

APPLICATION OF SALICYLIC ACID AND SOME FUNGICIDES AS SEED TREATMENT FOR CONTROLLING DAMPING-OFF AND ROOT ROT DISEASES OF SQUASH AND CANTALOUPE PLANTS UNDER FIELD CONDITIONS.

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ABSTRACT

The effect of salicylic acid (SA) individually or/and in combinations with three standard fungicides named Flutolanil (Moncut WP 30%), Telcolofos-methyl/thiram (Rhizolex 50%WP) and Carboxin-Thiram (Vitavax 200 WP) was studied for the field control of damping-off and root rot diseases of cantaloupe (*Cucumis melo* var. *cantaloupensis*) and squash (*Cucurbita pepo*), cvs *Askandarany* and *Mera*, respectively. The experiments were carried out in the years 2012 and 2013.

In vitro studies the effect of the aforementioned fungicides and salicylic acid on growth of *Rhizoctonia solani* and *Fusarium solani* was determined. The effective concentration was found to range from 50 to 200 ppm, and the higher concentration the greater inhibitory effect. The combination of salicylic and vitavax 200 and salicylic acid and Rizolex at 200 ppm showed the highest effect on *R. solani* and *F. solani* with the averages of 84.75, 86.79, 71.25 and 87.13%, respectively.

In field trials, soaking seeds in salicylic acid, either single or in combination with the fungicides in concern, significantly decreased both damping-off and root rot of squash and cantaloupe. In addition to increasing the numbers of survived plants, the used treatments increased shoot length as well as fresh and dry weights. The results showed that mixed salicylic acid application with fungicides to seeds of squash and cantaloupe significantly decreased disease symptoms by 70.32 and 64.07% for vitavax 200 and rhizolex treatments, respectively, for squash. The corresponding figures for cantaloupe were 62.89 and 55.90%, respectively in the second season 2013. Moreover, the treatments in concern increased the activities of peroxidase and polyphenoloxidase. It is noticed, however, a negative correlation between disease incidence and activities of both peroxidase and polyphenoloxidase in either squash or cantaloupe plants. Meanwhile, the treatments in concern increased the contents of Mn and Fe but decreased Zn of both tested crops

In general, the results indicated that especially the combined SA and standard fungicides showed a better response to fight damping-off and root rot diseases than the treatment alone. Thus the present study shows that the induction of defense related enzymes may be enhance resistance in squash and cantaloupe plants.

INTRODUCTION

Cantaloupe (*Cucumis melo* var. *cantaloupensis*) and summer squash (*Cucurbita pepo*) are considered of the major summer vegetable crops in commercial fields and under protected cultivation during winter in Egypt. They are considered a major source of essential nutrients such as vitamins, minerals, carbohydrates, antioxidants and anti carcinogenic substances,

which are important to human nutrition and health (Joseph, 1994). The cultivated area under Beheria Governorate conditions of cantaloupe and squash during 2013 season were 3023 and 8962 feddans yielded about 6-8 and 12-16 tons / feddan of fruits, respectively.

Cultivated vegetables are subjected to attack by several plant pathogenic fungi during different stages of plant growth from seed sowing up to seedling to flowering stages, and may to cause pre-emergence infection, thus forcing the farmer to replant the missed hills or dead plants. Moreover, root rot disease, produced by *Pythium spp.*, *Rhizoctonia spp.*, *Sclerotinia spp.* and *Fusarium spp.* cause widespread, serious economic losses both in greenhouse and field production systems under conditions favorable for the disease development (Celar, 2000 and Hibar *et al.*, 2006). In Egypt, *Alternaria solani*, *F. oxysporum*, *R. solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina* and *Pythium spp.* were isolated from diseased cucumber, cantaloupe, tomato and pepper plants grown in plastic houses and showing root rot disease symptoms (El-Mougy *et al.*, 2011 and Abdel-Kader *et al.*, 2012).

The most efficient and economically vital method for disease control is the use of resistant varieties (Wada, 2003). However, the development of resistant varieties is time consuming, until now; therefore fungicides have been described as the primary means of quick control (Olufolaju, 1993). Rukhsana Afzal *et al.* (2010) reported that the fungicidal seed treatment is highly effective, economical and easily applicable, as it can reduce the seed-borne mycoflora, improve seed germination and protecting seedlings for sufficient time. Abou El-Souod *et al.* (2005) reported the control of *R. solani*, *M. phaseolina* and *P. debaryanum* producing damping-off and root rot on *Phaseolus vulgaris* seedling with a group of fungicides.

A successful disease-control program could involve just a single practice, but the long term reduction of disease losses generally requires the application of several control measures. The management of soil-borne plant pathogens is particularly complex because these organisms live in or near the dynamic environment of the rhizosphere, and can frequently survive a long period in soil through the formation of resistant survival structures.

A new technology for plant disease control is based on the activation of the plant's own defense system with aid of low molecular weight synthetic molecules that induce systemic acquired resistance (SAR) in plants against a wide range of microbial pathogens. SAR and induced systemic (ISR) are two forms of the induced resistance. Plant defenses are preconditioned by prior infection or treatment that results in resistance (or tolerance) against subsequent challenge by pathogen (Vallad and Goodman, 2004). The ISR sensitizes the plant to respond rapidly after treatment. These responses include activation of peroxidase, polyphenoloxidase and phenylalanine ammonia lyase and phenols (Ragab *et al.*, 2009 and Abdel Aal *et al.*, 2012). Salicylic acid (SA) represents an interesting new opportunity in controlling fungal and bacterial diseases with in an environmental friendly integrated system (Ellis *et al.*, 2002; El-Khallal, 2007; Ali, *et al.*, 2009 and Yehia, *et al.*, 2011). Several investigators studied the effectiveness of these chemical inducers on root rot disease (Segarra *et al.*, 2006). Salicylic acid (SA) has

been used successfully to control some plant diseases such as root rot of cucumber (Chen-Chunquan *et al.*, 1999), root rot/wilt of sesame (Abdou *et al.*, 2001), root rot of wheat (El-Bana *et al.*, 2002) and root rot of tomato (El-Khallal, 2007 and Suprakash ojha, 2012). Farah bakhsh and Shams addin (2011) observed the improvement in maize vegetative growth following SA application under saline condition and concluded that the positive reaction of this material on plant growth may be due to the chemical changes in plants throughout avoiding the uptake undesired ions Na^+ and Cl^- and enhancing the uptakes of No_3^- , Mg^{+2} , Fe^{+2} , Mn^{+2} and Cu^{+2} .

The aim of work presented in this paper, was to assess the efficacy of salicylic acid (SA) on the induction of SAR in cantaloupe and squash plants compared to the action with three fungicides widely used for management of damping-off and root rot under field conditions. Also to identify some associated biochemical changes in treated plants.

MATERIALS AND METHODS

1- Isolation, purification and identification:

Roots of diseased Melon and squash plants were collected from field of Etay El-Baroud Agric. Res., Station, El Beheria Governorate, and washed with tap water to remove adhering soil particles. Small parts of infected roots were surface sterilized using sodium hypochloride solution (3%) for three minutes, washed with distilled sterilized water for several times, then dried using sterilized filter paper and transferred into petri-dishes containing potato dextrose agar medium (PDA). Plates were incubated at 25°C for three days. All grown fungi were purified using single spore or hyphal tip technique and identified according to Dhingra and Sinclair (1985), Booth (1977), Domsch *et al.* (1980) and Singh (1982).

2- *In vitro* experiment:

A laboratory study was performed to examine the sensitivity of *R. solani* and *F. solani* to salicylic acid (SA) which obtained from Sigma chemical Co. Aqueous solution of SA was dissolved in ethanol (95%) and added to distilled water at room temperature to prepare the stock suspensions. Sterile distilled water was also used to prepare stock preparations of three fungicides Telcolofos-methyl/thiram (rizolex-T WP 50%), Flutolanil (moncut WP 30%) and Carboxin-Thiram (vitavax-200 WP) at rates of 0, 50, 100, 200 ppm singly or in combinations with SA. The required concentrations were obtained by adding the appropriate amount of stock solution to 100 ml of autoclaved PDA before solidification. Discs (6 mm diameter) cut from the actively growing regions of four-day old *R. solani* and *F. solani* culture using cork borer were placed in the center of each petri dish. There were three replicates of nine dishes /treatment for each pathogen. Petri dishes were covered with Parafilm and incubated at 25°C for five days. The diameter of growth on petri dish was recorded in centimeter when mycelial growth of control covered the plates. The reduction percentage of growth (RG) ratio was calculated using the following formula (Amer, 1995):

$\text{RG}\% = [\text{RNT} - \text{RT}/\text{RNT}] \times 100$ Where:

RNT = Radius for non-treated media, and RT = Radius for treated media

3- Field experiment:

Salicylic acid and the three fungicides either single or in combinations were prepared in concentrations as mentioned in (Table, 1) by dissolving in distilled water with 1mL of Arabic gum/100 mL suspension. The seeds of cantaloupe cv. Mera and squash cv. Askandrany were soaked for 7 hours in any of the desired concentration, and then kept in cheesecloth bags for 12 hours before sowing. Germinated seeds were sown at 15 May in two successive growing seasons 2012 and 2013. The field plots (12m²) consisted of three terraces of 4 m long and 1m² in width, two seeds/hil were sown with 30-40 cm a spacing distance for hills in fields naturally infested with damping-off and root rot fungi. Untreated seed were used as control. All agricultural practices were carried out according to the recommendation of the Ministry of Agriculture and Land Reclamation, Egypt. Located at the experimented farm of Etay El- Baroud Agric., Res., Station, Beheria Governorate.

Table (1):Salicylic acid and fungicides used for controlling the studied fungi under field condition.

Commercial name	Common name	Chemical name	Application rate/Liter
Salicylic acid	Salicylic acid	o-hydroxybenzoic acid	1.0 g/L
Moncut WP 30%	Flutolanil	N-(3-(1-methylethoxy)-2-(trifluoromethyl)benzamide	2.0 g/L
Rizolex T WP 50%	Telcolofos-methyl/thiram	20%Telcolofos- methyl (0,2,6 dichloro-4methyl-phenyl 0,0 dimethyl phospho thioate) and 30% thiram.	3.0 g/L
Vitavax 200 WP 75%	Carboxin-Thiram	Carboxin: (5,6-Dihydro-2 methyl-N-phenyl-1.4-oxathiin-3-carboxamide) Thiram (tetramethyl thiuram disulfide)	2.0 g/L

Treatments used:

- 1- Check (Tap water)
- 2- Salicylic acid (SA)
- 3- Moncut WP 30% (Mo)
- 4- Rizolex WP 50% (Rz)
- 5- Vitavax 200 WP 75% (V200)
- 6- SA + Mo
- 7- SA + Rz
- 8- SA + V200

After 45 days from sowing, plant leaves were collected from each treatment for determining peroxidase and polyphenoloxidase activities. Growth parameters as shoot length (cm), fresh and dry weight/plant (g) were measured.

Disease assessment:

Pre- and post- emergence damping-off disease were assessed two and four weeks after sowing, then the survival plants was counted according to the following formula (Mona *et al.*, 2009):

$$\text{Pre-emergence (\%)} = \frac{\text{Total No. of ungerminated seeds}}{\text{Total No. of planted seeds}} \times 100$$

Post-emergence (%) = $\frac{\text{Total No. of rotted seedlings}}{\text{Total No. of planted seeds}} \times 100$

Healthy survival plants % = 100 - damping-off

4- Enzyme determinations:

Peroxidase assay:

Peroxidase activity was spectrophotometrically determined by measuring the oxidation of tetraguicual in the presence of H₂O₂ at 470 nm according to the method described by Kato and Shimizu (1987). A sample of 0.25 gm fresh weight was homogenized with phosphate buffer PH6 and centrifuged for 15 min at 4000 rpm. The enzyme activity was assayed by mixing 1 ml of crude enzyme extract, 0.3 ml. of 7.2 mM tetraguicual and 0.1 ml. of 11.8 mM H₂O₂. The enzyme activity was expressed as A₄₇₀/min/gm of fresh weight. Enzyme activity was expressed in units of μM of the substrate converted per min per gram fresh weight. Each value reported is the average of three replicates.

Polyphenoloxidase assay:

The activity of polyphenoloxidase was determined according to the method of Esterbaner *et al.*, (1977). 0.25 gm of fresh weight was homogenized in borate buffer, PH7. The homogenate was centrifuged at 4000 rpm for 15 min. one ml of crude enzyme extract mixed with 1.01 ml of 0.2 M sodium phosphate buffer PH7 and 1 ml of 10⁻³ M catechol. The reaction mixture was incubated for 1h. at 40°C. After briefly vortexing, the absorbance at 490 nm was then determined. The activity was expressed as A₄₉₀/min gm of fresh weight. Each value reported is the average of three replicates.

Micro elements analysis:

Accurately weighted sample (3g) in crucible was subjected to ashing in furnace for 4 hour at 550 C. After cooling in desiccators, 2.5mL of 6 N HNO₃ was added to crucible. The solution was analyzed for Fe, Mn and Zn by using Atomic Absorption Spectrophotometer (AAS-Perkin Elmer, Model analyst 800). The results were extrapolated while using a working standard of 1000 ppm for each of the species (Hussain *et al.*, 2009 and 2010).

5- Statistical analysis:

Data obtained were subjected to the statistical analysis according to the standard methods recommended by (Gomez and Gomez, 1984) using the computer program (costate). Means were compared using L.S.D. at the level 5% of probability and Duncan 's multiple range test at p < 0.05 level was used for means separation (Winer, 1971).

RESULTS AND DISCUSSION

1- *In vitro* experiments:

Data presented in Table (2) and illustrated in Figure (1) revealed that salicylic acid (SA) and fungicides, monocut (Mo), rizolex (Rz) and vitavax200 (V200) either individually or in combinations significantly reduced linear growth (cm) of both *R. solani* and *F. solani*. The lowest linear growth of *R. solani* was achieved by SA+V200 with the average of 3.52 cm followed by V200 and SA+Rz with an average of 3.90 and 4.12 cm, respectively. In

general, statistical analysis cleared that there are significant difference between the average linear growth values between the SA and fungicides treatments. In this regard Ghada and Abdel-Sattar (2013) who found that capritop, moncut, rizolex T and shirlan at 20 ppm reduced the linear growth of *F. solani*, *R. solani* and *Monosporascus cannonballus* isolated from diseased roots of cantaloupe. In this respect Saad, *et al.* (2014) reported complete inhibition of mycelial growth by vitavax 200 at 200 ppm and reached 95.59% at 100 ppm. Vitavax 200 inhibited the linear growth of *F. solani* by 24.15-100%, respectively. The combinations between SA and fungicides in the present study were more effective than using any of them individually. SA+V200 and SA+Rz at 200 ppm were the most effective where they recorded the highest reduction percentages of *R. solani* and *F. solani* with the averages of 84.75, 86.79%, 71.25 and 87.13, respectively. These results are in agreement with finding of Abdel-Monaim (2013) who mentioned that biocontrol agents combined with chemical inducers recorded the highest inhibition of growth of pathogenic fungi, especially in case of SA+ *Trichoderma viride* and SA+ *Bacillus megaterium*. The inhibitory effect produced by SA in the present study almost agrees with that found by Hilal *et al.* (2006) who found that salicylic acid (SA) completely prevented the mycelial growth of *Sclerotinia sclerotiorum* using the concentration 4.0g/L. Abdel-Monaim (2012) showed that the highest inhibitory effect on *F. oxysorum*. f. sp. *lycopersici* isolates was noticed when 200 ppm of H₂O₂ was added to liquid and soild media followed by SA at 200 ppm. In addition, obtained results by Abdel-Monaim *et al.* (2012) showed that the linear growth of *F. oxysorum*, *F. solani* and *R. solani* decreased by using SA at 200 ppm for the previous pathogens, while liner growth reduced from 90 mm in check treatment to 54.93, 48.60 and 65.45 mm in case of *F. oxysorum*, *F. solani* and *R. solani*, respectively.

Table (2): Effect of salicylic acid (SA) and fungicides individually or in combinations on linear growth (cm) of *R. solani* and *F. solani* under laboratory conditions.

Treatment	Conc. ppm	<i>R. solani</i>	*Mean	Inhibition (%)	<i>F. solani</i>	*Mean	Inhibition (%)
SA	0	7.58	5.62 a	-	8.00	5.52 a	-
	50	5.71		24.67	5.53		30.88
	100	5.17		31.79	4.51		43.63
	200	4.00		47.23	4.03		49.63
Mo	0	7.68	5.15 b	-	8.10	4.39 b	-
	50	5.17		32.68	4.32		46.67
	100	4.30		44.01	2.76		65.93
	200	3.43		55.34	2.37		70.74
Rz	0	8.12	4.36 cd	-	8.00	3.70 c	-
	50	3.50		56.89	3.03		62.13
	100	3.40		58.13	2.27		71.63
	200	2.40		70.44	1.50		81.25
V200	0	7.88	3.90 e	-	7.87	3.39 cd	-
	50	3.54		55.08	2.70		65.69
	100	2.40		69.54	1.70		78.40
	200	1.77		77.53	1.30		83.48
SA+Mo	0	7.94	4.62 c	-	7.94	4.25 b	-
	50	3.75		52.77	4.03		49.24
	100	3.50		55.92	2.99		62.34
	200	3.30		58.44	2.03		74.43
SA+Rz	0	8.00	4.12 de	-	8.00	3.52 cd	-
	50	3.33		58.38	2.80		65.00
	100	2.83		64.63	2.23		72.13
	200	2.30		71.25	1.03		87.13
SA+V200	0	7.41	3.52 f	-	7.80	3.26 d	-
	50	3.26		56.01	2.47		68.33
	100	2.27		69.37	1.73		77.82
	200	1.13		84.75	1.03		86.79
L.S.D. 0.05	Conc. = 0.217 Treat. = 0.288 C×T = 0.575				Conc. = 0.236 Treat. = 0.312 C×T = 0.625		

SA:Salicylic acid, Mo: Moncut WP 30%, Rz: Rizolex WP 50%, V200: Vitavax 200 75%

* Average diameter was calculated for each tested concentration based on three replicates

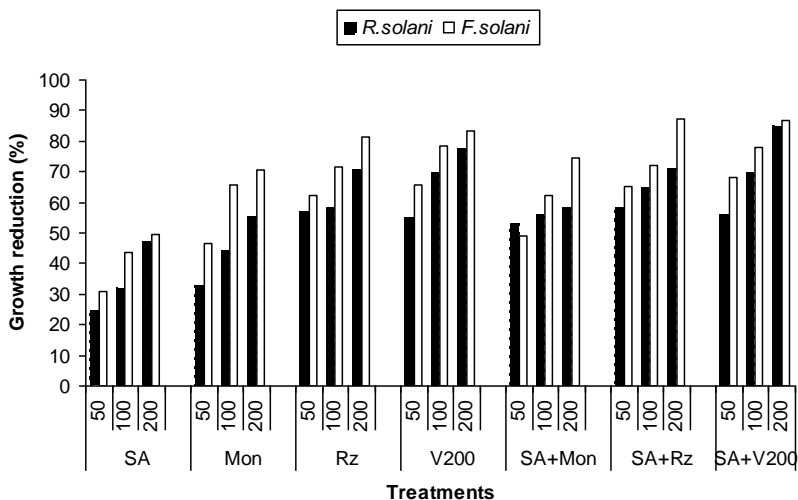


Fig. (1): Effect of salicylic acid (SA) and fungicides individually or in combinations on reduction percentages of mycelial growth of *R. solani* and *F. solani* under laboratory conditions.

2- Effect of SA and fungicides on disease incidence in field trials:

Table (3) shows that soaking squash and cantaloupe seeds in SA solution combined with fungicides being more effective than each of them alone. In the first season 2012, squash seed soaking in SA+Rz and SA+V200 was more effective to reduced total damping-off with the averages of 19.17 and 20.83% , respectively, and increased survival plants with the averages of 80.83 and 79.17, respectively. It is well established that SA naturally occurs in plants in very low amounts. It has been identified as an important signaling element involved in establishing the local and systemic disease resistance response of plants after pathogen attack (Alvarez, 2000). Salicylic acid levels often increase and induce the expression of pathogenesis related proteins and initials the development of systemic acquired resistance and hypersensitive response (Gruner *et al.*, 2003 and Kachroo *et al.*, 2005). Moreover, these results are in agreement with those reported by Nandakumar *et al.* (2001) who reported that the combined application methods of increased the durability of ISR in rice plants. The results of the present study confirmed the systemic nature of resistance against the pathogens which is induced through exogenous application of SA. However, Soad (2010) reported that all the combination treatments as Equation pro, Mancozeb plus ascorbic acid and salicylic acid were more effective in control of the early blight disease than the fungicide alone. Amel *et al.* (2010) found that, disease incidence (DI%) was highly significantly reduced up to 0% with dipping of tomato root seedlings in *Trichoderma harzianum* combination with SA and thiophanate methyl fungicide at half recommended dose compared with the control infected with *F. oxysporum*. The present study (Table, 3) shows that soaking cantaloupe seeds in SA+V200, SA+Rz and V200

decreased the total damping-off with the averages of 22.23, 25.56 and 27.78%, respectively that being expressed by the survived plants. In contrast single treatment with SA and Mo recorded the lowest effect on the total damping-off with the averages of 41.11 and 33.33 %, respectively. These results are in agreement with the finding of Harun and Craig (2007) who showed that in the artificially infestation with *Thielaviopsis basicola*, causing black root rot of cotton, decreased disease severity by the combination of fungicide (systhane) and Bion than using chemical alone, but these differences were not significant than the fungicide alone.

Table (3): Effect of seed soaking in SA and fungicides individually and/or in combinations on controlling damping-off, root rot of squash and cantaloupe plants under field conditions during growing first season 2012.

Tested plants	Disease incidence (%)	Treatment							
		Check	SA	Mo	Rz	V200	SA+Mo	SA+Rz	SA+V200
Squash Askandarany	Pre-emergence	39.17	24.17	20.00	18.33	16.67	15.83	12.50	12.50
	Post-emergence	16.67	13.33	9.17	10.00	9.17	11.67	6.67	8.33
	Total damping-off	55.84	37.50	29.17	28.33	25.84	27.50	19.17	20.83
	Efficiency %	-----	32.84	47.76	49.27	53.72	50.75	65.67	62.70
	Survival	44.16	62.50	70.83	71.67	74.16	72.50	80.83	79.17
L.S.D 0.05	Pre = 5.86 Post = 4.92 Survival = 5.52								
Cantaloupe Mera	Pre-emergence	36.00	27.78	22.22	21.11	18.89	21.11	16.67	15.56
	Post-emergence	16.67	13.33	11.11	10.00	8.89	10.00	8.89	6.67
	Total damping-off	52.67	41.11	33.33	31.11	27.78	31.11	25.56	22.23
	Efficiency %	-----	21.95	36.72	40.93	47.26	40.93	51.47	57.79
	Survival	47.33	58.89	66.67	68.89	72.22	68.89	74.44	77.77
L.S.D 0.05	Pre = 4.08 Post = 3.53 Survival = 5.26								

SA: Salicylic acid, Mo: Moncut WP 30%, Rz: Rizolex WP 50%, V200: Vitavax 200 75%

Table (4) showed similar trend in the second season 2013. Seed soaking in SA+V200 followed by SA+Rz were the best for reducing total damping-off with the averages of 15.83 and 19.16, respectively. Also treatment V200 decreased the total damping-off with the average of 22.50% for squash plants and revealed on the survival plants of the two previous treatments recorded 84.17, 80.83 and 77.50%, respectively. These results are in accordance with Abou El-Souod *et al.* (2005) who recorded that vitavax was the most effective against *R. solani*, *M. phaseolina* and *P. debaryanum* which causes damping-off and root rot on *Phaseolus vulgiris* seedling. These results confirm with those mentioned by Agostini *et al.* (2003), who reported that induced resistance products were effective for disease control but they may be more useful in an integrated program with standard fungicides. In several management strategies, the addition of resistance inducing chemicals in combination with biocontrol agents has significantly enhanced disease control. The result here in is in harmony with the opinion of (Shyamala and Sivakumar, 2012 and Abdel-Monaim, 2013). Moreover, cantaloupe seed

soaking in SA+V200, V200 and SA+Rz recorded the best effect in reducing total damping-off with the average of 17.77, 21.11 and 21.12 %, respectively and these results reflected on increased efficiency, which was recorded with the average of 62.89, 55.92 and 55.90% respectively.

Table (4): Effect of seed soaking in SA and fungicides individually and/or in combinations on controlling damping-off, root rot of squash and cantaloupe plants under field conditions during growing second season 2013.

Tested plants	Disease incidence (%)	Treatment							
		Check	SA	Mo	Rz	V200	SA+Mo	SA+Rz	SA+V200
Squash Askandarany	Pre-emergence	37.50	22.50	18.33	15.00	14.17	16.67	10.83	10.00
	Post-emergence	15.83	11.67	10.83	7.50	8.33	10.83	8.33	5.83
	Total damping-off	53.33	34.17	29.16	22.50	22.50	27.50	19.16	15.83
	Efficiency %	-----	35.93	45.32	57.81	57.81	48.43	64.07	70.32
	Survival	46.67	65.83	70.84	77.50	77.50	72.50	80.84	84.17
L.S.D 0.05	Pre = 3.85 Post = 4.42 Survival = 7.75								
Cantaloupe Mera	Pre-emergence	32.33	26.67	21.11	17.78	14.44	20.00	15.56	13.33
	Post-emergence	15.56	12.22	11.11	7.78	6.67	8.89	5.56	4.44
	Total damping-off	47.89	38.89	32.22	25.56	21.11	28.89	21.12	17.77
	Efficiency %	-----	18.79	32.72	46.63	55.92	39.67	55.90	62.89
	Survival	52.11	61.11	67.78	74.44	78.89	71.11	78.88	82.23
L.S.D 0.05	Pre = 2.82 Post = 3.12 Survival = 3.78								

SA: Salicylic acid, Mo: Moncut WP 30%, Rz: Rizolex WP 50%, V200: Vitavax 200 75%

2. a- Effect of SA and fungicides on growth parameters in field trials:

Table (5) shows the results of squash and cantaloupe seed soaking in SA solution combined with any one of the fungicides significantly improved squash and cantaloupe growth more than used each of the individually except in case of SA+Mo treatment. Also seed soaking in SA individually activated plant growth in both seasons 2012 and 2013. The highest fresh weights in the 2012 season were obtained from the combinations of SA+V200 and SA+Rz with the averages of 32.45 and 28.13 gm/ squash plant and 25.64 and 23. 73 gm/ cantaloupe plant, respectively. In case of dry weight the above two treatments had an averages of 4.09 and 3.07 gm/ squash plant and 3.48 and 3.03 gm/ cantaloupe plant, respectively. In the second season, the results were approximately the same as in the first season especially with SA+V200, SA+Rz and SA. These increases may be attributed to SA which plays an important role on various physiological processes in plants such as ion uptake, cell elongation, cell division, enzymatic activation and protein synthesis. In this respect, Sakabutdinova *et al.* (2003) reported that treatment of wheat plants with SA 0.05 M increased the level of cell division within the apical meristem of seedling roots which caused an increase in plant growth. In this regard, the exogenous application of SA had effected positively the growth and flowering of marigold plants (*Calendula officinalis* L.). Plants treated with 1 mM of SA showed the highest

leaf number, leaf dry mass and leaf area with the averages of 79.16, 69.24 and 59.95%, respectively (Pacheco *et al.*, 2013).

Table (5): Effect of seed soaking in SA and fungicides individually and/or combinations on fresh, dry weight and shoot length of squash and cantaloupe plants under field conditions during two successive seasons (2012 and 2013).

Treatment	Squash (Askandarany)						Cantaloupe (Mera)						
	2012			2013			2012			2013			
	Fresh weight (gm) plant	Dry weight plant (gm)	Shoot length (cm)	Fresh weight plant (gm)	Dry weight (gm)	Shoot length (cm)	Fresh weight plant (gm)	Dry weight (gm)	Shoot length (cm)	Fresh weight plant (gm)	Dry weight plant (gm)	Shoot length (cm)	
SA	27.00	3.01	33.25	30.82	3.65	39.00	19.51	3.04	30.75	23.75	3.31	32.75	
Mo	18.81	2.02	22.00	19.63	2.29	24.00	14.19	2.07	24.00	16.19	2.60	28.63	
Rz	21.06	2.59	26.00	27.22	2.92	30.63	17.83	2.72	28.25	20.68	3.21	32.33	
V200	22.53	2.81	31.00	30.83	3.54	37.00	16.36	2.77	29.00	18.61	3.08	31.38	
SA+Mo	20.64	2.15	26.25	22.21	2.34	27.00	15.91	2.63	25.25	22.98	2.79	27.00	
SA+Rz	28.13	3.07	36.63	32.49	4.15	40.00	23.73	3.03	31.00	25.85	3.47	34.33	
SA+V200	32.45	4.09	40.25	35.34	4.91	42.63	25.64	3.48	35.75	28.17	3.64	37.00	
Check	13.04	1.59	20.75	15.31	1.72	21.75	10.34	1.40	20.00	11.31	1.47	24.00	
L.S.D	0.05	4.14	0.524	3.00	3.18	0.349	2.820	1.43	0.347	1.698	1.42	0.448	1.85

SA: Salicylic acid, Mo: Moncut WP 30%, Rz: Rizolex WP 50%, V200: Vitavax 200 75%

2.b-Changes in polyphenoloxidase (PPO) and peroxidase (PO) activities:

Data in Table (6) show that all treatments either single or in combinations with SA have increased the activity of both PPO and PO over the check. Peroxidase activity was markedly increased after treatments with of SA+V200, SA+RZ and V200 followed by SA for squash plants with an average of 0.895, 0.795, 0.720 and 0.699 activity/min, respectively. Meanwhile, combinations of SA with V200 and either Rz or V200 individually recorded the highest increase in PPO enzyme activity with averages of 0.579, 0.545 and 0.507 activity/min respectively. In case of cantaloupe SA+V200 and SA+Rz had the highest values for peroxidase activity followed by SA with averages of 0.998, 0.861 and 0.670, respectively. The same trend being obvious in case of polyphenoloxidase where SA+V200 and SA+Rz had the highest values but Rz followed them with averages of 0.575, 0.560 and 0.533, respectively. The least activity however, was recorded for Mo treatment on both PPO and PO for squash and cantaloupe plants. The activity of both PPO and PO are naturally present in plant tissues. Polyphenoloxidase converts polyphenolic compounds to the oxidized form quinines which are thought to be inhibitory to pathogens invasion. Also it was found that the highest peroxidase activity was usually more active in resistant cultivars (Pradeep and Jambhale, 2002). Moreover, Amel *et al.* (2010) reported that combination of salicylic acid with *Trichoderma* as seedling

tomato root dipping (SA+T2) and Fungicide (thiophanate methyl) with its half recommended dose (SA+0.5F) increased PPO and POD activities. Similar results also gave evidence to the role of SA on tomato plants treated with different concentrations of SA had accesses in the activities of both peroxidase and polyphenoloxidase. The combined application of SA and *T. harzianum* enhanced activities of both enzymes in Fusarium infected tomato plants (Suprakash Ojha, 2012).

Table (6): Effect of seed soaking in SA and fungicides individually and/or in combinations on activities of peroxidase and polyphenoloxidase as optical density/minute/gm fresh weight in leaf extracts of squash and cantaloupe plants.

Tested plants	Enzyme activity	Treatment							
		Check	SA	Mo	Rz	V200	SA+Mo	SA+Rz	SA+V200
Squash Askandarany	Peroxidase* (PO)	0.345	0.699	0.558	0.645	0.720	0.635	0.795	0.895
	Polyphenol-oxidase** (PPO)	0.134	0.461	0.444	0.545	0.507	0.474	0.495	0.579
L.S.D 0.05		Po = 0.107 PPo = 0.091							
Cantaloupe Mera	Peroxidase* (PO)	0.383	0.670	0.518	0.617	0.619	0.653	0.861	0.998
	Polyphenol-oxidase** (PPO)	0.159	0.515	0.419	0.533	0.529	0.499	0.560	0.575
L.S.D 0.05		Po = 0.125 PPo = 0.093							

SA: Salicylic acid, Mo: Moncut WP 30%, Rz: Rizolex WP 50%, V200: Vitavax 200 75%

*Peroxidase activity expressed as change in absorbance at 470 nm/g fresh weight/15min.

**Poly phenol oxidase activity expressed as change in absorbance at 575 nm/g fresh weight/15min.

From the afore-mentioned data an opposite correlation between the PPO or PO enzyme activities and total disease incidence was recognized. Table (7) shows the values of correlation coefficient (r) confirmed the results obtained previously, where a negative correlation were noticed between the disease incidence and PPO and PO enzyme activities. This correlation reached 59.5 and 82.9% for squash PPO and PO enzyme activities and 71.3 and 74.7% for cantaloupe. These results are in harmony with the results mentioned by Pena and Ankue (1992) and Nawar and Kuti (2003) who indicated a positive relation between resistance and peroxidase activity. Peroxidase also produces free radicals and hydrogen peroxidase which are toxic to many microorganisms. Also, Morsy (2005) reported that the susceptibility of the four lentil cvs. was positively correlated with the activity of peroxidase, polyphenoloxidase and b 1-3 glucanase, where cv. Sinai 1 (more resistant to *R. solani* and *F. oxysporum* tested fungi) recorded the highest enzymes activity whereas cv. Giza 9 (highly susceptible) recorded the lowest enzymes activity. It could be generally concluded that salicylic acid mixing

with fungicides may be increased the fungicidal potentials against damping-off and root rot diseases along with enhancement of enzymes related resistance in squash and cantaloupe.

Table (7): Correlation coefficient between disease incidence % and each of peroxidase and polyphenol oxidase activities.

Variable	Linear correlation coefficient (r)	
	Squash	Cantaloupe
Peroxidase activity (AA ₄₇₀ Min/mg port.)	-0.595	-0.713
Polyphenol oxidase activity (Unit x100 Port.)	-0.829	-0.747

2. c- Micronutrients in squash and cantaloupe plants:

From Table (8), the application of salicylic acid either single or in combinations with fungicides caused a significant increase in the content of Zn, Fe and Mn, in squash and cantaloupe plants. The maximum values of Fe and Mn micronutrients contents were obtained with SA+V200, SA+Rz, SA and SA+Mo treatments in both squash and cantaloupe, contrary to SA treatment that decreased Zn content. These results are consistent with those reported by Fahad and Bano (2012) who observed that microelements of Co⁺³, Mn⁺², Cu⁺³ and Fe⁺² increased in roots of SA treated maize plants under salinity stress which may help in stimulating metabolic reaction of plants. These micronutrients also assist in enhancing enzymatic activity, Munns, (2005). On the other hand, Na⁺, Ni⁺³, Pb⁺⁴, and Zn⁺² content decreased in roots of SA treated in maize plants. Fatma (2006) found that SA applied at 10⁻⁴ and 10⁻⁵ M show increased the content of N, P, K, Fe, Mn and Cu in *Ocimum basilicum* and *Majorana hortensis*, except P (in case of basil) and Zn, Na and Cu content (in case of marjoam). Similarly foliar application of SA caused an increase in mean Mn uptake of chick pea shoot by about 7%. Meanwhile the effect of SA on mean Zn uptake in chickpea shoot was negligible. (Ghasemi-Fasaei, 2013). In retrospect, the preplanting soaking in SA may be recommended for extensive studies with other crops.

Table (8):Effect of seed soaking in SA and fungicides individually and/or in combinations on micro-elements content (ppm) of squash and cantaloupe plants.

Plant	Squash			Cantaloupe		
Micro-elements Treatment	Zn	Fe	Mn	Zn	Fe	Mn
SA	48.40	0.326	40.82	46.53	0.390	39.80
Mo	62.90	0.285	32.39	63.10	0.267	32.88
Rz	63.90	0.295	36.64	61.00	0.326	36.41
V200	60.40	0.298	36.74	63.93	0.317	34.40
SA+Mo	50.90	0.320	37.87	55.10	0.335	38.58
SA+Rz	50.65	0.402	37.60	53.10	0.339	45.87
SA+V200	50.63	0.438	54.16	52.10	0.462	54.94
Check	54.47	0.310	35.99	56.20	0.317	35.85
L.S.D 0.05	0.500	0.049	0.521	0.739	0.065	0.568

SA: Salicylic acid, Mo: Moncut WP 30%, Rz: Rizolex WP 50%, V200: Vitavax 200 75%

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إستخدام حمض الساليسليك و بعض المبيدات الفطرية كعامله بذرة لمكافحة أمراض سقوط البادرات و عفن الجذور لنباتات الكوسة والكتالوب تحت الظروف الحقلية

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تم إختبار حمض الساليسليك سواء كعامله منفردة أو مخلوطة مع ثلاثة مبيدات تجارية (مون كات - ريزولكس - فيتافاكس 200) ودراسة تأثيرها على مكافحة مرض موت البادرات وأعفان الجذور لنباتات الكوسة صنف إسكندراني والكتالوب صنف ميرا وذلك تحت ظروف الحقل خلال الموسمين الزراعيين 2012 و 2013 .

معمليا وجد أن إستخدام حمض الساليسليك سواء كعامله منفردة أو مخلوطة مع المبيدات (مون كات - ريزولكس - فيتافاكس 200) بتركيزات تتراوح من 50 إلى 200 ج ف م يقلل من معدل النمو المسليومي لفطري ريزوكتونيا سولاني وفيزاريوم سولاني مقارنة بالكنترول. وكذلك يزيد التثبيط المسليومي بزيادة التركيزات المستخدمة لكلا الفطرين في كل المعاملات. كما أدى خلط حمض الساليسليك مع الفيتافاكس 200 و ريزولكس SA+V200 و SA+Rz إلى أعلى نسبة نقص لنمو فطري ريزوكتونيا سولاني وفيزاريوم سولاني مقارنة بكل المعاملات وذلك عند التركيز 200 ج ف م بنسبة 71,25 , 86,79 , 84,75 و 87,13% على الترتيب.

في تجربة الحقل تم نفع بذور الكوسة والكتالوب في حمض الساليسليك و المبيدات الفطرية المستخدمة بالتركيزات الموصى بها منفردة أو مختلطة وتم تقييم تأثيرها على نسبة الإصابة بموت البادرات وأعفان الجذور ونسبة النباتات المتبقية بعد الإصابة. وجد أن كل المعاملات قللت بصورة معنوية من نسبة النباتات المصابة وبالتالي زادت من نسبة النباتات المتبقية بعد الإصابة كذلك أدت إلى زيادة في الوزن الرطب والجاف وطول النباتات. وجد أن نفع البذور في SA+V200 أو SA+Rz أعطى أعلى نسبة في تقليل نسبة النباتات المصابة بموت البادرات وأعفان الجذور بنسبة 70,32 و 64,07 لنبات الكوسة وبنسبة 62,89 و 55,90 لنبات الكتالوب على الترتيب في الموسم التالي 2013. أظهرت نتائج الدراسة بإستخدام حمض الساليسليك سواء كعاملات منفردة أو مخلوطة مع المبيدات كعامله بذرة إلى تحفيز بعض آليات الدفاع الكيمو حيوية مثل إنزيم البيروكسيداز والبولي فينول أوكسيديز وكانت معاملة البذرة مع SA+V200 أو SA+Rz أفضل المعاملات في هذا الصدد. وجد أن نفع البذور مع حمض الساليسليك والمبيدات منفردة أو ومخاليطها يزيد من محتوى النبات من عنصرى الحديد والمنجنيز وعلى النقيض من ذلك يقل المحتوى من عنصر الزنك.

بشكل عام , أغلب المعاملات المستخدمة وخاصة التي تم خلط حمض الساليسليك مع المبيدات الفطرية أدت إلى تقليل نسبة الإصابة مقارنة بتطبيق المعاملات منفردة وزيادة في نشاط إنزيمى الأوكسيديز والبولي فينول أوكسيديز المرتبطة بدفاع نباتات الكوسة والكتالوب ضد الإصابة بالمرض.



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