Efficacy of Salicylic Acid & 2, 6-Dichloroisonicotinic Acid as Systemic Acquired Resistance Activators *In vitro* on *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium oxysporum* f. sp. *radicis-cucumerinum* Radial Growth

Abdalla M. El-Alwany

Department of Plant Protection, Faculty of Agriculture, Benghazi University, Libya
alwany_a@yahoo.com

Abstract: *Fusarium oxysporum* f. sp. *lycopersici* (*F.o.l*) and *Fusarium oxysporum* f. sp. *radicic-cucumerinum* (*F.o.c*) the causal agents of fusarial vascular wilt on tomato and cucumber plants respectively. These two diseases constitute a great danger and threat to the cultivation of tomatoes, cucumbers, both in greenhouses or in open areas. Salicylic acid (SA) and 2,6-dichloroisonicotinic acid (INA), which have the ability to induce systemic acquired resistance in plants were used in this study to test their effect on radial growth of (*F.o.l*) and (*F.o.c*) in Petri dishes. Results showed that 500 ppm of SA and INA had the greatest radial growth of *F.o.l* and *F.o.c* compared to other concentrations 1000 and 2000 ppm significantly. Inhibition percentage measurements showed also SA and INA 500 ppm had the lowest inhibition percentage (6.8%), (28%) for *F.o.l* and (8.8%), (24.8) for *F.o.c*. respectively. Results of this study and many of other studies conducted for the induction of systemic acquired resistance by these two compounds proved that concentrations less than 500 ppm able to induce the systemic acquired resistance in plants, also their inhibitory influence on radial growth are very few or non-existent in many cases.

[Abdalla M. El-Alwany. **Efficacy of Salicylic Acid & 2, 6-Dichloroisonicotinic Acid as Systemic Acquired Resistance Activators *In vitro* on *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium oxysporum* f. sp. *radicis-cucumerinum* Radial Growth.** *Nat Sci* 2014;12(9):92-96]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 15

Keywords: Systemic acquired resistance; Salicylic acid; 2, 6-dichloroisonicotinic acid; *Fusarium oxysporum*

1. Introduction

Tomato (*Solanum lycopersicum* L., syn. *Lycopersicon esculentum*) and cucumber (*Cucumis sativus*) among the most important vegetables for human food and grown in all countries of the world in open fields and in protected cultivation (Celar, 2000 and Fritz, 2005). Arie *et al.* (2007) indicates that the cultivation of tomatoes bounded by many diseases caused by fungi, bacteria, viruses and nematodes. Saker *et al.* (2008) Showed that the major diseases on tomatoes produced from about 24 fungi, 7 bacteria, 10 viruses, 3 viroids and several nematodes. Tomato fusarial wilt is caused by *Fusarium oxysporum* f. sp. *Lycopersici* (Sacc.) W.C. Snyder H.N. Hansen is one of the most widespread economic diseases on tomato (*Lycopersicon escolentum* Mill.) and the severity of the disease lies in the three strains by their ability to infect a range of different varieties (Grattidge & O'Brien, 1982) to reduce tomato productivity by 25% (Farvel *et al.*, 2005). Fusarial wilt on cucumber caused by *Fusarium oxysporum* f. sp. *cucumerinum* is a soil borne disease transmitted through the soil and believed to be of major diseases soil dweller (Ye *et al.*, 2004), leading to a severe shortage in cucumber crop under greenhouse conditions (Shen *et al.*, 2008). Despite all the information available about fusarial wilt disease, the mechanism of disease, in case non-appropriate soil properties are still unclear (Ye *et al.*, 2006). Systemic acquired resistance (SAR) gained interest of many researchers and many studies carried out led to great discoveries represented in biochemical changes in host cell wall, phytoalexins and pathogenesis related proteins (PRs) production, and stimulate programmed cell death (hypersensitivity) (AL-korany and Faiadh, 2011). Chemical compounds that are used in the induction of (SAR) should not be have a toxic effect directly on pathogens either for the compound itself or one of its derivatives (Gozzo, 2003) as well have no toxic effects on plants and animals, have a wide range of defense, affect in small quantities, their prevention impact remains for a long time, and with low cost (Melvin and Muthukumaran, 2008). It has been found that the use of SA and its analogs such as INA and BTH is able to induce the genes expression responsible for the production of (PRs) which have importance in resistance process against viral, bacterial and fungal diseases in dicot plants (Pasquer, 2005). SA was used successfully to resist some plant diseases such as root rot and wilt of sesame (Abdou *et al.*, 2001), root rot in wheat (El-Bana *et al.*, 2002), root rot and wilt in lupine (Ali *et al.*, 2007 and Abdel-Monaim, 2008), fusarial wilt in tomatoes (El-Khallal, 2007) and fusarial wilt in chickpea (Night-Sarwar *et al.*, 2005). Edreva (2004) and Narusaka *et al.* (2006) recorded that INA is one of the essential synthetic compounds able to induce SAR. Gozzo (2003) reported that INA induces gene expression for SAR, sometimes before occurrence of infection in the plants and at other times, after pathogen attack only. This study aimed to isolation and identification the causal agent of wilt disease in tomato and cucumber and to test the effect of SA and INA as plant SAR activators on radial growth *In vitro*.

2. Material and Methods

Samples of infected tomato and cucumber plants were brought from plastic tunnels at Sidi-Faraj area/ Benghazi. Laboratory experiments were conducted in Department of Plant Production, Faculty of Agriculture / University of Benghazi.

Isolation and identification of *F.o.l* and *F.o.c*: *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium oxysporum* f. sp. *radicis-cucumerinum* were isolated from tomato and cucumber roots their plants exhibited fusarial wilt symptoms. The fungi were cultivated in sterile Petri dishes contains Potato Dextrose Agar Medium (P.D.A.), dishes were incubated overturned on 27±1°C. Identification was according to (Booth, 1971).

SAR activators preparation: Salicylic acid (SA) Sigma-Aldrich chemie-France and 2,6-dichloroisonicotinic acid (INA) Sigma Aldrich chemie-Germany, were purchased from Sigma-Aldrich chemicals company. Three concentrations 500 ppm, 1000 ppm and 2000 ppm from each chemical activator were prepared by dissolving in equal volumes from ddH_2O and ethyl alcohol, and adjusted to pH 7 with 1N NaOH.

Incorporation of PDA medium with chemical activators: 1 ml from each chemical concentration was added to sterile Petri dish 5 cm diameter by three replicates and distributed over an area of the dish, then sterile PDA medium was poured even cover the bottom of the dish and shaken gently to ensure mixing. 8 mm disks of *F.o.l* and *F.o.c* 3 days old were seeded centrally in the dishes then incubated at 27±1°C. Three dishes with chemical free media inoculated with fungal disks served as control for each fungus.

Radial growth measurement: When mycelial growth of control covered the plates, fungal growth diameters were determined. The percentage reduction of growth (RG) ratio was calculated according to the following formula (Amer, 1995):

$$RG (\%) = RNT - RT / RNT * 100$$

Where: *RNT* = Radius for non-treated media (control), and *RT* = Radius for treated media.

Statistical analysis: Experiment was conducted according complete randomized design (CRD), data were subjected to ANOVA (Gomez and Gomez, 1984), and significant differences were compared by Fisher's Least significant difference (FLSD) Test ($P \leq 0.05$). The package used for analysis was NCSS and GESS version 2007.

3. Results and Discussion

After incubation of *Fusarium oxysporum* f. sp. *lycopersici* and *Fusarium oxysporum* f. sp. *radicis-cucumerinum* cultures incorporated with chemical activator concentrations (treatments) and dishes free from chemical concentrations (control) on 27±1°C several days, radial growth for all treatments and control was measured. Results in Table (1) showed that 500 ppm exhibited higher significantly radial growth compared to other concentrations for both SA and INA in case of *F.o.l* and *F.o.c*. Radial growth values of this treatment in case of SA (4.66 cm), (4.56 cm) for *F.o.l* and *F.o.c,* respectively, were closer insignificantly to control (5 cm), meanwhile in case INA (3.60 cm), (3.76 cm) the values were differs significantly. Figs (1, 2) shows clearly that treatment (500 ppm) exhibited gradually decrease in radial growth with significant differences statistically, compared to other two treatments (1000 ppm) and (2000 ppm), this fact was agreed with Ozgonen *et al*. (2001) when he found inhibited effect of SA on *F.o.l* growth at high concentration over 600 ppm. Spletzer and Enydi (1999) also indicated that low concentrations of SA below 200 ppm had no antifungal inhibition for *Alternaria solani*.

Table 1. Radial growth average(±SE) for *Fusarium oxysporum* f. sp. *lycopersici* (*F.o.l*) and *Fusarium oxysporum* f. sp. *radicis-cucumerinum* (*F.o.c*) cultures incorporated with SA and INA concentrations.

Treatments	Radial growth average (±SE) /cm			
	Salicylic acid (SA)		2,6-dichloroisonicotinic acid (INA)	
	F.o.l	*F.o.c*	*F.o.l*	*F.o.c*
Control	5^{a}	5^{a}	5^{a}	5^{a}
500 ppm	4.66 ± 1.58^{a}	4.56 ± 1.21^{a}	3.60 ± 0.51^{b}	3.76 ± 0.54^{b}
1000 ppm	3.16 ± 0.55^{b}	3.70 ± 1.07^{b}	2.53 ± 0.55^{c}	2.96 ± 0.98^{c}
2000 ppm	2.13 ± 0.51^{c}	2.80 ± 0.54^{c}	1.46 ± 0.17^{d}	2.33 ± 0.12^{c}
FLSD (α=0.05)	1.01	0.80	1.20	0.69

Values followed by the same letter(s) in each column don't differ significantly according to FLSD test ($P \leq 0.05$).
Control: free from chemical activator. Average represents three replicates. ppm (part per million).

Measurements of inhibition percentage for both chemical activators and for both fungi showed that 500 ppm (Table 2) showed low percentage reduction of growth compared to other two concentrations (1000 ppm and 2000 ppm). It is clear from the measurements that the lowest percentage was for SA against INA for both *F.o.l* and *F.o.c.* Increasing concentrations of SA or INA in the growth medium resulted in increased inhibition of the growth of fungus, where it seems that high concentrations of toxic act also as hinder vital processes and building necessary compounds; coenzymes and nucleic acids of the fungus. These findings were not completely agreed with El-Mougy (2002) who found that *F.o.l* growth inhibited by increasing SA concentrations over 40 mM only. Measurements of INA indicate to high inhibition by increasing the concentration which shows the toxic effects of the compound at these concentrations, although many studies have indicate that INA has no antifungal effects for many fungi. One of these studies are mentioned Abdalhadi (2011) in a study of the impact of chemical activators to induce systemic acquired resistance against tomato diseases where, concentrations (500, 750, and 1000 µl/L) have no inhibitory effects on radial growth, hyphal length and hyphal thickness for *Alternaria solani* and *Fusarium solani*. It is also Kataria *et al*. (1997) demonstrated that INA and other chemical activators did not have inhibitory effect on the growth of *Rhizoctonia solani* when used at different concentrations as soil drench. Perhaps the toxic effect here is due to the fact that INA is an analogue of SA, where its inhibitory effect acts at high concentrations. All in all, it is clear from most of the studies conducted for the induction of systemic acquired resistance by chemical activators that concentrations less than 500 ppm able to induce SAR in plants, also the inhibitory influence on fungal growth is not mentioned. It seems also concentrations exceeded 500 ppm probably showed symptoms of phytotoxicity for many plants, this phenomenon was reported by Frey and Carver (1998), Rauscher *et al*. (1999) and Abdalhadi (2011).

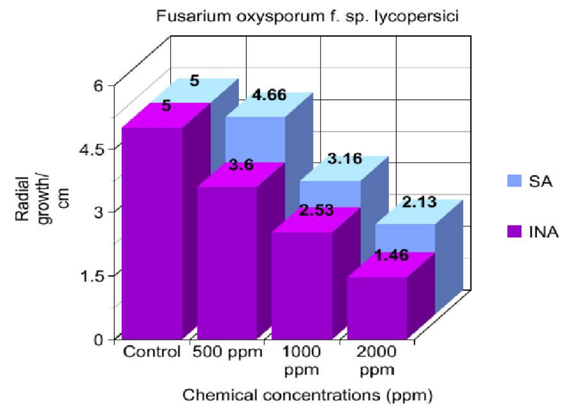

Figure 1. Effect of 500, 1000, 2000 ppm and control treatments for both SA and INA on radial growth of *Fusarium oxysporum* f. sp. *lycopersici*, where noticed gradual effect from high to low chemical concentration.

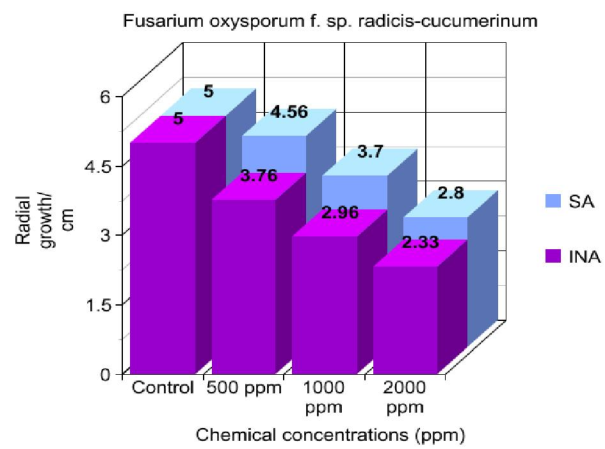

Figure 2. Effect of 500, 1000, 2000 ppm and control treatments for both SA and INA on radial growth of *Fusarium oxysporum* f. sp. *radicis-cucumerinum*, where noticed gradual effect from high to low chemical concentration.

Table 2. Reduction growth percentage of SA and INA concentrations on *Fusarium oxysporum* f. sp. *lycopersici* (*F.o.l*) and *Fusarium oxysporum* f. sp. *radicis-cucumerinum*.

Chemical concentrations (ppm)	Reduction growth (%)			
	F.o.l		***F.o.c***	
	(SA)	(INA)	(SA)	(INA)
500	6.8	28	8.8	24.8
1000	36.8	49.4	26	40.8
2000	57.4	70.8	44	53.4

References

1. Abdalhadi A. Studies on the induction of systemic acquired resistance (SAR) against some tomato fungal diseases by chemical activators. Ph. D. Thesis, Agricultural Botany Department, Faculty of Agriculture, Alexandria University, Egypt, 2011; 250.
2. Abdel-Monaim M. Pathological studies of foliar and root diseases of lupine with special reference to induced resistance. Ph. D. Thesis, Faculty of Agriculture, Minia University, Egypt, 2008.
3. Abdou El-S, Abd-Alla H, Galal A. Survey of sesame root rot/wilt disease in Minia and their possible control by ascorbic and salicylic acids. Assuit J. of Agric. Sci. 2001;32(3): 135-152.
4. Ali A, Ghoneem K, Eetwally M, Abdel-Hai K. Induce systemic resistance in lupine against root rot diseases. Pakistan J. of Biol. Sci. 2007; 12 (3): 213-221.
5. Al-korany J, Faiadh M. Efficiency of some chemical and biological induction compounds in the reduction of the tomato plant infection by the fungus *Fusarium oxysporum* schl. f.sp.*lycopersici*. J. Basrah Res. Sci. 2011; 37(4):19-30.
6. Amer M. Evaluation of adjuvants to enhance fungicide efficacy against plant pathogens. Ph. D. Thesis, Faculty of Agriculture and applied sciences, University of Gent, 1995; 200.
7. Arie T, Takahashi H, Kodama M, Teraoka T. Tomato as a model plant for plant-pathogen interactions. Plant Biotechnology 2007; 24: 135–147.
8. Booth C. The Genus *Fusarium*, Kew, Surrey. Commonwealth Mycol. Inst., 1971; 237.
9. Celar F. Cucurbit diseases. Bolezni bucinic. Sodobno Kmetijstvo, 2000; 33(4): 162-165. (c.f. Rev. of Pl. Path. 2000. 79: 10).
10. Edreva A. A novel strategy for plant protection: induced resistance. J. Cell Mol. Biol. 20043; 61-69.
11. El- Bana A, Ismaial A, Nageeb M, Galal A. Effect of irrigation intervals and salicylic acid treatments on wheat root rot and yield components. Proc. Minia 1 st Conf. for Agric. and Environ. Sci., Minia, Egypt, 2002; March25-28: 229-240.
12. El-Khallal S. Induction and modulation of resistance in tomato pants against Fusarium wilt disease by bioagent fungi (*Arbuscular mycorrhiza*) and/or hormonal elicitors (jasmonic acid and salicylic acid): 1- Changes in growth, some metabolic activities and endogenous hormones related to defense mechanism. Australian J. Basic and Appl. Sci. 2007; 1(4): 691-705.
13. El-Mougy N. In vitro studies on antimicrobial activity of salicylic acid and acetylsalicylic acid as pesticidal alternatives against some soilborne plant pathogens. Egy. J. Phytopathol. 2002; 30(2): 41-55.
14. Fravel D, Deahl K, Stommel J. Compatibility of the biocontrol fungus *Fusarium oxysporum* strain CS-20 with selected fungicides. Biol. Cont. 2005; 34: 165-169.
15. Frey S, Carver T. Induction of systemic resistance in pea to pea powdery mildew by exogenous application of salicylic acid. J. Phytopathol. 1998; 146:239–245.
16. Fritz M. Resistance induction in the pathosystem tomato – *Alternaria solani*. Ph. D. thesis, Giessen University, Germany, 2005; 130
17. Gomez K, Gomez A. Statistical procedures for agricultural research. John Wiley and Sons, New York, USA, 1984; 680.
18. Gozzo F. Systemic acquired resistance in crop protection: from nature to a chemical approach. J. Agric. Food Chem. 2003; 51: 4487-4503.
19. Grattidge R, O´brien R. Occurrence of a third race of Fusarium wilt of tomatoes in Queensland. Plant Disease 1982; 66:165-166.
20. Kataria H, Wilmsmeier B, Buchenauer H. Efficacy of resistance inducers, free-radical scavengers and an antagonistic strain of *Pseudomonas fluorescens* for control of *Rhizoctonia solani* AG-4 in bean and cucumber. Plant Pathology 1997; 46: 897-909.
21. Melvin J, Muthukumaran N. Role of certain elicitors on the chemical induction of resistance in tomato against the leaf caterpillar *Spodoptera litura* Fab. Not. Bot. Hort. Agrobot. Cluj. 2008; 36 (2): 71-75.
22. Narusaka M, Abe H, Kobayashi M, Kubo Y, Kawai K, Izawa N, Narusaka Y. A model system to screen for candidate plant activators using an immune-induction system in Arabidopsis. Plant Biotech. 2006; 23: 321–327.
23. Nighat-Sarwar M, Hayat-Zahid C, Ikramul-H. Jamil F. Induction of systemic resistance in chickpea against Fusarium wilts by seed treatment with salicylic acid and Bion. Pak. J. Bot. 2005; 37(4): 989-995.
24. Ozgnen H, Erkilic A. The effect of salicylic acid and endomycorrhizal fungus *Glomus etunicatum* on plant development of tomatoes and *Fusarium oxysporum* f. sp. *lycopersici*. Turk. J. Agric. 2001; 25: 25-29.
25. Pasquer F, Isidore E, Zarn J, Keller B. Specific patterns of changes in wheat gene expression

after treatment with three antifungal compounds. Plant Mol. Biol. 2005; 57: 693-707.

26. Rauscher M, Adam A, Wirtz S, Guggenheim R, Mendgen K, Deising H. PR-1 protein inhibits the differentiation of rust infection hyphae in leaves of acquired resistant broad bean. Plant J. 1999; 19: 625-633.
27. Saker M, Hussein H, Osman N, Soliman M. *In vitro* production of transgenic tomatoes expressing defensin gene using newly developed regeneration and transformation system. Arab J. of Biotech. 2008; 11(1): 59-70.
28. Shen W, Lin X, Gao N, Zhang H, Yin R, Shi W. Duan Z. Land use intensification affects soil microbial populations, functional diversity and related suppressiveness of cucumber *Fusarium* wilt in China's Yangtze River Delta. Plant Soil. 2008; 306: 117–127.
29. Spletzer M, Enyedi A. Salicylic acid induces resistance to *Alternaria solani* in hydroponically grown tomato. Phytopathology, 1999; 89: 722-727.
30. Ye S, Yu J, Peng Y, Zheng J, Zou L. Incidence of *Fusarium* wilt in *Cucumis sativus* L. is promoted by cinnamic acid, an autotoxin in root exudates. Plant Soil. 2004; 263: 143–150.
31. Ye S, Zhou Y, Sun Y, Zou L, Yu J. Cinnamic acid causes oxidative stress in cucumber roots, and promotes incidence of *Fusarium* wilt. Environ. Exp. Bot. 2006; 56: 255–262.

8/28/2014