



Assessment of plant activators against *Fusarium* wilt of Chilli under field conditions

Fatima Nasir¹, Muhammad Atiq^{*1}, Shahbaz Talib Sahi¹, Muhammad Rizwan Bashir², Nasir Ahmed Rajput¹, Irfan Ahmed³, Muhammad Sajid⁴, Abdul Jabbar⁵

¹Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan

²Oilseeds Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan

³Department of Forestry and Range Management, University of Agriculture, Faisalabad, Pakistan

⁴Department of Plant Pathology, Bahauddin Zakariya University, Multan, Pakistan

⁵Department of Agronomy, University of Agriculture, Faisalabad, Pakistan

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Abstract

The current research was conducted to find out the most appropriate concentrations of plant activators for appropriate management of *Fusarium* wilt disease under field conditions. Chilli is an important vegetable crop of solanaceae family after potato and tomato. *Fusarium* wilt disease of chilli is caused by *Fusarium oxysporum* f. sp. *capsici* which is a potential threat to the production of chilli in Pakistan. Four plants activators i.e. salicylic acid, KH_2PO_4 , benzoic acid and ascorbic acid were evaluated @ 0.5, 0.75 and 1% concentration for the management of *Fusarium* wilt disease under RCBD design in the research area of department of plant pathology. Out of four plant activators, minimum disease incidence was expressed by salicylic acid (26.681%) followed by KH_2PO_4 (30.719%), Ascorbic acid (33.381%) and Benzoic acid (38.737%) when the results were compared to the control. Salicylic acid at 1% concentration gave statistically significant results. After 3rd application of soil drenching (21 days interval), salicylic acid showed best results with 23.60% disease incidence. At 1% concentration of 3rd application of soil drenching salicylic acid exhibited the best result 13.76% followed by KH_2PO_4 (18.26%), ascorbic acid (20.40%), benzoic acid (28.70%) and control expressed the maximum disease incidence which is 80.43%. It is concluded that salicylic acid plays a significant role in the management of *Fusarium* wilt disease.

* Corresponding Author: Muhammad Atiq ✉ dratiq1@yahoo.com

Introduction

Chilli pepper (*Capsicum annum* L) is the cash and spice crop which belongs to the family Solanaceae (Khalid *et al.*, 2000; Khan *et al.*, 2006). Area under cultivation of chillies is 62.7 thousand hectares with 2.7 tons/ha average yield (GOP, 2013). It is a rich source of copper (14%), potassium (7%), calcium (1.5%), magnesium (6%), iron (13%), vitamin C (240%), vitamin B (39%) and vitamin A (32%) along with antioxidants like beta-carotenoids (Serra *et al.*, 2002). A number of fungal pathogens attack on chilli crop but *Fusarium oxysporum* f. sp. *capsici* which is causal agent of Fusarium wilt is the most destructive one. It causes 30 - 40% yield losses throughout the world while in Pakistan 53% yield losses were recorded. Wilting of leaves, epinasty, necrosis, vein clearing and chlorosis which ultimately results in death of the plant are typical symptoms of this disease (Roncero *et al.*, 2003; Agrios 2005).

Application of fungicides is a quick method to manage Fusarium wilt disease but fungicides are not eco-friendly and adversely affected human beings, microorganisms and environment with its toxic residues (Parker *et al.*, 1985). It has been observed that pathogen has developed genetic resistance against the fungicides (Sela-Buurlage *et al.*, 2001). While biological control method is the use of microorganisms which are isolated from the suppressive soils and these microorganisms provide protection to the plants against the *Fusarium* wilt disease but it is time consuming method (Alabouvette *et al.*, 1993).

Antioxidants like Jasmonic acid, salicylic acid and shikimic acid play a significant role in plant defense by stimulating the physiological processes which results in improved vegetative growth of plants (Agrios, 1997). Activators move through the plants systemically which lead to the expression of defense genes (Lu *et al.*, 2006). Jasmonic acid (JA) promotes the plant growth and development. Salicylic acid activates phenyl alanine ammonia lyase (PAL) which results in the development of resistance (Wen *et al.*, 2005).

Activators activate the enzymes that catalyzes the biosynthetic reactions which produces defense compounds like polyphenols, pathogenesis related proteins and alkaloids (Chong *et al.*, 2005).

Salicylic acid produced systemic acquired resistance at the site of infection (Kumar *et al.*, 2013) which results in the production of pathogenesis related proteins and higher level of H₂O₂ (Park *et al.*, 2009). Salicylic acid enhances the yield by inhibiting the ethylene production and catalase (CAT) activity (Leslie and Romani, 1986; Chen *et al.*, 1993). Benzoic acid inhibits the activity of polygalacturonase and polygalacturonic acid (Lyon and McGill, 1989).

Seed treatment with antioxidants increases the chlorophyll content and carotenoids (Abdel-Monaim, 2008). Plant activators do not have any pesticidal and antibiotic activity. Pathogens are unable to develop resistance because plant activators don't have direct interaction with pathogens (Huang and Hsu, 2003). That's why the present study was designed to evaluate the plant activators i.e. salicylic acid, benzoic acid, ascorbic acid and KH₂PO₄ against *Fusarium* wilt disease and their impact on yield.

Materials and methods

Collection of germplasm

Seed of susceptible chilli variety (*Capsicum annum* L), local was collected from the Vegetable Research Institute of Ayub Agricultural Research Institute (AARI), Faisalabad and sown in the pots which were placed in the research area of Department of Plant Pathology, University of Agriculture Faisalabad. When the seedlings were emerged these were transplanted on ridges under Randomized Complete Block Design (RCBD) in sick field with P×P distance 20 cm and R×R distance 60 cm with three replications. All the cultural practices were performed to keep the crop in healthy condition.

Preparation and application of plant activators

Four plant activators i.e. Salicylic acid, KH₂PO₄, Ascorbic acid and Benzoic acid were applied against *Fusarium oxysporum* f. sp. *capsici*.

Three different concentrations 0.5, 0.75 and 1% were prepared by weighing 0.5, 0.75 and 1 g on weighing balance (Sartorius Company TH-600) and dissolved in 100ml of distilled water. Control was treated only with distilled water. All the activators were applied at seedling stage through soil drenching method and these activators were transude to the depth of 10-15 cm for the inhibition of *Fusarium oxysporum*. Soil drenching was done three times after an interval of 7, 14 and 21 days (Abdel-Monaim and Ismail, 2010). Each treatment was repeated three times.

Data recording

Data regarding the *Fusarium* wilt disease was recorded according to the disease rating scale which was described by (Monaim and Ismail, 2010) 0= Immune, 1-20%= Resistant, 21-40%= moderately resistant, 41-50%= moderately susceptible, 51-70%= Susceptible and 71-100%= highly susceptible. The percentage of disease incidence was calculated by the following formula which was given by (Weitang *et al.*, 2004).

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

Statistical analysis

Data obtained from field trials parameters was subjected to randomize complete block design (RCBD) as described by Steel *et al.*, 1997. Least significant difference (LSD) design was applied to determine the significant differences. All the statistical tests were performed by using SAS/STAT statistical software (SAS Institute, 1990).

Results

All treatments, concentrations, drenching and the interactions between concentration and treatments, treatments and drenching, drenching and concentrations and treatments, concentrations and drenching showed significant results against *Fusarium* wilt disease (Table 1).

Table 1. ANOVA for *in vivo* evaluation of different plant activators against *Fusarium* wilt of chilli pepper caused by *Fusarium oxysporum* f. sp. *capsici* through soil drenching.

| Sources | DF | SS | MS | F | P |
|------------------|-----|---------|---------|---------|-------|
| Treatment (T) | 4 | 22914.0 | 5728.50 | 27604.8 | 0.00* |
| Concentration(C) | 2 | 2837.7 | 1418.86 | 6837.25 | 0.00* |
| Drenching (D) | 2 | 197.5 | 98.75 | 475.84 | 0.00* |
| TxC | 8 | 6233.8 | 779.22 | 3754.94 | 0.00* |
| TxD | 8 | 668.5 | 83.56 | 402.65 | 0.00* |
| CxD | 4 | 21.1 | 5.27 | 25.40 | 0.00* |
| TxCxD | 16 | 44.6 | 2.79 | 13.43 | 0.00* |
| Error | 88 | 18.3 | 0.21 | | |
| Total | 134 | 32935.9 | | | |

*= Significant.

Out of four plant activators, minimum disease incidence was expressed by Salicylic acid (26.681%) followed by KH_2PO_4 (30.719%),

Ascorbic acid (33.381%) and Benzoic acid (38.737%) while control had showed the maximum disease incidence 63.444% (Table 2).

Table 2. Comparative efficacies of different plant activators against *Fusarium* wilt disease of chilli pepper.

| Sr. # | Treatments | Disease incidence (%) |
|----------------|--------------------------|-----------------------|
| T ₁ | Salicylic Acid | 26.681 e |
| T ₂ | KH_2PO_4 | 30.719 d |
| T ₃ | Benzoic Acid | 38.737 b |
| T ₄ | Ascorbic Acid | 33.381 c |
| T ₅ | Control | 63.444 a |
| LSD | 0.2464 | |

*Mean values in this column having similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

The interaction between treatment and concentration showed that increase in concentration reduces the disease incidence like at 1% concentration Salicylic acid expressed 16.07% disease incidence followed by KH_2PO_4 , Ascorbic acid and Benzoic acid that showed 21.22, 23.133 and 30.578% disease incidence respectively. Control was treated only with distilled water that's why it showed the maximum disease

incidence 74.844%. At 0.5% and 0.75% concentrations maximum disease incidence was expressed by Benzoic acid (46.95 and 38.67%) followed by Ascorbic acid (43.55 and 33.45%), KH_2PO_4 (40.83 and 30.20%) while Salicylic acid expressed minimum disease incidence (37.522 and 26.44%) respectively as compared to control 62.522 and 74.844% (Table 3).

Table 3. Interaction of different treatments with their concentrations against *Fusarium* wilt disease of chilli.

| Sr. No. | Treatments | Concentrations | | |
|----------------|--------------------------|----------------|----------|----------|
| | | Soil drenching | | |
| | | 0.5% | 0.75% | 1% |
| T ₁ | Salicylic Acid | 37.522 h | 26.444 k | 16.078 n |
| T ₂ | KH_2PO_4 | 40.833 f | 30.200 j | 21.122 m |
| T ₃ | Benzoic Acid | 46.956 d | 38.678 g | 30.578 j |
| T ₄ | Ascorbic Acid | 43.556 e | 33.456 i | 23.133 l |
| T ₅ | Control | 52.967 c | 62.522 b | 74.844 a |
| | LSD | 0.4268 | | |

*Mean values in this column having similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

After 7 days interval of soil drenching, Benzoic acid (41.311%) showed maximum disease incidence while Salicylic acid (29.967%) showed minimum disease incidence followed by KH_2PO_4 (33.367%) and Ascorbic acid (36.311%) as compared to control (58.589%). After 2nd application of soil drenching (14 days interval), Salicylic acid exhibited 26.478% disease incidence while 30.756, 30.700 and 33.411%

disease incidence showed by KH_2PO_4 , Benzoic acid and Ascorbic acid respectively as compared to control 63.25%. After 3rd application of soil drenching (21 days interval), Salicylic acid indicated 23.60% disease incidence while 28.03, 36.20 and 30.42% disease incidence showed by KH_2PO_4 , Benzoic acid and Ascorbic acid respectively as compared to control was 67.48% (Table 4).

Table 4. Interaction of different treatments and soil drenching of activators against *Fusarium* wilt disease of chillies.

| Sr. No. | Treatments | Disease incidence | | |
|----------------|--------------------------|-------------------|--------------|---------------|
| | | Drenching I | Drenching II | Drenching III |
| T ₁ | Salicylic Acid | 29.967 i | 26.478 k | 23.600 l |
| T ₂ | KH_2PO_4 | 33.367 g | 30.756 h | 28.033 j |
| T ₃ | Benzoic Acid | 41.311 d | 30.700 e | 36.200 f |
| T ₄ | Ascorbic Acid | 36.311 f | 33.411 g | 30.422 h |
| T ₅ | Control | 59.589 c | 63.256 b | 67.489 a |
| | LSD | 0.4268 | | |

*Mean values in this column having similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

The interaction between concentration and soil drenching indicated that at 0.5% concentration of first application of soil drenching maximum disease incidence was observed 45.59%. At 0.75% and 1%

concentrations, disease incidence was 40.31 and 34.42% respectively. After second application of soil drenching at 0.5, 0.75 and 1% concentrations, disease incidence was 44.56, 38.28 and 32.72% respectively.

After 3rd application of soil drenching maximum disease incidence was expressed at 0.5% concentration 42.94% and when the concentration

increased to 0.75 and 1% disease incidence was reduced to 36.18 and 32.31% respectively (Table 5).

Table 5. Interaction between soil drenching and their concentrations against *Fusarium* Wilt disease of chillies.

| Sr. No. | Concentration | Drenching I | Drenching II | Drenching III |
|----------------|---------------|-------------|--------------|---------------|
| C ₁ | 0.5% | 45.593 a | 44.560 b | 42.947 c |
| C ₂ | 0.75% | 40.313 d | 38.280 e | 36.187 f |
| C ₃ | 1% | 34.420 g | 32.720 h | 32.313 i |
| | LSD | 0.3306 | | |

*Mean values in this column having similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

After 1st application of soil drenching at 0.5% concentration, Salicylic acid expressed minimum disease incidence 40 % followed by KH_2PO_4 (42.40%), Ascorbic acid (45.80%) and Benzoic acid (49.03%) as compared to control 50.73%. At 0.75% concentration, disease incidence reduced. Salicylic acid expressed 31.06% disease incidence while KH_2PO_4 , Benzoic acid and Ascorbic acid showed 33.53, 41.90 and 37.10% disease incidence while control had showed maximum disease incidence 57.96%. Salicylic acid expressed minimum disease incidence 18.83% at 1% concentration followed by KH_2PO_4 (24.16%), Ascorbic acid (26.03%) and Benzoic acid (33%) as compared to control 70.06%. At 0.5% concentration of 2nd application of soil drenching maximum disease incidence was expressed by Benzoic acid (47.03%) while Salicylic acid showed minimum disease incidence 37.70% followed by KH_2PO_4 (41.30%) and Ascorbic acid (44.10%) while control showed 52.66% disease incidence. At 0.75% concentration, salicylic acid, KH_2PO_4 , Benzoic acid and Ascorbic acid showed 26.100, 30.03, 39.03 and

33.16% disease incidence respectively as compared to control 63.06%. Salicylic acid exhibited 15.63% disease incidence followed by KH_2PO_4 , Ascorbic acid and Benzoic acid which showed 20.93, 22.96 and 30.03% disease incidence as compared to control 74.03%. After 3rd application of soil drenching all the treatments expressed good results as compared to first two applications of soil drenching. At first concentration, salicylic acid showed 34.86%, KH_2PO_4 (38.80%), Ascorbic acid (40.76%) and Benzoic acid (44.80%) disease incidence as compared to control which showed 55.50% disease incidence. At 0.75% concentration salicylic acid expressed 22.16% disease incidence followed by KH_2PO_4 (27.03%), Ascorbic acid (30.10%), Benzoic acid (35.10%) and control (66.53%). At 1% concentration of 3rd application of soil drenching salicylic acid exhibited the best result 13.76% followed by KH_2PO_4 (18.26%), Ascorbic acid (20.40%), Benzoic acid (28.70%) and control expressed the maximum disease incidence which is 80.43% (Table 6).

Table 6. Comparison of means of disease incidence of treatment, concentration and drenching against *Fusarium* wilt disease of chillies.

| Treatments | Drenching I | | | Drenching II | | | Drenching III | | |
|--------------------------|-------------|----------|-----------|--------------|----------|-----------|---------------|----------|-----------|
| | Conc. I | Conc. II | Conc. III | Conc. I | Conc. II | Conc. III | Conc. I | Conc. II | Conc. III |
| Salicylic acid | 40.0q | 31.1v | 18.8e | 37.7s | 26.1z | 15.6f | 34.8t | 22.2c | 13.7g |
| KH_2PO_4 | 42.4n | 33.5u | 24.7a | 41.30p | 30.3w | 20.9d | 38.8r | 27.3y | 18.3e |
| Benzoic acid | 49.3j | 41.9no | 33.0u | 47.1k | 39.1r | 30.1w | 44.8m | 35.1t | 28.7x |
| Ascorbic acid | 45.8l | 37.1s | 26.3z | 44.1m | 33.2u | 22.9b | 40.7p | 30.1w | 20.4d |
| Control | 50.7i | 57.9f | 70.1c | 52.6h | 63.1e | 74.1b | 55.5g | 66.5d | 80.4a |
| | LSD | 0.7392 | | | | | | | |

*Mean values in this column having similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

Discussion

Chilli (*Capsicum annuum*) is used as spice and vegetable crop all over the world which belongs to the family Solanaceae (Khan *et al.*, 2006). It ranks third in Solanaceae family after potato and tomato. Chillies have a great economic value in Pakistan (Khalid *et al.*, 2000). Many biotic and abiotic factors deteriorate the quality of chilli crop but *Fusarium* wilt disease is a potential threat for its production. It is caused by soil borne fungal pathogen *Fusarium oxysporum* f. sp. *capsici* (Sahi and Khalid, 2007). Several management strategies are used for the management of *Fusarium* wilt disease like cultural practices, use of resistant varieties, biological and chemical control. Application of fungicides causes environmental pollution and leaves toxic residues on the plant and biological control is a time consuming method that's why the present study was designed to evaluate different plant activators. It is a cost effective and eco-friendly approach for the management of this disease. Plant activators provide signals to the plant and activate the defense genes which provide resistance to the host plant against pathogens (Vidyashekar, 2004). When pathogen attacks on the host plant, it activates its defense system like hypersensitive response (HR), phytoalexins, phenolics, pathogenesis related proteins and production of reactive oxygen species (ROS) to combat against the pathogen (Garcia *et al.*, 2003; Almagro *et al.*, 2009). Plant activators activate the resistance genes of the plant which leads to the systemic acquired resistance (SAR). Activators are eco-friendly in nature and don't have direct interaction with pathogen that's why pathogen is unable to develop resistance against the activators (Huang and Hsu, 2003).

Salicylic acid (SA) brings hormonal changes in plant and causes accumulation of Indole acetic acid which promotes the growth and development of the plant and also results in resistance of the plant (Hayat *et al.*, 2005). SA acts as a signal molecule and it involves in signal transduction pathways which is activated by hormones (Aberg, 1981; Raskin, 1992, Gunes *et al.*, 2006). Application of plant activators activate the pathways which leads to stimulation of defense response (Kusumie *et al.*, 2006).

In present study, four plant activators (Salicylic acid, KH_2PO_4 , Benzoic acid and Ascorbic acid) were evaluated against *Fusarium* wilt disease of chillies and salicylic acid expressed minimum disease incidence. The current study is supported by (Mostafa, 2006) who evaluated salicylic, ascorbic, coumaric, benzoic acids, and propylgalate against the *Fusarium oxysporum* f. sp. *cumini* and Salicylic acid gave the best result. It is also supported by Ali *et al.*, (2000) and Sarwar *et al.*, (2005) who suggested that salicylic acid expressed minimum disease incidence against *Fusarium* wilt disease.

Salicylic acid is an important antioxidant compound which triggers the antioxidant enzymes which play a significant role in protection of plant (Erdei *et al.*, 1996; Bouchereau *et al.*, 1999; Rao and Davis, 1999). Application of SA results in the production of hydrogen peroxide which acts as a signaling compound and shows hypersensitive response at the site of infection. It is a part of reactive oxygen species and it inhibits the germination of fungal spores (Huynh *et al.*, 2001).

The present study is showing that increase in concentration of activators has inverse relationship with disease incidence. When the concentration of plant activators increases, it shows the minimum disease incidence. When concentration of salicylic acid increases, it also increases the activity of resistant (R) genes which help to manage the *Fusarium* wilt disease. The current study is supported by the findings of Howard *et al.*, 2000; Ye and Ng, 2002; Khan *et al.*, 2003.

Conclusion

Four plant activators are used in the current research. It is concluded that salicylic acid is statistically significant plant activator to minimize the losses and disease incidence caused by *Fusarium* wilt of chilli.

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