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Assessment of bio control agents for the management of fusarium wilt of cucumber under laboratory and cage house condition

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Abstract
Cucumber Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cucumerinum* is one of the most serious fungal diseases in cucumber production in the world. In this study, six bio-agents *viz*., *Trichoderma harzianum, Trichoderma viride, Trichoderma asperellum, Pseudomonas fluorescens, Bacillus subtilis* and *Aspergillus niger* were evaluated for their ability to inhibit the pathogen growth under *in vitro* and *in vivo* conditions. The pathogen which causes Fusarium wilt of cucumber, was isolated and identified as *Fusarium oxysporum* f. sp. *cucumerinum* (FOC). All the bioagents were obtained from Department of Plant Pathology, S.K.N. College of Agriculture, Jobner, Jaipur. The results of this study revealed that *Trichoderma harzianum* was found most effective in inhibiting the radial mycelial growth of the pathogen (85.90%) followed by *Trichoderma viride* (82.50%) *Trichoderma asperellum* (79.63%), *Bacillus subtilis* (68.52%) *Pseudomonas fluorescens* (64.81%) and *Aspergillus niger* (51.85%) compared to control under *in vitro* conditions by Dual Culture Method. Whereas under cage house condition *Trichoderma harzianum* was found most effective in reducing incidence of Fusarium wilt of cucumber.

Keywords: Cucumber wilt, *Fusarium oxysporum* f. sp. *cucumerinum*, bioagents

Introduction
Cucumber (*Cucumis sativus* L.) is one of the most important vegetable crops, which belongs to the family cucurbitaceae and it is one of the oldest cultivated vegetable dating back to 5,000 years (Wehner and Guner, 2004) [21]. Its ancestral *i.e*. Indian wild cucumber (*Cucumis sativus* var. *hardwickii*) probably originated in the foot hills of the Himalayas. It is cultivated from East ward to China and West ward to Asia Minor (Seshadri and Parthasarathy, 2002) [17]. It is the fourth most important cultivated vegetable crop in the world after cabbage, onion and tomatoes (Shetty and Wehner, 2002) [18]. Cucumber is an annual deep-rooted crop with tendrils and hairy leaves. The plants may have an indeterminate, determinate or a compact plant habit. The compact growth plants habit consists of shorter internode length than plants with indeterminate or determinate growth habit. Several flowering habits exist in cucumbers. Most cultivars are monoecious *i.e*. with separate male and female flowers in the same plant. It is grown for its tender fruits for fresh consumption as salad or as pickling cucumber for preservation, marinated with vinegar, salt, dill or other spices. The fruit of cucumber is said to have cooling effect, prevent constipation, check jaundice and indigestion (Nandkarni, 1927) [12]. Besides, the seeds of cucumber are used in ayurvedic preparations and raw fruits are also used in cosmetic preparations. It is reported that oil extracted from seed is good for brain and body. Nutritively, 100 g of edible portion of cucumber contains 96.3 g moisture, 2.5 g carbohydrates, 0.4 g protein, 0.4 g fat, 0.3 g minerals, 10.0 mg calcium, 0.4 g fiber, 1.5 mg iron and 2.0 mg vitamin C (Rai and Yadav, 2005) [16].

Leading producers of cucumber are China, Russia, Turkey, U.S, Ukraine, Egypt, Spain and India. In India, cucumber production is 1638.00 thousand MT with an area of 111.00 thousand hectares and productivity of 14.75 MT /hectare (Anonymous, 2019-2020) [3]. It is mostly grown in Haryana, Madhya Pradesh, Uttar Pradesh, Karnataka, Andhra Pradesh, Punjab, Assam, Telangana, Maharashtra and Kerala. In Rajasthan, it is mainly cultivated in Sikar, Tonk, Jaipur, Karuali, Dausa and Ganganagar districts. In Rajasthan, total area under cucumber cultivation is 1058.00 hectares with production of 3475.00 MT and productivity of 3.28 MT /hectare. (Anonymous, 2019-2020) [3]. Cucumber is a warm season crop but it is also grown in summer and rainy season. It requires 18 °C minimum temperatures for seed germination and 20-30 °C for growth and development of plant.

It requires sandy to sandy loam soil for early and good crop. Cucumber fruits become ready for first harvesting in about 60-70 days after sowing. Under protected cultivation, the optimum production of cucumber is in the slightly acid to neutral soils (*i.e*. pH 5.5-7.0) with a spacing of row to row 120 cm and plant to plant 20 cm.

Cucumber is affected by many fungal, bacterial, viral and nematode diseases. Among all Fusarium wilt, Powdery mildew, Angular leaf spot, Mosaic and Root knot nematode are most important diseases. *Fusarium oxysporum* is one of the most important phytopathogens causing Fusarium wilt disease in more than a hundred species of plants (Liu *et al*., 2004) [10]. Cucumber Fusarium wilt is one of the most serious fungal disease in cucumber production in the world (Jenkins & Wehner, 1983; Martinez *et al*., 2003) [8]. *Fusarium oxysporum* f. sp. *cucumerinum* is the most common pathogen on cucumber plants causing Fusarium wilt on cucumber and reduced the yield (Ogura, 1992) [13]. Epidemics of Fusarium wilt of cucumber often occurred in China and led to a major yield loss. Generally, it caused yield losses of ~10% to 30% and poor-quality products resulting in severe economic losses (Li *et al*., 2009). *Fusarium oxysporum* f. sp. *cucumerinum* was isolated from the infected roots of cucumbers and recorded in many areas (Huang, 1990; Huang *et al*., 1994) [6-7]. Fusarium wilt of cucumber is a serious disease in long term monoculture cropping. Symptoms of this disease include seedling damping off, plant stunting, yellowing and wilting of older leaves with brown vascular discolorations. The fungus attacks the cucumber plants at all stages of development. Vascular discoloration of stem and roots extending up to 8-10 nodes is of common occurrence (Owen, 1955; Vakalaunakis, 1993) [14].

Materials and Methods

The experiment was carried out in laboratory as well as in cage house during 2020-2021 at Department of Plant Pathology, S.K.N. College of Agriculture, Jobner, Jaipur (Rajasthan).

Testing of bioagents by Dual Culture Method

The efficacy of six bioagents *viz*., *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma asperellum*, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Aspergillus niger* were tested against *Fusarium oxysporum* f. sp. *cucumerinum* using Dual Culture Technique. In this method 20ml of autoclaved PDA was poured in each sterilized petri-plates and allowed for solidification, after solidification these plates were inoculated with 5 mm diameter mycelial bit taken from 7 days old culture of *Fusarium oxysporum* and antagonistic agents both were placed separately at equal distance on the periphery of petri-plates. Petri-plates containing PDA inoculated with pathogen alone is served as control. Inoculated petri-plates were incubated at 25 ± 2 °C in B.O.D. incubator for 7 days. Linear growth of pathogen as well as bio-agent was measured and per cent growth inhibition was recorded on 7th day of incubation.

Per cent growth inhibition was calculated by Vincent's (1947) formula.

$$\text{Per cent Growth Inhibition} = \frac{C - T}{C} \text{ X } 100$$

Whereas

C = Diameter of the colony in check (Average of both diagonals)

T = Diameter of colony in treatment (Average of both diagonals)

Cage house experiment

Efficacy of bioagents under pot condition (*In vivo*)

Commercial formulations of bio-agents those found most effective in *in vivo* were tested in pot conditions against Fusarium wilt disease of cucumber, by applying as seed treatment. Prior to sowing, these pots were sterilized with copper sulphate solution and filled with sterilized soil + vermicompost. These pots were inoculated with fungus inoculum multiplied on sorghum grains @ 20 g/pot. The pots were covered with polythene bags and kept in cage house. The seeds were treated with bio-agents (10 g/kg seeds). These treated seeds were separately sown in pots @ 10 seeds/pot with three replications. Surface sterilized seeds without bio-agents sown in inoculated sterilized soil served as check. The pots were watered as and when required. All the pots were maintained under identical conditions. An observation on per cent disease incidence was recorded 40 days after sowing. Per cent disease incidence and per cent control were calculated as follows:

$$\text{Per cent disease incidence} = \frac{\text{Number of wilted plants}}{\text{Total number of plants observed}} \times 100$$

The per cent disease control (PDC) was calculated by using the formula:

$$\text{Per cent disease control} = \frac{\text{Disease in control} - \text{Disease in treatment}}{\text{Disease in control}} \times 100$$

Results

Efficacy of bioagents against mycelial growth of *Fusarium oxysporum* by Dual Culture Technique (*In vitro*)

Antagonistic activity of *Trichoderma harzianum*, *Trichoderma asperellum*, *Trichoderma viride*, *Bacillus subtilis*, *Pseudomonas fluroscense* and *Aspergillus niger* were investigated in *in vitro* condition by using Dual Culture Technique on PDA medium. The results presented in table 1 showed that all bioagents were antagonistic to *Fusarium oxysporum* f. sp. *cucumerinum*.

Maximum inhibition of mycelial growth of fungus was recorded by *Trichoderma harzianum* (85.90%) followed by *Trichoderma viride* (82.50%) and *Trichoderma asperellum* (79.63%). Minimum inhibition of mycelial growth was recorded by *Bacillus subtilis* (68.52%), *Pseudomonas fluorescens* (64.81%) and *Aspergillus niger* (51.85%). Among the bio-agents used *T. harzianum* was found significantly superior followed by *T. viride* and *T. asperellum* being at par in inhibiting the mycelial growth of the fungus.

Efficacy of bioagents against Fusarium wilt of cucumber under pot condition (*In vivo*)

The efficacy of bio-agents which were found effective in *in vitro* were tested against *Fusarium oxysporum* in pots through seed treatment and the bio-agents used were, *T. harzianum* and *T. viride*. The results revealed that both bio-agents used were found significantly superior over control in reducing the per cent disease incidence at 40 DAS. Minimum disease

incidence was recorded with *T. harzianum* (33.33%) followed by *T. viride* (37.50%). T. harzianum was found significantly superior over other treatments in reducing the disease incidence. Maximum disease control over check was recorded with *T. harzianum* (38.47%) followed by *T. viride* (30.77%) over control at 40 DAS (Table 2)

Discussion

Antagonistic activity of *Trichoderma harzianum*, *Trichoderma asperellum*, *Trichoderma viride*, *Bacillus subtilis*, *Pseudomonas fluroscens* and *Aspergillus niger* were investigated in *in vitro* condition by using Dual Culture Technique on Potato Dextrose Medium (PDA). Maximum inhibition of mycelial growth of *Fusarium oxysporum* was recorded by *Trichoderma harzianum* (85.90%) followed by *Trichoderma viride* (82.50%) *Trichoderma asperellum* (79.63%), *Bacillus subtilis* (68.52%) *Pseudomonas fluorescens* (64.81%) and *Aspergillus niger* (51.85%). In general, fungal biocontrol agents are more effective in inhibiting mycelial growth of the pathogen as compared to bacterial biocontrol agents. The results obtained in present investigation are in accordance with Cho *et al*. (1989) [4] and Hamed (1999) [5] who reported *Trichoderma harzianum* as most effective bioagent against *Fusarium oxysporum* f. sp. *cucumerinum* and also justifies the findings of Akrami (2015) [1] who reported that a combination of three species of *Trichoderma* (*T. harzianum, T. asperellum and T. virens*) resulted in better protection against the Fusarium wilt and root rot of cucumber.

Among bioagents, *Trichoderma harzianum* (33.33%) was found most effective followed by *T. viride* (37.50%). The results obtained are in line with Ramesh and Singh (2017) [5] who found that talc formulated *Trichoderma* spp. were most effective in controlling *Fusarium oxysporum* f. sp *niveum* and also increased the yiled of watermelon

Table 1: Efficacy of bioagents against mycelial growth of *Fusarium oxysporum* f. sp. *cucumerinum* by Dual Culture technique on 7th day at 25 ± 2 °C

S. No	Bio-agents	Inhibition of mycelial growth* (%)
1	*Trichoderma harzianum*	85.90 (67.94)
2	*Trichoderma viride*	82.50 (65.27)
3	*Trichoderma asperullum*	79.63 (63.17)
4	*Bacillus subtilis*	68.52 (55.87)
5	*Pseudomonas fluorescense*	64.81 (53.61)
6	*Aspergillus niger*	51.85 (46.06)
7	Control	0.00 (0.00)
	SEm ±	0.87
	CD (p= 0.05)	2.67

*Average of three replications

Figures given in parenthesis are angular transformed value

Table 2: Efficacy bioagents as seed dresser against Fusarium wilt of cucumber under pot condition (*In vivo*)

Treatments	Dose	Disease incidence* at 40 DAS (%)	Per cent Disease Control (%)
T. harzianum	10 gkg-1	33.33 (35.26)	38.47
T. viride	10 g kg-1	37.50 (37.76)	30.77
Control	-	54.17 (47.39)	0.00
SEm±	1.04		
CD (p=0.05)	3.20		

*Average of three replications
Figures given in parenthesis are angular transformed values
DAS = days after sowing

Conclusion

Among six bioagents used *Trichoderma harzianum* was found most effective in inhibiting mycelial growth of *Fusarium oxysporum* f. sp. *cucumerinum* followed by *Trichoderma viride* and *T. asperellum*. *Aspergillus niger* was least effective in *in vitro* conditions by Dual Culture Method. Whereas *T. harzianum* was found most effective under pot condition.

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