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ACTIVATION OF *CAPSICUM ANNUUM* L. DEFENSE SYSTEM AGAINST FUSARIUM WILT THROUGH PLANT ACTIVATORS AND THEIR IMPACT ON HORTICULTURAL ATTRIBUTES

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ABSTRACT

Capsicum annuum L. member of family *Solanaceae* is an important vegetable crop throughout the world. Fusarium wilt of chilli caused by *Fusarium oxysporoum* f.sp. *capsici*, is a serious destructive disease, which reduces its yield and is a major threat to the economy. Plant activators play an important role to manage soil born disease. In current study, a pot experiment was carried out under greenhouse conditions in the research area of Plant Pathology Department, University of Agriculture, Faisalabad. Five plant activators K_2HPO_4, $CaCl_2$, Benzoic acid, Salicylic acid and Alpha-Tocopherol were used to manage the Fusarium wilt of chilli. Soil drenching of each plant activator was applied at three different concentrations 0.25%, 0.5% and 0.75% after transplantation. Results showed that the minimum incidence of disease, maximum length of shoot, length of root was revealed by K_2HPO_4 at 0.75% concentration. While benzoic acid exhibit maximum shoot fresh weight, maximum shoot dry weight, fresh root weight and dry root weight at 0.75% concentration. Maximum fresh leaf weight showed by salicylic acid whereas maximum dry leaf weight exhibited by alpha-tocopherol at 0.75% concentration. It was concluded that K_2HPO_4 at 0.75% concentration is effective to manage Fusarium *wilt* of chilli that can be used to manage disease in future.

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INTRODUCTION

Capsicum annuum L. is the second most important vegetable crop of *Solanaceae* family which is cultivated throughout the world. It is a dicotyledonous and perennial shrub with 60-80cm height (Quresh *et al.*, 2015). Chilli is known from the beginning of Western Hemisphere civilization and its history is very much related to the voyage of Columbus. He introduces pepper in Europe, and then it spreads towards Africa China, Japan and India. (Basu and De, 2003; Ali *et al.*, 2018).

It is a nutritionally important vegetable crop used in the human diet, rich and cheap source of nutrients, used as vegetable, fresh fruit, dry spice and pickled all over the world (Nawaz *et al.*, 2017; Madbouly and Abdelbacki, 2017). It has high pungency, strong flavor and high color, used in culinary, as spice (Mann, 2011). Medicinally, chilli is used to cure cough and cold, sore throat, asthma, sinus infection, cancer, regulate blood circulation, varicose veins, anorexia, liver congestion, haemorrhoids and helpful in digestion (Gantait *et al.*, 2012; Huang *et al.*, 2013; Yousaf *et al.*, 2017). It contains vitamin A, C, E, K and B6, riboflavin, betaine, thiamine, niacin, choline,

folic acid, folate, pantothenic acid, ascorbic acid and beta carotene (Ali *et al.*, 2018). Moreover, it contains calcium (Ca), copper (Cu), phosphorus (P), potassium (K), boron (B), iron (Fe), zinc (Zn), manganese (Mn), sodium (Na), magnesium (Mg), fiber, protein, fat, carbohydrate, capsaicinoids and phenolic compounds (Vega-Gálvez *et al.*, 2009; Baenas *et al.*, 2019).

Chilli is cultivated in 126 countries and covers1.8 million hectares worldwide. China is a leading producer of chilli that gives 50%, Mexico gives 7% and turkey gives 7% of the total world's production (Tesfaw, 2013). In the last 20 years, area under chilli cultivation increased to 35% (FAOSTAT, 2017). In Pakistan, agriculture is a dominating sector contributing about 20% to gross domestic product (GDP), vegetables are grown on large areas and facing low yield due to biotic and abiotic stresses (MOF, 2015–2016; FAOSTAT, 2016; Nawaz *et al.*, 2017). In 2017, Pakistan's total area under chillies cultivation was 14.81thousand ha and production was 65.1 million tons that has 3.20% share in GDP (FAOSTAT, 2017).

Throughout the world, reduction in quality and quantity has been seen due to the involvement of biotic factors that includes different plant pathogen (viruses, bacteria and fungi) (Nawaz *et al.*, 2016; Yousaf *et al.*, 2017; Hyder *et al.*, 2020). Different pathogen including *Rhizoctonia solani*, *Macrophomina phaseolina* and *Fusarium solani* are commonly attack on chilli and causing significant yield reduction (Madbouly and Abdelbacki, 2017). Annually 10-80% yield losses in chilli peppers occur due to disease (Ekundayo *et al.*, 2011). *Fusarium oxysporum* causes Fusarium wilt disease, that frightening for the chilli production. Fusarium wilt of chilli was first of all reported from New Mexico where 43% of the total chilli plants were affected with this disease. In western countries crop loss due to disease is 25% and in third world countries is 50% (Devika Rani *et al.*, 2007). This disease has consistently caused an annual yield loss of about 10-80% (Vanitha *et al.*, 2009). It has reported that three biological forms of *F. oxysporum* have ability to survive more than eleven years being unchanged morphologically, 50% of the Fusarium species considered toxigenic (Limón *et al.*, 2010; Wiemann *et al.*, 2013; Niehaus *et al.*, 2014). Infection of Fusarium wilt starts from roots and hyphae of fungus invades into vascular system of the plant. Hydrolytic enzymes and mycotoxins produced by pathogen cause cellular apoptosis in stem and roots. Foliar wilting starts due to blockage, ultimately death of the plant takes place after few days of infection (Pavlovkin *et al.*, 2004; Wu *et al.*, 2009).

Fruit production, growth, and quality are declined by the disease caused by *F. oxysporum.* It is difficult to tackle with this disease because *F. oxysporum* have ability to survive in soil for many years. Fungicides are available commercially that are used to control the outbreaks of wilt disease. Mycelical growth of this disease is suppressed by Ridmol MZ, Carbendizim, captan, and copper oxychloride (Sharma *et al.*, 2002). Fungicides like captan, carboxin and metalaxylare used to fight against the wilt causing pathogens (Chet *et al.*, 1982; Ram *et al.*, 2000). Fungicides are not environment friendly, excessive use of fungicides results in ecological pollution and can be an environmental hazard (Dubey *et al.*, 2007). Moreover *F. oxysporum* species have developed resistance against fungicides. Biological conversion is also caused by fungal pathogens for the detoxification of fungicides (Dekker, 1976).

Application of fungicides is dangerous for human health because it leaves toxic substances which remains on the plant. Eradication of fungal disease using antagonistic microorganism is known as biological control. This is contemplated as a feasible substitute, active method to chemical control and to fight with plant diseases. So, it is hypothesized that plant activators are helpful in the reduction of disease by activating the defense mechanisms of plant as well as re-strengthen the overall structure of plant including root and shoot length, fresh leaves and roots. By activating systematic acquired resistance, plants develop resistance against wide spectrum of pathogens by use of plant activator. Plant activator has no toxic effect on the parts of plants and pathogen has no ability to produce resistance because plant activators have no direct antibacterial or antifungal activity (Huang and Hsu, 2003; Ali *et al.*, 2014). Proteins and alkaloids related to defense compounds like polyphenols and pathogenesis are produced by biosynthetic reaction that are catalyzed by activating enzymes (Chong *et al.*, 2005). That's why present study was carried out with the objective to check the efficacy of different plant activators against the fusarium wilt of chilli.

MATERIALS AND METHODS

Pathogen Isolation, Identification and Purification

Diseased plants showing characteristic symptoms of wilt disease were collected and used to isolate the *F. oxysporum*. Small cuttings of plant root samples were

surface sterilized with 1% $HgCl_2$. The sterilized sample of the plant placed on a petri plate (two piece of infected plant sample per petri plate) containing PDA (Potato Dextrose Agar) medium. Plates were placed in the incubator for 48-72 hours at 25 °C for fungal growth, than hyphae of *F. oxysporum* were purified using PDA plates. Pathogen isolates were identified by microscopic study. Stereomicroscope was used to study the morphological characteristics of isolated fungi from infected plant samples. For the purification, mycelium was transferred on PDA media plates and incubated for 10-15 days.

Inoculum preparation

For inoculum preparation, culture was multiplied on PDA containing Petri plates. After maturation of culture, distilled water was poured into these plates and genetally mixed with a sterilized needle. Then this suspension was poured into the beaker and counts the spores with Hemocytometer. Spores of the inoculum were adjusted 1×10^6 spores/ml of the distilled water.

Pathogenicity test

Chilli seedlings (30 days old) were selected for pathogenicity of *F. oxysporum*. Chilli plants were inoculated with *F. oxysporum*. Symptoms appearance was noticed after one week of inoculation. To fulfill Koch's postulate re-isolation was carried out from the artificially diseased plants.

Sick field preparation

Three consecutive sprays of pure culture of *F. oxysporum* f. sp. *capsici* were carried out with an interval of 10 days. To produce maximum inoculum in the root zone of the plant, the spore suspension of 1×10^6 spores/ml of H_2O was inoculated. Healthy seedlings of chilli plants were transplanted to pots that contain contaminated soil with *F. oxysporum* f. sp. *capsici*.

Management of fusarium wilt of capsicum

The seedlings of Capsicum were collected from the vegetable section Ayub Agriculture Research Institute (AARI), Faisalabad. Pots were arranged under completely randomized design (CRD). Fifteen days old seedlings were transplanted in the pots and plant activators were applied two times, first after transplantation to pots and second soil drenching was applied after 20 days of transplantation and data regarding disease incidence of plant was taken after 5, 10, 15 and 20 days of applications. Three concentrations (0.25%, 0.5% and 0.75%) of each plant activator were used. The treatments were consisted on alpha-Tocopherol, Salicylic acid, Benzoic acid, $CaCl_2$, K_2HPO_4 and water served as negative control.

Evaluation of relative resistance or susceptibility to *Fusarium oxysporum*

Scale modified by Abdel-Monaim and Ismail (2010) was used to record the disease (Table 1).

Disease incidence was recorded by using the following formula;

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total No. of observed plants}} \times 100$$

Table 1: Disease rating scale used for disease evaluation (Abdel-Monaim and Ismail, 2010).

Disease Rating	Description	Reaction	Symbol
0	0	Immune	I
1	1-20%	Resistant	R
2	21-40%	Moderately Resistant	MR
3	41-50%	Moderately Susceptible	MS
4	51- 70%	Susceptible	S
5	70-100%	Highly Susceptible	HS

Data Analysis

Statistical analysis was carried out by using statistical software SAS/STAT (SAS Institute, 1990). Fisher's protected least significant difference (LSD) with the probability level 0.05% used for the separation of averages (Steel, et al., 1997).

Statistical software SAS/STAT software package was used for the preparation of ANOVA table, the interaction between different treatments and their combinations.

RESULTS

It was observed that K_2HPO_4 gave the least disease incidence 14.14% and maximum disease incidence was showed by $CaCl_2$ (22.05%) as compared to control

(85%). The interaction TxC (Treatment x Concentration) showed that K_2HPO_4 expressed minimum disease incidence 15.983%, 14.075% and 12.375% and maximum disease incidence was showed by $CaCl_2$ (23.86%, 21.92% and 20.39%) at 0.25%, 0.5% and 0.75% concentration respectively as compared to control (Figure 1 a, b). Interaction between treatments and time (Tr×T) expressed that K_2HPO_4 showed minimum disease incidence 18.40% after 5 days,14.54% after 10 days, 12.80% after 15 days and 10.70% after 20 days and maximum disease incidence was showed by $CaCl_2$ (26.10, 23.46, 20.76 and 17.90%) as compared to control. Interaction between treatments, concentration and time (TrxCxT) showed that minimum disease incidence was showed by K_2HPO_4 at 0.25%, 0.5% and 0.75% after 5 days (19.86, 18.36 and 17.36%), 10 days (16.76, 14.46 and 12.40%), 15 days (14.63, 12.93 and 10.83%) and 20 days and maximum disease incidence was showed by $CaCl_2$ after 5 days (27.36, 26.56 and 24.36 %), 10 days (25.47, 23.44 and 21.47%), 15 days (22.80, 20.20 and 19.30%) and 20 days (19.81, 17.47 and 16.44%) as compared to control (Figure 2 a, b).

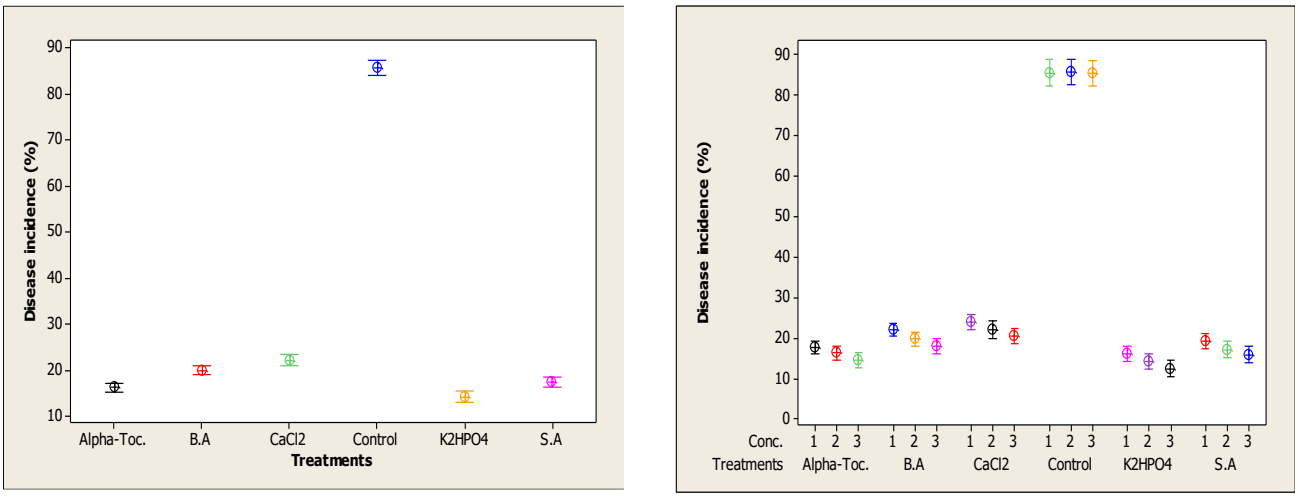


Figure 1 a. Effect of different plant activators on incidence of Fusarium wilt of chilli; b. Effect of interaction between treatment and concentration on incidence of Fusarium wilt on chilli.

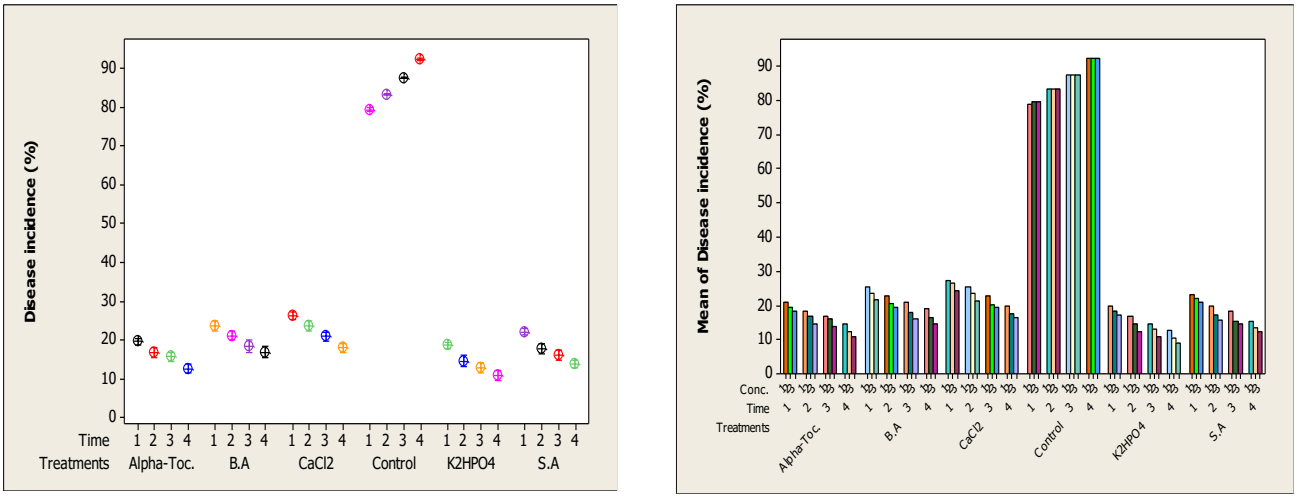
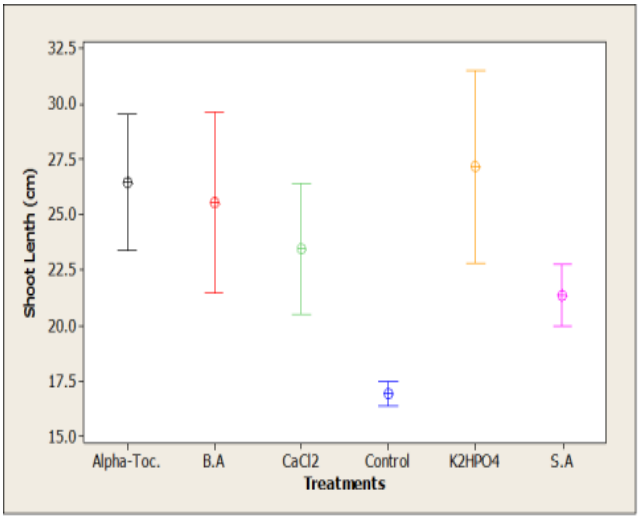


Figure 2 a. Effect of interaction between treatment and time on incidence of Fusarium wilt on chilli; b. Effect of interaction between treatment, time and concentration on incidence of Fusarium wilt on chilli.

The interaction between treatment and concentration (TxC) expressed that maximum shoot length (32.63cm) was observed at 0.75% concentration of K_2HPO_4 as compared to 0.5% (28.80cm) and 0.25% (20.00cm),

whereas alpha-tocopherol exhibit maximum root length (11.50, 13.90 and 17.83cm) at 0.25%, 0.5% and 0.75% concentration respectively as compared to control (Figure 3 a, b). Maximum shoot fresh weight (1.940g) and maximum shoot dry weight (0.59g) was observed at 0.75% concentration of benzoic acid (Figure 4 a, b).



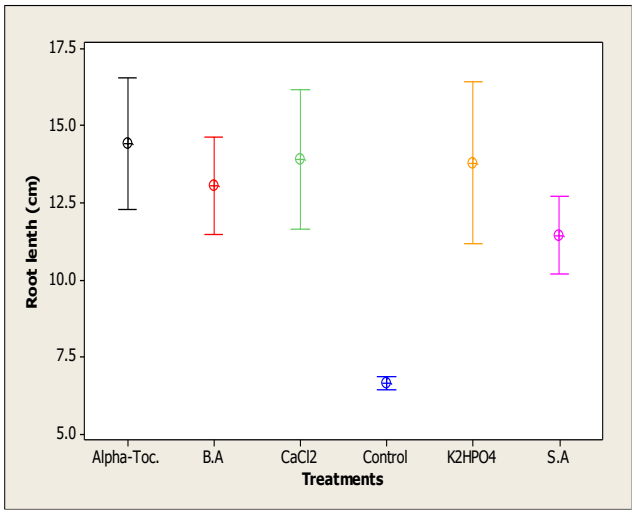
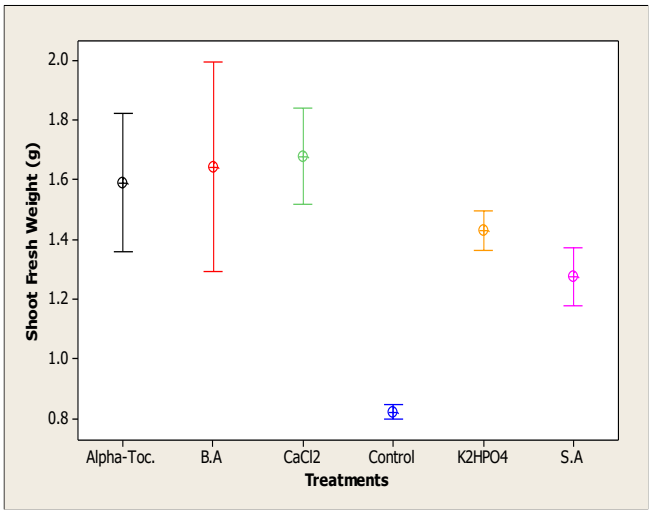


Figure 3 a. Effect of interaction between treatment and concentration on Shoot Length (cm) of chilli; b. Effect of interaction between treatment and concentration on Root Length (cm) of chilli



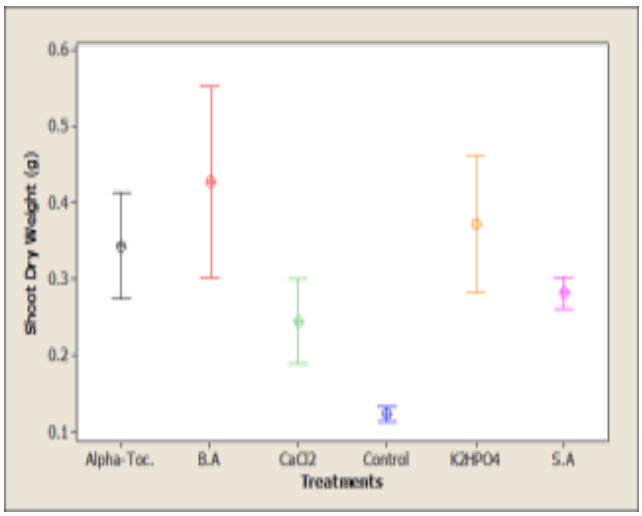


Figure 4 a. Effect of interaction between treatment and concentration on Shoot Fresh Weight (g) of chilli; b. Effect of interaction between treatment and concentration on Shoot Dry Weight (g) of chilli.

The interaction between treatment and concentration (TxC) was observed, benzoic acid expressed maximum root fresh weight (0.696, 0.610 and 0.470g) at 0.75%, 0.5% and 0.75% concentration and maximum root dry weight (0.373) was observed at 0.75% concentration of benzoic acid as compared to control (Figure 5 a, b). The interaction between treatment and concentration (TxC) expressed that maximum leaf fresh weight was at 0.75% (0.373), 0.5% (0.320) and 0.25% (0.290) concentration of alpha tocopherol that leaf dry weight was recorded maximum 0.080, 0.123 and 0.170 g at 0.25%, 0.5% and 0.75% concentration of alpha tocopherol respectively as compared to control (Figure 6 a, b).

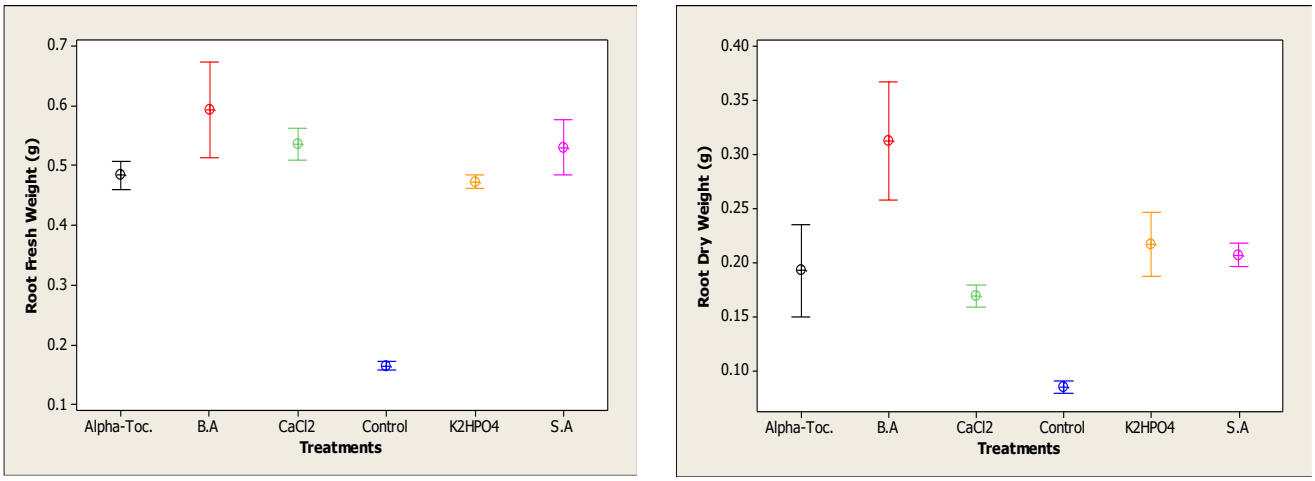


Figure 5 a. Effect of interaction between treatment and conc. Root Fresh Weight (g) of chilli; b. Effect of interaction between treatment and concentration on Root Dry Weight (g) of chilli.

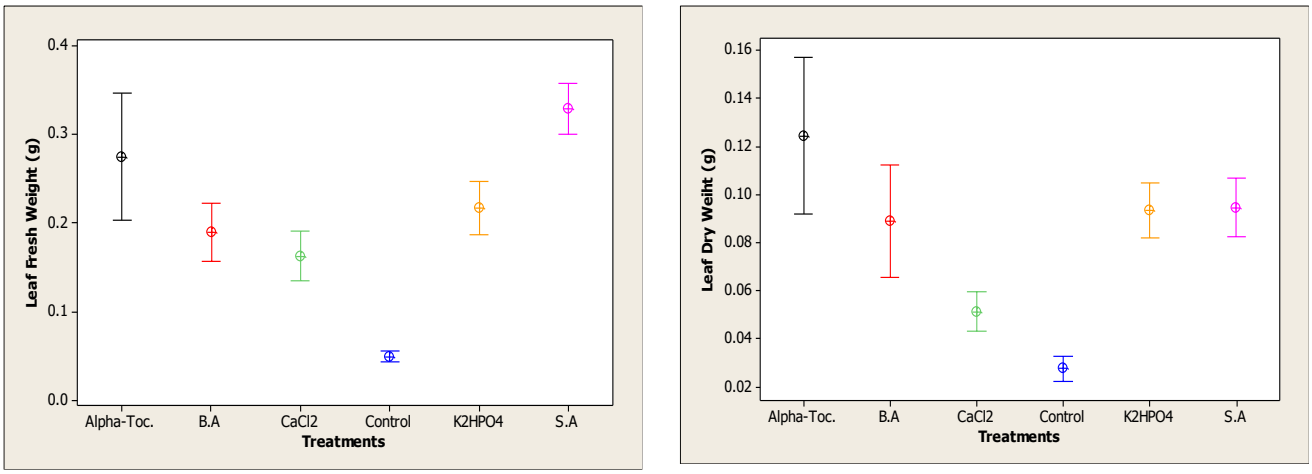


Figure 6 a. Effect of interaction between treatment and concentration on Leaf Fresh Weight (g) of chilli; b. Effect of interaction between treatment and concentration on Leaf Dry Weight (g) of chilli.

DISCUSSION

Fusarium wilt of chilli caused by *F. oxysporum* f. sp. *capsici* cause 70-100% yield loss and is a potential threat in chilli growing areas of Pakistan, under encouraging environmental circumstances (Ashfaq *et al.*, 2014). Wilting, chlorosis, yellowing of leaves and vascular discoloration are the distinguishing indications of this disease (Matthew *et al.*, 2006). Susceptible host, lethal pathogen and ecological factors subsidize headed for the outburst of disease (Naik *et al.*, 2008; Saremi and Amiri, 2010; Rekah *et al.*, 2001). The resistant host has less chance to get soil-borne diseases, using resistant cultivars not only the rate of disease incidence reduced, but environment can be saved avoiding toxicity of fungicide in soil (Russo and Howard, 2002; Devika Rani *et al.*, 2007; Naik *et al.*, 2007). Development of resistant cultivars by inserting and transferring the resistant gene following the molecular techniques is expensive and time taking but conventional breeding and screening of germplasm for resistance or susceptibility varieties is a substitute technique (Maruti *et al.*, 2014).

Various abiotic and biotic factors responsible for the growth of the plant influenced the normal growth (Mina and Dubey, 2010). The abiotic factors that are responsible for the development of infection or disease are maximum or minimum soil and air temperature (°C), wind speed, relative humidity %, soil moisture (%), and rainfall (mm) (Larkin and Fravel, 2002). Sudden change in climatic conditions, soil and other environmental dynamics are responsible for the disease development

and significant factor that influence the hosts susceptibility and resistance toward disease. Environmental factors are also responsible for the incidence of infection, growth, survival and reproduction of the pathogen and interaction of the host with the pathogen (Saremi *et al.*, 1999; Chakraborty and Pangga, 2004; Ghini *et al.*, 2008). Physiological functions of plant (uptake, assimilation and translocation of nutrients from root to shoot and their consumption) are influenced under pathogen attack (Stewart *et al.*, 2005; Suharja and Sutarno, 2009). Plant use nutrients to activate metabolism, regulate cellular functions and to develop resistance and tolerance against pathogen attack (Saikia *et al.*, 2009). Satisfactory accessibility of macro and micro nutrients lessen the incidence diseases in plants (Mahmood and Bashir, 2011) because many have role as active co-factors of enzymes and some are responsible for stabilizing the structure of proteins (Alabi, 2006).

Plant defense system fails to inactivate or delayed under pathogen attack and disease develops in response to plant pathogen interaction. The defense system is regulated by various growth regulators and stimulated by signal transduction molecules. Host plant initiates its defense response controlled by resistant genes under pathogen attack. In plants, growth regulator foliar application (non-toxic dosage) activate basal defense systems in response to pathogen (Jalali *et al.*, 2006) and plants that are susceptible to pathogen plant become resistant to disease. In plants salicylic acid is responsible to improve the growth and develop resistance by accumulating Indole acetic acid (Hayat *et al.*, 2005; Ye and Ng, 2002). Furthermore, salicylic acids in suitable amount activate the resistance genes and develop resistance in plant (Howard *et al.*, 2000; Khan *et al.*, 2003). Numerous plant species are known whose antifungal potential has been reported (Sridhar *et al.*, 2003; Duru *et al.*, 2003; Lee *et al.*, 2007). The plant contains natural ingredients, when an extract of plants at certain concentrations are used has beneficial effect, these are less phytotoxic, ecofriendly, harmless and biodegradable (Costa *et al.*, 2000; Sitara *et al.*, 2008). Biochemical and molecular study of medicinal plant for exploring or detection of metabolites, active ingredients and enzymes is required to use against Fusarium wilt of chilli (Singh *et al.*, 2004; Saxena *et al.*, 2005).

In the present study, five growth regulators (alpha-Tocopherol, Salicylic acid, Benzoic acid, $CaCl_2$ and K_2HPO_4) and water as a control were evaluated against Fusarium wilt of chilli pepper. From the results it was found that under the interaction of treatment and concentration, the minimum incidence of disease (12.37%) was observed at 0.75%, under the interaction of treatment and time minimum incidence of disease (10.70%) was observed at T_4. Similarly, the interaction of treatments, time and concentrations (T×C×D) expressed that the minimum incidence of disease (8.90%) was observed when K_2HPO_4 was applied. Under interaction of treatment and concentration it was observed that the maximum length of shoot (32.63 cm) and length of root (17.90cm) was shown by K_2HPO_4 at 0.75% concentration. The maximum shoot fresh weight (2.20g), maximum shoot dry weight (0.59g), the root, fresh weight (0.69g) and root dry weight (0.37g) was displayed by benzoic acid at 0.75% concentration. While alpha-tocopherol at 0.75% concentration maximum leaf dry weight (0.17g) and maximum leaf area (9.18 cm^2) was observed. Maximum leaf fresh weight (0.37g) was shown by salicylic acid at 0.75% concentration.

Outcomes of the present study are supported by the conclusions of Ali *et al.* (2000); Sarwar *et al.* (2005) and Hanieh *et al.* (2013), they evaluated dipotassium hydrogen ferric chloride, orthophosphate, calcium chloride salicylic acid, hydrogen peroxide, metalaxyl, and indole acetic acid and stated that salicylic acid showed significant resistance against Fusarium wilt. Through the production of reactive oxygen species salicylic acid activates defense of plant by changing composition of cell wall and by producing phytoalaxins against fungi (Bashir *et al.*, 1997; Colson-Hanks *et al.*, 2000; Agrios, 2005; Govindappa *et al.*, 2011) and by producing proteins of different kind (Hayat *et al.*, 2007). El-Yazeid (2011) and Biswas *et al.* (2012) reported similar results who evaluate hydrogen peroxide, salicylic acid, calcium chloride, dipotassium hydrogen orthophosphate, metalaxyl, ferric chloride and indoleacetic acid *F. oxysporum* attack and reported that on tomato plant minimum incidence of wilt disease was observed under salicylic acid used in various concentrations. Salicylic acid application compensates the production of hydrogen peroxide a ROS that inhibit the fungal spore germination by pormoting antifungal activity of plant. In plant cell wall and phenoxyradicals produced during phenolpolymerization (Inbar *et al.*, 1998; Huynh *et al.*, 1996). The fugal defense system is weekend by Lipid peroxidation disintegration of membrane permitted by action of ROS necrosis induces the phytoalaxins

production and Lipo-geneses metabolites alter the expression and function of defense gene of pathogen (Lee *et al.*, 2007). Antioxidants save the plant from oxidative stress strength the defense system in plants by partially reducing production of ROS (Torres-Castillo *et al.*, 2013; Sitara and Hasan, 2011).

CONCLUSION

Results of present study showed that the minimum incidence of disease, maximum length of shoot, length of root was revealed by K_2HPO_4 at 0.75% concentration. While benzoic acid exhibit maximum shoot fresh weight, maximum shoot dry weight, fresh root weight and dry root weight at 0.75% concentration. Maximum fresh leaf weight showed by salicylic acid whereas maximum dry leaf weight exhibited by alpha-tocopherol at 0.75% concentration. It was concluded that K_2HPO_4 at 0.75% concentration is effective to manage Fusarium *wilt* of chilli, that can be used to manage disease in future.

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CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

AUTHORS CONTRIBUTIONS

Muhammad Atiq supervised and reviewed overall research experiment; Sameen Adil conduct research trials; Nasir A. Rajput conceive the research idea; Shahbaz T. Sahi edited manuscript; Muhammad Usman helped in research trials and manuscript write-up; Shahid Iqbal helped in manuscript write-up; Shahid A. Chand recorded data; Ahmad Nawaz help in conducting research trials; Asif M. Arif performed data Analysis; Azeem Akram arranged data for statistical analysis while Hamza Shahbaz helped in manuscript write-up.