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# ***In-vitro* evaluation of novel fungicides against leaf spot and flower blight of marigold by different techniques**

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## **ABSTRACT**

Leaf spot and flower blight caused by *Alternaria zinniae* is serious disease of marigold causing yield loss of 50-60 % in crop. Many previously used fungicides showed resistance against the pathogen. So, the present investigation was carried out with an objective to study the efficacy of new fungicides available in the market using poison food technique, well diffusion technique and disc diffusion technique. Three fungicides such as azoxystrobin +tebuconazole, tebuconazole + sulphur, Metalaxyl were evaluated at the concentration of 0.1%, 0.15%, 0.2% respectively. The present investigation revealed that Azoxystrobin + Tebuconazole recorded maximum mycelial growth inhibition of 96.85% followed by Tebuconazole + Sulphur 96.40% and Metalaxyl of 95.50% in poison food technique. Similarly, Azoxystrobin + Tebuconazole showed the highest inhibition of mycelial growth (88.10%), followed by Tebuconazole + Sulphur (86.36%), and Metalaxyl (84.38%) in well diffusion technique. In a same manner Azoxystrobin + Tebuconazole showed the highest inhibition of mycelial growth (77.73%), followed by Tebuconazole + Sulphur (75.10%), and Metalaxyl (72.25%) in disc diffusion method. Further the effectiveness of new fungicides may be tested under field condition against *Alternaria zinniae*.

**Key words:** Fungicides, Marigold, *Alternaria*, Azoxystrobin + Tebuconazole.

## **Introduction**

The marigold flower often referred to as genda phool is a member of the family Asteraceae. It is a native of Mexico and America. There are fifty different types of annual and perennial herbaceous plants in India. With a yield of 70,000 metric tonnes, marigolds are produced on 8000–10,000 hectares of land in India (Negi *et al.*, 1998). The shelf life and length of flowering of marigold flowers are very long. The plant grows swiftly and produces yearly flowers, and the flower spreads quickly as well. The plant can grow from 6 inches to 3 feet tall. In India, it is

mostly used for ornamental and therapeutic uses. In addition to other illnesses, it is used to treat rheumatism, colds, and bronchitis. Each portion of the plant is prized for its therapeutic properties; the leaves, for example, are commonly used as an antibacterial, to treat kidney problems, and to treat piles. The flowers of marigold have many ayurvedic uses for treating fever, scabies, liver issues, and eye issues in addition to other conditions. The plant's shoots are frequently used to produce tea in Mexico. The flower's bioactive substance has fungicidal and insecticidal effects. Due to their phenolic and antioxidant activity, the leaves and flowers have medicinal character-

istics and are equally important to the pharmaceutical industry (Tripathy and Gupta, 1991; Khalil *et al.*, 2007). The demand for marigold essential oil in the perfume business is enormous (Naik *et al.*, 2003). The main marigold-growing regions in Odisha are Dhenkanala, Koraput, Sambalapur, Sundergah, and Balasore.

Diseases brought on by nematodes, fungi, viruses, and bacteria are the most frequent cause of yield loss because they significantly harm crops and reduce output. Marigold plants are susceptible to *Alternaria* leaf spot, *Fusarium* wilt, flower blight, wilt, and stem rot. The most dangerous diseases, blossom blight and leafspot, are brought on by *Alternaria zinniae*.

We continue to use conventional fungicides to address the disease. As a result, plant diseases have evolved resistance to specific fungicides. Plant disease control consequently becomes more difficult. Fungicides are more efficient than using biological or plant-based ingredients. There are now several new fungicides available on the market, and they are quite effective against different pathogens. However, this pathogen has not been tested against by these fungicides. The department of plant pathology, Siksha o Anusandhan (deemed to be) university conducted research on this aspect using novel group of fungicides by deploying different techniques like poison food method, Well diffusion method and Disc diffusion method.

## Materials and Methods

The experiment was carried out in the Department of Plant Pathology, Institute of Agricultural Sciences in the Year 2021. The techniques like poison food technique, well diffusion technique and Disc diffusion technique are used to evaluate three novel fungicides in *in-vitro* condition. The list of fungicides with recommended concentrations was mentioned in Table 1.

**Table 1.** Different fungicides with their concentration

Treatment	Chemical name	Concentration (%)
T1	Azoxystrobin + Tebuconazole	0.1
T2	Tebuconazole + Sulphur	0.15
T3	Metalaxyl	0.2

### Poison Food Technique

Three fungicides were bought from the market and

tested at recommended concentrations. The required chemical concentrations were prepared and mixed into sterilised, cooled potato dextrose agar medium.

Twenty millilitres of media were put into 90 mm sterilised petri dishes, and each plate was inoculated with a 5 mm test fungus mycelia disc that was actively growing. For each treatment, three replications were kept. These plates were incubated for seven days at 25±1°C, after which the colony diameter was measured. Vincent's formula was used to calculate the percent inhibition over control (1947).

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

I = Per cent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

### Well Diffusion Method

Fungal spores were spread thoroughly on Potato dextrose agar Medium. A 6 mm well was created in the centre of each medium plate using the sterilized cork borer. Recommended doses of various fungicides were poured into the wells. The plates were incubated for 5 days at 25 °C ±2. Antifungal activity of fungicides was evaluated by measuring zone of no growth (cm). In control treatments, sterilized water was used instead of fungicides. Percentage inhibition was calculated using the following formula.

$$MGI (\%) = DC/DT \times 100$$

DT = diameter of the fungal colony (cm) in the blank Petri dish

DC = diameter of the fungal colony (cm) on fungicide treated growth medium.

### Disc Diffusion Method

In this method, spores of *Alternaria* sp. were distributed uniformly under aseptic conditions on the surface of (PDA agar). Then a disc of 6 mm diameter was cut from the pre sterilized Whatman filter paper No 1, saturated with recommended dose of the fungicide and placed on agar plates. These Petri plates were first refrigerated for 2 hours to allow diffusion of the compounds presents in the fungicides into the growth medium and then incubated at 25 °C ± 2 for 5 days. Sterilized water was used as control. Growth inhibition was assessed by the presence of opaque

halo around the disc of filter paper. Percentage inhibition was calculated using the following formula.

$$\text{MGI (\%)} = \text{DC} / \text{DT} \times 100$$

DT = diameter of the fungal colony (cm) in the blank Petri dish

DC = diameter of the fungal colony (cm) on fungicide treated growth medium.

## Results

### Poison Food Technique

It had been found from Table 2 that Azoxystrobin + Tebuconazole showed the highest inhibition of mycelial growth (96.85%), followed by Tebuconazole + Sulphur (96.40%), and Metalaxyl (95.50%).

### Well Diffusion Method

It had been found from Table 3 that Azoxystrobin + Tebuconazole showed the highest inhibition of mycelial growth (88.10%), followed by Tebuconazole + Sulphur (86.36%), and Metalaxyl (84.38%).

### Disc Diffusion Method

It had been found from Table 4 that Azoxystrobin + Tebuconazole showed the highest inhibition of mycelial growth (77.73%), followed by Tebuconazole + Sulphur (75.10%), and Metalaxyl (72.25%).

## Discussion

*Alternaria zinniae* causes leaf spot and blossom blight in marigolds, resulting in yield losses of 50-60% in the crop. Many previously used fungicides have been shown to be resistant to the disease. As a result, the current study was carried out with the goal of determining the efficacy of new fungicides available on the market using the poison food technique, well diffusion technique and disc diffusion technique. Three fungicides including azoxystrobin + tebuconazole, tebuconazole + sulphur, Metalaxyl were tested against the pathogen. Azoxystrobin + Tebuconazole showed the greatest inhibition of mycelial growth (96.85%), followed by Tebuconazole + Sulphur (96.40%), and Metalaxyl (95.50%). Similarly, Azoxystrobin + Tebuconazole

**Table 2.** *In vitro* evaluation of fungicides by Poison food technique

Treatment	Chemical name	Concentration	Growth inhibition
T1	Azoxystrobin+Tebuconazole	0.1	96.85
T2	Tebuconazole +Sulphur	0.15	96.40
T3	Metalaxyl	0.2	95.50
	CD	0.05	
	SE (M)	0.21	

**Table 3.** *In vitro* evaluation of fungicides by well diffusion method

Treatment	Chemical Name	Concentration	Growth Inhibition (%)
T1	Azoxystrobin + Tebuconazole	0.1	88.1
T2	Tebuconazole + Sulphur	0.15	86.36
T3	Metalaxyl	0.2	84.38
	SE (M)	0.08	
	CD	0.24	

**Table 4.** *In vitro* evaluation of fungicides by disc diffusion method

Treatment	Chemical Name	Concentration	Growth Inhibition (%)
T1	Azoxystrobin + Tebuconazole	0.1	77.73
T2	Tebuconazole + Sulphur	0.15	75.1
T3	Metalaxyl	0.2	72.25
	SE (M)	0.256	
	CD	0.538	

showed the highest inhibition of mycelial growth (88.10%), followed by Tebuconazole + Sulphur (86.36%), and Metalaxyl (84.38%) in well diffusion technique. In a same manner Azoxystrobin + Tebuconazole showed the highest inhibition of mycelial growth (77.73%), followed by Tebuconazole + Sulphur (75.10%), and Metalaxyl (72.25%) in disc diffusion method. The previous research work showed that mancozeb was the effective fungicide against the disease as Sunita *et al* (2010) reported that Mancozeb showed maximum growth inhibition of the pathogen. Mancozeb (0.2%) and Carbendazim (0.05%) spray can be used to control the disease at regular intervals (Aktar and Shamsi, 2015). Similarly, Yadav *et al.* (2013) were tested different concentration of systemic fungicides in *in-vitro* condition and revealed that Hexaconazole was found most effective with highest inhibition of radial growth (98.21%) followed by Propiconazole (97.32%) and Difenconazole (91.23%)

### Conclusion

The *in vitro* study on the effect of fungicides on the radial growth of *Alternaria zinniae* revealed that Azoxystrobin + tebuconazole (0.1%) recorded maximum inhibition followed by tebuconazole + sulphur and Metalaxyl in all the three methods of evaluation of fungicides. These fungicides may be trailed under

field condition to know more about its efficacy against the pathogen so that it can be recommended in the farmer field condition.

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