



Targeting *Fusarium oxysporum* f.sp. *lycopersici*: *In vitro* Efficacy of Aqueous Rhizome Extracts from Different *Curcuma* spp.

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Abstract

An *in vitro* study was performed with aqueous rhizome extracts of four different *Curcuma* species, namely, *Curcuma longa*, *C. aromatica*, *C. caesia* and *C. amada*, against fusarium wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici*. The rhizome extracts were prepared in water and were tested at 1%, 2% and 5% concentrations using the poisoned food technique. Among all treatments, the 5% extract of *C. longa* showed the highest mycelial growth inhibition of 74.07% over control, followed by *C. caesia* at 5% and 5% *C. aromatica*. All extracts were more effective at higher concentrations, indicating a dose-dependent response. The results suggest that aqueous extracts of *Curcuma* spp. have strong antifungal potential and serve as eco-friendly and easily available alternatives for managing *Fusarium* wilt of tomato.

Keywords: Aqueous extract, *Curcuma* spp., *Fusarium oxysporum*, *In vitro* efficacy, Tomato

Introduction

In modern farming, one of the main problems for industries is the efficient management of plant diseases, which causes yearly crop losses of 20-40% (Fenu and Mallocci, 2021). The two main categories of pathogenic organisms that cause the most damaging plant diseases, impacting both natural and production systems, are fungi and oomycetes (Kamoun et al., 2015; Pham et al., 2017). *Fusarium* wilt is one of the most destructive fungal diseases of a wide range of economically important crops, such as tomatoes, legumes, bananas, etc. It causes significant yield loss in the infected crops. Due to the increasing and indiscriminate use of fungicides, the fungicidal residue on fruit, soil and the development of resistance by the pathogens have become a matter of great concern for researchers. As a result, more efforts are being directed towards safer alternatives, such as the use of botanicals to control the pathogens. In tomato (*Solanum lycopersicum* L.), *Fusarium* wilt is caused by the pathogen *Fusarium oxysporum* f.sp. *lycopersici*, which results in 10-90% yield loss in temperate regions. It causes 90% of crop losses with repeated infection, especially in the same growing season in greenhouses (Vignesh et al., 2021). The pathogen

can survive in the soil as chlamydospores, which remain viable for many years (Fu et al., 2017). Temperature plays an important role in the development of *Fusarium* wilt diseases. Kaur et al. (2022) reported that the growth of *Fusarium* wilt pathogens is usually greatest at 23-27 °C peaking at 28 °C and is inhibited above 33 °C and below 17 °C.

Agrios (1988) reported that the initial symptoms produced due to *Fusarium oxysporum* infection were vein clearing in the outer margin of the leaves, followed by epinasty, yellowing and marginal necrosis of older leaves; wilting of leaves and stem, defoliation and finally death of the entire plant. On the dissection of the main stem of infected plant, dark brown longitudinal stripes can be seen.

Curcuma spp., members of the Zingiberaceae family, are rich in natural chemicals and exhibit a variety of bioactivities. The importance of turmeric as an antimicrobial agent has undergone substantial transformation with the identification of the antioxidant and antibacterial capabilities inherent in its phenolic components (Alolga et al., 2022). The turmeric extract inhibits the growth of fungi by altering the morphology of hyphae, which can cause them to appear collapsed, break down the plasma membrane, damage

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the mitochondria, remove cytoplasm, fold the nuclear membrane and thicken the cell wall. These changes are attributed to the chemical components of the spice extract (Murugesh et al., 2019).

Normally aqueous extract is prepared from water infusion or decoctions mainly because of the polarity of water helps in extracting the polar compounds very efficiently (Abubakar and Haque, 2020); whereas, solvent are made from organic solvents or aqueous organic mixtures having a broader range of polarity which has the ability to target specific compound of class (Arya et al., 2025). The main advantage of aqueous extract is that water is non toxic and non inflammable and leaves no residues with low cost and availability. Apart from this water provides a good polar bioactives like phenolics, flavonoid, tannins and saponins which are identified as pure water soluble (Abubakar and Haque, 2020). The limitations of aqueous extract are lower selectivity and more impurities including thermal and hydrolytic dehydrations (Ncama et al., 2025). Moreover aqueous extract have microbial stability issues and poor recovery of non polar compounds (Abubakar and Haque, 2020). Compared to aqueous extract solvent extract having higher extraction, better stability and handling with access to lipophilic bioactives compounds (Arya et al., 2025) and disadvantage are toxicity, residues, higher cost with potential degradation of sensitive compounds.

The present study was performed to explore and compare the *in vitro* efficacy of four different *Curcuma* spp. namely: *Curcuma longa* L., *Curcuma aromatica*, *Curcuma caesia* Roxb. and *Curcuma amada*. By disrupting the growth of mycelia, *C. longa* has been said to have harmful effects on fungi implicated in the degradation of agricultural products (Ferreira et al., 2013). Similarly, *C. aromatica* (wild turmeric) and *C. amada* (Mango ginger) possess significant antimicrobial activity due to the presence of terpenoids and essential oils (Dosoky and Setzer, 2018). *C. caesia* Roxb. i.e., black turmeric, which is native to northeast India, has a characteristic bluish colour rhizome is used in native medicines due to its antimicrobial activity.

Materials and Methods

Geographical Location

The experiment was carried out in the Nanotechnology laboratory, Department of Plant Pathology, Assam Agricultural University, Jorhat-13. The experiment site is situated at 26°43' N latitude, 94°12' E longitude and 86.8 meters above mean sea level. The experiment was carried out from the month of March to May, 2024.

Plant Materials

Fresh rhizomes of turmeric (*C. longa* L.), wild turmeric (*C. aromatica*), black turmeric (*C. caesia* Roxb.) and mango ginger (*C. amada*) were taken from the Horticulture Experimental Farm, Assam Agricultural University, Jorhat-13 maintained in All India Coordinated Research Project on Medicinal and Aromatic Plants and Betelvine, which authenticate the planting material.

Test Organism

The fungal culture of *Fusarium oxysporum* f.sp. *lycopersici*,

which is the causal organism of *Fusarium* wilt of tomato, has been taken from the Nanotechnology laboratory, Department of Plant Pathology, Assam Agricultural University, Jorhat with the accession number PV156109.

Maintenance of the Culture

The pure culture of the pathogen was maintained throughout the period of investigation on potato dextrose agar (PDA) slant by routine sub-culturing at regular intervals and preserved in a refrigerator at 4 °C for further study.

Preparation of Plant Extract

The plant extracts were prepared by using the standard protocol given by Ekefan et al. (2018) with slight modification. The collected rhizomes were washed properly to remove the soil present on the surface. Then the rhizomes were left to air dry. The outer skin of the rhizomes was peeled off and the rhizomes were cut into small pieces. The pieces were then dried at 60 °C for 3 hrs. 5 g of dried rhizomes were taken and ground properly with the help of a mortar and pestle. 25 ml of deionized water (1:5 w/v) was added to the ground powder and then the mixture was stirred and left for 2 hours and subsequently filtered using sieve cloth and then through Whatman No. 1 filter paper to get the aqueous extract of the rhizome. The extract was then stored at 4 °C in refrigerator for further use.

Preparation of Different Concentrations of Rhizome Extract

The stock solutions of different curcuma species which was kept in 4 °C in refrigerator was used for making different concentration of solutions and it was considered as 100 percent. From these different concentrations like 1%, 2% and 5% was prepared for further conducting the experiments.

Antifungal Assay of the Aqueous Rhizome Extract

The antifungal activity of the prepared extract was performed by using the poisoned food technique (Nene and Thapliyal, 1993). Firstly, fresh and young fungal cultures were prepared by subculturing from the original culture of the pathogen on new PDA media. The 5-day-old culture was used for the poisoned food assay. The antifungal efficiency of the aqueous extract was tested at 3 different concentrations of 1%, 2% and 5%. The desired concentrations of the plant extracts were prepared on PDA media and the PDA containing extracts were poured into sterilized petri dishes having a diameter of 9.0 cm. After the solidification of the media, a mycelial dish was cut out at the margin of the mycelial colony with the help of a sterile stainless steel cork borer of 7 mm and placed in the middle of the petri dishes. Commercially available copper oxychloride (COC) at standard dose, i.e., 0.3%, is used as a chemical control to compare the results and a negative control was also taken where only the pathogen was present without any treatment. The experiment was performed in Completely Randomized Design (CRD) with five replications per treatment. Inoculated plates were kept in incubator at 27±2 °C. The results were taken when complete growth on the negative control plate occurred. The per cent inhibition of the mycelial growth was calculated by the formula of Vincent (1947).

$$I = (C-T/C) \times 100$$

Where,

I = Inhibition of mycelial growth

C = Growth in control

T = Growth in treatment

Statistical Analysis

The gathered data on mycelial growth and its suppression were subjected to analysis of variance (ANOVA) in OPSTAT software. We compared the calculated value of F to the tabulated values at the 5% level of significance for the right degree of freedom. The Least Significant Difference (LSD) test was used to find the differences between the means of the treatments.

Results and Discussion

All the extracts of *Curcuma* spp. showed a statistically significant ($p=0.05$) inhibitory effect on the mycelial growth of the pathogen compared to the untreated control. However, extracts at higher concentrations (5%) showed better mycelial growth inhibition. In the *in vitro* test, T_3 : *Curcuma longa* extract 5% showed the highest inhibition of 74.07% with a radial colony diameter of 2.33 cm (Table 1; Figure 1) over control. This was followed by the treatment T_9 : *Curcuma caesia* extract 5% with 66.29% (3.03 cm) mycelial growth inhibition over control (Table 1; Figure 1). In comparison, the chemical fungicide T_{13} : COC at 0.3% showed the overall maximum growth inhibition of 80.74%.

Diseases resistant to fungicides are on the rise and antifungal treatments currently in use are highly toxic. This is a major public health concern globally. There is an urgent need to develop new antifungal therapies that have novel mechanisms of target. In this sense, compounds derived from natural sources have been proposed as potential antifungal agents due to their different biological activities, low toxicity and wide availability (Hermann *et al.*, 2022). Curcumin is a natural compound found in turmeric (*Curcuma longa*) and is a biodegradable and effective plant-based antifungal alternative (Qi *et al.*, 2024). Curcumin is a strong inhibitor of *Fusarium oxysporum* and its inhibitory action is not limited to this genus but also to other genera like *Rhizoctonia* and *Alternaria* (Chen *et al.*, 2018, Qi *et al.*, 2024; Firmansyah *et al.*, 2024).

The evaluated extracts of different *Curcuma* spp. possess significant mycelial growth inhibition of the tested pathogen of tomato *i.e.*, *Fusarium oxysporum* f.sp. *lycopersici*. The findings of the *in vitro* test were aligned with those of Muthomi *et al.* (2017), who showed that ethanolic extract of turmeric can significantly reduce the growth of *Fusarium oxysporum* f.sp. *lycopersici*.

The findings of the study closely aligned with the findings by Cruz *et al.* (2024). They observed that the secondary metabolite of *Curcuma longa* significantly suppresses the growth of *Fusarium* spp. The antifungal activity of *C. longa* is due to the presence of higher amount of its primary active compound, curcumin, along with the volatile sesquiterpenes like ar-turmerone, turmerone and turmerone (Chen *et al.*, 2018). These lipophilic molecules can easily

Table 1: Percent mycelial inhibition of the tested pathogen

Treatment No.	Treatment name	Mycelial growth	Percent mycelial inhibition over control
T_1	<i>C. longa</i> extract 1%	7.73 ^c	14.07
T_2	<i>C. longa</i> extract 2%	3.60 ^{gh}	60.00
T_3	<i>C. longa</i> extract 5%	2.33 ^j	74.07
T_4	<i>C. aromatica</i> extract 1%	8.23 ^b	8.51
T_5	<i>C. aromatica</i> extract 2%	6.55 ^d	27.22
T_6	<i>C. aromatica</i> extract 5%	3.46 ^h	61.48
T_7	<i>C. caesia</i> extract 1%	7.93 ^c	11.85
T_8	<i>C. caesia</i> extract 2%	5.23 ^f	41.85
T_9	<i>C. caesia</i> extract 5%	3.03 ⁱ	66.29
T_{10}	<i>C. amada</i> extract 1%	8.16 ^b	9.25
T_{11}	<i>C. amada</i> extract 2%	5.50 ^e	38.88
T_{12}	<i>C. amada</i> extract 5%	3.73 ^g	58.51
T_{13}	Chemical (COC) 0.3%	1.73 ^k	80.74
T_{14}	Control	9.00 ^a	0.00
SE± (d)			0.054
CD			0.109
CV (%)			1.559

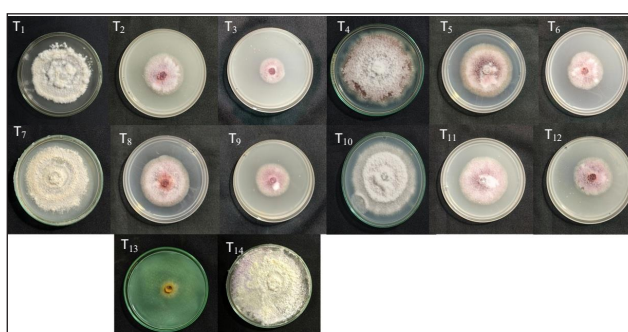


Figure 1: *In vitro* efficacy test of different treatments against *Fusarium oxysporum* f.sp. *lycopersici*

penetrate through the fungal plasma membrane and result in intercellular leakage and disruption of mitochondrial respiration.

Similarly, the higher antifungal efficiency of *Curcuma caesia*

is due to the high concentration of camphor, ar-turmerone and 1,8 -cineole in its dark rhizome (Ibrahim *et al.*, 2023). These compounds disrupt cell wall integrity, thereby restricting fungal growth. The gradual drop in efficiency was observed at lower concentrations across all the different extracts imply that a minimum threshold concentration of these active phytochemicals is necessary to disrupt fungal enzymes and metabolic processes.

Conclusion

From the experimental findings it was clear that aqueous extracts of *Curcuma* spp. possess significant antifungal properties, especially at higher concentrations against *Fusarium oxysporum* f.sp. *lycopersici*. In our experiment extracts with 5% concentration showed the highest potential for suppressing mycelial growth under *in vitro* conditions. The antimicrobial property of the extracts is due to the presence of antioxidants and active compounds such as curcumin. The procedure of preparation of aqueous extract is very simple cost-effective and relies on locally available resources. Consequently, these extracts offer a practical and eco-friendly option for farmers to manage *Fusarium* wilt. This approach eventually helps to reduce the use of chemicals while supporting long-term soil and crop health. Future research work will focus on validating these botanicals under greenhouse and field conditions to evaluate their practical performance *in vivo*. Before evaluating the antifungal activity of different *Curcuma* spp. under greenhouse and field conditions, the major phytochemical compounds responsible for this activity should be identified and analysed.

Author's Contribution

M. Bora performed the whole experiment. P.K. Kaman drafted the whole manuscript. D. Senapoty did the final checking of the manuscript. S. Sharma did the statistical analysis. B. Kalita did the data interpretation and photograph arrangement and B.B. Bhuyan did the proof reading.

Ethical Statement

The authors declare that no generative artificial intelligence tools were used in drafting or preparing this manuscript. The manuscript was written, interpreted and revised solely by the authors, who take full responsibility for its content.

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