To know the antagonistic activity, six bio-agents namely *trichoderma harzianum,t. viride*, *T. koningii*, *T. longibrachiatum*, *T. hamatum* and *Chaetomiumglobosum* were screened against the pathogen *in vitro* and observed that *T. harzianum* was more effective in suppressing the growth of the pathogen. It is safe and ecofriendly method of management of pathogen.

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