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Assessment of Antifungal Property of Garlic Extracted in Different Solvents against the Leaf Spot and Flower Blight of Marigold

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ABSTRACT

Alternaria zinniae causes marigold leaf spot and blossom blight in tropical and subtropical climates, which reduces yields by 50–60%. Agronomic control techniques are challenging to deploy, and chemical controls may affect ecosystems. This study aimed to investigate the effectiveness of various solvent extractions of garlic against the test pathogen. Methanol, ethanol, acetone, acetic acid, chloroform, and water extraction of garlic were tested against the test fungus at 10% and 20% concentrations for this purpose. We discovered that the ethanol extract of garlic showed an inhibition of 87.84% at 10% concentration, followed by the methanol extract of garlic (86.49%). Garlic solvent extraction using acetone and acetic acid revealed an inhibition of 85.81% and 83.11%, respectively. These outcomes were all on par. Garlic solvent extraction in chloroform, however, had the least inhibition. In the instance of a 20% concentration, the same tendency had been noted. *Allium sativum's* ability to combat marigold flower disease may be explained by the presence of the phenol component, though more research is necessary. The effectiveness of garlic solvent extraction against marigold flower blight could also be tested in the field.

Key words : *Marigold, Garlic, Methanol, Ethanol, Acetone, Acetic acid, Chloroform, Flower blight*

Introduction

One of the most significant flowers traditionally used for decorating in India is the marigold, which belongs to the Asteraceae family. It is a South American and Mexican native. The flower was brought from Portugal to India around the 16th century, and it has since become naturalised in several agroclimatic zones. There are over 33 species of marigold, although just a few, like the African and French marigold, are significant. The three most widely cultivated species, *Tagetes erecta* (African marigold), *T. patula* (French marigold), and *T. tenuifolia* (striped marigold), are produced commercially throughout India in an area of roughly 8000–10,000 ha, with a combined flower production of almost 70,000 mt (Negi *et al.*, 1998). 3000 t of marigold meal were exported from Peru in the mid-to-late 1980s.Outside of Latin America, North America and Western Europe, mainly Spain and Portugal, are the main importers of marigold meal and its extracts (www.Prota.org). However, the yearly global demand for Tagetes essential oil is roughly 10 t, and *T. erecta*'s flower, which is widely grown in America, supplies an important yellow colourant (www.chemicalweekly.com).

In addition to being widely utilised in religious and social gatherings, it is also valued as a cut flower for interior decorating, bedding, hanging baskets, and garlands. The phenolic and antioxidant

properties of the leaves and blossoms provide them therapeutic significance, and they are both crucial to the pharmaceutical business (Tripathy and Gupta 1991; Khalil *et al.,* 2007). When grown as an intercrop, it has bioactive substances like thiophenes that are commonly used as insecticides (especially against *Aedes aegypti* and *Anopheles stephensi* adults and larvae; Wells *et al.,* 1992), fungicides, and also found to be helpful in the control of nematode populations of *Meloidogyne* and *Pratylenchus*species (Vasudvan *et al.,* 1997). *Alternaria solani* was less common when marigold and tomato were interplanted.(Gómez-Rodrí-guez *et al.,* 2003).

The crop has progressively become more vulnerable to a greater variety of soil-, seed-, and air-borne diseases as its area has increased. When compared to other diseases, *Alternaria zinnia*'s leaf spot and flower blight, which causes 60% disease severity in African marigolds, have become important restrictions (Sen, 1996). According to reports, the infection is a seedborne one (Dhiman and Arora, 1990).

Chemicals which are used in augmenting the plant diseases have much adverse environmental effects. At Present many plant extracts have been used for the disease management of plants. Out of all those plants, Garlic (*Allium sativum*) plays a significant role both in human and plant disease management. Keeping in mind of these facts, the investigation on "Evaluation on fungi toxic potential of garlic extract 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

Collection of Garlic bulbs : The garlic bulbs had been collected from the local markets near the Institute of Agricultural Sciences, Bhubaneswar, Odisha.

Extraction of bioactive material

Fresh garlic bulbs were gathered and washed with tap water before distillation. These bulbs were allowed to organically dry out in the open. 100 grammes of fresh material were cut into smaller pieces and then crushed with 100 ml of various solvents, including methanol, ethanol, acetone, acetic acid, chloroform, and water (control) (1:1 w/v), in a pestle and mortar that had been surface-sterilized. After that, shaken in a Rotary Shaker for 12 hours. Whatman's no. 1 filter paper was used to filter the extracts. For 10 minutes, the extracts were centrifuged at 1500 rpm. A stock solution was then created using the acquired supernatant.

Test Organism

Prior to inoculation *Alternaria zinniae* were sub-cultured onto the fresh Potato dextrose agar media to obtain a more vigorous population. The stock cultures were incubated at 37 ºC for 24 h.

Screening for antifungal activity by Poison Food Technique

To explore the antifungal mechanism of plant extracts in vitro, the poisoned food technique was used. 6 ml and 12 ml of the stock solution (Methanol, Ethanol, Acetone, Acetic Acid, Chloroform and water extracts) were combined with 60 ml of sterilised molten PDA medium, respectively, to generate 10 and 20% concentrations. To ensure that the extract was evenly dispersed, the medium was firmly shaken. 20 mL of medium was added to each sanitised Petri plate.

Cut 5 mm diameter discs of mycelium off the edge of an actively growing culture with a sterile cork borer disc, which were then deposited in the centre of each Petri plate. To maintain the controls, a single pathogen was cultured on PDA dishes.Each treatment was repeated four times, with plates incubated at 28 °C until the control dishes attained their radial growth maximum. The percent inhibition over control was calculated using Vincent's formula (1947).

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

Where,
I = Percent inhibition of mycelium
C = Growth of mycelium in control
T = Growth of mycelium in treatment

Statistical analysis

The results obtained in present study were statistically analysed with one-way analysis of variance in completely randomized design.

Results

The effect of five solvents of garlic with water extract as control were evaluated against the test fungus (*Alternaria zinniae*) as per the procedure described under the "Materials and Methods" which is

presented in Table 1 and Figure 1 and 2.

It was found from the Table 1, Figure 1 and 2 that, at 10% concentration the ethanol extract of garlic was found to have the highest microbial inhibition of 87.84 percent, followed by the methanol solvent extract of garlic (86.49 percent). The chloroform solvent extract of garlic showed the least inhibition. Same trend was also observed at 20% concentration also.

Discussion

In this study we have evaluated the different solvent extraction of garlic over water extract of garlic as control against the leaf spot and flower blight of marigold. We have found that at 10% concentration ethanol extract of garlic has shown an inhibition of 87.84% followed by methanol extract of garlic (86.49%). Acetone and Acetic acid solvent extraction of garlic have shown an inhibition of 85.81% and 83.11% respectively. All these results were at par. But chloroform solvent extraction of garlic had shown least inhibition. The same trend had been reported in case of 20% concentration. The phenolic compound that is extracted from the garlic in the solvents like methanol, ethanol, acetone and acetic acid may be the reason of growth inhibition of the fungus. But that phenolic compound may not be extracted in the chloroform extraction of garlic that is why it had shown least inhibition.

Conclusion

Chemical controls can have a negative effect on ecosystems, and agronomic control methods might be difficult to modify. Chemical controls are exceedingly dangerous with repeated use, hasten biodegradation, and damage the environment. The bulk of chemicals are now completely prohibited or have their usage severely restricted, and there is an ur-

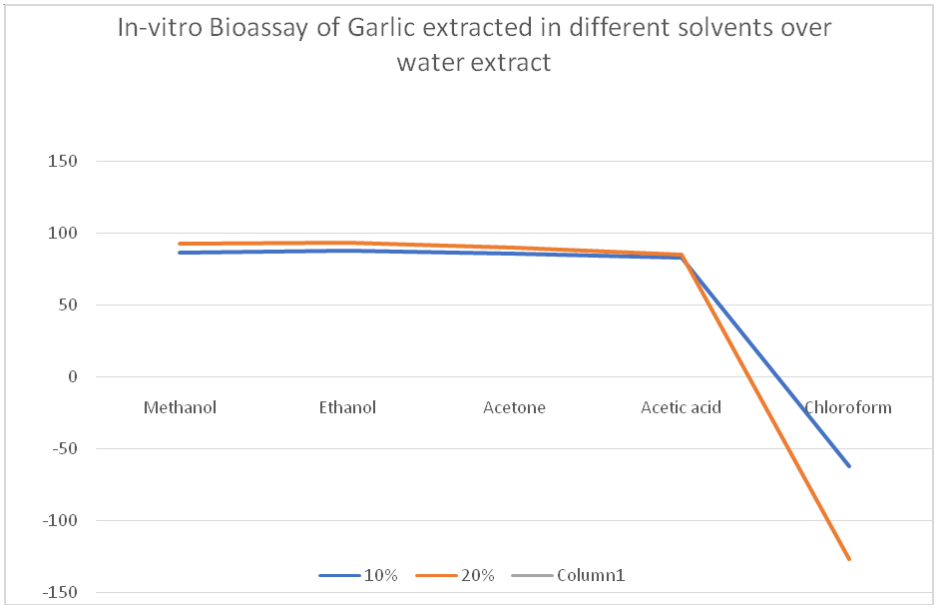

Fig. 1.

Table 1. In-vitro Bioassay of Garlic extracted in different solvents over water extract

Treatment Number	Solvents used for extraction of Garlic	Growth Inhibition	
		10% concentration	20% concentration
1.	Methanol	86.49	91.96
2.	Ethanol	87.84	92.86
3.	Acetone	85.81	89.29
4.	Acetic acid	83.11	84.82
5.	Chloroform	-62.16	-126.79
SE (m)	1.811	1.315	

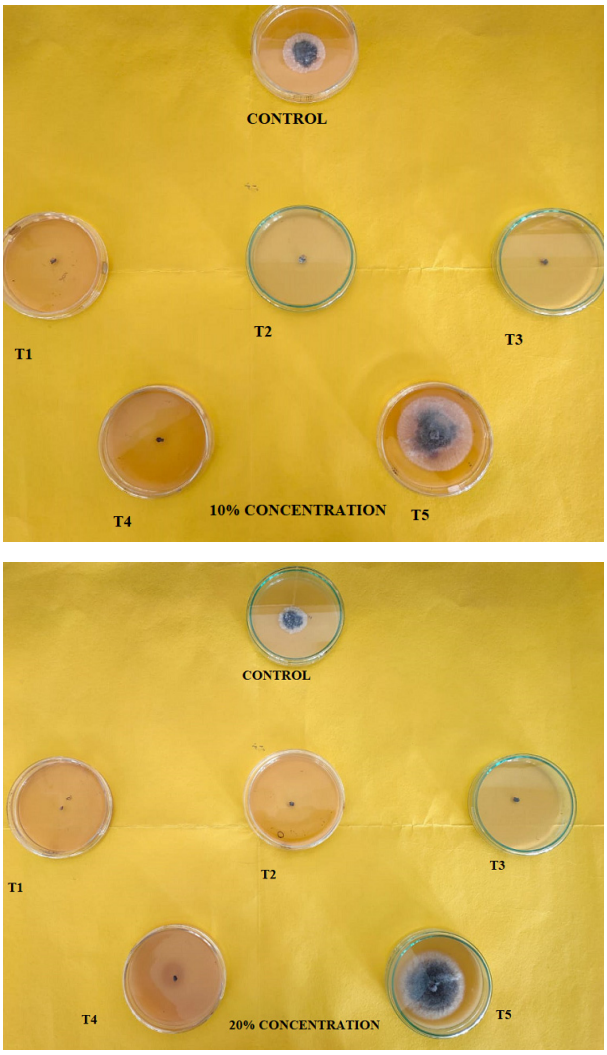

Fig. 2. Growth Inhibition

gent need for tools that are both efficient and kind to the environment. The potential use of garlic for the prevention and treatment of flower blight and marigold leaf spot has been supported by in vitro research from earlier studies. Garlic was extracted using several solvents in vitro, and the results showed that at a 10% concentration, the ethanol extract had the highest level of microbial suppression (87.84%), followed by the methanol solvent extract of garlic (86.49 percent). However, this *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

Rakesh Roshan Satapathy had done all the experiments in the laboratory of Department of Plant Pathology, Institute of Agricultural Sciences, Sikhsha O Anusandhan (Deemed to be) University. Dr Kartik Chandra Sahu 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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