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## ***In vitro* evaluation of bio-agents and botanicals against *Alternaria tenuissima* causing leaf spot and flower blight in marigold**

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### **Abstract**

Leaf spot and flower blight caused by *Alternaria tenuissima* is one of the important diseases of marigold, which causes upto 50 to 60% losses in flower yield. In this present investigation, different botanicals and bio-agents were evaluated under laboratory condition. The results revealed that, *T. harzianum* showed maximum (76.30%) growth inhibition of the pathogen followed by *T. koningii* (71.52%), *T. longibrachiatum* (70.13%), *T. viride* (69.07%), *Bacillus subtilis* (55.18%) and *Pseudomonas fluorescens* (41.85%). Among botanicals, turmeric rhizomes was found most effective showing 82.38 percent inhibition of mycelial growth of *A. tenuissima* followed by dhatura leaves showed 67.22 percent inhibition. Less than 50 percent growth inhibition was recorded with neem leaves (49.51%), ginger rhizomes (48.55%), ardusi leaves (35.48%), garlic cloves (30.41%) and kadvi mehendi leaves (26.08%).

**Keywords:** Marigold (*Tagetes* spp.), *Alternaria tenuissima*, leaf spot, flower blight, *in vitro* evaluation, bio-agents, *Trichoderma harzianum*, *Trichoderma* spp., *Bacillus subtilis*, *Pseudomonas fluorescens*, botanicals, medicinal plant extracts, poisoned food technique, dual culture technique, biological control, growth inhibition

### **1. Introduction**

Marigold (*Tagetes* spp.) is one of the most economically valuable flowering plants belonging to the Asteraceae family. In India, Madhya Pradesh, Karnataka, Gujarat, Andhra Pradesh, Haryana, West Bengal, Maharashtra, Chhattisgarh, Sikkim and Tamil Nadu are the major marigold growing states. The total area of production under marigold cultivation is 66.13 thousand hectares with a total production of 603.18 thousand tonnes (Anonymous, 2017) [1]. In Gujarat, the marigold growing region is concentrated in and around the districts of Ahmedabad, Anand, Navsari, Vadodara, Kheda, Gandhinagar, Surat and Bharuch with an output of 84414 metric tonnes over an area of 8818 ha (Anonymous, 2017) [1]. Although the area under marigold cultivation is growing year after year but average flower production yield per unit area fluctuates as a result of pest and disease incidence. Leaf spot and flower blight of marigold is one of the most damaging and economically important diseases and leads to low productivity, resulting in a 50-60 percent loss of flower yield (Cotty *et al*, 1983) [3].

### **2. Materials and Method**

#### **2.1 Evaluation of different bioagents against *A. tenuissima* *in vitro***

##### **Dual culture technique**

The test organism (antagonist) and the pathogen were grown on PDA medium and from seven days old culture, a 5mm disc of the test organism (antagonist) was cut aseptically from the periphery of actively growing culture and placed at one end of the Petri plate containing 20ml PDA and in the opposite direction and approximately 70mm away from the disc of antagonist, a similar disc of the pathogen was placed aseptically.

Whereas, in case of bacterial antagonist's evaluation, the bacteria were grown on nutrient agar and from 24 hours old colony, bacteria was streaked at the one end of the Petri plate containing NA+PDA and in the opposite direction 70mm away from the antagonist, 5 mm disc of the pathogen was aseptically placed.

Three repetitions of each treatment were kept and the plates with only pathogen served as a control. The plates were incubated at average mean temperature ( $27\pm 2^{\circ}\text{C}$ ) and the radial growth of the test organism and pathogen was measured after 24 hours interval till control plate covered completely with mycelium of the pathogen and the Percent Growth Inhibition (PGI) was calculated by using the formula given by Vincent (1947).

$$\text{PGI} = \frac{100 (\text{DC} - \text{DT})}{\text{DC}}$$

Where,

PGI = Percent growth inhibition

DC = Average diameter of mycelial colony in control set (mm)

DT = Average diameter of mycelial colony of treated set (mm)

## 2.2 Evaluation of different locally available medicinal plants against *A. tenuissima* in vitro

### Poisoned food technique

The effect of plant extracts of seven medicinal plant species were tested *in vitro* by poisoned food technique to know their inhibitory effect on the growth of pathogen. Healthy fresh plant parts i.e., leaves, cloves or rhizomes were taken (Table 1) and washed thoroughly with clean water and finally rinsed with sterilized distilled water. Fifty grams (50g) of respective plant parts (Table 1) were minced with the help of grinder by adding 50ml sterilized water. The extracts were filtered through double layers sterilized muslin cloth and collected in 150ml conical flasks and plugged with non-absorbent cotton. The filtered phytoextracts were autoclaved at  $1.2 \text{ kg cm}^{-2}$  pressure for 20 minutes. The sterilized extracts were individually added in previously sterilized PDA at 10 percent (i.e. 2ml phytoextract + 18ml PDA per plate) in the conical flasks and mixed thoroughly at the time of pouring in the previously sterilized Petri plates. All the plates containing phytoextracts were inoculated by placing the 5mm mycelial disc from efficiently grown seven days old pure culture of pathogen with sterilized forcep and incubated at average mean temperature ( $27\pm 2^{\circ}\text{C}$ ). Three repetition of each treatment were maintained and the plates without phytoextract served as control. The observations on colony diameter (mm) were recorded periodically until control plate fully covered with the growth of pathogen and the Percent Growth Inhibition (PGI) was calculated as per formula given by Vincent (1947).

$$\text{PGI} = \frac{100 (\text{DC} - \text{DT})}{\text{DC}}$$

Where,

PGI = Percent growth inhibition

DC = Average diameter of mycelial colony in control set (mm)

DT = Average diameter of mycelial colony of treated set (mm)

## 3. Results and Discussion

### 3.1 Evaluation of bio-agents against *A. tenuissima* in vitro

Out of six antagonists, the least growth of the pathogen was observed in the treatment *Trichoderma harzianum* (21.33mm). This was significantly superior in its efficacy against the pathogen over rest of the antagonists screened. The next best treatment in order of merit was *T. koningii* (25.63mm) which was statistically at par with *T. longibrachiatum* (26.88mm) and *T. viride* (27.83mm) effective against pathogen where the antagonist overgrew the small colony of the pathogen restricting its further growth. Both bacterial antagonists, *Bacillus subtilis* (40.33mm) and *Pseudomonas fluorescens* (52.33mm) were showed higher average diameter of pathogen and proved moderately effectiveness in reducing the mycelial growth of the pathogen.

*T. harzianum* showed maximum (76.30%) growth inhibition of the pathogen and appeared to be the most superior in its efficacy over all the antagonists screened. This was followed in sequence by *T. koningii* (71.52%), *T. longibrachiatum* (70.13%), *T. viride* (69.07%), *Bacillus subtilis* (55.18%) and *Pseudomonas fluorescens* (41.85%) were in decreasing order for percent inhibition of pathogen.

It was appeared from this study that all antagonists tested by dual culture was effective against *A. tenuissima* and were very useful as potential biocontrol agent. From the results of the present investigation and above discussion it was concluded that *T. harzianum* was best antagonists of *A. tenuissima*.

Our findings are in line with the work of Shindhe (2017) [5] reported that maximum inhibition of mycelial growth of *A. alternata* causing leaf spot and flower blight of marigold in *T. harzianum* UAHS-1 (82.30%) and UAHS-2 (79.22%) followed by *T. viride* - GKVK (76.48%), *T. viride* - DWD (74.37%) and also found that *Pseudomonas fluorescens* and *Bacillus subtilis* were moderately effective against *A. tagetica*.

**Table 1:** Inhibitory effect of different bio-agents against *A. tenuissima* in vitro

| Sr. No.        | Name of Bio-agents                                  | Average colony diameter of pathogen (mm) | Growth inhibition (%) |
|----------------|-----------------------------------------------------|------------------------------------------|-----------------------|
| T <sub>1</sub> | <i>Trichoderma viride</i> Pers, Ex. Fr. NAU isolate | 27.83                                    | 69.07                 |
| T <sub>2</sub> | <i>Trichoderma harzianum</i> Rifai. NAU isolate     | 21.33                                    | 76.30                 |
| T <sub>3</sub> | <i>Trichoderma longibrachiatum</i> Junagadh isolate | 26.88                                    | 70.13                 |
| T <sub>4</sub> | <i>Trichoderma koningii</i> Junagadh isolate        | 25.63                                    | 71.52                 |
| T <sub>5</sub> | <i>Bacillus subtilis</i> Ell. NAU isolate           | 40.33                                    | 55.18                 |
| T <sub>6</sub> | <i>Pseudomonas fluorescens</i> NAU isolate          | 52.33                                    | 41.85                 |
| T <sub>7</sub> | Control                                             | 90.00                                    | 00.00                 |
| S. Em. ±       |                                                     | 0.727                                    |                       |
| CD at 5%       |                                                     | 2.225                                    |                       |
| CV %           |                                                     | 3.098                                    |                       |



Fig 1: Evaluation of different bio-agents against *A. tenuissima* in vitro

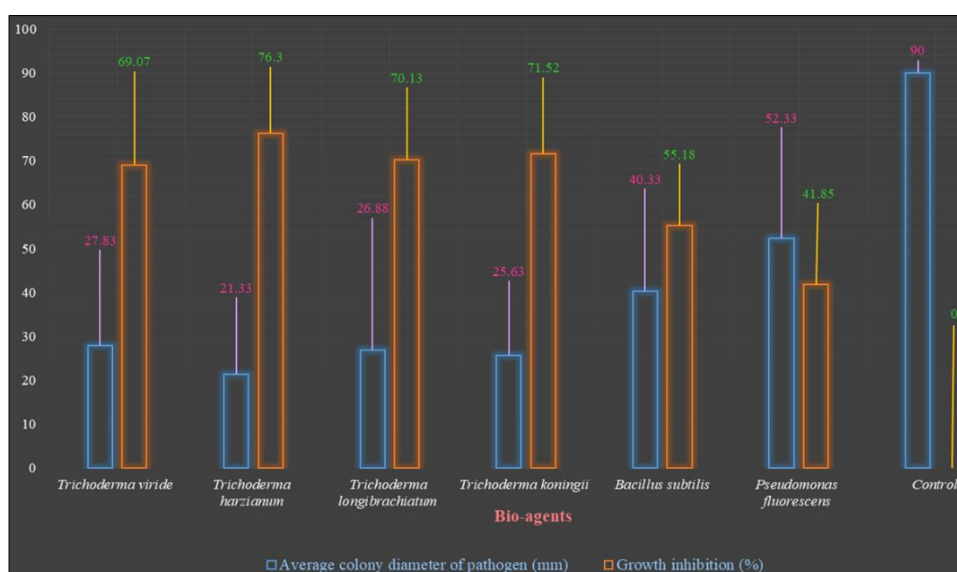


Fig 2: Inhibitory effect of different bio-agents against *A. tenuissima* in vitro

### 3.2 Evaluation of different locally available medicinal plants against *A. tenuissima* in vitro

Result obtained from the effect of test phytoextracts on radial mycelial growth and inhibition of the test pathogen over untreated control were recorded and presented in the Table 2, Fig 3 and Fig. 4. Mycelial growth was measured after 12 days of incubation. Mycelial growth of the test fungus revealed that all the phytoextract showed inhibition activity against the targeted fungus and suppressed the mycelial growth.

Among effective phytoextracts, least mycelial growth of *A. tenuissima* was recorded in the extract of turmeric rhizome (15.85mm) which was proved to be significantly superior. Next best phytoextract in order of merit was dhatura leaves (29.50mm) followed by neem leaves (45.44mm) and ginger rhizome (46.30mm) and rest of phyto-extracts, arduisi leaves, garlic cloves and kadvi mehendi leaves showed comparatively less inhibitory effect against pathogen.

Among seven plant extracts evaluated, turmeric rhizomes was found most effective showing 82.38 percent inhibition of mycelial growth of *A. tenuissima*, followed by dhatura

leaves (67.22%). Less than 50 percent growth inhibition was recorded with neem leaves (49.51%), ginger rhizomes (48.55%), arduisi leaves (35.48%), garlic cloves (30.41%) and kadvi mehendi leaves (26.08%).

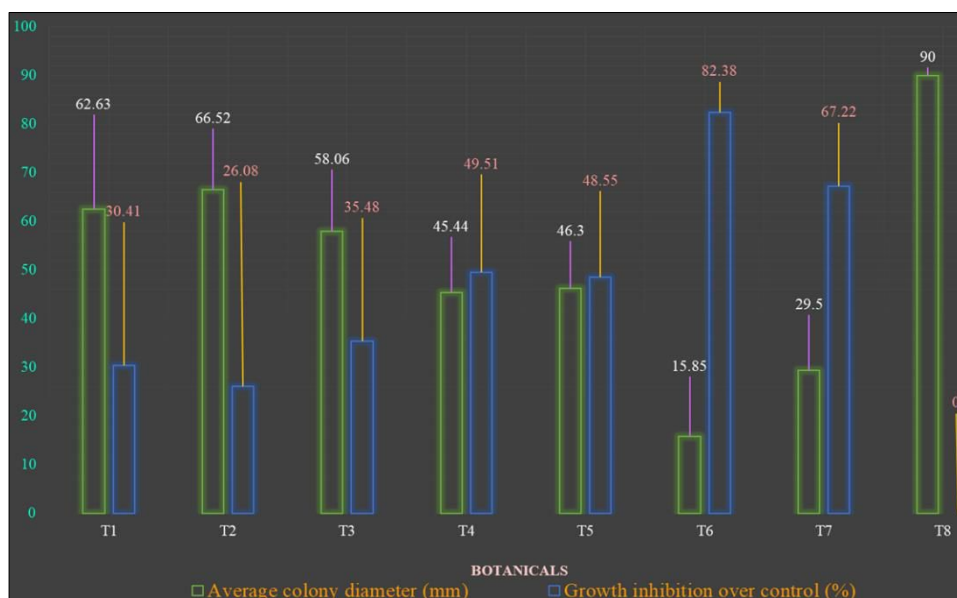
Result obtained in present investigation are in line with the findings of, Barnwal *et al.* (1998) [2] revealed that bulb extract of garlic was the most effective against *A. tenuissima* causing blight of marigold followed by mint and dhatuta extract while, Patel (2008) [4] evaluated the efficacy of ten phytoextract as fungitoxic botanicals against *A. alternata* in vitro by means of poisoned food technique and revealed that rhizome extract of turmeric (62.19%) was excellent in inhibition of mycelial growth of pathogen.

### 4. Conclusion

*T. harzianum* showed maximum (76.30%) growth inhibition of the pathogen and appeared to be the most superior in its efficacy over all the antagonists screened. Turmeric rhizomes was found most effective showing 82.38 percent inhibition of mycelial growth of *A. tenuissima* followed by dhatura leaves showed 67.22 percent inhibition.

**Table 2:** Inhibitory effect of different locally available medicinal plants against *A. tenuissima* in vitro

| Sr. No.        | Botanicals    | Scientific name                   | Plant parts used | Average colony diameter (mm) | Growth inhibition over control (%) |
|----------------|---------------|-----------------------------------|------------------|------------------------------|------------------------------------|
| T <sub>1</sub> | Garlic        | <i>Allium sativum</i> L.          | Cloves           | 62.63                        | 30.41                              |
| T <sub>2</sub> | Kadvi Mehandi | <i>Lawsonia inermis</i> L.        | Leaves           | 66.52                        | 26.08                              |
| T <sub>3</sub> | Ardusi        | <i>Adhatoda vasika</i> Nees.      | Leaves           | 58.06                        | 35.48                              |
| T <sub>4</sub> | Neem          | <i>Azadirachta indica</i> L.      | Leaves           | 45.44                        | 49.51                              |
| T <sub>5</sub> | Ginger        | <i>Zingiber officinalis</i> Rosa. | Rhizome          | 46.30                        | 48.55                              |
| T <sub>6</sub> | Turmeric      | <i>Curcuma longa</i> L.           | Rhizome          | 15.85                        | 82.38                              |
| T <sub>7</sub> | Dhatura       | <i>Datura metel</i> L.            | Leaves           | 29.50                        | 67.22                              |
| T <sub>8</sub> | Control       | -                                 | -                | 90.00                        | 00.00                              |
| S. Em. ±       |               |                                   |                  | 0.994                        |                                    |
| CD at 5%       |               |                                   |                  | 3.004                        |                                    |
| CV %           |               |                                   |                  | 3.323                        |                                    |

**Fig 3:** Evaluation of different botanicals against *A. tenuissima* in vitro**Fig 4:** Inhibitory effect of different locally available medicinal plants against *A. tenuissima* in vitro

## References

- Anonymous. Horticultural statistics at a glance 2017. New Delhi: Horticulture Statistics Division, Department of Agriculture, Cooperation & Farmers Welfare, Ministry of Agriculture & Farmers Welfare, Government of India; c2017. p. 481.
- Barnwal MK, Jha DK, Dubey SC. Evaluation of different plant extracts against blight of marigold. J Res Birsa Agric Univ. 1998;10:221-223.
- Cotty PJ, Misaghi IJ, Hine RB. Production of zinnitol by *Alternaria tagetica* and its phytotoxic effect on *Tagetes erecta*. Phytopathology. 1983;73(9):1326-1328.

4. Patel VP. Investigation on leaf spot and fruit rot (*Alternaria alternata* (Fr.) Keissler.) of bottle gourd (*Lagenaria siceraria* (Mol.) Standl.) under South Gujarat condition. Navsari (India): Navsari Agricultural University; c2008. p. 82.
5. Shindhe M. Studies on leaf spot and flower blight of marigold caused by *Alternaria tagetica* (Shome and Mustafee). Shivamogga (India): University of Agricultural and Horticultural Sciences; c2017. p. 90.