ECO-FRIENDLY MANAGEMENT STRATEGY AGAINST FUSARIUM WILT OF TOMATO CAUSED BY *Fusarium oxysporum f. sp lycopersici* (SACC.)

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ABSTRACT

Fusarium wilt of tomato caused by *Fusarium oxysporum f. sp lycopersici* (Sacc.), is the most devastating disease of tomato that cause quantitative and qualitative losses. Plant extracts have antimicrobial potential properties by decreasing the severity of various phytopathogens. The present study investigated the effect of various concentrations of garlic aqueous extract against Fusarium wilt of tomato. The target causal agent was isolated from infected tomato stems and leaves, collected from the District Mohmand of Pakistan during May-June 2019. The experiment was laid out in Completely Randomized Design (CRD) with five treatments replicated eight times under standard conditions in The Department of Plant Pathology, The University of Agriculture Peshawar during June-September 2019. The tested garlic aqueous extract concentrations were 5 ml, 10 ml, 15 ml and 20 ml per liter of media. Results revealed that the highest mycelial growth inhibition was found in higher concentrations of garlic extract in the plates (20 ml/litre) followed by 15 ml, 10 ml and 5 ml liter^{-1} of media after twelve days which was significantly ($P<0.05$) different from the mycelial growth recorded in control plates. Similarly, biomass of the mycelia was significantly ($P\leq0.05$) reduced as compared to the control treatment. It is concluded that the present research findings will be helpful in the management of Fusarium wilt of tomato in the field. Furthermore, it is suggested to conduct an experiment to prepare garlic extracts under more sophisticated techniques to enhance its efficacy and to determine the bio-active compounds in the garlic against the disease.

Keywords: Garlic, extract, radial growth, inhibition, management.

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INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) belongs to the family *Solanaceae* or Nightshade, is considered the utmost vital vegetable in the world which contributes to the world economy in terms of GDP in trade (Babalola *et al*., 2010). The origin of tomato is believed to be Central and South America especially Mexico, from where it spread to the Europe in the 16th century and then to the rest of the world (Osman, 2016). However, most tomatoes are grown for the processing industry, which makes tomatoes the world's leading vegetable for processing. The yield of tomato Crop is relatively lower as compared to the international market (Mari *et a*l., 2007). It is mostly consumed as a fresh vegetable and is capable of being transformed into a variety of

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products, including ketchup and different kinds of sauces. It has a healthy amount of vitamin C (31 mg per 100 g), vitamin A, calcium, and iron (Srivastava and Kulshrestha, 2013). Tomatoes naturally contain lycopene, a powerful antioxidant that helps prevent the development of certain cancerous tumors (Adenuga *et al*., 2013). It is categorized as the most important vegetable in terms of quality and quantity globally sharing 16% of the total vegetables. It is also a very fast-growing product with a 49% increase in its production from 2000 and 2016 (Lazaretto and Marios, 2014).

The top five largest tomato production countries are China, USA, India and Turkey and combined account for approximately 70% of global production (Sharma *et al*., 2005). Due to its seasonal production in several regions of the world, it is accessible all year long. The "Food and Agriculture Organization Corporate Statistical Database" (FAOSTAT) estimates that 182 million metric tonnes of tomatoes were produced worldwide on a surface area of 4.8 million ha (FAO, 2017). An average yield of 10.2 thousand tonnes per hectare was achieved by Pakistan's tomato farming, which covered 63.2 thousand hectares and resulted in a production of 60.1 thousand tonnes. The three main producing provinces are Punjab with 8.3 thousand ha, Baluchistan with 10.8 thousand ha, and Khyber Pakhtunkhwa (KP) with 13.0 thousand ha (Anonymous, 2018). Tomato productivity significantly drops annually due to the attack of several pathogens, pests and other climatic-related factors. One of these causes is the soil-borne phytopathogen *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.), which causes fusarium wilt, is a significant biotic factor in the KP province of Pakistan. The disease was for the first time reported and described by G.E Massee in 1895 (Rekah *et al*., 2000). The fact that at least 32 nations have previously reported the disease on a global scale demonstrates its significance, which is more severe in countries with warm temperatures (Maurya et al., 2019). The disease adversely exaggerated tomato production in Florida and the southeastern states of the United States. This pathogen has been found to have three physiological races: race 1, race 2, and race 3. Race-1 is the most distributed race of the pathogen and has been reported from nearly every geographical area. Although race-2 was first documented in Ohio in 1940, it did not become widespread or of economic concern until in the United States and in several other countries, i.e., Australia, Brazil, Great Britain, Mexico, Morocco, the Netherlands, and Iraq reported the pathogen with devastating impact on the economic sector (Chang et al., 2018). Soon after, race-3 was reported in Brazil (Gonçalves et al., 2016). Thereafter, it has been found in Australia, in Florida and California. Nonetheless, the development and usage of resistant cultivars have aided in minimizing disease-related losses in tomato crop (Heydari and Pessarakli, 2010). Meanwhile, the pathogen has gradually overcome tomato cultivar resistance. To overcome this disease and to minimize yield losses in tomatoes, integral approaches have been adopted such as fungicidal compounds like as benomyl, captafol, imazalil, thiram, and others were used to disinfect soil and planting materials from the pathogen (Mng'omba *et al*. 2012), cultivation of resistant varieties and crop rotation (Smith *et al*., 1988). Chemical fungicides have been widely used, which has resulted in environmental and toxicological issues (Gurjar *et al*., 2012) which are now a major concern for the world health organization. Scientists throughout the world are forcing to apply microbial (Bowers and Locke, 2004) and botanical fungicides as an alternative to synthetic fungicides. According to the reports, plant extracts and essential oils have highly effective antimicrobial activity against a variety of pathogens, including foliar pathogens and soil-borne fungal phytopathogens (Singha *et al*., 2011; Bowers and Locke, 2004).

Keeping in view the importance of tomato and the damages caused by tomato wilt fungi as well as the health hazards of fungicides used against *F. oxysporum,* the current experiment was directed to find out the toxicity of garlic aqueous extracts in various concentrations against *F. oxysporum* f. sp *lycopersici* under laboratory condition.

MATERIAL AND METHODS

Collection and Isolation of Pathogen

The plants of tomato showing typical disease symptoms of tomato wilt were collected from various fields of district Mohmand for the isolation of the pathogen. Infected tomato leaves samples were thoroughly washed with sterile distilled water (SDW), surface disinfected with mercuric chloride (0.1%) for 30 seconds and rinsed with sterilized distilled water (SDW) to get rid of the chemical particles. The infected tissues were carefully removed and placed on potato dextrose agar (PDA) in Petri-dishes, sealed with parafilm, labeled and incubated at 27 ºC. The pathogen was confirmed and identified by using the taxonomic keys of Nelson et al (1994). Pure cultures were maintained on PDA media for further studies.
To get the pure culture for further studies, the stock culture of the pathogen was plated on potato dextrose agar (PDA) media under aseptic conditions. A plug of 0.8cm from the margin of actively growing colony of the fungus was excised and transferred to the center of a fresh plate in a laminar flow unit (LFU). The plates were sealed and incubated at 27 ºC for fungal growth (Fig 3.1 a, b).

Preparation of Garlic Extract

Garlic bulbs were procured from a local market in Peshawar. After removal from the bulb, each clove was manually peeled. The clove was then macerated using a lemon juice extractor. The extract was then sieved through muslin cloth to get the pure liquid extract. This extract was then used in different concentrations for further studies.

Experimental layout

Five different concentrations (0, 5, 10, 15, and 20 ml) of garlic extract were tested. PDA media was amended with different concentrations of the extract after sterilization at concentrations per 500 ml of PDA using a magnet stirrer.
Under the aseptic condition, a 0.8 cm plug was placed in the middle of each plate. Parafilm was used to seal the plates, which were then incubated at 27 °C for a week. Data were measured on numerous parameters at the interval of four days for twelve consecutive days. Followed by incubation, the radial growth was assessed a few days after. The colony diameter was measured from the underside of the plate along two perpendicular lines using a ruler. The measurements were then averaged to obtain the mean value. In the experiment, eight replicates were used in the completely randomized design (CRD).

Biomass calculation

The bio-mass was usually taken on the first day of the experiment after the sealing of the plates which include the weight of the plate, the media, and the plug. Similarly, it was also taken on the final day of the experiment and then by subtracting the initial bio-mass from the final bio-mass and got the original bio-mass.

Percent Growth Inhibition

The percent mycelial growth inhibition in each treatment was collected through the following formula:

$$\text{Percent Growth Inhibition} = \frac{A - B}{A \times 100}$$

Where
A=Growth in control plates and
B=Growth of *Fusarium* in treated plates.

Statistical Analysis

Data were subjected to Analysis of Variance (ANOVA) test using a completely randomized design (CRD). Means were separated by LSD using the statistical software, Statistix 8.1 (Steel and Torrie, 1984).

RESULTS

Morphological Characteristics

Morphological identification of *F. oxysporum* was done by observing oval to ellipsoid/sickle-shaped spores. Three types of spores were found in the culture of Fusarium i.e., micro-spores, macro-spores and Chlamydospores. While the cultural characters were observed as delicate white to pink, and sparse to abundant white cottony mycelium with dark red to

pink color was found on the undersurface growth of the PDA plate (Figure 1).

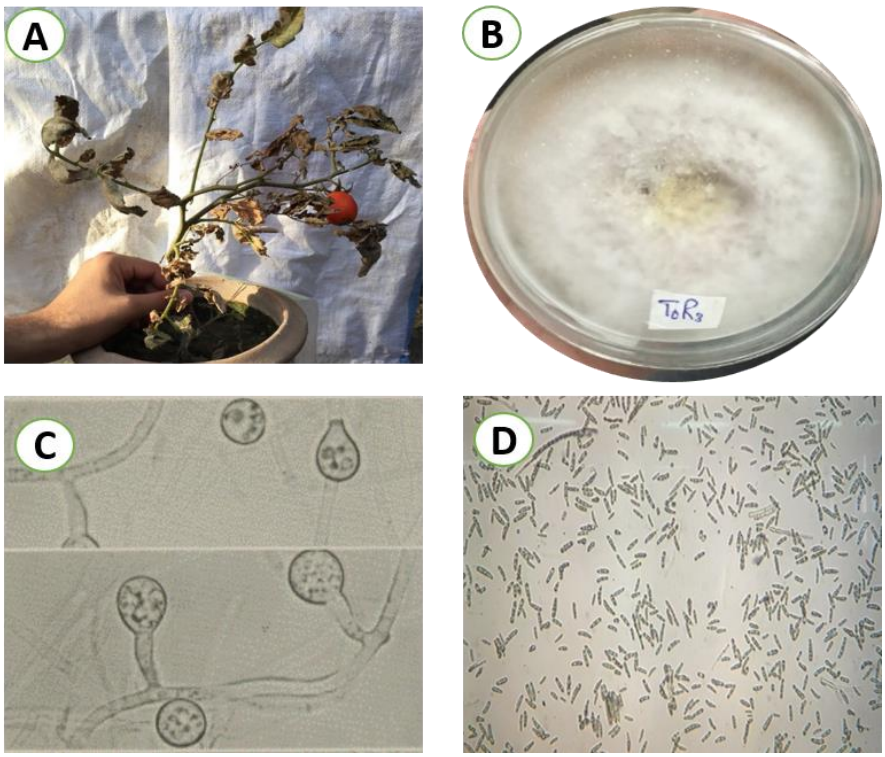

Figure 1. Various characteristics of tomato wilt; A). Tomato wilted plant of tomato, B). Isolated culture of *F. oxysporum f. sp. Lycopersic*, C- D). its morphological characteristics study under microscope

Influence of garlic extracts on mycelial growth

Results regarding the effect of garlic extract on the mycelial growth of the fungus after 4, 8 and 12 days of intervals are presented in Figure 2 and 3. The effect of the extracts was found significant for various concentrations (i.e., 5, 10, 15, and 20 ml) of garlic extract (P=0.00) concerning restricting the growth of the pathogen assessed after four days of incubation at 27ºC. There was a progressive decreasing trend in the colony diameter of the pathogen as the concentration of the extract was increased with time intervals ranging from 1.33 to 6.13 cm. The higher growth retardation was observed at the 20 ml concentration which was closely followed by 15 ml control plates and has shown a higher colony diameter (6.13cm) of the pathogen.

The effect of the extract was also significant for various concentrations (i.e., 5, 10, 15, and 20 ml) of garlic extract (P=0.00) concerning restricting the growth of the pathogen assessed after eight days of incubation at 27 ºC. There was also a progressive decreasing trend in the colony diameter of the pathogen when the concentration of the extract was increased which ranged from 4.69-8.99 cm. The higher growth retardation was observed at the 20 ml concentration which was closely followed by the 15 ml concentration of the extract whereas the control plates which were not amended with extract showed a higher colony (8.99 cm) of the pathogen.

The effect of the extract was also significant for various concentrations of garlic extract (P=0.00) concerning restricting the growth of the pathogen when data were assessed after 12 days of incubation at 27 ºC. There was also a progressive decreasing trend in the colony diameter of the pathogen when the

concentration of the extract was increased which ranged from 5.09 to 8.99 cm. The higher growth retardation was observed at the 20 ml concentration which was closely followed 15 ml concentration of the extract. The control plates which were not amended with extract showed a higher colony (8.99cm) of the pathogen.

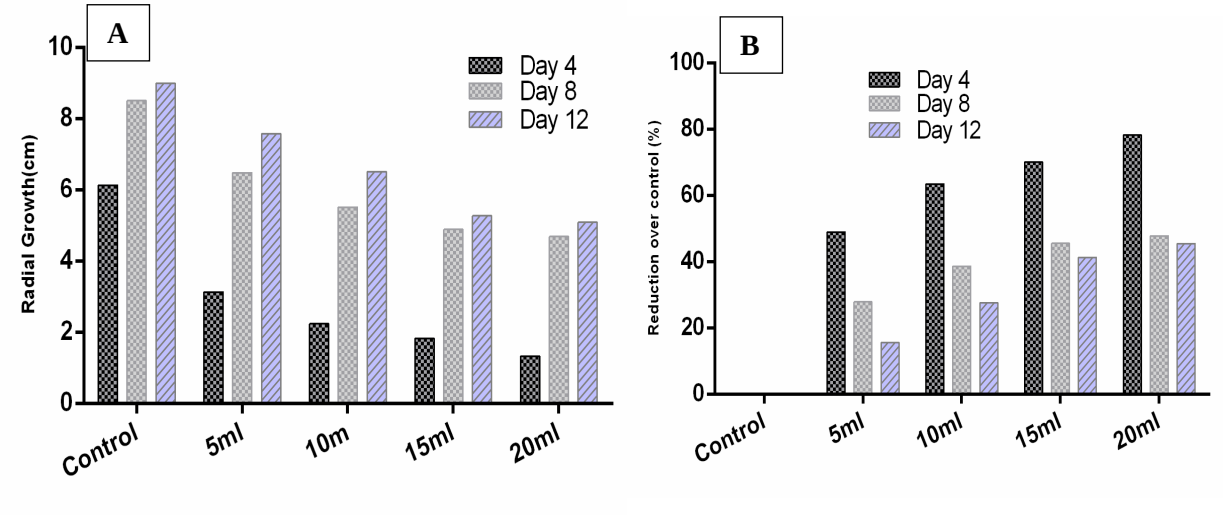

Figure 3. Effect of garlic aqueous extract for control of tomato wilt: A). Mycelial radial growth (cm) of the fungus, and B). Percent reduction over control after 4, 8 and 12 days of treatment application.

Effect on mycelial bio-mass

The effect of the extract on the mycelial bio-mass was also significantly reduced by using different concentrations (i.e., 5, 10, 15, and 20 ml) as compared to the negative control treatment (Figure 4). The effect of extracts on mycelial biomass was found in the range of 0.6362 to 3.7687 g. The mean data shows that the highest mycelial bio-mass reduction was observed in 20 ml concentration (0.6362 g) followed by 15 ml concentration (0.7800 g), whereas the lowest mycelial bio-mass reduction was recorded in 5 ml concentration (1.8013 g) followed by 10 ml concentration (1.3150 g) as compared to negative control treatment (3.7687 g).

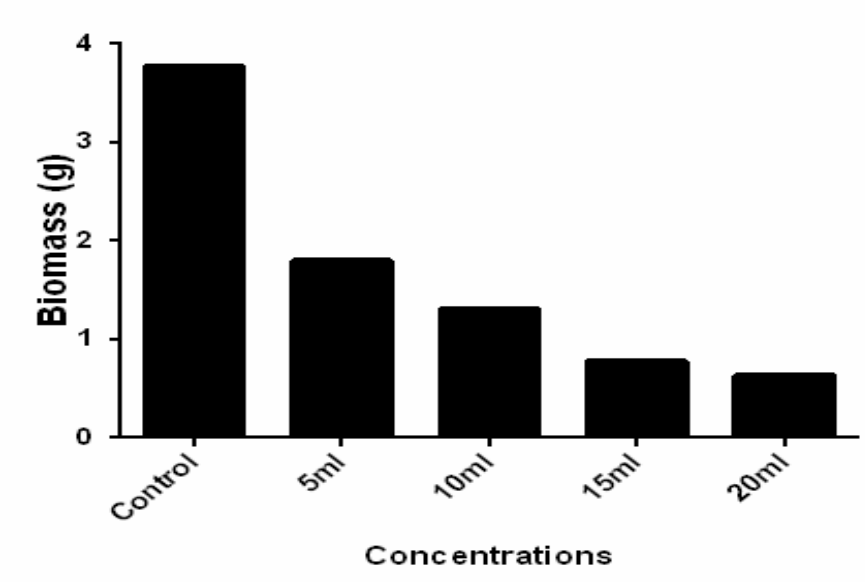

Figure 4. Effect of garlic aqueous extract over mycelial biomass (gm) of *F. oxysporum f. sp. Lycopersic* after 12

days.

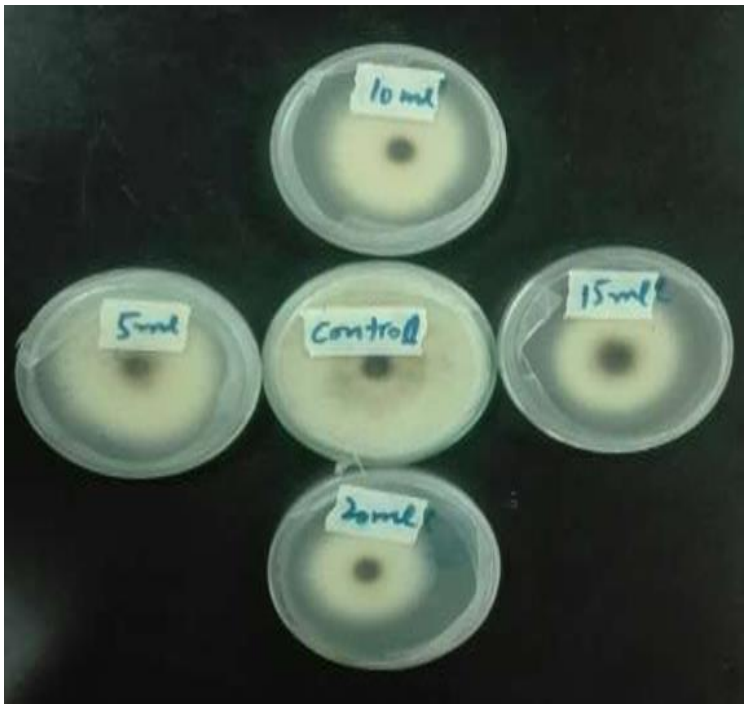

Figure 5. Effect of garlic extract with concentrations of 0ml, 5ml, 10ml, 15ml, 20ml against *F. oxysporum* f. sp. *Lycopersici.*

DISCUSSION

To reduce the residual effect of chemical fungicides, alternative strategies are urgently required for combating the attack of various fungi on different crops. Many scientists have reported the usefulness of plant extracts in controlling different phytopathogens (Siripornvisal, 2010; Amini et. al., 2012). Fusarium wilt, a devastating disease of tomato caused by *F. oxysporum* f. sp. *lycopersici*, has been controlled by the aqueous extract which exhibits some levels of antifungal activity. These extracts have biodegradable active ingredients that are selective in their toxicity. (Awuah 1989; Hussani and Deeni 1991). Taking this into account, in the present experiment, garlic extracts in different concentrations were screened against *F. oxysporum* and shown that garlic bulb extracts inhibited mycelial growth of the pathogen ranged from 48.94- 78.3% as related to the control during the first four days of incubation. However, overall, the growth inhibition at the highest three concentration levels (10, 15 and 20%) did not differ significantly from one another. The current study results are in line with those results obtained by Kumar *et al*., 2018 exhibited 100% fungal growth inhibition treated with the extracts. Our results showed clearly the potential of garlic extracts for control of *Fusarium* wilt in tomatoes. Furthermore, as time passed the percentage of growth inhibition decreased gradually. We suggest that this would be appropriate against fusarium wilt in the field. However, the reduction against fungi toxicity over time by the increase in mycelial growth of the fungus after four days of incubation on PDA media, suggests that the effect of the extract under field conditions would probably reduce its efficacy/toxicity maximum within a week time and in this way, fungal infection in crops will need several applications of the treatment till harvest. Plant extract is considered the main combating strategy against various phytopathogen while in meantime it is eco-friendly, and improve the beneficial microbial community of a plant and its

productivity.

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