Efficacy of different bio-agents and organic amendments against *Fusarium oxysporum* f. sp. *capsici in vitro*

ABSTRACT

Aims: With an objective to find out the efficacy of different bio-agents and organic amendments against *Fusarium oxysporum* f. sp. *capsici in vitro* condition. *Fusarium oxysporum* f. sp. *capsici* is a major soil-borne fungal pathogen causing wilt in chilli (*Capsicum annuum* L.), leading to significant yield losses worldwide.

Study design: CRD (Completely Randomized Design) and FCRD (Factorial Completely Randomized Design)

Place and Duration of Study: Department of Plant Patahology, C. P. College of Agriculture, SDAU, Sardarkrushinagar during *kharif*, 2023-24.

Methodology: This study evaluated the *in vitro* efficacy of different bio-agents *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* against *F. oxysporum* f. sp. *capsici*. Dual culture assays were conducted to assess percent inhibition of mycelial growth as well as efficacy of different organic amendments *viz.* mustard cake, neem cake, karanj cake, castor cake, cotton cake, poultry manure were tested *in vitro* by using poisoned food technique against *F. oxysporum* f. sp. *capsici*.

Results: Results indicated that all bio-agents significantly reduced mycelial growth compared to the control. *Trichoderma harzianum* showed the highest inhibition (79.72%), followed by *Trichoderma viride* (72.77%), *Bacillus subtilis* (50.00%) and *Pseudomonas fluorescens* (40.55%). Among six organic amendments, the highest inhibitory effect was recorded with neem cake at 5 and 10 per cent concentrations with growth inhibition of 46.15 and 50.09, respectively. The next effective amendment was cotton cake which recorded 43.00 and 44.84 per cent radial growth inhibition at 5 and 10 per cent concentrations, respectively. The mustard cake was observed as least effective in inhibiting the mycelial growth of 33.18 and 35.29 per cent at 5 and 10 per cent concentrations, respectively. Among the others, next better radial growth inhibition was observed in karanj cake with 41.88 and 45.18 per cent at 5 and 10 per cent concentrations, respectively. The poultry manure recorded 38.54 and 44.16 per cent radial growth inhibition at 5 and 10 per cent concentrations, respectively followed by castor cake with 35.01 and 37.68 at 5 and 10 per cent concentrations, respectively. These findings suggest that bio-control agents and organic amendments have potential as integrated disease management tools against *F. oxysporum* f. sp. *capsici* in chilli.

Conclusion: Among the evaluated antagonists, *Trichoderma harzianum* proved most effective, showing 79.72% growth inhibition, followed by *T. viride* (72.77%), while bacterial bio-agents exhibited comparatively lower efficacy. Among six organic amendments, the highest mean growth inhibition of 48.12 per cent was recorded with neem cake followed by cotton cake (43.92%).

Keywords: Chilli, Wilt, Bio-agents

1. INTRODUCTION

The chilli (*Capsicum annuum* L.) popularly known as “Mirchi”. Chilli shrubs are perennial and short lived, woody at the base belonging to the family Solanaceae. Chilli is locally known as red pepper, hot pepper, cayenne pepper, sweet pepper, etc. The word capsicum has been derived from the Greek word Kapsimo meaning to burn. It has been growing in India, Korea, Pakistan, Turkey and China since ancient times. Chilli has high nutritional value containing, Vitamins A, C, and E, folic acid, calcium, potassium and antioxidants (flavonoids, capsaicinoids, and carotenoids) are all abundant and 75.4 KJ in case of red chilli. Total carbohydrate content is 4.3 g in case of green chilli and 4.0 g in case of red

chilli. Green chilli contains 0.9 g of protein and red chilli contains 0.8 g of proteins. The amount of vitamin A found in green chilli, red chilli and spices or cayenne are 530 IU, 428 IU and 2185 IU respectively. The amount of calcium found in green chilli, red chilli and spices are 8.1 mg, 6.3 mg and 7.8 mg, respectively (Chakrabarty *et al*. 2017). Chilli is regarded as one of the world's most important commercial crops. Chilies, both green and dried, play a significant role in our daily diet. It give our foods the necessary pungency, colour, taste and flavour. Chilies activate our taste ssenses and as a result, enhance the flow of saliva, which contains the enzyme amylase, which aids in the digestion of starchy or cereal carbohydrates into the readily assimilated sugar glucose. Chilli's pungency is due to the presence of 'Capsaicin', which cosmetics and treatments for heart conditions. Chilli is grown throughout india, accounting for nearly 46% of the country's total acreage and production. Indian chilli occupied 7.33 lakh hectares (18.11 lakh acres) in 2019-20, with a yield of 17.64 lakh tonnes. Andhra Pradesh (49%), Karnataka (15%), Maharashtra (6%) and Tamil Nadu (3%) are the top chilli-growing states in India, accounting for almost 75% of total planted area. Despite the fact that India is the world's biggest producer, the average yield of chilli is quite low (1.11 t/ha dry chilli) when compared to industrialized countries such as the United States, China and others, where the average output ranges from 3 to 4 t/ha. Chilli is sold in more than 144 countries. India exported chilli worth 78.81 million dollars in the fiscal year 2020-2021 (April- November). In the years 2020-2021 (April-November), the total volume of exports was roughly 42 million (Anonymous., 2021). India sold 349 thousand tonnes of fresh weight equivalent chilli for a total value of USD 62 million. Panchamahal, Kheda, Mehsana, Rajkot, Amreli, Surendranagar, Dahod, Bhavnagar and Gondal are the major chilli-growing districts in Gujarat. Chilli is an indian spice that is used throughout the country. In India, production and productivity of chillies estimated are 19.14 lakh tonnes and 2576 kg/ha in year 2020-2021 respectively. In North Gujarat, chilli was cultivated in 1953 hectares with an annual production of 3718 MT and productivity of 1.90 MT/ha in year 2022-2023 (Anonymous., 2023). In the system of fungi, the genus *Fusarium* is classified in the class Hyphomycetes which belongs to sub-phylum Deuteromycotinan (Zemankova and Lebeda., 2001). *Fusarium* wilt caused by *Fusarium oxysporum* is one of the fungal disease that become a severe problem in the last decade (Anonymous., 2005). The pathogen is usually soil-borne and disease growth is aided by dry weather and abundant soil moisture. *Fusarium oxysporum* and its various formae speciales have been characterized as causing symptoms like leaf chlorosis, vascular discoloration and wilting of chilli wilt. *Fusarium* wilt results in considerable losses in terms of yield and quality of plants having agricultural and horticultural importance. The yield loss due to the disease reported were 10-80 per cent, worldwide, depending on the prevailing climatic conditions.

2. MATERIAL AND METHODS

2.1 Antagonistic effect of different bio-agents against *F. oxysporum* f. sp. *capsici* by dual culture technique:

Different antagonist bio-agents *viz*. *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated for their antagonistic activity against *F. oxysporum* f. sp. *capsici in vitro* by dual culture technique (Dennis and Webster, 1971). The tested bio agents and *F. oxysporum* f. sp. *capsici* were grown separately on potato dextrose agar (PDA) medium. The mycelial disc (5 mm diameter) of pathogen and fungal antagonists were placed on the same plate, 6 cm away from each other. To test for antagonistic bacteria, a 5 mm of the mycelial disc of pathogen cultures were placed on one side of a Petri plate containing PDA medium. A loopful of bacteria was streaked 3 cm

away from the disc of *F. oxysporum* f. sp. *capsici* on the same plate. Paired cultures were incubated at 27±2 °C. The plates inoculated only with test pathogen served as control. Four repetitions were maintained for each antagonist. The antagonistic fungal culture was maintained on PDA culture media and bacterial cultures were maintained on nutrient agar (NA) media. The assay for antagonism was performed on PDA media on Petri plates by the dual culture method. Inhibition zone were measured at 24 hours interval till the colony in the control plate covered with mycelium of pathogen. Per cent Growth Inhibition (PGI) were calculated by using the formula suggested by Vincent (1947).

$$PGI = \frac{C - T}{C} X\ 100$$

Where:-

PGI= Per cent growth inhibition
C = Colony diameter in control (mm)
T = Colony diameter in treatment (mm)

2.2 Efficacy of different organic amendments against *F. oxysporum* f. sp. *capsici in vitro*.

Efficacy of different organic amendments *viz.* mustard cake, neem cake, karanj cake, castor cake, cotton cake, poultry manure at 5 and 10 per cent, respectively were tested *in vitro* by using poisoned food technique to know their inhibitory effect on the growth of *F. oxysporum* f. sp. *capsici.* All the amendments were crushed to make a fine powder. Fifty grams powder of each amendment was taken into 250 ml flask and 150 ml water added to the flask. All these flasks were plugged with cotton and allowed for decomposing the material for 15 days. After 15 days, the material was strained with a muslin cloth to obtain the extract. The strained liquid was autoclaved at 1.045 kg/cm² pressure for 20 minutes and considered as per cent concentration (standard solution). The measured quantity of a standard solution of the organic amendments were incorporated separately in melted sterilized PDA medium in conical flasks aseptically at the time of pouring the medium to obtain desired concentrations. The medium was shaken well to give uniform dispersal and then poured about 20 ml in each sterilized Petri plate. After solidification of the medium, the petri-plates were inoculated in the centre by placing seven days old mycelial discs and then incubated at 28±2°C temperature. A control was also maintained by growing the pathogen on soil amendment free medium. Per cent Growth Inhibition (PGI) were calculated by using the formula suggested by Vincent (1947).

$$PGI = \frac{C - T}{C} X\ 100$$

Where:-

PGI= Per cent growth inhibition
C = Colony diameter in control (mm)
T = Colony diameter in treatment (mm)

3. RESULTS AND DISCUSSION

3.1 Antagonistic effect of different bio-agents against *F. oxysporum* f. sp. *capsici* by dual culture technique:

The results presented in (Table-1, Figure-.1, Plate-I) revealed that the significant difference in the growth inhibition of all the antagonist. Among the tested fungal and bacterial antagonist, the fungal antagonist found superior over bacterial antagonist. It was recorded that *Trichoderma harzianum* showed maximum growth inhibition (79.72%) which was statistically followed by *T*. *viride* (72.77%). In case of bacterial bio-agents *Bacillus subtills* (50.00%) was potential antagonists followed by *Pseudomonas fluorescens* (40.55%).

Table 1. Efficacy of different bioagents against *F. oxysporum* f. sp. *capsici in vitro*

Sr. No.	Name of bioagents	Growth inhibition (%)
1	*Trichoderma viride*	58.56 (72.77)
2	*Trichoderma harzianum*	63.23 (79.72)
3	*Pseudomonas fluorescens*	39.53 (40.55)
4	*Bacillus subtilis*	44.98 (50.00)
5	Control	4.05 (0.50)
	Mean	42.07 (48.70)
	S. Em. ±	0.79
	C. D. at 5%	2.42
	C. V. %	3.79

These findings were close enough with similar results were shown by Barari (2016) when working with tomato wilt, he found that *T. harzianum*, isolate N-8, was found to effectively inhibit the radial mycelial growth of the *F. oxysporum* f. sp. *lycopersici*. Jat *et al*. (2017[b]) proved that different bioagents against coriander wilt caused by *Fusarium oxysporum* f. sp. *coriandri* and got similar results, which indicated that *Trichoderma harzianum* was very effective in inhibiting growth of *F. oxysporum* f. sp. *coriandri*, where inhibition zone formation was significantly superior (83.69%), followed by *Trichoderma viride* with (81.17%) mycelial growth inhibition. Virani (2018) also evaluated different bioagents against *F. oxysporum* f. sp. *vasinfectum* and got similar results, which indicated that *Trichoderma harzianum* was very effective in inhibiting growth of *F. oxysporum* f. sp. *vasinfectum*, where inhibition zone formation was highest (87.50%), followed by *Trichoderma viride* (85.40 %).

3.2 Efficacy of different organic amendments against *F. oxysporum* f. sp. *capsici in vitro*.

The results presented in (Table-2, Figure-2., Plate-II) revealed that all organic amendments at 5 and 10 per cent concentrations inhibited the growth of the pathogen (*F. oxysporum* f. sp. *capsici*) significantly as compared to control. The highest inhibitory effect was recorded with neem cake at 5 and 10 per cent concentrations with growth inhibition of 46.15 and 50.09, respectively. The next effective amendment was cotton cake which recorded 43.00 and 44.84 per cent radial growth inhibition at 5 and 10 per cent concentrations, respectively. The mustard cake was observed as least effective in inhibiting

the mycelial growth of 33.18 and 35.29 per cent at 5 and 10 per cent concentrations, respectively. Among the others, next better radial growth inhibition was observed in karanj cake with 41.88 and 45.18 per cent at 5 and 10 per cent concentrations, respectively. The poultry manure recorded 38.54 and 44.16 per cent radial growth inhibition at 5 and 10 per cent concentrations, respectively followed by castor cake with 35.01 and 37.68 at 5 and 10 per cent concentrations, respectively.

Table 2. Efficacy of different organic ammendments against *F. oxysporum* f. sp. *capsici in vitro*

Tr. No.	Treatments	Growth inhibition (%)		
		Concentration (%)		Mean
		5%	10%	
T1	Mustard cake	35.15 (33.18)	36.42 (35.29)	35.79 (34.23)
T2	Neem cake	42.77 (46.15)	45.03 (50.09)	43.90 (48.12)
T3	Karanj cake	40.30 (41.88)	42.21 (45.18)	41.26 (43.53)
T4	Castor cake	36.26 (35.01)	37.85 (37.68)	37.05 (36.34)
T5	Cotton cake	40.95 (43.00)	42.01 (44.84)	41.48 (43.92)
T6	Poultry manure	38.35 (38.54)	41.62 (44.16)	39.99 (41.35)
T7	Control	4.05 (0.50)	4.05 (0.50)	4.05 (0.50)
	Mean	33.98 (34.03)	35.60 (36.82)	-
	S. Em. ±	Treatment	Concentration	Treatment× Concentration
		0.27	0.14	0.38
	C. D. at 5%	0.78	0.41	1.11
	C. V. %	2.10		

Similar findings were also reported by earlier research workers. Yelmame *et al*. (2010) reported the effect of organic amendments and concluded that among tested amendments, neem cake was the most effective in controlling the wilt disease in chilli. Vahunia *et al*. (2017) evaluated the efficacy of organic amendments and revealed that neem cake was the best in inhibiting mycelial growth for the control of wilt pathogen in castor. Theradimani *et al*. (2018) evaluated the efficacy of organic amendments and revealed that neem cake was the best in inhibiting mycelial growth for the control of wilt pathogen in tomato.

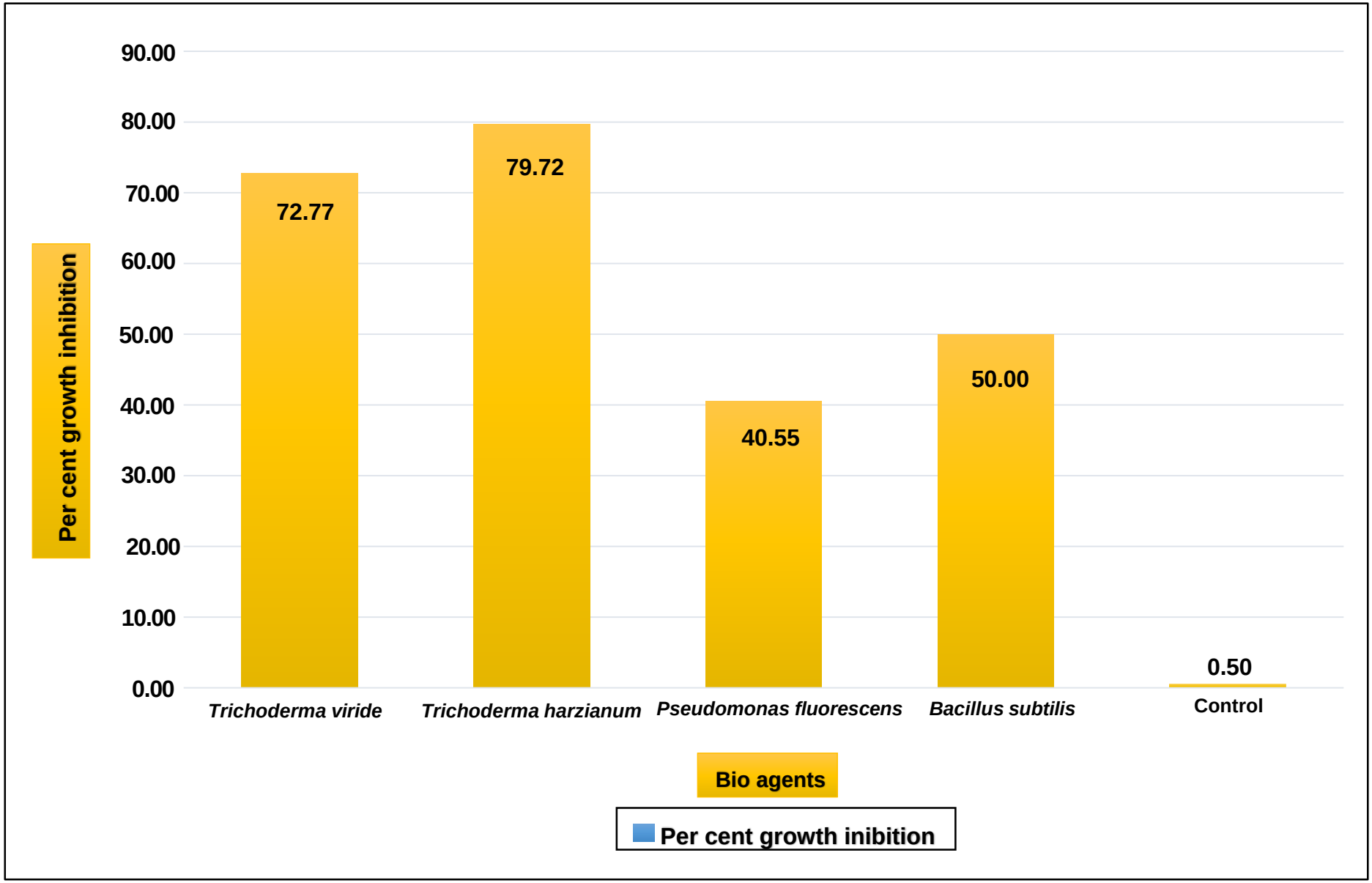


Figure 1: Efficacy of different bioagents against *F. oxysporum* f. sp. *capsici in vitro*

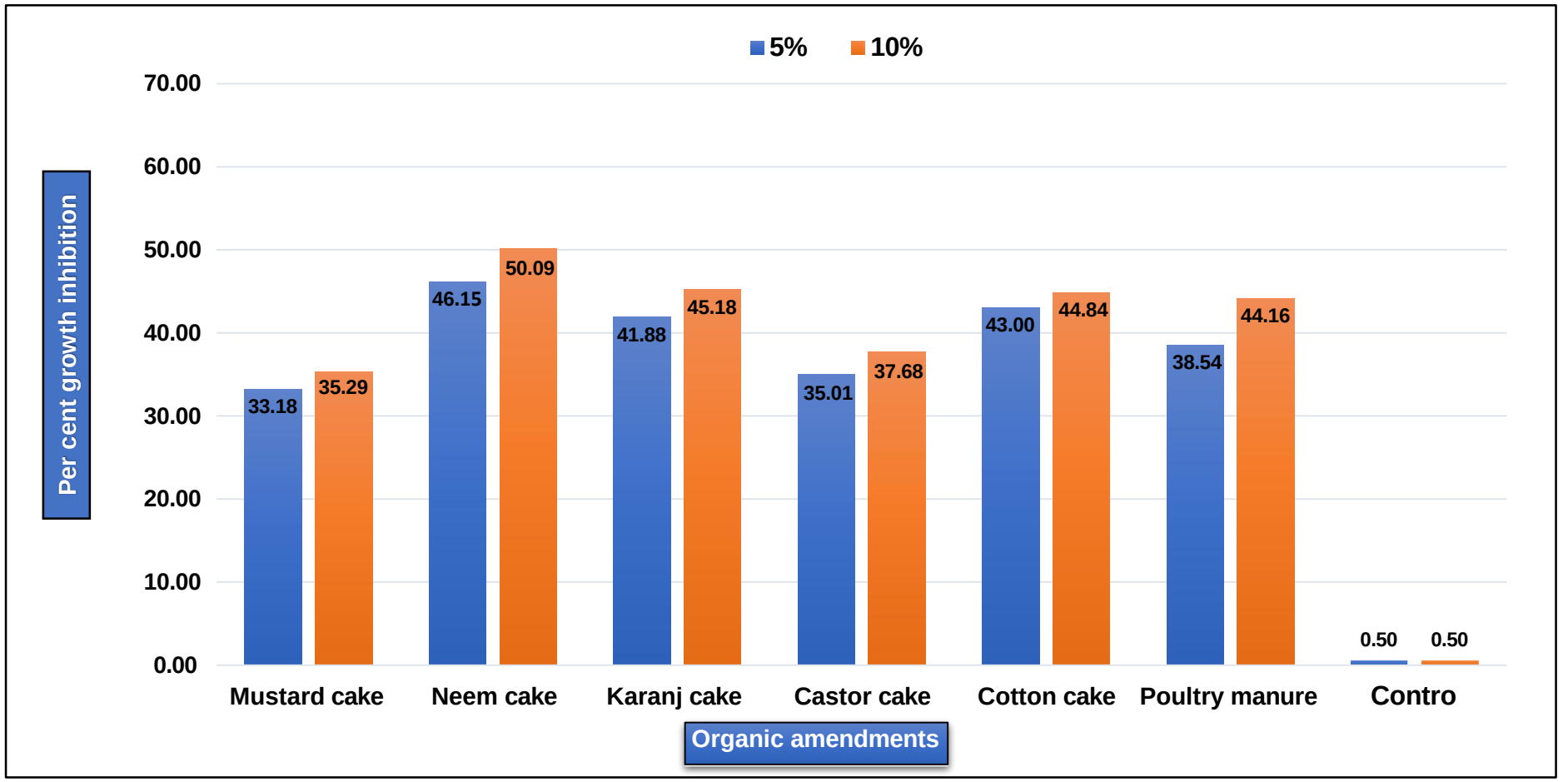


Figure 2.: Efficacy of different organic amendments against *F. oxysporum* f. sp. *capsici in vitro*

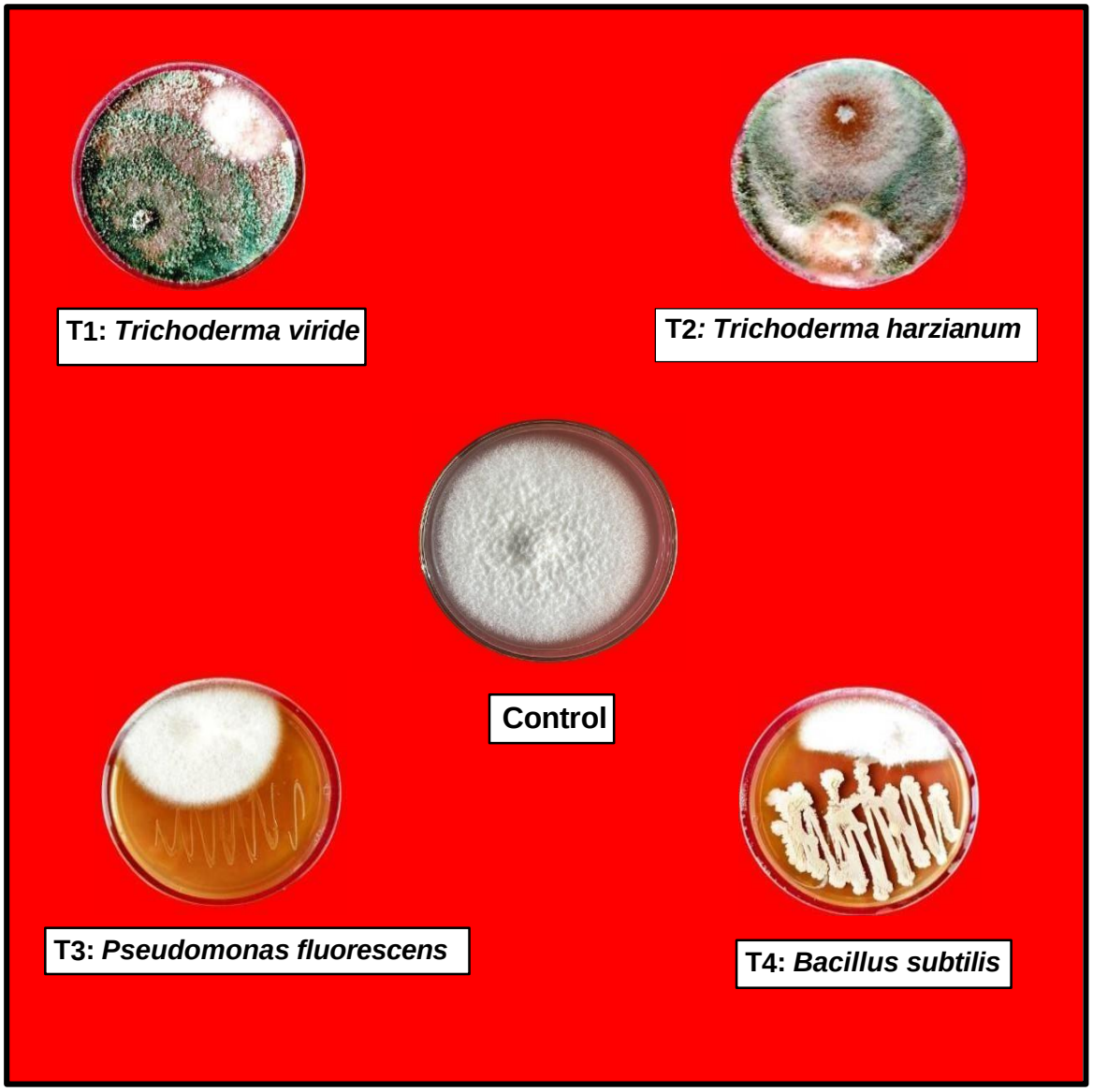
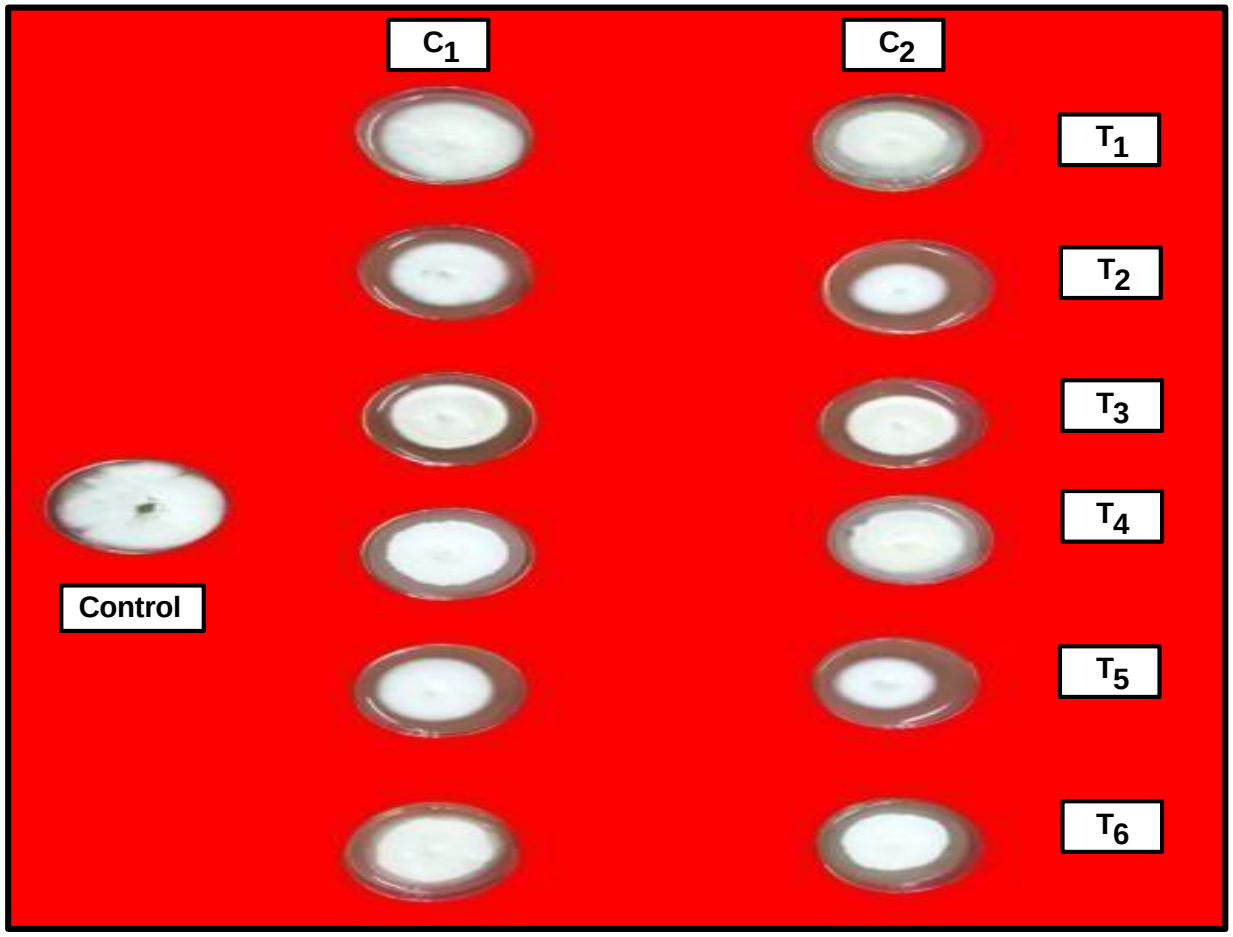


Plate I: Efficacy of bio-agents against *F. oxysporum* f. sp. *capsici in vitro*

Plate 2: Efficacy of different organic amendments (at 5 and 10 %) against F. oxysporum f. sp. capsici in vitro



T_1- Mustard cake
T_2- Neem cake
T_3- Karanj cake
T_4- Castor cake
T_5- Cotton cake
T_6- Poultry manure
T_7- Control

C_1- 5%
C_2- 10%

CONCLUSION

Based on present study it can be concluded that chilli is one of the important spice and vegetable crops, suffering seriously from wilt diseases. Among the evaluated antagonists, *Trichoderma harzianum* proved most effective, showing 79.72% growth inhibition, followed by *T. viride* (72.77%), while bacterial bio-agents exhibited comparatively lower efficacy. Among six organic amendments, the highest mean growth inhibition of 48.12 per cent was recorded with neem cake followed by cotton cake (43.92%).

REFERENCE

Anonymous, (2005). Annual Report of Network Project on Wilt of chilli. ICAR, India, New Delhi, pp. 1-7.

Anonymous, (2021). District wise estimated area and production of horticultural crops for the year 2020-21. Directorate of Horticulture, Ministry of Agriculture and Farmers Welfare, Government of Gujarat, pp. 1-11.

Anonymous, (2023). District wise estimated area and production of horticultural crops for the year 2022-23. Directorate of Horticulture, Ministry of Agriculture and Farmers Welfare, Government of Gujarat, pp. 1-1.

Barari, H. (2016). Biocontrol of tomato *Fusarium* wilt by *Trichoderma* species under *in-vitro* and *in-vivo* conditions. Cercetari Agronomice in Moldova, 1(165), 91-98.

Chakrabarty, S., Islam, A. K. M. & Islam, A. (2017). Nutritional benefits and pharmaceutical potentialities of chilli: A Review. Fundamental and Applied Agriculture, 2(2), 227-232.

Dennis C & Webster J. (1971). Antagonistic properties of species-groups of Trichoderma: I. Production of non-volatile antibiotics. Transactions of the British Mycological Society, 57(1), 25-IN3.

Jat, M. K., Ahir, R. R., Choudhary, S., Kakraliya, G. L. (2017[b]). Management of coriander wilt using biocontrol agents. International Journal of Chemical Studies, 5(2), 523-525.

Theradimani, T.; Susitha, S. and Amudha, C. (2018). Biocontrol of *fusarium* wilt in tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*. International Journal of Current Microbiology and Applied Sciences, 7(9), 420-429.

Vahunia, B., Singh, P. and Vaja, S. J. (2017). Eco-friendly management of castor (*Ricinus communis* L.) wilt caused by *Fusarium oxysporum* f. sp. *ricini* by organic manure/cakes *in vitro*. Trends in Biosciences, 10(45), 9253- 9255.

Vincent J. M. (1947). Distortion of fungal hyphae in the presence of certain inhibitors. Nature,150: 840.

Virani, A. B. (2018). Characterization of *Fusarium oxysporum* f. sp. *vasinfectum* [(Atk.) Snyder & Hansen] causing wilt of cotton and its management. M. Sc. (Agri.) thesis submitted to J. A. U., Junagadh.

Yelmame, M. G., Mehta, B. P., Deshmukh, A. J. and Patil, V. A. (2010). Evaluation of some organic extract in *in vitro* to control *Fusarium solani* causing chilli wilt. International Journal of Pharma and Biosciences, 1(2), 1-4.

Zemankova, M. and Lebeda, A. (2001). *Fusarium* species, their taxonomy, variability and significance in plant pathology–a review. Journal of Plant Protection Sciences, 37(1), 25-42.