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Effect of different plant extract on seedling diseases of Chillies, caused by *Fusarium* f. sp. *Capsici*

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Abstract:

Seedling disease caused by Fusarium spp. are the most serious threats in man crops including chilli. Present study determined eco-friendly antifungal compounds from different plant extracts against most predominant Fusarium spp. in chillies. The pathogenic fungus Fusarium oxysporum f. sp. capsici was isolated and identified from naturally infected chili seeds collected from local markets with highest frequency (56%), followed by Aspergillus sp. (19%), Fusarium spp. (10), Macrophomina phaseolina (7%), Alternaria sp. (3%), Rhizoctonia solani (3%) and Phoma capsici (2%), respectively. The results regarding effectiveness of different plant extracts on germination of chili seeds, plant growth (shoot, root and total plant length and weight) and mortality of seedlings showed highly significant difference

at P < 0.01 and 0.05. In case of plant extracts, the maximum seed germination, plant length and weight (79.00%, 6.8250 cm and 6.8250 mg) was recorded by using Acasia followed by Neem (75.50%, 6.6750 cm and 0.3388 mg), Giant Milk Weed (69.50%, 6.500 cm and 0.3027 mg) and Australian Acasia (68.00%,cm and 0.2760 mg). Whereas, significantly minimum mortality (5.500 and 16.500 at 15 and 30 DAS) was recorded in Acasia followed by Neem (9.5000 and 27.500 at 15 and 30 DAS), Giant Milk Weed (11.000 and 32.00), Australian Acasia (18.00 and 33.000), and thorn apple (20.500 and 36.000) at 15 and 30 DAS respectively. Significantly maximum mortality (27.500 and 46.000 at 15 and 30 DAS) was recorded by using inoculated untreated chilli seeds followed by uninoculate untreated chilli seeds (20.000 and 43.00 at 15 and 30 DAS). Based on the findings, it is suggested that use of plant extract not only reduce the seedling diseases but also helpful in reducing the health hazards caused by chemical pesticides.

Key words: Chillies; Seedling diseases; *Fusarium* spp.; Plant extract

INTRODUCTION

Chillies (*Capsicum annuum*. L.) belong to the Family Solanaceae (Baloch, 2009), is one of the most important spice crops occupies high commercial value. Chilli fruits are rich in vitamin A and C; and also contain appreciable amount of calcium, phosphorus and iron, while hot type chillies are a source of the digestive stimulant, capsaicin (Khoso, 1994). Production of chilli has been reduced due to the many a biotic and biotic factors, especially fungal disease problems. Several pathogenic fungi may be involved in causing diseases in chilli. *Fusarium* spp. causes seed rots, seedling rot, root rot and *Fusarium* wilt (Hashmi, 1989; Hashmi and Thrane, 1990; Mushtaq and Hashmi, 1997).

Effective, safe, economical and easy control management of seedling diseases of chillies, caused by *Fusarium* spp is still under question. Pawar (2011) stated that the use of extracts of

different plant parts i.e. leaf, stem, root, fruit and seeds against fungi is practiced since a long time. Channa, *et al.* (2008a and b) studied effect of plant extracts on plant height, yield, mortality and incidence of root rot of okra. The effect of Neem *(Azadiracta indica*) leaf extract against *Fusarium oxysporum*, the causal agent of wilt have also been evaluated by Hassanein *et al*. (2010). They reported that germination of seeds was highest and disease incidence was obtained lowest in pots treated with the pathogen and irrigated with aqueous neem extract. However, the work done in the prvious studies has been limited to one or two plant or plant part extracts. Thus the present study have been planned to observe the effect of different plant extracts on the pathogen towards the development of eco-friendly antifungal compounds for controlling most predominant *Fusarium spp*. study in chilies.

MATERIALS and METHODS

The experiment on effect of plant extracts on the pathogen towards the development of eco-friendly antifungal compounds for controlling most predominant *Fusarium* spp. in chilies was conducted in the department of Plant Pathology, FCPT, SAU, Tandojam.

Collection of seed

Chilli seeds were collected from the commercial markets of Hyderabad, Tando jam and Tando Allayar.

Isolation and identification of disease causal agent

Isolation was done from chili seeds under aseptic conditions through standard isolation method. The seeds were surface sterilized by dipping in 5% Bleach (Sodium hypochlorite, NaOCl) for about 4 minutes and passed twice from distilled sterile water for 3 minutes to remove the traces of surfactant. Five seeds per plate were transferred to Petridishes, containing

sterilized potato-dextrose agar medium. All the plates were incubated at 25 ± 1^{O}C for 7 days. The growth of the fungi was observed daily for preparation of subculture from grown fungus to meet the requirements of pure culture for identification. The fungi isolated were identified by studying their typical mycelial growth, produced on the potato-dextrose agar medium and observing conidial morphology, using standard diagnostic keys developed by Booth (1971) and Barnett and Hunter (1972). Isolates were further confirmed using internet database of fungi.

Antifungal assay of plant extracts

Effect of different plant extracts on the pathogen towards the development of eco-friendly antifungal compounds for controlling most predominant *Fusarium* spp. in chilies was evaluated in pot experiments. Initially chili seeds were dipped in spore suspension of *Fusarium* sp. for 5 minutes. The suspension was prepared by mixing fresh mycelial growth of the fungus over one Petri dish in 200ml water. These artificially infested Chilli seeds were then treated with different plant extracts i.e. Acasia, Neem, Giant Milk Weed, Australian Acasia, and Thorn apple and sown in earthen pots containing 2 kg steam sterilized soil. While uninfested seeds were dipped in tape water and sown in same way as control for comparison of plant extracts effects.

The data was recorded on frequency of fungi associated with chili seeds, germination and damping off percentage of seedlings , plant growth (shoot, root and total plant height and weight) and mortality (%)of chili seedling, artificially inoculated with *Fusarium oxysporum* f. sp. *capsici* and treated with different plant extracts.

Experimental design

The experiments for the effect of plant extracts on the pathogen towards the development of eco-friendly antifungal compounds

for controlling most predominant *Fusarium* spp. in chilies were arranged as completely randomized design by sowing 50 seeds per replication (pot) and four replications (pots) per treatment (five of plant extracts, , uninoculated and inoculated control). The data was analysed through analysis of variance using computer software "Students edition of Statistix".

RESULT AND DISCUSSION

Frequency (%) of fungi isolated from naturally infected chili seed

Fusarium oxysporum f. sp. *capsici* was isolated and identified with highest frequency (56%) from naturally infected chili seeds collected from local markets, followed by *Aspergillus* sp. (19%), *Fusarium* spp. (10), *Macrophomina phaseolina* (7%), *Alternaria* sp. (3%), *Rhizoctonia solani* (3%) and *Phoma capsci* (2%), respectively (Figure 1). Other researchers also isolated and identified similar fungi from chili seeds. Kamara-Keita, *et al.* (2002) identified as *Phytophthora sp., Fusarium oxysporum sp. and Alternaria alternata* sp. Pérez-Moreno, *et al.,* (2002) identified *Phytophthora capsici*, *Fusarium* sp. and *Rhizoctonia* sp. Lopez *et al.,* (2002) conducted seed quality tests and identifed *Aspergillus* spp., *Penicillium* spp., *Alternaria* spp., *Trichoderma* spp., *Fusarium* spp. and *Rhizoctonia* spp. Gherbawy and Yaser (2003) isolated 31 species belonging to 16 genera, the most frequently isolated fungi were *Aspergillus flavus*, *A. niger*, *A. terreus*, *Fusarium oxysporum*, *Penicillium jensenii* and *Trichoderma harzianum*. *F. oxysporum* was the most common. Sharfun-Nahar (2004) isolated 47 fungal species. Telang (2010) found total eighteen fungi to be associated with the seeds of Chilli varieties. Sitara and Hasan (2011) isolated a total of 19 genera and 38 species of fungi, of these *Fusarium moniliforme*, *F. oxysporum* and *F. solani* were more frequently isolated

Effect of different plant extracts on chili growth

Seed germination: Effect of different plant extracts on percent of seed germination showed that significantly maximum seed germination (79.00%) was recorded by using Acasia fallowed by Neem (75.50%), Giant Milk Weed (69.50%) and Australian Acasia (68.00%). Significantly minimum seed germination (45.50%) was recorded and inoculated but treated with thorn apple (66.50 and 67.00%), respectively (Figure 2). Meneses (2002) also investigated the fungicidal activity of various wild plants against the mould species, inculde *Fusarium poae and F. moniliforme*. On the other side, Devi, *et al.* (2008) evaluated the antifungal activity of *Calotropis procera* against *Fusurium* sp.. Hernandez *et al..,* (2004) evaluated the effect of 3% neem and 27.5% Di Tera (an extract of *Myrothecium verrucaria*) on the germination of *Capsicum annuum*. Hassanein, *et al.* (2010) studied the effect of Neem *(Azadirachta indica*) leaf extract against *Fusarium oxysporum*, the causal agent of wilt, and reported that germination of seeds was highest in pots containing the negative control (soil free of pathogen) and in pots irrigated with the aqueous neem extract. Sahoo *at el* (2010) used aqueous leaf extract of different concentrations to investigate their effects on germination. The aqueous leaf leachate was found to have both stimulatory and inhibitory effect on germination. Khair and Nadia (2011) tested the antifungal activity of aqueous extracts of 22 of plant species; all plant extracts have an antifungal activity at concentrations of 2.5%.

Seedling Height (cm): Effect of different plant extracts on seedling height showed that significantly maximum shoot length (2.125cm) was recorded by using acasia followed by giant milk weed and neem (4.900cm), *Australian acasia* (4.700 cm) and thron apple (4.600 cm). Significantly minimum shoot length was recorded in inoculated untreated (3.275 cm) followed

by uninoculated untreated (4.200 cm) chili seeds. The maximum root length (1.775 and 1.700cm) was recorded in case of treating the seed with neem and acasia, respectively; followed giant milk weed (1.600cm), *Australian acasia* (1.500cm) and thron apple (1.350cm). The minimum root length was recorded in inoculated untreated chili seed (0.975 cm) and uninoculated untreated chili seed (1.025). However, significantly maximum total plant length was recorded in case of acasia (6.825 cm) followed by neem (6.675 cm), giant milk weed (6.500 cm), *Australian acasia (*6.200 cm) and thron apple (5.950 cm). The minimum total plant length was recorded in inoculated untreated chili seed (4.250cm) followed by uninoculated untreated chili seeds (2.225 cm) (Figure 3). Muthukumar *et al*., (2007) reported that the results obtained from screen house application of *A. pavonina, L. leucocephala* and *Eucalyptus* spp., @ 0.1 and 1% w/w showed significant control of *Fusarium* spp., and enhanced plant growth in term of shoot length, shoot weight, root length and root weight. Telang (2010) screened root stems, leaf and bark extracts of some common and easily available plants for the Bio-control of the seed mycoflora of the Chilli. The extracts of all the test plants were found to be inhibitory in more or less degree for the incidence of seed mycoflora while with a few exceptions, they were found to be stimulatory for seed germination.

Seedling weight (mg): Effect of different plant extracts on seedling weight showed that significantly maximum shoot weight (0.294 mg) was recorded by using acasia followed by giant milk weed (0.2600 mg), *Australian acasia* and neem (0.247 mg) and thron apple (0.217 mg). Significantly minimum shoot length was recorded in inoculated untreated and uninoculated untreated (0.0923 mg) chili seeds. The maximum root length (0.0918 and 0.0910 mg) was recorded in case of treating the seed with neem and acasia, followed giant milk weed (0.0428 mg), *Australian acasia* (0.033 mg) and thron apple

(0.025 mg).The minimum root length was recorded in inoculated untreated chili seed (0.0183 mg) followed by uninoculated untreated chili seed (0.020 mg). Similarly, the maximum total plant weight was recorded in case of acasia (0.3862 mg) followed by neem (0.3388 mg), giant milk weed (0.3027 mg), *Australian acasia (*0.276 mg) and thron apple (0.243 mg). The minimum total plant length was recorded in inoculated untreated chili seed (0.1105 mg) followed by uninoculated untreated chili seeds (0.182 mg) (Figure 4). Zainab-Mushtaq *et al*., (2009) examined effect of seed powder of *Azadirachta indica,* A. Juss, *Adenanthera pavonina* L., *Leucaena leucocephala* (Lam.) de Wit and *Eucalyptus* spp., in the control of *Fusarium* spp. The results obtained from screen house application of *A. pavonina, L. leucocephala* and *Eucalyptus* spp., @ 0.1 and 1% w/w showed significant control and enhanced plant growth in term of shoot length, shoot weight, root length and root weight. Similarly, Hassanein, *et al*. (2010) studied the effect of Neem *(Azadirahcta indica*) leaf extract against *Fusarium oxysporum*, the causal agent wilt, and reported that there was significant gradual increase in growth parameters (shoot and root length, number of leaves, fresh and dry weight of shoots and roots), when the plants were sprayed and irrigated with aqueous Neem extract with the greatest improvement. It has been found stimulatory and inhibitory effect on shoot, root elongation and dry matter of receptor plants.

Mortality of chili seedlings

Effect of different plant extracts on mortality (%) of chili seedlings showed that significantly minimum mortality (5.500and 16.500 at 15 and 30 DAS) was recorded in Acasia followed by Neem (9.5000 and 27.500 at 15 and 30 DAS), Giant Milk Weed (11.000 and 32.00), Australian Acasia (18.00 and 33.000), and thorn apple (20.500 and 36.000) at 15 and 30 DAS respectively. Significantly maximum mortality (27.500 and

46.000 at 15 and 30 DAS) was recorded by using inoculated untreated chili seeds followed by uninoculate untreated chili seeds (20.000 and 43.00 at 15 and 30 DAS), respectively (Figure 5). Chowdary *et al.,* (2000) reported that mortality of plants was not observed in the combined application of *T. viride* chestnut compound and neem cake, whereas, plant mortality was 11.4 and 13.6% in combined application of *T. viride* and neem cake, and *T. viride* and chestnut compound treatments, respectively. While, Hegde *et al*., (2001) determined in vitro efficacy of the plant extracts (0.3% chilli, ocimum, neem, onion, *Clerodendrum*) and observed that all the tested plant extracts exhibited inhibitory activity against the pathogen.

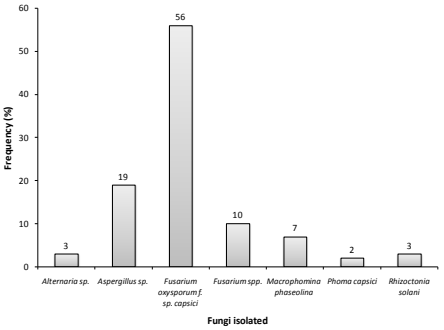
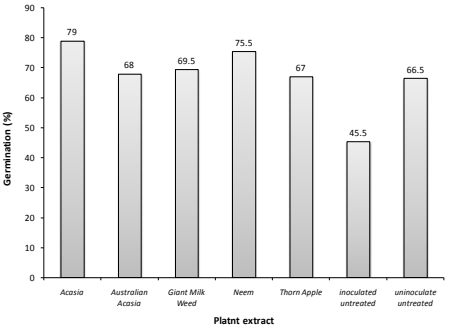
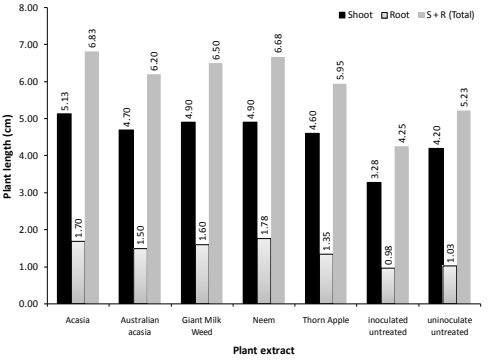


Figure 1. Frequency (%) of fungi isolated and identified from naturally infected chili seeds collected from local markets.



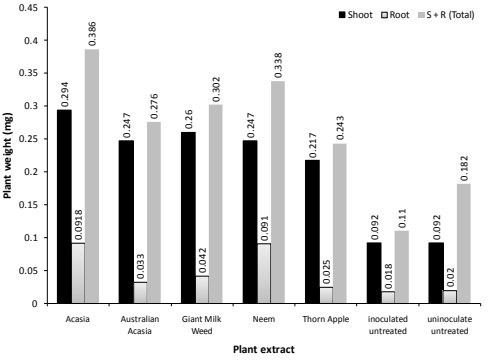
Alpha 0.01 = 7.1388
Alpha 0.05 = 5.2434

Figure 2. Effectiveness of different plant extracted on seed germination of chili seeds, artificially inoculated with *Fusarium oxysporum* f. sp. *capsici*



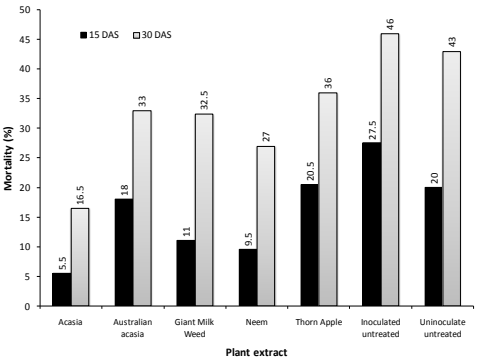
Alpha 0.01 = 0.2118 0.1329 0.248
Alpha 0.05 = 0.1556 0.0976 0.182

Figure 3. Effectiveness of different plant extracted on plant root, shoot and total plant height of chili seedling, artificially inoculated with *Fusarium oxysporum* f. sp. *capsici*



Alpha 0.01 = 7.855E-03 7.095E-03 0.0119
Alpha 0.05 = 5.769E-03 5.211E-03 8.735E-03

Figure 4. Effectiveness of different plant extracted on plant root, shoot and total plant weight of chili seedling, artificially inoculated with *Fusarium oxysporum* f. sp *capsici*



Alpha 0.01 = 9.1642 6.7311
Alpha 0.05 = 6.5679 4.8241

Figure 5. Effectiveness of different Plant extracted on Seedling mortality of chili seedling, artificially inoculated with *Fusarium oxysporum* f. sp. *capsici*

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