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## **Efficacy of Some Medicinal Plant Extracts, Oil and Microbial Antagonists Against *Fusarium* spp. Affecting Brinjal and Guava Crops**

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### **ABSTRACT**

Aim of this study was to evaluate the efficacy of medicinal plants as well as fungal antagonists against pathogenic fusaria. Pathogenic fusaria viz., *Fusarium solani* f. sp. *melongena* and *F. oxysporum* f. sp. *moniliforme* causing brinjal and guava wilt are also responsible for significant reduction in yield and quality. *Fusarium* strains isolated from different sites and identified by Phase Contrast Microscope (PCM) and Scanning Electron Microscope (SEM). The oil and extracts of plant were extracted by hydro distillation in Clevenger apparatus and Soxhlet apparatus. Mycelial growth inhibition was determined by food poison method. *In vitro* efficacy of five medicinal plants viz., *Azadirachta indica* (oil and leaf extract), *Psidium guajava* (leaf extract), *Eucalyptus camaldulensis* (bark extract), *Trachyspermum ammi* (seed extract) and *Ocimum sanctum* (leaf extract) and three microbial antagonists viz., *Trichoderma harzianum*, *T. atroviride* and *T. longibrachiatum* were tested using 4, 8 and 12  $\mu$ L against both the test fungi on 3rd, 5th and 7th day of inoculation. Among all the medicinal plants, *O. sanctum* (leaf extract), *T. ammi* (seed extract) and *A. indica* (leaf extract) showed 100% inhibition of mycelial growth of *F. solani* and *F. moniliforme* at 12  $\mu$ L concentration on 7th day followed by *A. indica* (oil), *P. guajava* and *E. camaldulensis*. Among different bioagents, *T. longibrachiatum* against *F. solani* and *F. oxysporum* f. sp. *moniliforme* was found significantly superior to the rest in infecting the growth and showed 100% inhibition at 8 and 12  $\mu$ L concentrations on 3rd, 5th and 7th day while *T. harzianum* against both the test fungus was most effective and completely inhibited the mycelial growth at 12  $\mu$ L concentration on all three days followed by *T. atroviride*.

**Key words:** Wilt disease, *Fusarium solani*, *Fusarium moniliforme*, medicinal plants, fungal antagonists

### **INTRODUCTION**

*Fusarium solani* f. sp. *melongena* and *Fusarium oxysporum* f. sp. *moniliforme* are responsible for damaging brinjal (*Solanum melongena*) and guava (*Psidium guajava*) crops in India (Alabouvette *et al.*, 2009; Neela *et al.*, 2014). *Fusarium* spp. produce mycotoxins in food besides causing root rot and wilt (Stumpf *et al.*, 2013). Chemicals used in the control of diseases pollute the environment and affect the properties of medicinal plants so that to avoid the hazardous effects of chemicals, natural products of some plants and microbial antagonists have been used to control plant disease (Momin and Nair, 2001; Agbenin *et al.*, 2004; Akkopru and Demir, 2005).

Essential oils and extract of medicinal plants as well as antimicrobial agents have been found to be effective as antifungal agent (Sridhar *et al.*, 2003; Bokhari and Perveen, 2012; Neela *et al.*, 2014; Enespa and Dwivedi, 2014). The oil and extract of *A. indica* (*Meliaceae*) showed *in vitro* antimicrobial effect against *F. solani* and *F. moniliforme* (Allameh *et al.*, 2002; Moslem and El-Kholie, 2009). The antifungal effects against *F. solani* and *F. moniliforme* of seed extract of *T. ammi* (Parekh and Chanda, 2006; Singha *et al.*, 2011), bark extract of *E. camaldulensis* (Tang *et al.*, 2012), leaf extract of *P. guajava* (Joseph and Priya, 2011) and the leaf extract of *O. sanctum* (Dissanayake, 2014) have also been studied.

On the other hand, the antagonistic fungi especially *Trichoderma* spp. viz., *T. harzianum*, *T. atroviride* and *T. longibrachiatum* have been widely used against *F. solani* and *F. oxysporum* (Dubey *et al.*, 2007; Jegathambigai *et al.*, 2009; Hossain *et al.*, 2013). This study indicates that biopesticides and biocontrol agents are the only alternative, economical and eco-friendly means for the management of wilting in these crops.

## MATERIALS AND METHODS

**Sample collection and isolation of mycoflora:** The rhizospheric, non-rhizospheric soil and plant samples (leaf, stem and root) of brinjal and guava (healthy and wilt infected) were collected from crop field of Siddhauri (Sitapur) and Bijnor (Lucknow) Uttar Pradesh, India, respectively. Samples were taken in sterilized polythene bags (to avoid aerial contamination) separately. After sampling, the soil (0-15 cm depth) and plant parts were brought to the laboratory for further studies. Soil samples were sieved ( $\pm 0.78$  mm) and air dried (oven) for 3-5 days at 18°C. Fungal colonies were isolated following serial dilution technique (Benson, 2002). The samples of soil were shaken in 9 mL of sterile distilled water separately for 10 min and left for 20 min. A dilution series was prepared from  $1 \times 10^3$  to  $1 \times 10^6$ . An aliquot of (1 mL) was spread on Czapek-dox agar (CZA, 20 mL) plates, replicated thrice and incubated at  $28 \pm 2^\circ\text{C}$  for 10 days in culture room. Fungal colonies were isolated from plant samples (root, leaf and stem) by pour plate method (Aneja, 2003). The colonies were purified and identified by available literatures (Hildebrand, 1938; Booth, 1985).

**Identification of isolated mycoflora by phase contrast microscope:** The isolated fungi were identified on the basis of their colour, morphological characters as well as sporulating structure and conidia under microscope (40x and OLYMPUS CX41, Tokyo). The isolated pathogenic fusaria (*F. solani* and *F. oxysporum* f. sp. *moniliforme*) and bioagents i.e., *Trichoderma* species (*T. harzianum*, *T. atroviride* and *T. longibrachiatum*), *Penicillium* spp. (*P. chrysogenum*, *P. italicum*, *P. citrinum* and *P. oxalicum*) and *Aspergillus* spp. (*A. niger*, *A. flavus*, *A. luchuensis* and *A. sulphureus*) were purified by hyphal tip technique (Ho and Ko, 1997) and identified on the basis of cultural and morphological characters (Hansen, 1926; Leslie and Summerell, 2006; Nasr-Aboul and Abdul-Rahman, 2014).

**Scanning electron microscope:** The culture of fungal strain cropped from the mid exponential phase from petri plates prepared in Czapek-Dox agar medium and fixed into a (2.5 mL) eppendorf tube which consisted of 2.5% of glutaraldehyde (LobaChemi, INDIA) in Millipore water for 1-2 h at room temperature. Fixed culture was washed twice with Millipore water and post-fixed with 2% osmium tetra oxide stain (0.5 mL for 1 h). The culture was subsequently dehydrated using a series of 10, 30, 50, 70 and 90% ethanol in Millipore water for 5 min. The final dehydration in 100% of ethanol was carried out for 10 min. The dehydrated culture drop was fixed at the cover slip and

then dried overnight in an oven and desiccators till mounting. The specimens were mounted over stainless steel stub onto the sample holder with double-stick carbon adhesive tapes and coated with platinum using a sputter coater prior to viewing using a scanning electron microscope of JEOL (JSM 6490 LV) JAPAN.

**Extraction of essential oil:** The fruits of neem (*A. indica*) were powdered (800 mesh size) using domestic model grinder and were subjected to hydro distillation in Clevenger apparatus for 6 h in accordance with European pharmacopeia procedure (Maissonneuve, 1983). Yellow-colored oil (yield 2.2%) with characteristic odor and sharp taste was obtained. It was dried over anhydrous sodium sulfate to remove traces of moisture and stored in refrigerator at 4°C until further use.

**Acetone extract:** *Psidium guajava* (leaves), *E. camaldulensis* (bark), *A. indica* (leaves), *O. sanctum* (leaves) and *T. ammi* (seeds) were used for the extraction. Fresh plant materials were washed thoroughly under running tap water followed by sterilized distilled water. The samples were dried at room temperature and then powdered (800 mesh size) using domestic model grinder (Maharaja, Whiteline, India). Extract was obtained by extracting 20 g of dried plant samples with 900 mL of acetone for 6 h in a Soxhlet apparatus. The extract was concentrated up to 24 mL. The remaining acetone was evaporated by placing the samples in a vacuum drier under reduced pressure. The extracts of the samples obtained were stored in a refrigerator at 4°C until further use.

**Mycelial growth inhibition by poisoned food technique:** Different concentrations (4, 8 and 12 µL) of acetone extracts of *P. guajava*, *E. camaldulensis*, *A. indica*, *O. sanctum*, *T. ammi* and cultural filtrate of fungal antagonists viz., *T. harzianum*, *T. atroviride* and *T. longibrachiatum* were incorporated into Czapek's Dox agar medium in sterilized petri dishes. The petri dishes containing medium devoid of the extracts with same amount of distilled water served as control. The blocks of 4 mm diameter of test pathogens, cut from actively growing margin of seven days old culture, were placed at the centre of the petri dishes separately containing different concentration of the poisoned medium and incubated at 25±2°C for 7 days. The average diameter of the fungal colonies was measured on the 3rd, 5th and 7th day of incubation and percentage of mycelial growth inhibition was calculated using the following formula (Rao and Srivastava, 1994):

$$\text{Percentage mycelial growth inhibition} = \frac{gc - gt}{gc} \times 100$$

where, gc is growth of mycelial colony in control set after incubation period subtracting the diameter of inoculum disc, gt is growth of mycelial colony in treatment set after incubation period subtracting the diameter of inoculum disc.

**Statistical analysis:** Statistical analysis of data was performed by one way Analysis of Variance (ANOVA) followed by DMRT (Duncan Multiple Range Test) significant at  $p \leq 0.05$ .

## RESULTS AND DISCUSSION

**Isolated and purified pathogens:** Fungal growth occurred on all symptomatic tissue cut from the plants and serially diluted (soil), plated on CZA medium. Microscopic examinations of cultures

revealed that both fungi (*F. solani* and *F. moniliforme*) had similar characteristics. Under the microscope, variable conidiophores and two kinds of conidia were apparent. The large macroconidia were hyaline, several celled (5-6 cells) and boat shaped with slightly pointed ends. The microconidia were one celled, ovoid, numerous, borne singly and also hyaline. Some intermediate 2-3 celled hyaline conidia were also seen under the microscope. The fungus was identified as *F. oxysporum* f. sp. *moniliforme* and *F. solani*. In contrast, *F. oxysporum* f. sp. *moniliforme* was isolated from most of the diseased plant samples. Pure culture of *F. solani* and *F. moniliforme* was maintained on CZA slants at 4°C.

**Identified mycoflora:** A total of 25 fungal isolates were isolated from wilt affected and healthy soils+plant parts by soil dilution agar plating method. All fungal isolates were maintained in pure cultures by using standard techniques. Twenty one fungal isolates were identified as, *Fusarium solani*, *Penicillium* spp. (*P. citrinum*, *P. oxalium*, *P. chrysogenum* and *P. italicum*), *Aspergillus flavus*, *A. niger*, *A. sulphuriosus*, *A. leuchuensis*, *Cheatomium globosum*, *Humicola grisea*, *Cladosporium herbarium*, *Fusarium moniliforme*, *Curvularia geniculata*, *Alternaria alternata*, *Fusarium coeruleum*, *Fusarium semitectum*, *Fusarium longipes*, *Trichoderma viride*, *T. harzianum* and white sterile mycelium. The biocontrol agents used as substitute of pesticides.

**Comparative analysis of antifungal activity:** The recorded mean fungal radial growth (mm) for each treatment presented in Table 1, 2 and Fig. 1-3 indicates the inhibition zones of oil, extracts

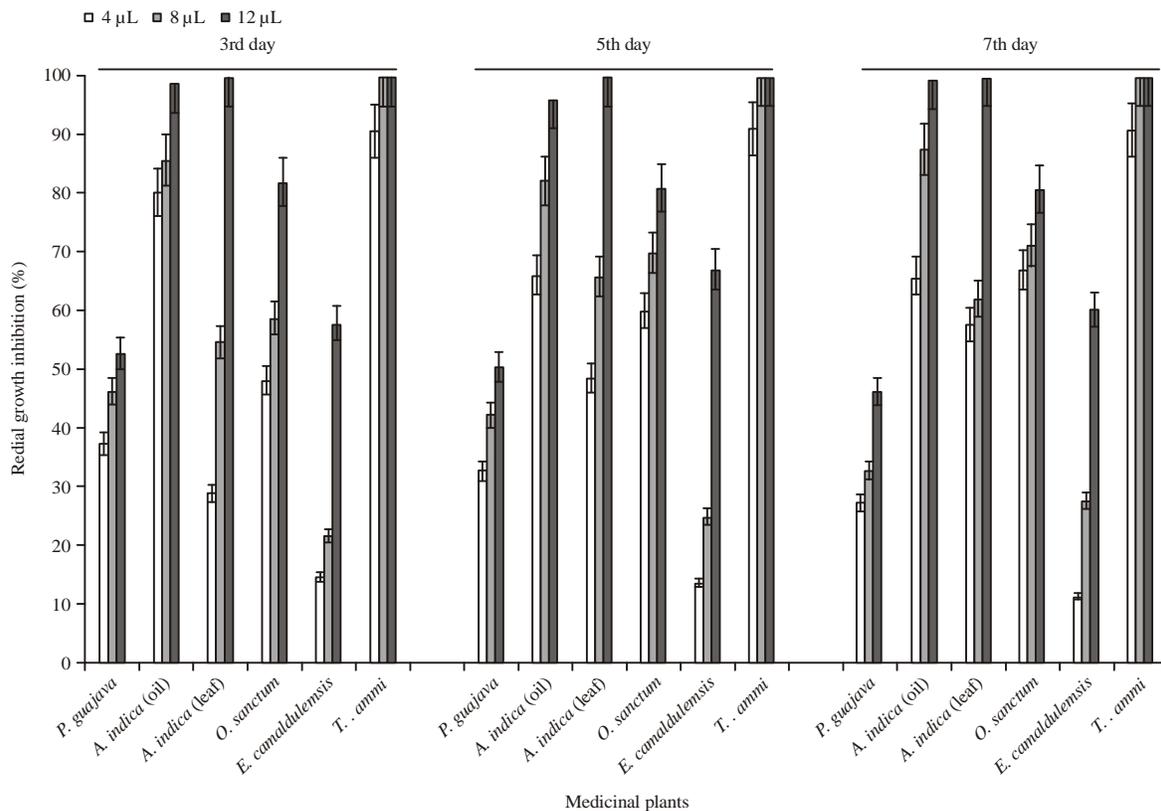


Fig. 1: Radial growth inhibition of *F. solani* f. sp. *melongena* through antagonistic medicinal plant extracts on 3rd, 5th and 7th day of incubation

and antagonists. All the medicinal plant, oil and extracts showed significant reduction in the growth of the test pathogens. Among the different extracts, *A. indica* (oil) against *F. oxysporum* f. sp. *moniliforme* and *F. solani* was most effective and inhibited the mycelial growth at the concentration of 12 µL. Growth of *F. solani* was inhibited at 4 µL concentration by *P. guajava* (leaves), *A. indica* (oil and leaf), *O. sanctum*, *E. camaldulensis* (bark) and *T. ammi* (seeds) by 37.47, 80.39, 29.06, 48.28, 14.76 and 90.79% on 3rd day; 32.82, 66.12, 48.69, 60.08, 13.81 and 91.19% on 5th day and 27.44, 66.07, 57.69, 67.06 and 11.41, 91% on 7th day, respectively. On the other hand, at 8 µL concentration of the foresaid medicinal plants inhibited the mycelial growth of *F. solani* by 46.42, 85.81, 54.79, 58.81, 21.75 and 100% on 3rd day; 42.31, 82.34, 66.00, 70.03, 25.11 and 100% on 5th day and 32.88, 87.67, 62.14, 71.39, 27.78 and 100% on 7th day, respectively, whereas, maximum inhibition i.e., 52.88, 98.92, 100, 81.99, 58.04 and 100% on 3rd day; 50.51, 96.10, 100, 81.10, 67.17 and 100% on 5th day and 46.31, 99.59, 100, 80.87, 60.34 and 100% on 7th day was recorded at 12 µL concentration (significant at 5%) (Table 1 and Fig. 1).

Table 1: Efficacy of medicinal plant extracts against *F. solani* f. sp. *melongena* and *F. oxysporum* f. sp. *moniliforme* at different concentrations on 3rd, 5th and 7th day of inoculation

| Medicinal plants                               | Concentration (µL) | Fungal pathogen | Days after inoculation/mean colony diameter (mm) |                         |                         |
|--|--------------------|-----------------|--|-------------------------|-------------------------|
|  |                    |                 | 3rd  | 5th                     | 7th                     |
| <i>Psidium guajava</i> (leaf extract)          | 4                  | Fsm             | 24.23±0.02 <sup>c</sup>                          | 41.20±0.64 <sup>b</sup> | 59.03±0.76 <sup>a</sup> |
|  |                    |                 | 20.76±1.08 <sup>c</sup>                          | 35.38±0.33 <sup>b</sup> | 54.60±0.78 <sup>a</sup> |
|  |                    |                 | 18.26±0.61 <sup>c</sup>                          | 30.35±0.27 <sup>b</sup> | 43.68±0.92 <sup>a</sup> |
|  | 8                  | Fom             | 35.58±0.63 <sup>c</sup>                          | 51.15±1.12 <sup>b</sup> | 70.37±0.33 <sup>a</sup> |
|  |                    |                 | 30.67±0.39 <sup>c</sup>                          | 43.35±1.05 <sup>b</sup> | 55.33±0.32 <sup>a</sup> |
|  |                    |                 | 21.40±1.23 <sup>c</sup>                          | 33.21±1.40 <sup>b</sup> | 47.95±0.75 <sup>a</sup> |
| <i>Azadirachta indica</i> (oil)                | 4                  | Fsm             | 7.60±1.02 <sup>a</sup>                           | 20.78±1.25 <sup>b</sup> | 27.60±0.78 <sup>c</sup> |
|  |                    |                 | 5.50±1.67 <sup>a</sup>                           | 10.83±0.45 <sup>b</sup> | 10.03±0.42 <sup>b</sup> |
|  |                    |                 | 0.42±0.72 <sup>a</sup>                           | 2.39±1.38 <sup>b</sup>  | 0.33±0.58 <sup>a</sup>  |
|  | 8                  | Fom             | 14.82±1.01 <sup>c</sup>                          | 34.14±0.12 <sup>b</sup> | 26.44±0.67 <sup>a</sup> |
|  |                    |                 | 13.33±0.38 <sup>c</sup>                          | 19.87±0.79 <sup>b</sup> | 15.10±0.10 <sup>a</sup> |
|  |                    |                 | 0.00±0.00 <sup>c</sup>                           | 0.00±0.00 <sup>b</sup>  | 0.00±0.00 <sup>a</sup>  |
| <i>Azadirachta indica</i> (leaf extract)       | 4                  | Fsm             | 27.49±0.47 <sup>c</sup>                          | 31.47±1.75 <sup>b</sup> | 34.42±0.38 <sup>a</sup> |
|  |                    |                 | 17.52±1.34 <sup>c</sup>                          | 20.85±0.78 <sup>b</sup> | 30.80±6.64 <sup>a</sup> |
|  |                    |                 | 0.00±0.00 <sup>c</sup>                           | 0.00±0.00 <sup>c</sup>  | 0.00±0.00 <sup>a</sup>  |
|  | 8                  | Fom             | 9.67±0.51 <sup>b</sup>                           | 13.35±0.54 <sup>a</sup> | 14.99±0.15 <sup>a</sup> |
|  |                    |                 | 0.00±0.00 <sup>b</sup>                           | 0.00±0.00 <sup>a</sup>  | 0.00±0.00 <sup>a</sup>  |
|  |                    |                 | 0.00±0.00 <sup>b</sup>                           | 0.00±0.00 <sup>a</sup>  | 0.00±0.00 <sup>a</sup>  |
| <i>Ocimum sanctum</i> (leaf extract)           | 4                  | Fsm             | 20.04±1.94 <sup>c</sup>                          | 24.48±2.51 <sup>b</sup> | 26.79±0.75 <sup>a</sup> |
|  |                    |                 | 15.96±0.93 <sup>c</sup>                          | 18.38±0.41 <sup>b</sup> | 23.28±0.49 <sup>a</sup> |
|  |                    |                 | 6.98±1.09 <sup>c</sup>                           | 11.59±1.29 <sup>b</sup> | 15.56±0.54 <sup>a</sup> |
|  | 8                  | Fom             | 30.49±0.35 <sup>c</sup>                          | 34.98±0.14 <sup>b</sup> | 40.66±0.45 <sup>a</sup> |
|  |                    |                 | 13.97±1.71 <sup>c</sup>                          | 28.33±1.97 <sup>b</sup> | 35.90±1.83 <sup>a</sup> |
|  |                    |                 | 0.00±0.00 <sup>c</sup>                           | 0.00±0.00 <sup>b</sup>  | 0.00±0.00 <sup>a</sup>  |
| <i>Eucalyptus camaldulensis</i> (bark extract) | 4                  | Fsm             | 33.03±0.06 <sup>c</sup>                          | 52.86±0.70 <sup>b</sup> | 72.07±0.36 <sup>a</sup> |
|  |                    |                 | 30.32±0.59 <sup>c</sup>                          | 45.93±0.07 <sup>b</sup> | 58.75±0.71 <sup>a</sup> |
|  |                    |                 | 16.26±0.42 <sup>c</sup>                          | 20.13±0.12 <sup>b</sup> | 32.26±0.25 <sup>a</sup> |
|  | 8                  | Fom             | 35.70±0.79 <sup>c</sup>                          | 58.82±0.78 <sup>b</sup> | 66.27±0.30 <sup>c</sup> |
|  |                    |                 | 33.74±0.49 <sup>c</sup>                          | 54.18±0.07 <sup>b</sup> | 62.13±0.14 <sup>a</sup> |
|  |                    |                 | 29.71±0.32 <sup>c</sup>                          | 44.12±0.13 <sup>b</sup> | 59.39±0.36 <sup>a</sup> |
| <i>Trachyspermum ammi</i> (seed extract)       | 4                  | Fsm             | 3.57±0.37 <sup>b</sup>                           | 5.40±0.62 <sup>a</sup>  | 7.32±0.35 <sup>a</sup>  |
|  |                    |                 | 0.00±0.00 <sup>b</sup>                           | 0.00±0.00 <sup>a</sup>  | 0.00±0.00 <sup>a</sup>  |
|  |                    |                 | 0.00±0.00 <sup>b</sup>                           | 0.00±0.00 <sup>a</sup>  | 0.00±0.00 <sup>a</sup>  |
|  | 8                  | Fom             | 13.27±0.73 <sup>b</sup>                          | 14.07±0.06 <sup>a</sup> | 17.19±0.30 <sup>a</sup> |
|  |                    |                 | 0.00±0.00 <sup>b</sup>                           | 0.00±0.00 <sup>a</sup>  | 0.00±0.00 <sup>a</sup>  |
|  |                    |                 | 0.00±0.00 <sup>b</sup>                           | 0.00±0.00 <sup>a</sup>  | 0.00±0.00 <sup>a</sup>  |
| Control  | Fsm                | Fom             | 38.75±1.51 <sup>a</sup>                          | 61.33±1.42 <sup>b</sup> | 81.35±1.21 <sup>c</sup> |
|  |                    |                 | 38.01±1.04 <sup>a</sup>                          | 62.64±1.10 <sup>b</sup> | 81.62±1.34 <sup>c</sup> |

Values shown are the Mean±SE of 3 replicates. Different letters represents the statistical differences between treatments, significance level was determined at p<0.05, Fsm: *Fusarium solani* f. sp. *melongena*; Fom: *Fusarium oxysporum* f. sp. *moniliforme*

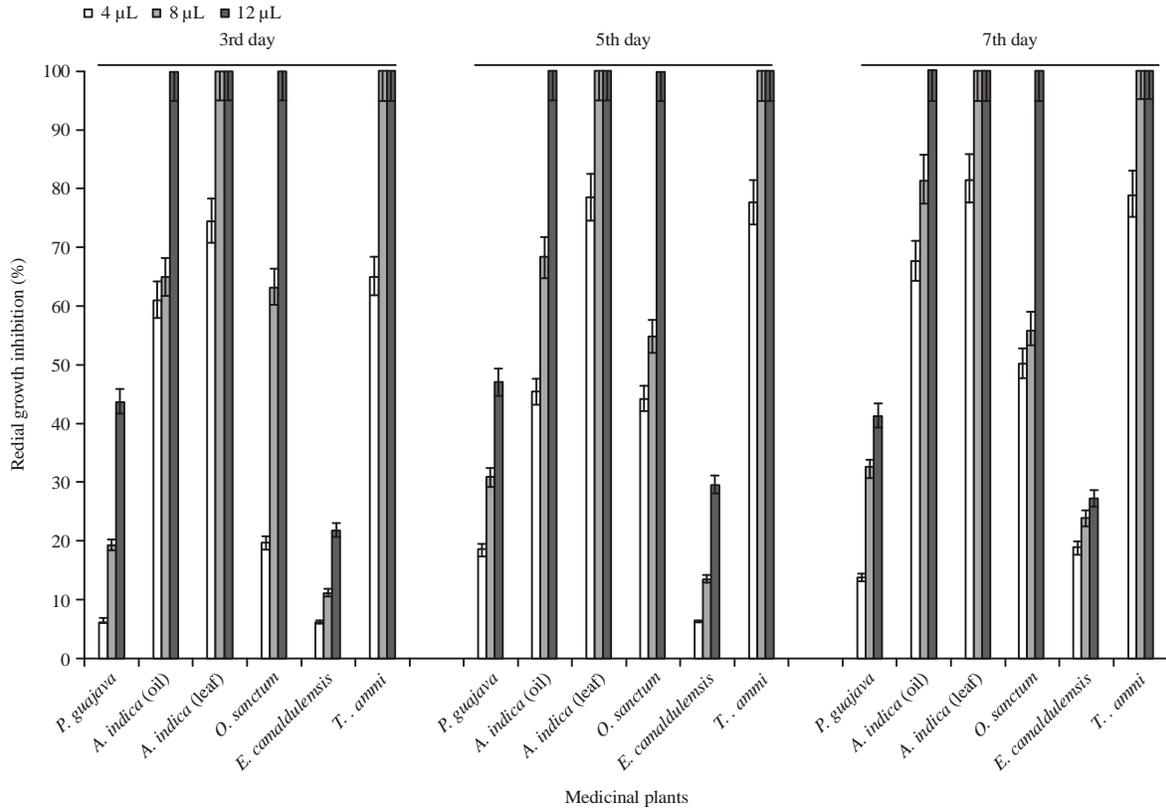


Fig. 2: Radial growth inhibition of *F. oxysporum* f. sp. *moniliforme* through antagonistic medicinal plant extracts on 3rd, 5th and 7th day of incubation

*Psidium guajava* (leaves), *Azadirachta indica* (oil and leaf), *Ocimum sanctum* (leaves), *Eucalyptus camaldulensis* (bark) and *Trachyspermum ammi* (seeds) at 4 μL concentration inhibited the mycelial growth of *Fusarium oxysporum* f. sp. *moniliforme* by 6.37, 61.01, 74.55, 19.76, 6.07 and 65.07% on 3rd day; 18.34, 45.49, 78.70, 44.16, 6.11 and 77.55% on 5th day and 13.78, 67.6, 81.63, 50.17, 18.81 and 78.94% on 7th day, respectively. On the other hand, at 8 μL concentration of foresaid medicinal plants, the mycelial growth was inhibited by 19.31, 64.93, 100, 63.24, 11.23, 100% on 3rd day; 30.79, 68.28, 78.7, 44.16, 13.5 and 77.55% on 5th day and 32.21, 81.49, 100, 56.01, 23.88 and 100% on 7th day, respectively. Maximum percentage inhibition i.e., 43.69, 100, 100, 100, 21.84 and 100% on 3rd day; 46.98, 100, 100, 100, 29.56 and 100% on 5th day and 41.25, 100, 100, 100, 27.23 and 100% on 7th day was recorded at 12 μL concentration (significant at 5%, Table 1 and Fig. 2).

The extract and oil of all medicinal plants were suppressive to the radial growth of *F. solani* and *F. oxysporum* (Table 1). *A. indica* (oil and extract), *T. ammi* (seeds) and *O. sanctum* (leaves) showed highest mycelial growth inhibition (100%) followed by *P. guajava* (leaves) and *E. camaldulensis* (bark). With the increasing concentration of medicinal plant extracts and oil, there was increase in percent inhibition of the mycelial growth of *F. solani* and *F. oxysporum*. These findings are in conformity with Hossain *et al.* (2013) reported that aqueous leaf extract of *A. indica* was highly effective in reducing the mycelial growth of *F. oxysporum* by 51.48, 53.70 and 55.19% at 10.0, 20.0 and 40% concentration, respectively. Sridhar *et al.* (2003) also reported that *T. ammi*

seed oil inhibited the mycelial growth 100% at higher concentration (90 and 135 ppm) and at lower concentration the inhibition was 44 and 85% against *F. oxysporum* at 25 and 45 ppm concentration, respectively. Antifungal activities and chemical properties of *Azadirachta indica* leaf extract on the growth of *Aspergillus*, *Rhizopus*, *Alternaria solani* and *Alternaria brassicae* are also reported by Jarvis and Morgan (2000), Hanaa *et al.* (2011) and Dissanayake (2014). Similar results were reported by Joseph and Priya (2011) in guava leaf extract against *Aspergillus niger* and *Aspergillus aculeatus*.

The percentage inhibition of *F. solani* and *F. oxysporum* at different concentration of culture filtrate of *T. harzianum*, *T. atroviride* and *T. longibrachiatum* is presented in Table 2. The data revealed a significance increase ( $p < 0.001$ ) in colony growth reduction of *F. solani* and *F. oxysporum* f. sp. *moniliforme* with increasing the concentration of culture filtrates. *T. harzianum*, *T. atroviride* and *T. longibrachiatum* filtrates inhibited the growth of *F. solani* by 77.55, 33.75, 100% and by 100, 73.68 and 100% in case of *F. oxysporum* f. sp. *moniliforme*, respectively at 12  $\mu$ L concentration on 3rd day; on 5th day 79.89, 48.91 and 100% against *F. solani* and 100, 74.98, 100% against *F. oxysporum*. However, on 7th day the percent reduction was 81.97, 44.68, 100% against *F. solani* and 100, 71.42 and 100% against *F. oxysporum*, respectively. At 8  $\mu$ L concentration, the foresaid antagonists inhibited the growth of *F. solani* by 73.34, 20, 81.08, 91.24, 67.55 and 100% by *F. oxysporum* respectively on 3rd day, whereas on 5th day 73.91, 32.61, 85.32% against *F. solani* and 91.49, 67.54 and 100% against *F. oxysporum* and on 7th day the percent reduction was 73.78, 38.94, 86.48% against *F. solani* and 89.79, 62.02 and 100% against *F. oxysporum*, respectively. On the other hand, at lower concentration (4  $\mu$ L), *T. harzianum*, *T. atroviride* and *T. longibrachiatum* were least effective and inhibited the growth of *F. solani* and *F. oxysporum* by 22.58, 13.99, 69.88, 62.29, 57.89 and 78.08% on 3rd day, 58.48, 29.89, 72.82, 73.93, 58.49 and 80.32% on 5th day and 43.45, 35.25, 72.55, 74.67, 53.44 and 77.12% on 7th day, respectively (Table 2 and Fig. 3).

Table 2: Efficacy of fungal antagonists against *F. solani* f. sp. *melongena* and *F. oxysporum* f. sp. *moniliforme* at different concentrations on 3rd, 5th and 7th day of inoculation

| Fungal antagonists                 | Concentration ( $\mu$ L) | Fungal pathogen | Days after inoculation/mean colony diameter (mm) |                               |                               |
|------------------------------------|--------------------------|-----------------|--|-------------------------------|-------------------------------|
|                                    |                          |                 | 3rd  | 5th                           | 7th                           |
| <i>Trichoderma harzianum</i>       | 4                        | Fsm             | 30.00 $\pm$ 1.00 <sup>c</sup>                    | 38.67 $\pm$ 2.08 <sup>b</sup> | 46.00 $\pm$ 1.00 <sup>a</sup> |
|                                    |                          |                 | 10.33 $\pm$ 0.58 <sup>c</sup>                    | 16.00 $\pm$ 1.00 <sup>b</sup> | 21.33 $\pm$ 1.53 <sup>a</sup> |
|                                    |                          |                 | 8.67 $\pm$ 0.58 <sup>c</sup>                     | 12.33 $\pm$ 0.58 <sup>b</sup> | 14.67 $\pm$ 1.53 <sup>a</sup> |
|                                    | 12                       | Fom             | 14.33 $\pm$ 0.58 <sup>c</sup>                    | 16.33 $\pm$ 1.53 <sup>b</sup> | 20.67 $\pm$ 2.08 <sup>a</sup> |
|                                    |                          |                 | 3.33 $\pm$ 0.58 <sup>c</sup>                     | 5.33 $\pm$ 0.58 <sup>b</sup>  | 8.33 $\pm$ 0.58 <sup>a</sup>  |
|                                    |                          |                 | 00.00 $\pm$ 0.00 <sup>c</sup>                    | 0.00 $\pm$ 0.00 <sup>b</sup>  | 0.00 $\pm$ 0.00 <sup>a</sup>  |
| <i>Trichoderma atroviride</i>      | 4                        | Fsm             | 33.33 $\pm$ 0.58 <sup>c</sup>                    | 43.00 $\pm$ 2.00 <sup>b</sup> | 52.67 $\pm$ 2.08 <sup>a</sup> |
|                                    |                          |                 | 31.00 $\pm$ 1.00 <sup>b</sup>                    | 41.33 $\pm$ 1.15 <sup>b</sup> | 49.67 $\pm$ 0.58 <sup>a</sup> |
|                                    |                          |                 | 25.67 $\pm$ 1.15 <sup>c</sup>                    | 31.33 $\pm$ 1.53 <sup>b</sup> | 45.00 $\pm$ 1.00 <sup>a</sup> |
|                                    | 12                       | Fom             | 16.00 $\pm$ 1.00 <sup>c</sup>                    | 26.00 $\pm$ 1.73 <sup>b</sup> | 38.00 $\pm$ 1.73 <sup>a</sup> |
|                                    |                          |                 | 12.33 $\pm$ 0.58 <sup>c</sup>                    | 20.33 $\pm$ 0.58 <sup>b</sup> | 31.00 $\pm$ 1.00 <sup>a</sup> |
|                                    |                          |                 | 10.00 $\pm$ 0.00 <sup>c</sup>                    | 15.67 $\pm$ 0.58 <sup>b</sup> | 23.33 $\pm$ 1.53 <sup>a</sup> |
| <i>Trichoderma longibrachiatum</i> | 4                        | Fsm             | 11.67 $\pm$ 1.53 <sup>c</sup>                    | 16.67 $\pm$ 1.15 <sup>b</sup> | 22.33 $\pm$ 0.58 <sup>a</sup> |
|                                    |                          |                 | 7.33 $\pm$ 1.15 <sup>c</sup>                     | 9.00 $\pm$ 1.00 <sup>b</sup>  | 11.00 $\pm$ 1.00 <sup>a</sup> |
|                                    |                          |                 | 0.00 $\pm$ 0.00 <sup>c</sup>                     | 0.00 $\pm$ 0.00 <sup>b</sup>  | 0.00 $\pm$ 0.00 <sup>a</sup>  |
|                                    | 12                       | Fom             | 8.33 $\pm$ 0.58 <sup>b</sup>                     | 12.33 $\pm$ 0.58 <sup>a</sup> | 18.67 $\pm$ 1.15 <sup>a</sup> |
|                                    |                          |                 | 0.00 $\pm$ 0.00 <sup>b</sup>                     | 0.00 $\pm$ 0.00 <sup>a</sup>  | 0.00 $\pm$ 0.00 <sup>a</sup>  |
|                                    |                          |                 | 0.00 $\pm$ 0.00 <sup>b</sup>                     | 0.00 $\pm$ 0.00 <sup>a</sup>  | 0.00 $\pm$ 0.00 <sup>a</sup>  |
| Control                            | Fsm                      | Fsm             | 38.75 $\pm$ 1.51 <sup>a</sup>                    | 61.33 $\pm$ 1.42 <sup>b</sup> | 81.35 $\pm$ 1.21 <sup>c</sup> |
|                                    |                          | Fom             | 38.01 $\pm$ 1.04 <sup>a</sup>                    | 62.64 $\pm$ 1.10 <sup>b</sup> | 81.62 $\pm$ 1.34 <sup>c</sup> |

Values shown are the Mean $\pm$ SE of 3 replicates. Different letters represents the statistical differences between treatments, significance level was determined at  $p \leq 0.05$ , Fsm: *Fusarium solani* f. sp. *melongena*, Fom: *Fusarium oxysporum* f. sp. *moniliforme*

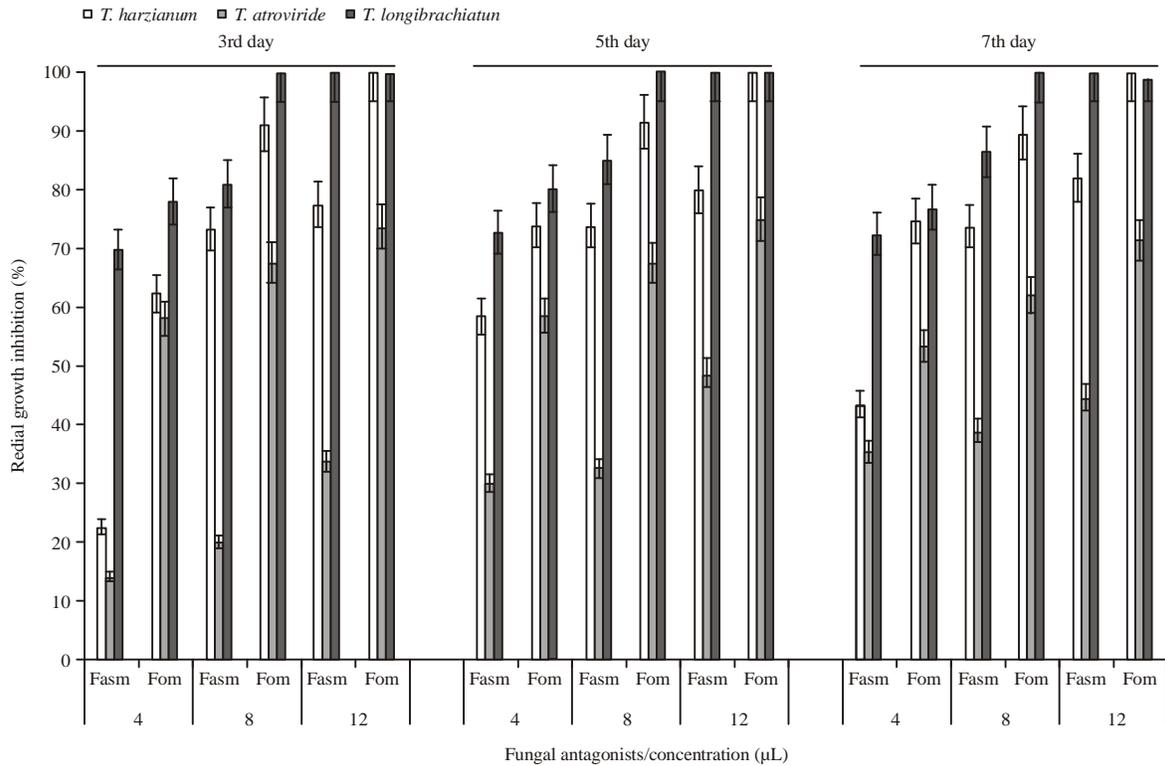


Fig. 3: Radial growth inhibition of *F. solani* f. sp. *melongena* and *F. oxysporum* f. sp. *moniliforme* through fungal antagonists on 3rd, 5th and 7th day of incubation

The results indicate that *T. harzianum*, *T. atroviride* and *T. longibrachiatum* are deleterious bioagents against wilt pathogen of brinjal and guava. It has been established that *Trichoderma* spp. inhibit pathogenic invasion through phenomena of mycoparasitism, antibiosis, competition (Freeman *et al.*, 2004), lysis of pathogenic hyphae (Kumar *et al.*, 2011), coiling, penetration (Yigit and Dikilitas, 2007), production of organic metabolites (Manzinger *et al.*, 2002) and volatile inhibitory substance-acetaldehyde are wide range of phenomena attributed to biocontrol potential of *Trichoderma* spp. (Upadhyay and Mukhopadhyay, 1986; Enespa and Dwivedi, 2014). There have been numerous recent attempts to use *Trichoderma* spp. against soil borne pathogens viz., *Sclerotinia*, *Fusarium*, *Pythium* and *Rhizoctonia* (Harman *et al.*, 2004; Bokhari and Perveen, 2012; Dubey *et al.*, 2015).

## CONCLUSION

Based on present study, it is concluded that certain biologically active compounds, extracts, essential oils and microbial antagonists are cheaper source and effective biopesticide for soil-borne pathogenic *Fusarium* species as an environment friendly. Thus, it can be recommended that the use of *Trachyspermum ammi*, *Azadirachta indica* (oil and leaves), *Ocimum sanctum* (leaves), microbial antagonists viz., *T. longibrachiatum* and *T. harzianum* followed by *Psidium guajava*, *Eucalyptus camaldulensis* and *T. viride* may be exploited as environmental safe alternatives to check the aforesaid fusaria.

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