Integrated Management of *Fusarium* wilt of Chilli caused by *Fusarium solani*

Abstract:

Chilli is an important spice cum vegetable crop from the Solanaceae family grown around the world for its pungent flavor. Fusarium wilt of chilli is one of the most important diseases as it drastically reduces the yield. In the present experiment a total of 4 different botanical extracts and 4 isolates of *Trichoderma* were evaluated against the growth of wilt pathogen *Fusarium solani.* Among themneem oil and Tr1 isolate of *Trichoderma* recorded better results hence they were selected and further studied under pot culture conditions alone in combinations with panchagavya on the incidence of *Fusarium* wilt of chilli.Out of 7 different treatments tested, highest germination (93.24%) was recorded from T7. And, highest percent disease inhibition of *Fusarium* wilt (69.56%) was recorded from T7. On a similar note, plant growth parameters such as improved plant height, dry weight, no. of flowers/ fruits per plant and fruit length were recorded from plants treated with T7. It was clear the IDM strategies perform better in reducing disease as compared to that of chemical control besides improving yield and growth of treated plants.

Introduction

Chilli, scientifically identified as *Capsicum annuum* L. (chromosome number 2n=24), is a member of the Solanaceae family. This plant holds significance as a crucial vegetable and spice crop cultivated worldwide. Chilli was first introduced into India by Portuguese traders in the year 1584 **(Atiq et al. 2022)**. India holds the distinction of being the leading global producer and consumer of chilli, with an impressive total production of approximately 4.221 billion tonnes. In India, the states of Andhra Pradesh, Karnataka, Tamil Nadu, and Maharashtra collectively contribute to around 75% of the total cultivated area dedicated to chilli. Additionally, chilli cultivation extends to Haryana, covering an estimated 13.290 thousand hectares and yielding a total production of 141.650 thousand tonnes**(NHB)**. Chilli production is being affected by so many constraints which include diseases among which wilt disease caused by *Fusarium* spp. play a very crucial role. Wilt symptoms initiate with a mild yellowing of older leaves, progressing to younger leaves, leading to chlorosis and dryness.

This is subsequently followed by gradual withering and the eventual demise of the entire plant **(Khan *et al*., 2018)**.*Fusarium* spp. cause morphological changes in plants, including chlorosis, leaf fall, necrosis, seedling crumbling, stunting, and wilting. Severe water stress, primarily caused by vessel occlusion, manifests as the wilting symptoms.These alterations contribute to a yield loss ranging from 10% to 80% in the global production of chili peppers**(Loganathan *et al*., 2013)**.The fungus penetrates the plant through its roots and progresses from within the roots to the cortex. It produces microconidia in this region, which enter the sap stream and ascend. Additionally, the mycelia from germinated spores cause the clogging of vascular vessels, ultimately resulting in plant death due to wilting. Moreover, the fungus produces multiseptated macroconidia, which serve to infect neighbouring plants. It resides in the soil surrounding affected plants and reproduces through chlamydospores**(Smith, 2007)**. For decades, chemical agents have been widely used without discrimination to decrease the occurrence and contain the spread of this plant disease, leading to a variety of ecological problems.The agricultural sector is currently transitioning towards ecological methods of disease control, with chemicals either prohibited or subject to restrictions in their usage. Plant extracts offer an effective solution for managing *Fusarium* wilt disease, serving as an alternative to depending solely on fungicides as they strong anti-fungal properties. Various botanicals such as neem, garlic, datura leaf extract, and different plant oils are successfully employed in disease management strategies. This is attributed to their environmentally friendly nature and their lack of adverse effects on non-target organisms **(Enespa and Dwivedi, 2014)**. Previously studies conducted by **Singh *et al*. (2017)**and**Sitara *et al*. (2011)** clearly stated that neem oil significantly reduced the incidence of *Fusarium* wilt when tested under field conditions. Liquid organic formulations are dynamic organic inputs derived from the fermentation of animal products over a specific period. These formulations are abundant in micro and macro nutrients, as well as plant growth-promoting hormones**(Suchith Kumar and Singh, 2021)**.The application of organic liquid formulations such as panchagavya to plants has a notable positive impact on their growth and development. Additionally, these formulations exhibit suppressive effects on various plant diseases **(Balraj *et al*., 2014)**. Indeed, the use of biocontrol agents (BCAs) in the biological control of plant diseases has demonstrated effectiveness and is often perceived as a safer and environmentally friendly alternative to chemical fungicides as they are ecofriendly, compatible with various organic practices and show very low to no resistance **(Brimmer and Boland, 2003)**.*Trichoderma* spp. is indeed well-known and widely studied biocontrol agents that have demonstrated effectiveness in managing plant diseases and promoting plant growth. *Trichoderma* is a genus of fungi commonly found in soil, and some species within this genus have beneficial properties for plants **(Savazzini*et al*., 2019)**. The main mechanisms behind inhibition of pathogens by antagonistic microorganisms include competition, secretion of cell wall degrading enzymes, secreting antimicrobial metabolites**(Carmona-Hernandez, 2019)**.Keeping in view of the effectiveness of above mentioned liquid organic formulations, botanical extracts and bio control agents, an alternative sustainable management strategy must be developed for the management of *Fusarium* wilt of chilli under field conditions.

Material and methods

A survey was conducted in chilli fields of Kakinada district in Andhra Pradesh. The disease was identified based on the symptoms and the pathogen responsible for *Fusarium* wilt was isolated from the tissues of the infected plants which were collected separately. A total of 4 different botanical extracts*viz.*, Neem oil, neem leaf extract, garlic bulb extract and eucalyptus leaf extract were assayed against *Fusarium* spp. invitroby employing poisoned food technique at two different concentrations i.e., 0.1% and 0.2%. Similarly, 4 isolates of *Trichoderma*i.e., Tr1, Tr2, T3, Tr4 were tested against *Fusarium* spp. *invitro*by employing dual culture technique.

Integrated management of *Fusarium* wilt of chilli under pot culture

After observing outcomes from in vitro studies, Tr1 derived from *Trichoderma* spp. and neem oil extract from botanical sources were specifically chosen. These selections underwent testing in experimental field conditions in combinations with liquid organic preparation, Panchagavya, to investigate both their individual and collective impact on the occurrence of wilt disease in chili caused by *Fusarium* spp. The experiment was laid in randomized block design with a total of 10 treatments and 3 replications w

The treatments were applied as seed treatments before sowing.The treatments were as follows.

T1- Neem oil, T2 - Panchagavya, T3 –*Trichoderma*spp., T4 – Neem oil + Panchagavya, T5 – Neem oil + *Trichoderma* spp., T6 – Panchagavya + *Trichoderma*spp., T7 – Neem oil + Panchagavya + *Trichoderma*spp., T8 – Carbendazim, T9 – inoculated control, T10 – Healthy control

Rate of application: Neem oil @ 0.2%, Panchagavya @ 3%, Trichoderma spp. @ 4g/kg and Carbendazim @ 2g/kg

Chilli seeds of local variety were sown in plug trays following treatments and transplanted to pots with sterilized soil after 4 weeks. The intensity of the disease (Percent disease incidence) was calculated and compared with that of controls (healthy and inoculated) 30 days after transplantation.

$$\text{Percent disease incidence} = \; = \frac{\text{No. of infected plants}}{\text{Total number of plants observed}} \text{ X } 100$$

Observations on growth and yield parameters

Growth and yield characters such asplant height (cm), biomass (g),no. of flowers per plant,no. of fruits per plant, fruit lengthwas recorded from each treatment.

Statistical Analysis

Statistical analysis has been performed by employing ANOVA on the data obtained from all the experiments. Critical difference and Standard error were worked out for in vitro (P= 0.01) studies and in vivo (P= 0.05) studies and results were interpreted accordingly. The statistical analysis was carried out in OPSTAT website of Hisar Agriculture University, Hisar **(Sheoran*et al*., 1998)**.

Results and Discussion

Among the four botanical extracts tested, highest inhibition of the mycelial growth of the pathogen was recorded by neem oil (50.37%) at 0.2% concentration (Table 1). Followed by neem leaf extract (45.80%) at 0.2% concentration. However, garlic bulb extracts and eucalyptus leaf extracts recorded significantly lower and similar percent inhibition of mycelial growth of pathogens at both the tested concentrations i.e., 0.1% and 0.2%.Similar works were earlier performed by**Khokhar *et al*. (2014)** in which they tested the effect of five different botanicals extracts on the mycelial growth of *Fusarium verticillioides* and reported that neem oil at 0.2% concentration recorded 52.2% inhibition.

Singh *et al*. (2017)tested the effect of 12 botanicals at 4 different concentrations and reported that neem oil and garlic oil at 10% concentration completely inhibited the growth of chilli wilt pathogen*Fusarium oxysporum.* Presence of antimicrobial compounds might be responsible for the inhibitory effect of plant extracts against plant pathogens. Anti-fungal activity of neem products might be due to the presence of compounds such as nimbin, nimbidin, salannin, azadirachtin etc. **(Ramprasad, 2005; Manea *et al*., 2022; Wasim *et al*., 2023)**.

Table 1.Effect of different botanical extracts on the mycelial growth of *Fusarium*sp. by employing poisoned food technique

S. No	Concentration	Colony Diameter (mm)*				Percent Inhibition			
		Neem oil	Neem leaf extract	Garlic bulb extract	Eucalyptus leaf extract	Neem oil	Neem leaf extract	Garlic bulb extract	Eucalyptus leaf extract
1	0.1	46.36	50.73	57.66	58.03	35.89	29.86	20.27	19.76
2	0.2	35.89	39.2	55	55.66	50.37	45.80	23.96	23.04
3	Control	72.33	72.33	72.33	72.33	0.0	0.0	0.0	0.0
CD at 5%		3.31	1.77	3.96	5.74	-			
SE (m)±		0.93	0.50	1.12	1.62				

Four different *Trichoderma* isolates were isolated from the rhizosphere region of healthy chilli plants. All the tested isolates of *Trichoderma* significantly reduced the growth of chilli wilt pathogen *in vitro*. Among the isolates highest radial growth inhibition of the pathogen was recorded by Tr1 at 63.91% followed byTr4 (56.27%) and Tr2 (55.06%) which were significantly on par with each other. However, the least radial growth inhibition of the pathogen was record by Tr2 at 53.05% (Table 2). Based on the morphological studies, the isolated Tr1 was identified as *Trichoderma asperellum*.The inhibitory effect of different *Trichoderma* spp. (*Trichoderma longibrachiatum*, *T. harzianum* and *T. atroviride*) against *Fusarium solani* was proved earlier by **Boureghda*et al*. (2009)**. Similarexperiments were earlier performed by **Patel *et al*. (2023)** in which they tested the antagonistic activity of 6*Trichoderma* isolates and reported thatBVVT-1 isolate recorded highest inhibition of radial growth of *Fusarium* spp. It was earlier reported by **Islam *et al*. (2022)** that *Trichoderma harzianum* recorded a remarkable 81.67% mycelial growth inhibition of *Fusarium oxysporum* in dual culture technique after 7 days.Similarly, studies conducted by **Mishra *et al*. (2017)**revealed that among different Trichoderma isolates tested, CA-09 recorded maximum growth inhibition of *Fusarium oxysporum*f.sp. *capsici*. They further reported that the main mechanisms behind the inhibition of radial growth of pathogens might be due to competition, mechanical obstruction and hyper parasitism. *Trichoderma* spp. produces a range of enzymes, including chitinase, cellulase, β-1-3-glucanase, and protease, along with secondary metabolites. These substances contribute to the breakdown of the cell walls in soil-borne pathogens, leading to the inhibition of their mycelial growth**(Saravanakumar *et al*., 2016; Li *et al*., 2016)**.

Table 2. Effect of different *Trichoderma* spp. on the radial growth of *Fusarium* spp. by employing dual culture technique

S. No.	*Trichoderma* spp.	Radial Growth* (mm)	Percent inhibition
1	Tr1	23.93	63.91
2	Tr2	31.13	53.05
3	Tr3	29.80	55.06
4	Tr4	29.00	56.27
5	Control	66.32	-
CD at 5%		3.55	-
SE (m)±		1.11	

Effect of different treatments on germination per cent and incidence of *Fusarium* wilt

A total of 10 different treatments were tested under pot culture conditions on the incidence of *Fusarium* wilt of chilli. Among all the treatments assayed,highest germination (93.24%) was recorded from the plants treated with T7 which is a combination of Neem oil, panchagavya and *Trichoderma*. This was followed bytreatments T6 and T3 which recorded germination of 84.23% and 83.34% respectively. Lowest germination of 74.86% was recorded by treatment T1 in which the seeds were treated with neem oil alone (Table 3). However, the seeds treated with carbendazim recorded 89.2% germination which is statistically on par with T7. Inoculated control and healthy control resulted in to germination of 58.23% and 72.54% respectively.

All the treatments significantly reduced the incidence of *Fusarium* wilt under pot culture conditions. Among the treatments, highest disease inhibition was recorded from T7 which was 69.56% in which the plants are treated with a combination of neem oil, panchagavya and *Trichoderma*. Similarly, treatments T6, T5 and T3 recorded percent disease inhibition of 58.92, 46.89 and 35.84 respectively. The lowest percent disease inhibition was recorded from the treatments T2 and T1 which are statistically on par with each other. However, plants treated with carbendazim recorded highest percent disease inhibition of 73.95.

Effect of different treatments on various growth parameters of chilli

A total of 7 different treatments were tested under pot culture conditions along with a chemical check and two controls. All the treatments significantly increased the growth treated plants. Among them, highest plant growth parameters such as plant height (62.8 cm), dry weight (38.12), no. of flowers per plant (21), no. of fruits per plant (41) and fruit length (9.5 cm) was recorded from the plants treated with T7 which is a combination of neem oil, panchagavya and *Trichoderma asperellum*(Table 4). This was followed by T6 in which the seeds were treated with panchagavya and *T. asperellum.* However, the lowest growth parameters were recorded from the plants in which the seeds were treated with Neem oil alone (T1). Although plants treated with carbendazim recorded higher growth characteristics, they were statistically on par with T7. This shows that the combination of panchagavya + *T. asperellum* + neem oil performed superior to that of a chemical fungicide (carbendazim) in terms of decreased disease incidence and improved plant growth promotion.

Table 3. Effect of different treatments on germination and incidence of *Fusarium* wilt disease at 60 DAT

Treatment No	Treatments	Germination (%) *	Percent disease incidence (%) *	Percent efficacy of disease control (%) *
1	Neem oil	74.86	24.54	10.24
2	Panchagavya	77.21	23.17	15.25
3	*Trichoderma asperellum*(Tr1)	83.34	17.54	35.84
4	Neem oil + Panchagavya	78.32	21.22	22.38
5	Neem oil + *T*.asperellum(Tr1)	80.23	14.52	46.89
6	Panchagavya + *T.asperellum* (Tr1)	84.23	11.23	58.92
7	Neem oil + Panchagavya + *T.asperellum* (Tr1)	93.24	8.32	69.56
8	Carbendazim	89.2	7.12	73.95
9	Inoculated control	58.23	39.32	-
10	Healthy control	72.54	27.34	-
CD at 5%		3.477	-	
SE (m)±		1.161		

*Mean of 3 replications

Table 4.Effect of different treatments on various growth parameters of chilli

Treatment No	Treatments	Plant Height (cm)	Dry weight (g)	Flowers per plant	Fruits per plant	Fruit Length (cm)
1	Neem oil	49.3	29.81	15	35	7
2	Panchagavya	50.03	30.54	15	35	7
3	*Trichoderma asperellum*(Tr1)	51	31.51	16	36	7.9
4	Neem oil + Panchagavya	53.23	33.74	17	37	8
5	Neem oil + *T.*asperellum(Tr1)	53.96	34.47	18	38	8.7
6	Panchagavya + *T.asperellum* (Tr1)	56.96	37.05	20	40	9.1
7	Neem oil + Panchagavya + *T.asperellum* (Tr1)	62.8	38.12	21	41	9.5
8	Carbendazim	55.60	36.09	19	39	8.3
9	Inoculated control	43.87	31.38	15	35	7.6
10	Healthy control	50.30	30.81	15	35	7.1
CD at 5%		2.7	1.02	0.66	1.97	0.32
SE (m)±		0.91	0.34	0.22	0.63	0.11

Similar experiments were performed by **Islam *et al.* (2022)** in which they tested the effect of *Trichoderma harzianum* under greenhouse conditions and reported that the treated plants recorded reduced incidence (38.46%) of chilli*Fusarium* wilt.They further reported the increase in growth parameters such as shoot length, root length and vigour index etc. of the treated plants.*Trichoderma* releases diverse secondary metabolites, such as gibberellins, indole-3-acetic acid, siderophores, etc., into the soil surrounding the roots. These substances could potentially contribute to enhanced plant growth while minimizing disease susceptibility **(Zhou *et al*., 2018)**.**Bhat *et al*. (2016)** previously tested the effect of *T. viride*under field conditionsat two different places on the incidence of fusarium wilt of chilliand reported that the treated plants recorded reduce incidence of disease (10.8% and 38.5%)in both the seasons.On a similar note, *Trichoderma* spp. when applied as soildrenching the disease incidence to 24.7% as compared to that of 83% in control. Besides disease control the treated plants also recorded increased shoot length, root length, fresh weight and dry weight **(Anjum**

***et al*., 2020)**. The current findings align with those documented in prior research, demonstrating that *Trichoderma* spp. has proven effective in enhancing the growth of lettuce, tomato, and pepper plants**(Vinale*et al*., 2006)**.*Trichoderma* spp. have been noted for their ability to generate diverse plant growth-promoting substances, as well as to solubilize phosphorus and other minerals like iron (Fe), manganese (Mn), and magnesium (Mg). This process enhances nutrient uptake, subsequently contributing to improvements in various growth and yield parameters in treated plants **(Vinale*et al*. 2008)**.

Panchagavya, an organic product, is advocated for enhancing crops in organic agriculture. It finds application as a foliar spray, in soil application combined with irrigation, and as a seed treatment**(Sangeetha and Thevanathan, 2010)**.Panchagavya has played a pivotal role in conferring resistance to pests and diseases, leading to a notable increase in overall yields **(Tharmaraj*et al*., 2011)**. Studies conducted by **Rao *et al*. (2015)** clearly indicated thatpanchagavya when applied in soil and as spray on chilli plants significantly improved various growth parameters such as plant height, number of branches, number of flowers, number of fruits and fruit length as compared to that of control. Panchagavya exhibits the capability to supply plant hormones macro and micronutrients, contributing to the heightened ascorbic acid content in chili fruits as previously confirmed by **Shailaja *et al*. (2014)** and **Madhukumar *et al*. (2018)**.The increased plant growth and yield characteristics observed in treated plants can be attributed to the presence of essential nutrients and plant growth-promoting substances, such as IAA, GA3, and cytokinins**(Perumal *et al*., 2006)**.Panchagavya was found to reduce the incidence of *Fusarium wilt* caused by *Fusarium oxysporum*and *Fusarium solani***(Rathore and Patil, 2019)**. In addition to its plant growth-promoting capabilities, the antifungal activity of Panchagavya is credited to the existence of ammonia toxicity and antagonist microbes. These factors play a crucial role in inhibiting plant pathogens **(Mehta *et al*., 2014)**.

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