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COMPARATIVE EFFICACY OF CHEMICAL FUNGICIDES, PLANT EXTRACTS AND BIO-CONTROL AGENTS AGAINST *FUSARIUM SOLANI* UNDER LABORATORY CONDITIONS

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ABSTRACT

Fusarium is a large genus of filamentous fungi widely dispersed in soil and causes huge yield losses in crop plants. In present study different fungicides, plant extracts and bio-control agent were tested *in-vitro* by food poisoned method against *Fusarium solani*. Ten different fungicides *viz*., Carbendazim, Fosetyl-aluminium, Thiophanate-methyl, Propineb, Metiram, Copper oxychloride, Mandipropamid, Myclobutanil, Difenoconazole and Penconazole were tested at three concentrations that are 100, 1000 and 10,000 ppm. All fungicides showed varied effects against *F. solani.* However, Carbendazim was highly effective at low as well as at medium and high concentrations, which reduced 100% mycelial growth followed by Mandipropamid, Thiophanate-methyl and Fosetyl-aluminium. Among plant extracts [citrus (*Citrus hystrix*), neem (*Azadirachta indica*), garlic (*Allium sativum*), onion (*Allium cepa*), dhatura (*Datura stramonium),* calotropis *(Calotropis procera),* peppermint *(Mentha piperita),* fennel (*Foeniculum vulgare*), ginger *(Zingiber officinale)* and chili *(Capsicum annuum)*], higher dose of *A. indica* and *C. procera* extracts showed maximum inhibition followed by *C. hystrix* and *C. annuum* extracts. Among seven fungal biocontrol agents, only *Penicillium citrinum*, *Trichoderma pseudokoningii* and *Aspergillus flavus* appeared highly effective to reduce mycelial growth of *F. solani*.

Keywords: Bio-control, Chemical control, Non-chemical control, *In-vitro*, Soil-borne

INTRODUCTION

Fusarium is largest genus of filamentous fungi widely distributed in agricultural soils. It contains large number of destructive plant pathogens such as *F. avenaceum*, *F. eumartii*, *F. oxysporum* and particularly *F. solani,* which is potential cause of vascular wilt, root rot and fruit rot as well as influence seed germination in different host plants. In sponge gourd, bottle gourd, squash, pumpkin reported to cause crown, foot rot, fruit rot and significant decrease in seed germination (Shakir and Mirza, 1992; Shakir *et al.,* 1995; Zitter, 1996). This can be seed-borne both internal and external, survive more than 1-2 years in seed (Watt, 2006). Crop plants are highly susceptible to attack of *F. solani* during the pre- and post-emergence stages (Nawar, 2007). It easily develops in several types of soil, especially on light sandy soils and can cause root rot disease in different crops in various sparts of the world (Celar, 2000). Under drought stress, the losses could reach 95% of production in some fields (Rojo *et al*., 2007).

Although currently a variety of techniques and methods have been known to control plant pathogens, in which few has been proved to satisfactory. The use of chemical based fungicides is most effective and reliable method to control the pathogens. However, chemicals are highly toxic to targeted pathogen, plants, products, human beings and other form of life.

Biological control based on botanical products as well as use of antagonists is the popular alternative of chemical control and in most of the cases confined to experimental research. The aim of research on biological control is to reduce the pathogen density and activity (Baker and Cook, 1974), production of harmless and/or beneficial

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microorganisms and subsequent analysis of their bio-control potential. Use of plant secondary metabolites against pathogen has gained attention of scientist. Particularly the use offresh plant extracts of different plant parts are frequently exploited during last three decades to control the plant diseases (Siddiqui *et al*., 2004*;* Amadioha and Uchendu, 2003; Sahayaraj *et al*., 2006; Haikal, 2007; Mohana and Raveesha, 2007; Joseph *et al*., 2008). Antifungal compounds from plant origins are efficient, less toxic and more environmental friendly (Lee *et al*., 2007).

Bio-control agents are more specialized and specific to targeted pathogens. The bio-control agents of some plant pathogens have been found effective and gaining importance as alternate to chemical control method. Therefore, the present study was carried out to compare the efficacy of different fungicides, plant extracts and bio-control agents against *F. solani*.

MATERIALS AND METHODS

Isolation and purification of fungi: The affected lentil roots showing symptoms of root rot were surface sterilized through 5% Sodium hypochlorite (commercial bleach) for 2 minutes and then placed in sterilized Petri plates containing freshly prepared potato dextrose agar (PDA) medium. Five pieces of roots were placed in each Petri plate. These Petri plates were incubated at 25±2°C for five days. Different fungal colonies were seen, from which *F. solani* were identified, purified and multiplied on PDA.

Effect of different fungicides on *F. solani*: Different systemic and contact fungicides were tested by food poisoned method. Different concentrations i.e. 10000, 1000 and 100 ppm were prepared by serial dilution method. Non-amended fungicide PDA medium served as negative control. After solidifying of the medium, 5 mm disc from one week old pure culture of *F. solani* was placed in the center of Petri plates and incubated at 25±2^{0}C. Experiment was replicated five times. The radial colony growth of *F. solani* was recorded after 24 hours till the upper surface in control treatment was fully covered with the mycelium of the test fungus.

The percent inhibition (PI) of *F. solani* over control was calculated by using the following formula:

$$PI = \frac{(A - B)}{A} \times 100$$

Where;

A: Colony growth of the *F. solani* in control plates.

B: Colony growth of the *F. solani* in treated plates.

Preparation of plant extracts: Plants used in experiment were collected from field area surrounding Campus of Sindh Agriculture University Tandojam and local market of Tandojam town. Extracts were prepared from freshly leaves, bulbs, seeds and rhizomes of different plants including citrus (*Citrus hystrix*), neem (*Azadirachta indica*), garlic (*Allium sativum),* onion (*Allium cepa),* dhatura (*Datura stramonium*), calotropis *(Calotropis procera),* peppermint *(Mentha piperita),* fennel (*Foeniculum vulgare*), ginger *(Zingiber officinale)* and chili *(Capsicum annuum)*. Fifty gram of each plant material (freshly leaves, bulbs, seeds and rhizomes) were washed with tape water and then grinded in pestle mortar. After grinding, strain them through muslin cloth by adding 250 ml of distilled water.

Effect of plant extracts on *F. solani*: The prepared extracts were evaluated for their effect on radial colony growth of *F. solani* by adding at the time of pouring in the sterilized PDA medium at 1, 2 and 4 ml /15ml separately. Other procedures were the same as described above.

Effect of bio-control agents on *F. solani*: Different antagonists including *Gliocladium virens*, *Trichoderma harzianum*, *T. polysporum*, *T. pseudokoningii*, *Stachybotrysatra*, *Acrophialophora fusispora*, *Aspergillusniger*, *A. flavus*, *Penicillium citrinum*, *Paecilomyces variotii* and *P. lilacinus* were tested *in-vitro* conditions against *Fusarium solani*. The bio-control agents were obtained from Culture Collection Center, Department of Agriculture & Agribusiness Management, University of Karachi, Pakistan. In dual culture plate essay, a 5 mm diameter disc of test fungus and antagonistic agent were placed near the opposite edge of a Petri plates containing PDA medium. The plates without antagonists serve as negative control. The plates were arranged in CRD with five replications. These plates were incubated at 25±2^{0}C. The colony growth of bio-control agents and *F. solani* were recorded and the percent inhibition was calculated. The interactions between pathogen and each antagonist were recorded as described by (Yaqub and Shahzad, 2005).

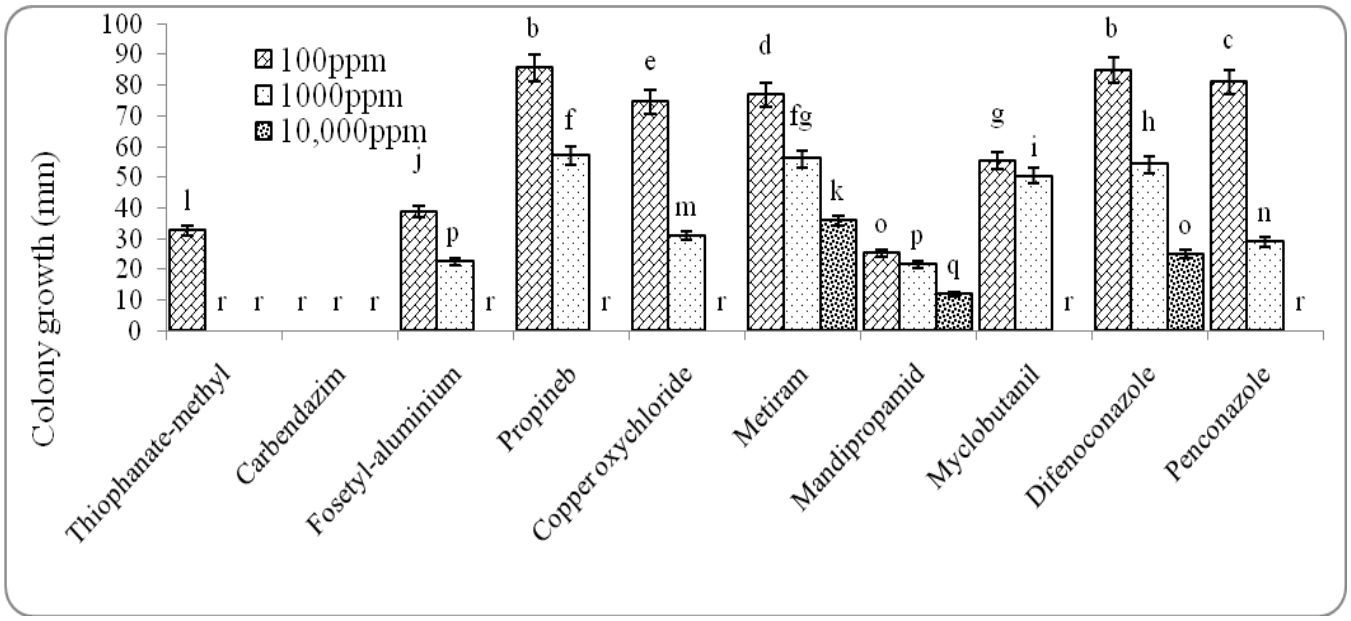
STATISTICAL ANALYSIS

After that the data was analyzed by ANOVA using Statistix 8.1 software. Least significant differences (LSD) were calculated using significant level at P = 0.05.

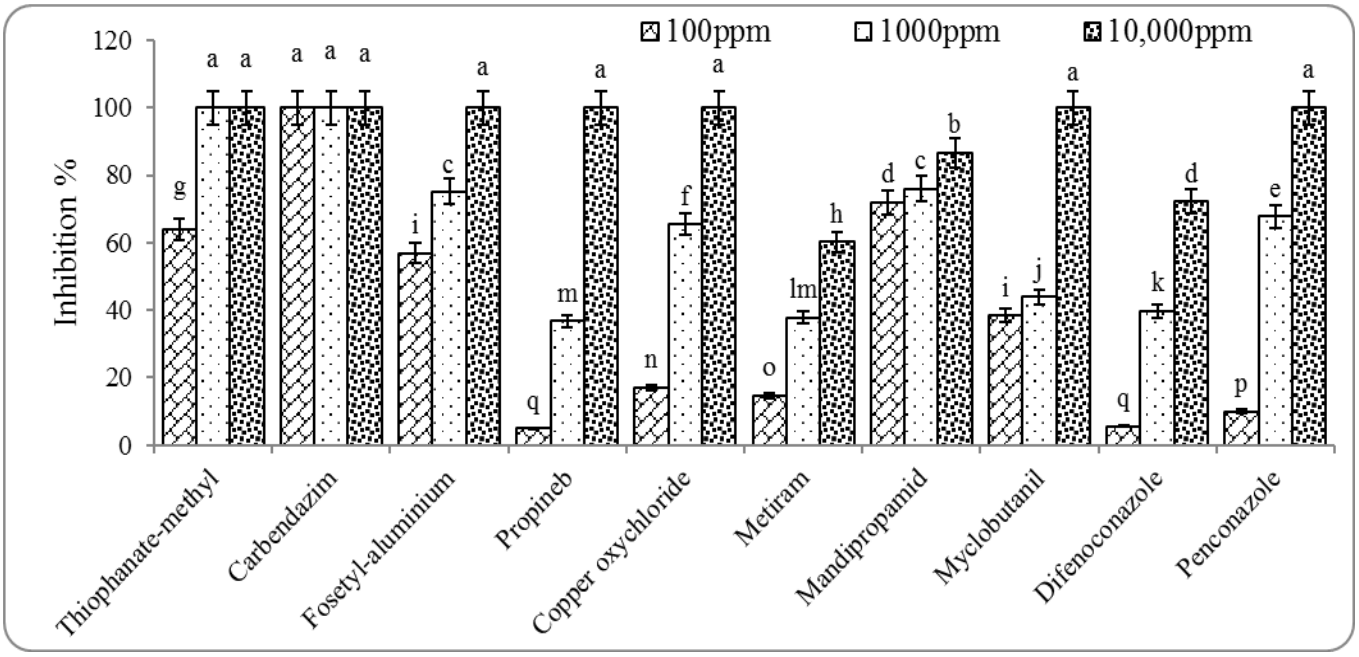
RESULTS

Effect of different fungicides on *F. solani*: Ten different fungicides Fosetyl-aluminium, Propineb, Carbendazim, Copper oxychloride, Metiram, Thiophanate-methyl,

Mandipropamid, Myclobutanil, Difenoconazole and Penconazole were tested by food poisoned method. The effectiveness of all the tested fungicides varied significantly in reducing *in vitro* growth of *F. solani.* Generally, higher concentration appeared more effective as compared to the lower concentrations. At 10,000 ppm of Fosetyl-aluminium, Propineb, Carbendazim, Copper oxychloride, Thiophanate-methyl, Myclobutanil and Penconazole the *F. solani* unable to grow. Among, different fungicides, Carbendazim appeared highly effective as its all concentrations completely inhibited the colony growth of test pathogen. However, Mandipropamid, Fosetyl-aluminium, Penconazole and Copper oxychloride remained moderately effective which inhibited 75.99, 75.10, 67.77 and 65.55% growth, respectively (Figure 1).



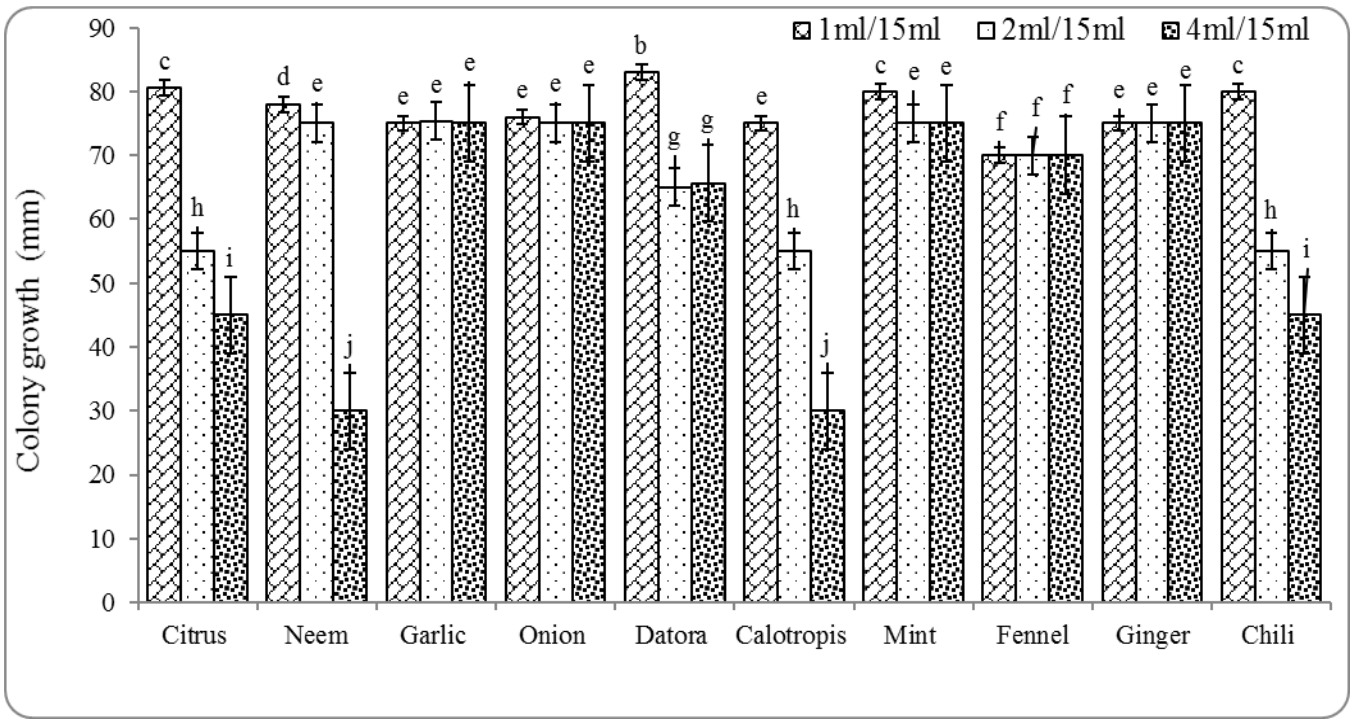
(a)



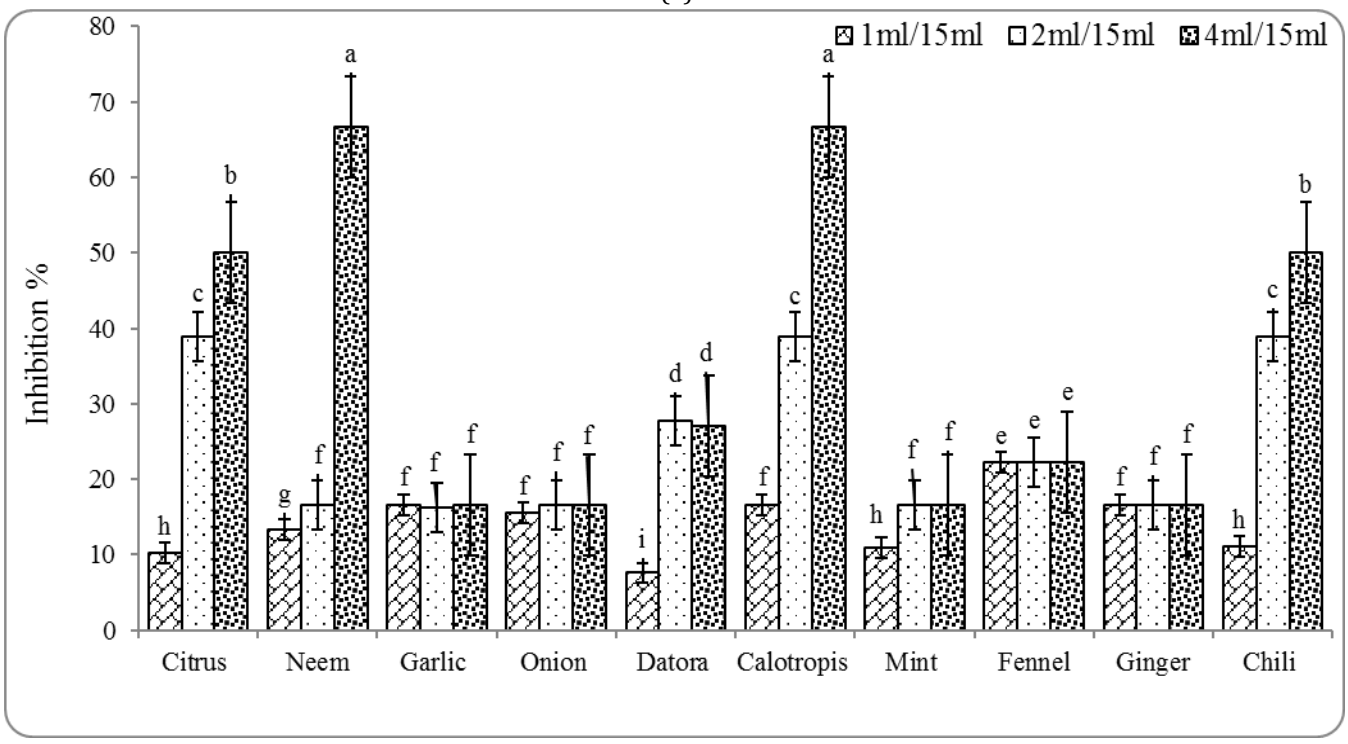
(b)

Figure 1. Effect of different fungicides on (a) colony growth and (b) inhibition (%) of *F. solani.*

Effect of different plant extracts on *F. solani*: The plants extracts of citrus, neem, garlic, onion, dhatura, calotropis, mint, fennel, ginger and chili found comparatively less effective than fungicides against *F. solani.* Only higher dose (4ml/15ml) of neem, calotropis, chili and citrus extracts brought ≥50% inhibition in the colony growth of test pathogen. All other treatments caused less than 50% reduction in the growth of *F. solani* (Figure 2).



(a)



(b)

Figure 2. Effect of different plant extracts on (a) colony growth and (b) inhibition (%) of *F. solani.*

Effect of bio-control agents on *F. solani*: All antagonists such as *G. virens*, *P. variotii*, *T. harzianum*, *T. polysporum*, *T. pseudokoningii, A. flavus* and *P. citrinum* caused more or less reduction in the colony growth of *F. solani.* Among these antagonistic fungi *P. citrinum*, *T. pseudokoningii* and *A. flavus* produced maximum reduction in the growth of *F. solani* followed by *G. virens* and *T. polysporum. P. citrinum* and *T. pseudokoningii* showed D type of interaction, which means colonies of the *F. solani* and these bio-control agents intermingled. While, *A. flavus* showed C type interaction, which mean mycelium of bio-control agent and test pathogen met each other; no further growth either of the pathogen (*F. solani*) or the bio-control agent was observed (Table1).

Table 1. Effect of different bio-control agents on mycelial growth of *F. solani.*

S. No	Bio-control agent	Incubation time (hours)	Diameter of pathogen in interaction (mm)	Diameter of bio-control agent (mm)	Type of interaction
1	*T. harzianum*	96	40.6 b	49.6	D
2	*T. polysporum*	96	30.6 c	59.4	C
3	*T. pseudokoningii*	72	15.4 f	74.6	D
4	*G. virens*	72	22.0 d	68	D
5	*P. variotii*	144	40.8 b	49.2	C
6	*A. flavus*	72	17.2 e	72.8	A
7	*P. citrinum*	72	14.4 f	75.6	D
8	*F. solani* (control)	144	51.8 a		

DISCUSSION

The use of chemical based fungicides is most effective and reliable method to control the pathogens. In order to find out fungicides which are effective even at low doses, ten different fungicides such as Fosetyl-aluminium, Propineb, Carbendazim, Copper oxychloride, Metiram, Thiophanate-methyl, Mandipropamid, Myclobutanil, Difenoconazole and Penconazole were tested against *F. solani*. Fungicides like Fosetyl-aluminium, Propineb, Carbendazim, Copper oxychloride, Thiophanate-methyl, Myclobutanil and Penconazole appeared highly effective and *F. solani* failed to grow at their higher dose. While, Mandipropamid, Fosetyl-aluminium, Penconazole and Copper oxychloride found moderately effective against the *F. solani*. Testing of fungicides against the destructive fungal pathogens is the common practice throughout the world. There are other reports which also confirmed the highly effective nature of Carbendazim against *F. solani* (Hiremath *et al*., 1981; Singh, 1988; Chattannavar *et al*., 2006). Methoxyethyl mercury chloride (100 ppm), Thiram (1000 ppm) and Carbendazim (50 ppm) were also effectively reduced growth and sporulation of *F. solani* (Rathnamma, 1994).

We investigated antifungal activity of ten plant extracts under laboratory conditions. The results revealed that only higher dose of *Azadirachta indica* and *Calotropis procera* extracts caused maximum inhibition fallowed by *Citrus hystrix* and *Capsicum annuum* extracts. Extracts of *Artemessia annua, A. indica, Eucalyptus globules, Ocimum sanctum* and *Rheum emodi* found effective against *F. solani* f. sp. *melongenae* (Joseph *et al*., 2008). In another study bulb extract of *A. sativum* and leaf extract of *A. indica* caused maximum inhibition of mycelial growth of *F. solani* and *R. solani* (Mallesh and Narendrappa, 2009). Yelmame *et al*. (2010) found inhibitory effect against *F. solani* with the use of neem cake.

Among antagonistic fungi *Penicillium citrinum*, *Trichoderma pseudokoningii, Aspergillus flavus* and *Gliocladium virens* proved as potential bio-control agents against *F. solani*. Similarly, Gupta (1999) found *T. pseudokoningii* highly effective against *F. solani* causing cutting rot in mulberry. Rini and Sulochana, (2007) found that *T. pseudokoningii* TR17 and *T. harzianum* TR20 were two most antagonistic isolates against *R. solani*. Similarly, Bajwa and Javaid (2007) evaluated the effect of five species of *Trichoderma* (*T. viride, T. harzianum, T. areoviride, T. pseudokoningii* and *T. koningii*) and found that the effect of all the tested *Trichoderma* species except *T. koningii* was statistically significant against the test pathogenic fungal species. The inhibition in the growth of *F. solani* was also achieved through the use of *A. niger, T. viride, Gliocladium* sp., *T. harzianum* and *P. citrinum* (Ainbikapathy *et al*., 2002). *G. virens, T. hamatum, P. fluorescens* and *B. cepacia,* also reduced the Fusarium wilt of tomato compared to control(Larkin and Fravel, 1998).The non-aflatoxigenic strains strains of *Aspergillus*

flavus were also extensively tried and used as bio-control agent (Abbas *et al*., 2011; Ehrlich and Cotty, 2004).

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