



RESEARCH ARTICLE

# *Trichoderma asperellum* behave as antagonist to control leaf spot and flower blight of Marigold

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## Abstract

*Alternaria zinniae* causes leaf spot and blossom blight in marigolds, resulting in yield losses of 50-60 % in tropical and subtropical climates. Chemical controls can have a negative influence on ecosystems and agronomic control approaches are difficult to execute change to Chemical control are highly toxic, enhance biodegradation and cause environmental pollution after repeated use. Potential adverse effects on the earth and prolonged use have prompted a complete exclusion or limited utilization of most chemicals and an urgent need for eco-friendly and efficient tools. *In vitro* evidence for the possible use of *Trichoderma* spp. for biocontrol of marigold leaf spot and flower blight has been reported in previous investigations. In this study, we used a dual culture approach to investigate the antagonistic and mycoparasitic properties of 4 *Trichoderma asperellum* strains against *Alternaria zinniae* *in vitro*. The strain 2 of *Trichoderma asperellum* was found to have the highest microbial inhibition of 91.23 %, followed by the strain 1 of *Trichoderma asperellum* (81.58 %). This trail should be done under the field condition to get more efficacy of the result so that it can be recommended to the farmers.

## Keywords

Flower blight, Marigold, Dual culture, *Trichoderma asperellum*

## Introduction

Marigold (*Tagetes erecta* L.), a member of the genus *Tagetes* in the Asteraceae family, is native to Mexico and America and is also known as Genda phool. There are fifty different types of annual and perennial herbaceous plants in India. Marigolds are farmed on 8000-10000 ha of land in India. Marigold was grown in 70000 metric tonnes (1). The flower spreads swiftly since each flower in cultivation has a longer blooming duration and a beautiful flower with a long shelf life, and they are also known for being a fast-growing and annual flowering plant. The plant can grow to be 6" tall or 3' tall. In India, it is mostly used for ornamental and therapeutic purposes. It's used to cure a variety of ailments, including rheumatism, colds and bronchitis.

Every component of the plant is essential. Each part of the plant has medicinal characteristics; for example, the leaves are often used as an anti-biotic, to treat kidney disorders and to treat piles. The flower's composition is more ayurvedic, making it useful for fever, scabies, liver disorders and eye difficulties. Teas made from the plant's shoots are popular in Mexico. The flower's bioactive component possesses insecticidal and fungicidal capabil-

ities. Because of their phenolic and antioxidant activity, both the leaves and flowers have medicinal characteristics and are very much important in the pharmaceutical industry (2, 3). In the perfume industry, marigold essential oil is in high demand (4). The principal marigold-growing districts in Odisha are Dhenkanala, Koraput, Sambalpur, Sundergah and Balasore. Productivity is higher, except during the hot summer months.

Diseases produced by fungus, viral and bacterium, as well as nematodes, are the most common causes of yield loss, causing severe damage and yield loss. Flower blight, wilt and stem rot, *Alternaria* leaf spot and *Fusarium* wilt are all fungal diseases that harm marigold plants. Blossom blight and leafspot are the most devastating diseases caused by *Alternaria zinnia* (5) causing yield loss of 50-60%.

The ubiquitous and disfiguring disease, *Alternaria zinniae*, also affects other plants, including China aster, lettuce, sunflower, tobacco and tomato. On seeds and in soil, the *Alternaria* fungus spends the winter. On the upper leaf surface, tiny, rounded reddish brown dots with white to greyish white centres develop. Later, the lesions grow to be uneven, dark reddish brown or purple and rather large (up to 10 mm in diameter). An older spot's centre could disappear, leaving a jagged hole. Leaves with severe infection turn brown and dry.

Chemicals which are used in augmenting the plant diseases have much adverse environmental effect. In the present day scenario, many antagonistic micro-organisms are evolving which can be used for the augmentation of plant diseases. Keeping in mind of these facts, the investigation on Evaluation on fungi toxic potential of some bio-control agents against plant pathogens was undertaken in the Department of Plant Pathology, Institute of Agricultural Sciences, Siksha o Anusandhan (deemed to be) University Bhubaneswar, Odisha.

## Materials and Methods

### Soil sample collection

The soil sample was collected from the marigold field of instructional farm of Institute of Agricultural Sciences, Balasore, Sambalpur and Koraput. The denotations of the different soil sample are described in Table 1.

**Table 1.** Denotation of soil samples

Place of Collection of Soil sample	Denotations	Treatment No
Instructional Farm (Institute of Agricultural Sciences)	C1	T1
Balasore	C2	T2
Sambalpur	C3	T3
Koraput	C4	T4

### Isolation and Identification of *Trichoderma*

There are several methods for isolating *Trichoderma*; nevertheless, serial dilution of samples is one of the most popular methods mentioned in the literature. This method is

straightforward, cost-effective and suitable for big samples. Soil samples are taken, dried in the air, then milled into powder. Dissolve 10 g of powdered soil sample in 90 ml distilled water to make a sample stock solution. Following that, serial dilutions of samples were made as follows: 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-5</sup>. One ml of each prepared dilution is uniformly put on a suitable medium in a petri dish and incubated at 28 °C for 7 days. The cultures were then forwarded to the IARI's ITCC (Indian Type Culture Collection) in New Delhi for identification.

### Isolation and Maintenance of *Alternaria zinnia*

*Alternaria zinnia* had been isolated from the flower blight sample and maintained on the potato dextrose agar with repeated sub-culturing.

### In-vitro Assay using Dual culture technique

Twenty ml of sterilised and cooled potato dextrose agar was placed onto sterile petri dishes and allowed to harden in the dual culture technique. By leaving a 3-4 cm space between the pathogen and antagonist inoculations, the pathogen was infected on one side of the petri dish and the antagonist was inoculated on the opposite side of the same plate. Actively growing cultures were employed for this. Each treatment was carried out 5 times. The radial growth of the pathogen was measured after the required period of incubation, i.e. after the control plate had grown to a diameter of 90 mm. The % inhibition over control was calculated using the standard methods (6).

$$I = \frac{(C - T) \times 100}{C}$$

I = Per cent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

## Results

### Identification of *Trichoderma*

According to the report of Indian Type Culture Collection (ITCC), the 4 samples had been identified as the different strains of *Trichoderma asperellum*. The respective Identity number had been described in the Table 2.

**Table 2.** Identification of Cultures with Identification Number

Place of Collection of Soil sample	Denotations	Fungus Identified	ID Number (ITCC)
Instructional Farm (Institute of Agricultural Sciences)	C1	<i>Trichoderma asperellum</i>	11666.22
Balasore	C2	<i>Trichoderma asperellum</i>	11667.22
Sambalpur	C3	<i>Trichoderma asperellum</i>	11668.22
Koraput	C4	<i>Trichoderma asperellum</i>	11669.22

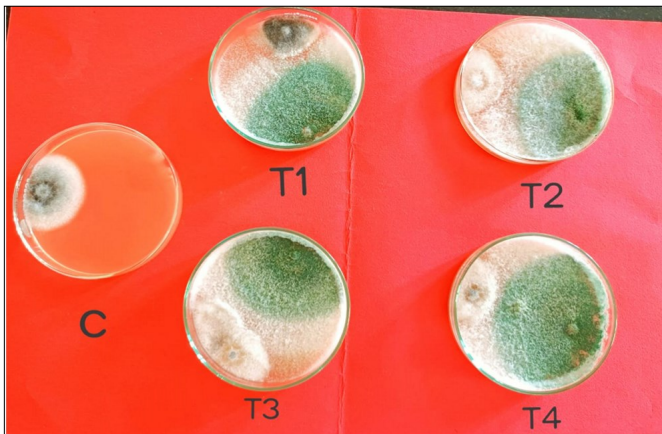
### In-vitro Assay

The effect of four strains of *Trichoderma asperellum* were

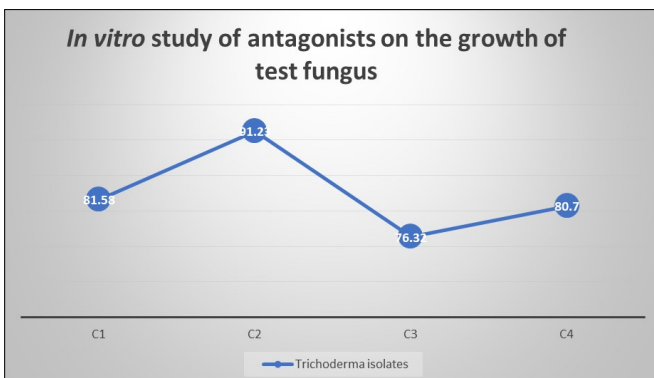
evaluated against the test fungus (*Alternaria zinniae*) as per the procedure described under the Materials and Methods which is presented in the Table 3, Fig. 1 and Fig. 2.

**Table 3.** *In vitro* study of antagonists on the growth of test fungus

Treatment No	Denotations	Antagonists	Growth Inhibition (%)
T1	C1	<i>Trichoderma asperellum</i>	81.58
T2	C2	<i>Trichoderma asperellum</i>	91.23
T3	C3	<i>Trichoderma asperellum</i>	76.32
T4	C4	<i>Trichoderma asperellum</i>	80.70
		SE(m)	3.289



**Fig. 1.** Effect of different antagonists on the test pathogen.



**Fig. 2.** Graphical Representation of different antagonists on the test pathogen.

It was found from the Table 3, Fig. 1 and 2 that, the strain 2 of *Trichoderma asperellum* was found to have the highest microbial inhibition of 91.23 %, followed by the strain 1 of *Trichoderma asperellum* (81.58 %). The strain 4 has showed an inhibition of 80.7 % and the strain 3 has inhibition of 76.32 %.

## Discussion

In this study, we evaluated the antagonistic and mycoparasitic capabilities of 4 strains of *Trichoderma asperellum* against *Alternaria zinniae* *in vitro* using dual culture technique. Microbial inhibition was found to be highest in strain 2 of *Trichoderma asperellum*, with 91.23 %, followed by strain 1 of *Trichoderma asperellum* (81.58 %). The strain 4 has showed an inhibition of

80.7% and the strain 3 has inhibition of 76.32 %. In all the treatments, the *Trichoderma* species grew much faster than the tested fungi and inhibited the growth of pathogen. In dual culture screening, *Trichoderma asperellum* was found to be the most potent in reducing the growth and colonization of pathogen. So far there are very limited published research work on the leaf spot disease in different species of marigold. In this aspect, the present work might be the pioneer work on biological control of leaf spot disease of marigold, caused by *Alternaria zinniae*.

## Conclusion

Agronomic control methods are challenging to implement modification to, and chemical controls can have a harmful impact on ecosystems. After frequent usage, chemical controls are extremely hazardous, accelerate biodegradation and pollute the ecosystem. The full ban on or restricted use of the majority of chemicals and the urgent need for environmentally friendly and effective tools are results of potential negative effects on the environment and continuous use. Previous studies have provided *in vitro* evidence for the potential use of *Trichoderma* spp. for the bio-control of marigold leaf spot and flower blight. The *in-vitro* evaluation of 4 strains of *Trichoderma asperellum* revealed that the strain 2 of *Trichoderma asperellum* was found to have the highest microbial inhibition of 91.23 %, followed by the strain 1 of *Trichoderma asperellum* (81.58 %). However, *in-vitro* result should be evaluated under field condition to know more about its efficacy so that it can be recommended for the farmers use.

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## Authors contributions

RRS had done all the experiments in the laboratory of Department of Plant Pathology, Institute of Agricultural Sciences, Siksha O Anusandhan (Deemed to be) University. KCS had overall guided in all the experiments. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** – Authors do not have any conflict of interests to declare.

**Ethical issues:** None.

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