POTENTIAL OF PLANT EXTRACTS AND FUNGICIDES FOR MANAGING Fusarium oxysporum f. sp. lycopersici

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ABSTRACT

Fusarium oxysporum f. sp. lycopersici (FOL) is a highly destructive fungal pathogen of tomato crop causing wilt disease which may reduce 10 to 90% yield. In Pakistan, tomato is widely grown in Sindh province, major territories are district Hyderabad, Tando Allahyar and Mirpurkhas. Thus, surveys of these territories were conducted to record intensity of the disease and confirm etiology. Furthermore, potential of different botanical pesticides and commercially available fungicides were tested to inhibit mycelial growth of the causal fungus. The experiment was laid down with complete randomized block design with three replications. The results showed that the disease was occurring in all locations with the range of 8-47 % incidence. F. oxysporum f. sp. lycopersici was predominantly isolated from the collected disease sample during survey and pathogenic nature of the fungus was tested on the tomato Golo variety through soil drenching method. The disease incidence of 30 and 42 % (72 % as total) was recorded in inoculated tomato plants at 20 and 40 DAI, respectively. Maximum (67 %) inhibition of the fungal growth was found by neem seed extracts at higher dose of 8 % concentration followed by 63 % with neem seeds and Eucalyptus at 6 and 8 %, respectively. Alternatively, the Nativo 75 WG fungicide was found most effective in reducing the redial mycelial growth of target fungus followed by Topsin M at 1000 ppm whereas Aliette and Melodedue fungicides were found least effective under in-vitro conditions.

Keywords: Tomato, Fusarium oxysporum, Incidence, Botanical pesticides, Fungicides.

INTRODUCTION

Tomato is considered highly nutritious and widely grown vegetables in 144 countries of the world. Among them China, United States, Turkey, India and Egypt are the top most tomato producing countries of the world in metric ton production. The total area under cultivation of tomato is 45, 82,438 thousand ha. Whereas, the production 15051381 thousand tones and productivity of 32.8 tones/ha has been recorded in the world (FAO, 2016).

It is the second major vegetable, produced in Pakistan after potatoes (Mirza, 2007). In Pakistan tomato crop is grown on area of 62.6 thousand hectares which produces 587.1 thousand tons with an average yield of 9.4 thousand tons per hectare (GOP, 2016). Sindh province is major tomato producing region of Pakistan, where the crop is grown on an area of 27.9 thousand hectares with total production of 206.5 thousand tones and 7.4 thousand tones yield per hectare (GOP, 2016). This average yield is lowest as compare to other provinces of Pakistan (GOP, 2016). It is very important vegetable, having much nutritional value at comparatively low prices as compare to other vegetables. It has 95.3% water, 0.07% calcium and niacin, Vitamin A, C, E and rich source of nutrients like Na, K, Fe, and antioxidants especially lycopene and salicylate. All of have great importance in human metabolic system. Tomato fruit is commonly consumed in our daily life in different modes, such as vegetables,
salad, ketchup, chatni and other dishes (Afzal et al., 2013; Chohan and Ahmad, 2008).

The yield per hectare is lower as compared to other countries due to attack of several pathogens that are responsible for damaging the quantity and quality of the crop every year. Among them, *Fusarium oxysporum* f.sp. *lycopersici* is one of destructive soil borne fungal pathogens, causing wilt disease in tomato (Rai et al., 2011). In culture it produces white aerial mycelium often with pink, orange, red, blue, violet or purple pigmentation developing with age. It can also produce slimy clump of spores (sporodochia) and blue sclerotia in culture (Windles, 1992). *Fusarium oxysporum* f.sp. *lycopersici* can produces three types of asexual spores, macro and micro conidia and chlamydospores depending on culture conditions. Macro conidia are usually produced abundantly as long, sickle-shaped, thin-walled, spores with several septa. Micro conidia are also usually abundant and are smaller single- celled, oval-shaped spores. The production of micro conidia on short monopodialites is a distinguishing characteristic. Chlamydo spores are thick-walled, round; dormant spores are formed singly or in pairs. It is typically produced in 2-4 weeks on old culture (Nelson, 1983) It was first reported in England in 1895 by G.E Massee. The fungus is prevailing in 32 countries where the environmental conditions are warm (Mui- Yun Wong, 2003). The symptoms produced by *Fusarium* wilt disease of tomato include drooping, yellowing, wilting, and dying of the lower leaves, often on one side of the plant. These symptoms may appear on successively younger leaves with one or more branches being affected and others remaining healthy. After a few weeks, browning of the vascular system may be observed by slicing the stem open lengthwise with a knife. This brown discoloration inside the stem can be found from the roots to the top of the plant. Plant growth is stunted, and, under warm conditions, the plant may die (Kenneth et al., 2014). 10 to 90% losses in yield occurs due to the attack of *Fusarium* wilt disease of tomato (Singh and Kamal, 2012). Management of this fungus is difficult due to their endophytic growth and longer persistence in soil according to Borroro et al. (2006) and Haridon et al. (2011). In Pakistan the fungus is managed using integrated practices such as, cultivation of resistant varieties, systemic induced or acquired resistance and application of bio-control agents (Hussain et al., 2013; Sultana et al., 2013; Shafique et al., 2015; Hussain et al., 2013; Fatima and Anjum, 2017). Even though, such practices are efficiently and safely managing the fungal development but are either time consuming or unavailable for commercial purpose. Additionally, genetic variation among the fungus population may overcome the effectiveness of such practices and lead to develop epidemics in this region. Alternatively, application of plant extracts and fungicides are choice of practices for farmers to manage the yield losses. Previously, worker determined potential of such plant extracts and fungicides (Sultana and Ghaffar, 2013; Fareed et al., 2015). However, the samples of tomato plant showing *fusarium* wilt disease were collected from different region of Sindh Pakistan that may change its behavior against tested plant extracts and fungicides. Therefore, in this study, we determined effectiveness of various plant extracts and fungicides under *in-vitro* conditions.

**MATERIALS AND METHODS**

**Disease incidence %**: *Fusarium* wilt disease incidence was recorded from different locations of tomato growing districts of Sindh viz., Hyderabad (Neon Shehar, Bodani, Bhitae Masjid and Jeay Shah), Tando Allahyar(Darya khan Nahiyon, Dhigano Bazdar, Ilyas Khanzada ,Javed Khanzad,), and Mirpurk has(Bashirabad, Kachhelo farm, Mian saleem, Dehal gar) during 2015-2016. The disease incidence % was recorded from each location by using the following formulae:

\[
\text{Disease incidence %} = \frac{\text{Number of diseased plant observed}}{\text{Total number of plants observed}} \times 100
\]

**Isolation and identification of *F. oxysporum* f.sp. *lycopersici***: Isolation was done from 200 small root pieces showing tomato wilt disease symptoms. The roots were cut from adjoining areas of the specimen from diseased and healthy areas of the plants. Root pieces were washed under tap water for about 30 minutes to remove any dirt or soil particle. The root pieces were dipped in 0.01% HgCl2 for about 2 minutes and then passed from two washes of distilled sterile water for 2-3 minutes each. The treated root pieces were dried completely and then transferred to Petri dishes containing sterilized potato-dextrose agar medium with
five pieces per plate. All the plates were kept at 25 ± 1°C for 7 days. The fresh growth of the fungi was transferred to freshly prepared potato-dextrose agar medium for sub culturing. The growth was sub-cultured/multiplied whenever needed during the entire study. The fungi isolated were identified by studying their typical mycelial growth produced on the potato dextrose agar medium and conidial morphology using standard diagnostic keys as described by Barnett and Hunter, (1972) and Ellis, (1971).

Pathogenicity test: The Pathogenicity test of Fusarium oxysporum f. sp. lycopersici was done on three weeks-old seedlings of tomato variety golo at rate of 3 seedlings per plate. The obtained values were statistically analyzed similar as above.

Purification of F. oxysporum f. sp. lycopersici: The isolated fungus, Fusarium oxysporum f. sp. lycopersici was maintained on potato dextrose agar (PDA) slants having optimum growth at pH 7.0 and temperature 25+1 0C. Sub culturing was done at monthly intervals to maintain the stock cultures and preserved at 15 °C. Moreover, sub culture was maintained in sterilized Petri plates containing PDA for further studies.

Efficacy of plant extracts to inhibit mycelial growth of F. oxysporum f. sp. lycopersici: Bitter apple leaves (Citrullus colocynthis), eucalyptus leaves (Eucalyptus obliqua) and neem leaves and seeds (Azardica indica) and Ginger rhizome (Zingiber officinalis) were tested at four concentrations (2, 4, 6 and 8 %) against F. oxysporum f. sp. lycopersici using food poisoning method described by Singh and Tripathi (1994). The extract was prepared as 100 grams of fresh leaves were poured with 250 mL of water for 3-4 days and kept in shaking incubator for two days. The extracts were filtered by using Whatmann filter paper No.1 and then concentrated in vacuum at 40°-50°C using a rotary evaporator. Evaporation of solvent in the rotary evaporator results crude extract from different solvents and these extracts were used against the target pathogen. There were three replications for each concentration and plates without amendment of extracts served as control. Inhibition % was calculated using following formula given by Sundar et al. (1995). The obtained values were statistically analyzed using computer software STATISTIX v.8.1.

\[
\text{PI}=\left(100 \times \frac{X-Y}{Y}\right)\%
\]

Pl= Percent inhibition in mycelial growth
X= Mycelial growth in control plate
Y= Mycelial growth in treated plate

RESULTS AND DISCUSSIONS

Disease incidence in different locations: The major tomato growing locations of district Hyderabad, Tando Allahyar and Mirpurkhas were surveyed to estimate the disease incidence of Fusarium wilt disease. The disease was found at all localities surveyed with range between 8.3 to 47.0 incidences (Figure 1). In district Hyderabad the maximum disease incidence % was found at Bodani (47.0) followed by Neonshehar (43.0) and...
there was non-significant variation in incidence at Jeay Shah and Bhitae Masjid (38.667 and 36.3), respectively. Whereas in district Tnado Allahyar, the maximum incidence of 25.3 was recorded at Dhigano Bazdar and 12, 10 and 8.3 % were recorded at Darya khan Nahiyon, Ilyas Khanzad and Javed Khanzad, respectively. In district Mirpurkhas 22 % incidence was recorded at Kachhelo farm and there was non-significant difference at other three localities. The results are showing that the disease is more prevalent in district Hyderabad as compare to Tando Allahyar and Mirpurkhas. Such variation among incidence values at different localities of the same region is reported by Jiskani et al. (2007) among damping off disease of tomato. This indicates there is adequate variation in the region for susceptibility of host plants to pathogens or infection potential of the pathogens. This might be due to favourable environmental conditions, management practice of the growers, involvement of different fungal races or preference of grown cultivars.

Figure 1. Disease incidence % of major tomato growing areas of Sindh

![Figure 1. Disease incidence % of major tomato growing areas of Sindh](image)

**Isolation and pathogenicity of F. oxysporum f.sp. lycopersici:** The diseased symptoms were confirmed by isolating *F. oxysporum* f.sp. *lycopersici* from randomly collected specimens, which was identified taxonomic keys based on morphological characteristics as described by Barnett and Hunter, (1972) and Ellis, (1971). The pathogenicity test showed that 18 plants (30 %) were diseased at 20 day of inoculation, infection increased to 25 plants (42 %), whereas in control no plant showed the disease symptoms (Figure 2). This result confirmed pathogenic nature of *F. oxysporum* f. *lycopersici*, similar as Ignjatov et al. (2012) confirmed pathogenic potential of different Fusarium isolates with artificial inoculation in soil and plants showed wilting symptoms.

Figure 2. Pathogenicity test of *F. oxysporum* f. *lycopersici* on local tomato variety Golo

![Figure 2. Pathogenicity test of *F. oxysporum* f. *lycopersici* on local tomato variety Golo](image)
Efficacy of plant extracts against *Fusarium oxysporum* f.sp. *lycopersici*: Bitter apple leaves, eucalyptus leaves and neem leave as well as seeds and Ginger rhizome were tested at four concentrations like 2, 4, 6 and 8 % against the fungus under in-vitro conditions and showed various responses to inhibit mycelial growth. In general, higher concentration showed more inhibition of mycelial growth (Figure 3). The neem seed extracts were found the most effective at all applied doses as compare to other plant extracts. The maximum (66.2) % inhibition was found by neem seed extracts followed by 63.3, 58.5, 55.9 and 55.6 % by Eucalyptus, neem leaves, ginger rhizome and bitter apple respectively, at 8% concentrations. The minimum inhibition of 35.6% and 40.0 % of the fungal growth was found in extracts of bitter apple and ginger rhizome at 2% concentrations (Figure 3). Chohan et al. (2011) assessed effects of five medicinal plants against *F. oxysporum* f.sp.gladioli under in-vitro and they found *A. indica* extract was most effective to inhibit mycelial growth at higher concentration. In another study Shafique et al. (2015) determined potential of extracts prepared from *Eucalyptus citriodora* fruit, bark and leaves at various concentrations against *F. oxysporum*, which is significantly suppressed leaf extract as compare to other remaining parts.

![Figure 3. Effect of different plant extracts on the mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici*.](Image)

![Figure 4. Effect of different fungicides on the mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici*.](Image)
Efficacy of different fungicides against *Fusarium oxysporum* f. sp. *lycopersici*: Cabriotop, Melodedue, Nativo, Topsin-M and Allieate fungicide widely available for commercial purpose amended at different concentrations in PDA inhibited growth of the fungus. The Nativo was found the most effective fungicides (write digital values) for reducing the redial mycelial colony growth of target fungus followed by Topsin-M at 1000 ppm (write digital values) where as Allieate (provide value) and Melodedue (write digital values) fungicides were found least effective as compare to other fungicides. The Cabriotop was found as moderated effective fungicide (value) in reducing the mycelial growth of target fungus under in-vitro conditions (Figure 3). Sultana and Ghaffar, (2013) found that Allieate, Benlate and Carbendazim fungicides completely inhibit the colony growth of *F. oxysporum* @ 100 ppm whereas Mancozeb, Ridomil, Topsin-M and Vitavax completely inhibited the colony growth at 1000 ppm. Fareed et al. (2015) showed Ridomil as effective to control growth of cucumber fusarium wilt causing fungus as to compare to Score, Copper oxychloride, Cabriotop, and Antracol. This is indicating that *F. oxysporum* infecting different crops or in different regions may chance its response to fungicides.

**CONCLUSION**

It is concluded that the Fusarium wilt disease of tomato is prevalent in all major tomato growing areas of Sindh with different ranges. As for as its management the fungicides were found most effective as compare to plant extracts. The Native and Topsin-M Fungicides were the most effective fungicides followed by Neem seed and Eucalyptus plant extracts at their higher concentrations.

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