Effects Sprayed Solution of Salicylic Acid to Prevent of Wilt Disease Caused by *Fusarium oxysporum*

Dina. Y. M. Yousif*

* Department of Biology., College of Science, Mustansiriyah University. Baghdad-Iraq.

dr.dinayousif33@gmail.com , dr.dinayousif@uomustansiriyah.edu.iq

Abstract. The current search aimed to detective the effect of sprayed solution of salicylic acid on plant and leaves of sweet green pepper (*Capsicum annuum*) for control the pathogen *Fusarium oxysporum* comparer with control plant and leaves. Results indicated that, the spray of salicylic acid at concentration 0.5 g/L is effecting the fungal infection through prevent transport fungus *F. oxysporum* to the neighboring green pepper plant. The number of dead green pepper plant after sprayed with solution of salicylic acid and only water they were (4, 6, and 3) (8, 9, and 10) respectively. While the experimental infection of green pepper leaves after inoculated the fungus as local spot by scorching small spots of these leaves with the aid of hot nail. These spots were then exposed to the 0.5 g/L aqueous solution salicylic acid before and after the inoculation of the fungus. The spray of salicylic acid before 24 and 48 hour prevent the development of disease and make a good protection of the mention leaves from infection with this fungus, the diameter of leaves lesion (1,1.5 cm) respectively. while the ability of fungus to grow after 24 and 48 hours from salicylic acid treatment was markedly reduce as compared with control, such treatment show slow growth of pathogen infect.

Key words: Biological control, *Fusarium* wilts, Salicylic acid (SA)

1.Introduction

Fusarium wilt is widespread, which considered one of the most important plant disease[1] caused by many forms of the soil-inhabiting pathogenic fungus such as *Fusarium oxysporum* which belongs to class Hyphomycetes [1,2,3] consider one of the most important soil-borne plant pathogen (saprophyte ). Fusarium wilt, which is also called “yellows”. This self-explanatory name indicates the major symptom of the disease [2,3,4]. A deadly vascular wilting syndrome in plants around the world. importance disease, which is particularly severe in countries with warm climate [5,6].

Different managements were used to control this disease [7,8,9]. Unfortunately, most these managements have side effect due to the development of resistant strains of pathogens against various chemical fungicides [10,11,12].
Salicylic acid is monohydroxybenzoic acid [13]. It is a natural phenolic compound from White willow (Salix alba), that affects a variety of biochemical and molecular events associated with induction of disease resistance [14,15,16]. That the phenolic compounds present fungitoxic, antibacterial and anti virotical activities [16,17,18].

2. Materials & Methods

2.1. Fungi Isolates & Preparation.

*F. oxysporum* was obtained from my pervious study [19]. For preparation fungal inoculum, the isolates of (*F. oxysporum*) Figure (1) were grown on potato dextrose agar (PDA) for 72 hr. at 2 ± 28°C. The inoculum was prepared by flooding the surface of the one agar with 10 ml of sterile distilled water and scraping the sporulated aerial mycelium with a loop. The suspensions obtained were used as inoculum in these experiments.

2.2. Plant Preparation.

One and two month old plants of sweet green pepper (*C. annuum*) grown in a row (Cork box), using Peat moss (which sterile by use autoclave at 121 o C for 1 hr. and steam pressure at 15 pound/inch²).

2.3. Preparation Solution of Salicylic acid.

Plants of sweet green pepper (*C. annuum*) were sprayed with solution of salicylic acid at concentration 0.5 gm /L.

2.4 Effect of SA to prevent transport fungus *F. oxysporum* to plant of sweet green pepper grown in line.

The plant was placed in cork box (90 X 60 cm) contain sterile Peat moss and grown in a row (one after each other), spray the lift line with solution of salicylic acid at concentration 0.5 gm /L, while the right line spray with water only as control treatment. The plant leaved for 24 hr., and then placing inoculum at the converging end of the plant rows. Three replications were made. Kept the treatment in Greenhouse conditions for 2 weeks with irrigation from time to time. In addition to that, record all note about symptom and disease development with count of dead plant 'figure 2'.

2.5 Effect spray SA on Green pepper leaves before and after inoculum with pathogen *F. oxysporum*

Two month old plants of sweet green pepper (*C.annuum*) scorching a small spots of their leaves with the aid of hot nail. These spots were then spray with 0.5 g/L aqueous solution salicylic acid before and after 24 and 48hr. from inoculated with two drops of inoculum fungus *F. oxysporum* as local spot. Kept the treatments in greenhouse conditions for 10 days with irrigation from time to time.

3. Result & Discussion

When one-month plant of sweet green pepper grown in a row (in a pot) were sprayed with 0.5 g/L solution of salicylic acid the fungal inoculum was placed beside the first plant in the raw. The infection spread in a row slowly to the neighboring plants, whereas in the untreated row the infection spread much faster to the neighboring plant Figure (2A). After one week from inoculum with pathogen (*F. oxysporum*), were appear on most plants that spray with water only, first noticed on the lower (older) leaves causing the foliage to wilt and turn yellow Figure (2B).
Other disease symptoms which include: vein clearing and leaf epinasty, followed by stunting, the lower leaves, successive, yellowing, progressive wilting, defoliation and, finally, death of the plant [20,21]. While, symptoms on root include: weakly, brownish primary and secondary root [21, 22, 23,24] as see in 'figure 3'. Disease fungi (F. oxysporum) enter through the roots and interfere with the water conducting vessels of the plant. As the infection spreads up into the stems and leaves it restricts water flow causing the foliage to wilt and turn yellow [20, 22, 23, 24, 25] as see in 'Figure 3'.

'Figure 4' showed the number of dead green pepper plant after sprayed solution of salicylic acid at 0.5 g/L per each replication. They were: (4, 6, and 3). While showed the number of dead green pepper plant after sprayed with only water, they were: (8, 9, and 10).

Data in 'figure 5' reveal that the leaves of sweet green pepper (C. annuum) which treatment with (SA) either before 24 or 48 hr. from inoculum with pathogen (F. oxysporum) that reduction of the disease lesion comparing with control. SA prevent growth of the pathogen completely from the first day of treatment and did not show any symptom disease in local spot figure (6). Salicylic acid reduces and prevent growth the pathogen through prevent the metabolism activity of fungus. In addition, the period time before inoculum with pathogen was enough to induced resistance against many necrotic or systemic fungal pathogens in leaves of plants [33]. While the diameter of leaves lesion which spray with (SA) either after 24 or 48 hr. from inoculum with F. oxysporum were (1,1.5 cm) respectively as seen in 'figure 5'. leaves lesion of sweet green pepper show slow growth of the pathogen and then stopped completely in sixth day as see in 'figure 5'. Growth was observed in the control leaves, the happen development of lesion to success infection after sixth day from start the treatment. There increase in lesion diameter was (2.2 cm). Symptom disease as the following characteristic: brownish soft lesion and start the leaves turn yellow, 'figure 6'.

\[ \text{SA} \]

\[ \text{Figure 1. } F. \text{ oxysporium under light microscope 40X show the mycelium and conidia (note macroconidia and microconidia) } \]

\[ \text{Figure 2. One month old plants of sweet green pepper (C. annuum) grown in a row, inoculum with pathogen (F. oxysporum),} \]
AS = Spray the lift line with solution of salicylic acid at concentration 0.5 gm /L.
C = Spray the right line with water only.

**Figure 3.** Root plants of sweet green pepper (*C. annuum*), inoculum with pathogen (*F. oxysporum*).

A= Spray with solution of salicylic acid.
B= Spray with water only (note the soft rot root with brownish).

**Figure 4.** Number of dead green pepper plants.

SA = Spray with solution of salicylic acid and inoculum with pathogen (*F. oxysporum*).
Control = Spray with only water and inoculum with pathogen (*F. oxysporum*).
Figure 5. Show the diameter of lesion (cm) by pathogen (*F. oxysporum*) in days.
A = Green pepper leaves inoculum with pathogen after 24hr. spray with (SA).
B = Green pepper leaves inoculum with pathogen after 48 hr. spray with (SA).
C = Green pepper leaves inoculum with pathogen before 24hr. spray with (SA).
D = Green pepper leaves inoculum with pathogen before 48hr. spray with (SA).
E = Inoculum with *F. oxysporium* only (control -).

Figure 6. Green pepper leaves with a small spots.
a = Spray SA after 24 hr. inoculum with *F. oxysporum*.
b = Spray SA after 48 hr. inoculum with *F. oxysporum*.
c = Spray SA before 24 hr. inoculum with *F. oxysporum*.
d = Spray SA before 48 hr. inoculum with *F. oxysporum*.
e = Spray with water only (control +).
f = Inoculum with *F. oxysporium* only (control -).

Fariduddin *et al.*, [34] reported that exogenous application of salicylic acid enhanced the net photosynthetic rate, Co2 assimilation and water use efficiency in *Brassica juncea*. Salicylic acid (2-hydroxybenzoic acid) consider as endogenous and exogenous elicitor, plays a crucial role in plant growth and development, and serves as an endogenous signal to activate certain immune responses and to establish disease resistance [14,16,18] by induction processes of systemic acquired resistance (SAR) [26,27]. Various defense-related stimuli have been shown to trigger enhanced SA levels in local and systemic plant tissues. Exogenous application of SA can stimulate particular enzymes catalyzing biosynthetic reactions to produce defense compounds [28,29], and induce reactive oxygen intermediates (ROI) production, pathogenesis-related (PR) gene expression and immunity against various pathogens with bio trophic or hemi bio trophic lifestyles.
[29,30,31]. In another words, it's can be affirmed that SA acts in two ways, by inducing resistance in sweet green pepper, and also by the fungitoxic action on the pathogen [30,31,32]. SA is known as an antioxidant compound which is involved in prohibition of the activity of reactive oxygen species.

References

[1] Both C. 1977 Fusarium laboratory Guide to the Identification of the Major Species. 1st Ed. Commonwealth. Mycological Institute, kew, Surrey England.
[2] Leslie, J. F. and Summerell. B. A. 2006 The Fusarium Laboratory Manual. Blackwell, Ames, IA.
[3] Nelson P. E. 1983. Fusarium species. An Illustrated Manual for Identification. The Penn St. University Press.
[4] Groenewald, S. 1988. Biology, pathogenicity and diversity of Fusarium oxysporum f.sp. cubense. University of Pretoria etd. 153: 2-3.
[5] Agrios GN. Plant pathology 5th Ed. Burlington: Elsevier Academic. 2005:922.
[6] Jun ma, L.; Geiser, D.; Proctor, R.; Rooney, A.;Donnell, K.; Trail,F.;Gardiner, D.; Manners,J. and Kanzan,K. 2013. Fusarium pathogenomics. Annu.Rev. Microbi.; 67: 399-416.
[7] Reid, T. C., Hausbeck, M. K. and Kizilkaya, K. 2002. Use of fungicides and biological control in the suppression of Fusarium crown root rot of asparagus under greenhouse and growth chamber conditions. Plant Disease, 86: 493-498.
[8] Abada, K. A. and Ahmed, M. A. 2014. Management Fusarium wilt of sweet pepper by Bacillus strains. American Journal of Life Sciences 2 6-2: 19-25
[9] Amini, Jahanshir and Sidovicho, Dzhallilov Fevzi. 2010. The effects of fungicides on fusarium oxysporum f. Sp. Lycopersici associated with fusarium wilt of tomato. Journal of plant protection research Vol. 50, No. 2: 172-178.
[10] Rajput,abdul qayoom ; arain, m. H; pathan, m. A .; jiskani, m. M. And lodhi, a. M. 2006. Efficacy of different fungicides against fusarium wilt of cotton caused by Fusarium oxysporum f. Sp. Vasinfecum. Pak. J. Bot. Vol. 38, Issue 3: 875-880.
[11] Gnanamanickam, S.S. 2002 Biological Control of Crop Diseases. New York. Basel: Marcel Dekker, Inc.; 158.
[12] Ahmed. S. Dwaish, Dina. Y. M. Yousif and Siham. N. Lefta (2016). "Use of Spirogyra Sp. Extract Against Multidrug Resistant Bacterial Pathogens." International Journal of Advanced Research, Volume 4, Issue 7, 575-579.
[13] Arberg, B. 1981. Plant growth regulators. Monosubstituted benzoic acid. Swedish Agriculture Research, 11, 93–105
[14] Chandra, A., & Bhatt, R. K. 1998. Biochemical and physiological response to salicylic acid in relation to the systemic acquired resistance. Photosynthetica, 35, 255–258
[15] Fariduddin Q, Hayat S, Ahmad A. 2003 Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in Brassica juncea. Photosynthetica. 41: 281–284.
[16] Hayat Q, Hayat S, Irfan M. Ahmad A. 2010 Effect of exogenous salicylic acid under changing environment: A review. Environmental and Experimental Botany 6: 14-25.
[17] Hayat S, Fariduddin Q, Ali B, Ahmad A. 2007 Effect of salicylic acid on growth and enzyme activities of wheat seedlings. Acta Agron. Hung 53:433–437.
[18] Eraslan F, Inal A, Gunes A, Alpaslan M. Impact of exogenous salicylic acid on growth, antioxidant activity and physiology of carrot plants subjected to combined salinity and boron toxicity. Sci. Hort. 113: 120-128.
[19] Yousif, Dina. Y. M.; Dwiash, Ahmed. S. and Shafiq, Shatha Ali. 2015. Antifungal activity of algal spirogyra sp. Against fungal *Fusarium oxysporum*. *World Journal of Pharmaceutical Research*. Volume 4, Issue 1, 1620-1628.

[20] Fletcher, J. T. 1994. *Fusarium* stem and fruit rot of sweet peppers in the glass house. *Plant Pathology*, 43: 225-227