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INDUCTION OF RESISTANCE IN CUCUMBER PLANTS AGAINST ROOT-ROT AND DAMPING OFF DISEASES USING DIFFERENT CHEMICALS

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ABSTRACT: Diseases are one of the most important factors affecting cucumber production especially in greenhouses. Roots of cucumber plants were subjected to fungal diseases specially damping- off and root-rot diseases. This work was directed to study the inducing of resistance in cucumber plants against these fungal diseases using three different chemicals namely: ethephon, acetylsalicylic acid, and phosphate salts.

Acetyl Salicylic Acid (ASA) was the most effective inducer causing the highest percentages of healthy survival plant followed by ethephon and potassium phosphate mono-basic (K_2HPO_4). Seed soaking technique for all tested chemicals, was more effective than foliar application. Untreated plants recorded the least percentages of healthy survival plants.

Activity some enzymes such as peroxidase (PO) and polyphenol oxidase (PPO), also, free and total phenol levels, which played an important role in plant resistance, were also determined.

All treatments stimulated po activity, the highest activity was recorded for ASA, followed by K_2HPO_4 then ethephon.

The most effective treatment in increasing ppo activity was, ASA followed by, ethephon and K_2HPO_4, respectively.

The values of free and total phenol were the highest in ASA treatment followed by K_2HPO_4 and ethephon treatments, respectively.

Key words: Cucumber plant, chemical induced resistance, plant extract induced resistance, biotic agent induced resistance.

INTRODUCTION

Cucumber (*Cucumis stativus L.*) is one of the most important vegetable crops in the Arab Republic of Egypt and other parts of the world. The economic importance of this crop appears in both local consumption and exportation purposes.

Several fungal diseases attacked cucumber plants during all growing stages causing a considerable reduction in either number of cucumber plant/ area or yield / feddan. Both damping-off [*Fusarium solani* (Mart.) Sacc., *Rhizoctonia salani* (Kuhn) and *Pythium* spp.] and downy mildew [*Pseud-peronospora cubensis* (Berk and Curt.) Rostow], powdery mildew [*Sphearotheca fuliginea* (Schecht) pallacci], are the most important diseases in cucumber plants specially under greenhouse conditions. Non-chemical control offers a logical alternative to synthetic fungicides for the control of soilborne and folliage diseases are needed.

The side effects of fungicides, however, necessitate the search for new and safe methods, including disease resistance induction in hosts. Many reports have been published on the use of chemical inducers for inducing resistance in host plants against soil born fungi (Orion and Hoestra, 1974; Bovio *et al.*, 1987; Okuno *et al.*, 1991 and Gottstein and Kuc, 1989).

In this research induced resistance by seed soaking and foliar treatments with different chemical inducers namely ethephon, acetyl salicylic acid and dipotassium hydrogen phosphate K_2HPO_4 were investigated.

Some biochemical aspects such as peroxidase activity, polyphenol oxidase activity, phenolic compounds (free and total phenols) were also investigated as influenced by the tested inducers.

MATERIALS AND METHODS

Infestation Soil

The previously isolated pathogenic causal organisms of cucumber root-rot disease (*Pythium* sp; *Fusarium solani* Mart (Sacc.) and *Rhizoctonia solani Kühn)* were used. Plastic pots (20 cm in diameter) were filled with autoclaved soil and used in three replicates for each particular treatment. The pots were divided into eight groups as follows:

Group (1) was infested with *Pythium* sp.

Group (2) was infested with *Fusarium solani.*

Group (3) was infested with *Rhizoctonia solani.*

Group (4) was infested with *Pythium* sp. + *F. solani*

Group (5) was infested with *Pythium* sp. + *R. solani*

Group (6) was infested with *F. solani*+ *R.solani.*

Group (7) was infested with *Pythium* sp. + *F. solani*+ *R.solani.*

Group (8) was un-infested soil to serve as a control.

Ten disinfected seeds on cucumber (cucumis stativus L.) var. Bablon were cultivated in each pot of the eight groups. Inoculum was prepared by inoculation each of the tested fungi in 500 ml conical flask containing 200 ml autoclaved potato borth liquid medium and incubated at 20℃ for 10-15 days for *Fusarium solani* and *Rhizoctonia solani.* The fungal mat of each fungus was blended in the blender to obtain small fragments of the fungal mycelium into small parts. The fungal mycelium fragments were adjusted to be 10^5 cfu/ml (colony forming unit) using sterilized water and heamocytometer. Pots were singly infested by the fungal mycelium at the rate of 5% (v/w). In case of infestation with an equal amount of three causal organisms to adjust the combined inoculum to be 5% then adding it to the pots. The percentage of pre-emergence damping- off; post-emergence damping- off; root- rot and healthy plants were recorded after 10, 20, 30 and 45 days from sowing, respectively.

Tested Inducers

The following chemicals were tested for inducing resistance in cucumber plants c.v. Bablon which considered as susceptible host to soil born pathogens.

1. **Ethephon:** Was tested as seed soaking in aqueous solution of *400, 600 and 800 ppm.* Meanwhile, *300, 400 and 500 ppm* were tested as foliar application at the 1st leaf growth stage.
2. **Acetylsalicylic acid (ASA):** Was tested as seed soaking in aqueous solutions of 5, 10 and 15 mM. Meanwhile, 2.5, 5.0 and 7.5 mM were tested as a foliar application.
3. **Phosphate salts:** K_2HPO_4 was tested as foliar and seed soaking applications at the concentrations of 50, 100 and 150 mM.

Seed Treatment with the Tested Inducrers

Cucumber seeds (Bablon c.v.) were soked in solution of each chemical for 24hrs and placed between two filter paper for another 24hrs, then sown in the pots. Seeds were soaked in tap water for the same period and used as a control (Aly *et al., 1988).*

Effect of the Tested Inducers on Some Biochemical Aspects in Diseased and Healthy Cucumber Plants

The effect of ethephon (400 ppm), Acetyl salicylic ocid (10mM), and K_2HPO_4 (100 mM) which proved itself to be more effective on oxidative enzymes activity (peroxidase and polyphenol oxidase) and phenolic compounds (free, and total phenols) was pursued.

Foliar application

Foliar application with the tested selected inducer was carried out at the 1st leaf growth stage, while a set of plants were sprayed with tap water only and used a control.

Plant samples of each experiment (seed and foliar application) were taken before challenge and 5, 10 and 20 days after challenge.

Determination of oxidative enzymes activities

One gm. of leaf tissue from healthy and infected plants was used to determine peroxidase and polyphenol oxidase activites. Each sample was cut into small pieces and grinded in a porcelain mortar by the pestle in the presence of purified sand and 2ml.of buffer phosphate (PH 7.0) as described by Goldschmidt *et al.* (1968).

The obtained extract was quantitatively completed to 10 ml. then centrifuged at 5000 rpm for 15 minutes according to the methods desribed by Malik and Singh (1980). The resulted supernatant was used to determine peroxidase and polyphenol oxidase activities.

Peroxidase activity

Peroxidase activity was evaluated according to the method described by Allam and Holis (1972) as follows: the reaction mixture contained 0.5 ml. phosphate buffer (PH 7.0); 0.2 ml. peroxidase enzyme (sample extract), 0.3 ml. of 0.05 m pyrogallol, 0.1 ml. of 1.0 % (v/v) H_2O_2 and distilled water to obtain final volume of 3.0 ml. The reaction mixture incubated at 30°C for 5 minutes, then the reaction

were inactivated by adding 0.5 ml of 5.0% (v/v) H_2SO_4 (Kar and Mishra, 1976) and the absorbance was recorded at wave length of 425 nm. One unit of provides activity was expressed as the change in absorbance at 425 nm/minute/ 1.0 gm fresh weight.

Polyphenol oxidase activity

The polyphenol oxidase activity was quantitatively determined in sample according to the method decreased by Matta and Dimonal (1963). The reaction mixture contained 0.2 ml. of polyphenol enzyme (sample extract), 1.0 ml of phosphate buffere (PH 7.0); 1.0 ml of 10^{-3} M catechol and completed with distilled water up to 6.0 ml. The reaction mixture was incubated for 30 minute at 30°C. The absorbance of the puspuragallin formed was measured at 420 nm. One unite of polyphenol oxidase was expressed at the change in absorbance at 420 nm/30 minutes/1.0 gm fresh weight.

Determination of phenolic compounds

Free and total phenols were determined using the colorimetric methods as described by Snell and Snell (1953).

Preparation of folin-Denis reagent

The phenol reagent was prepared by adding 100 gm. sodium tungestate and 25gm sodium molybdate to 700 ml. of distilled water then 50 ml. phosphoric acid 85% and 100 ml HCL were attached to reflex condenser. The mixture was left to boil gently for 10hr in a water bath then left to cool. Twenty five ml. lithium sulphate and 50 ml. distilled water were added few drops of bromine were also added and the mixture was heated again without reflex condenser. Finally, the mixture was completed to one liter with distilled water.

Extraction

Extraction was conducted using 70% Ethanol as an extraction on boiling water bath for 12-15 hrs. Five gms. of fresh cucumber roots were taken from each sample separately and put in beaker covered with conical flasks containing cool water, which changed occasionally to minimized evaporation. Small amount (2 drops) of ethanol were added to the samples at different periods to substitute evaporated ethanol. The combined ethanolic extract and the extracted samples were filtered using filter paper No.1. Filtrates

were evaporated to near dryness in a mild water bath at 60°C. The dried residues were redissolved in 10 ml. of 50% isopropanol and quantitively transferred to glass vials and kept at 1°C.

Determination of free phonols

One ml. of sample extract was put in a sterilized test tube, 1 ml. distilled water, 1 ml. folin-Denis reagent and 3 ml. Na_2 CO_3 20% (Weight), were added. The colour density was read using (spectronic-20) spectrophotometer at 520 nm.

Determination of total phenols

One ml of sample extract was treated with 0.25 ml. HCL and boiled in water bath for 10 minutes then cooled. One ml. of folin-Denis and 6 ml. Na_2 CO_3 were added. The mixture was completed to 10 ml. with distilled water and the colour density was read at 520 nm using the same apparatus.

Standard curve

One gm. of catechol was dissolved in distilled water and the volume was made up to one liter.

Different volumes from catechol solution were taken and raised to 100 ml. with distilled water in volumetric flasks.

One ml. of the different catechol concentrations were taken separately in test tubes, 1 ml distilled water, 1 ml folin-Denis regnant and 3 ml Na_2 CO_3 were added. The mixture was completed to 10 ml with distilled water, and then treated as shown in the determination of free phenols. Finally, the relationship between the reading at 520 nm and the known concentration of catechol were determined.

Statistical Analysis Procedures

Statistical analysis of all the previously designed experiments has been carried out according to the procedures "ANOVA" reported by Sndecor and Cochran (1980). Treatment means were compared by the least significant difference test "L.S.D" at 5% level of probability.

RESULTS AND DISCUSSION

Inducted Resistance by Ethephon

Results in Table 1 indicated that all tested concentration induced systemic aquired resistance (SAR) against soil born fungi infesting cucumber. The most effective concentration was ethephon 600 ppm as seed soaking application.

Ethephon reduced the disease incidence by 53.3; 67.1 and 63.3% for the three tested concentration respectively as compared with untreated control (24.8%). Also, these applications decreased the

Table 1. Damping-off incidence in cucumber plants descended from seeds soaked in different concentrations of ethephon

Concentrations ppm	Artificial infested soil		Pre-emergence damping-off %	Post emergence damping-off %	Root rot %	Healthy/ survival %
0.0	*Pythium sp. (P.s)*		40	23.3	3.3	26.7
	Fusarium solani (F.s)		40	26.7	3.3	30.0
	Rhizoctonia solani (R.s)		46.7	20.0	3.3	30.0
	P.s + F.s		53.3	20.0	6.6	20.0
	P.s + R.s		53.3	20.0	0.0	20.0
	F.s + R.s		56.7	16.7	6.6	20.0
	P.s + F.s + R.s		46.7	20.0	6.6	26.7
average			48.1	20.9	12.7	24.8%
400	*Pythium sp. (P.s)*		20.0	13.3	6.6	60.0
	Fusarium solani (F.s)		20.0	13.3	3.3	63.3
	Rhizoctonia solani (R.s)		23.3	13.3	3.3	60.0
	P.s + F.s		26.7	13.3	10.0	50.0
	P.s + R.s		30.0	13.3	10.0	46.7
	F.s + R.s		33.3	13.3	10.0	43.3
	P.s + F.s + R.s		30.0	13.3	10.0	50.0
average			29.0	13.3	7.1	53.3%
600	*Pythium sp. (P.s)*		13.3	6.6	6.6	73.3
	Fusarium solani (F.s)		13.3	10.0	6.6	70.0
	Rhizoctonia solani (R.s)		10.0	13.3	6.6	70.0
	P.s + F.s		13.3	13.3	10.0	63.3
	P.s + R.s		16.7	13.3	6.6	63.3
	F.s + R.s		13.3	13.3	10.0	63.3
	P.s + F.s + R.s		16.7	10.0	6.6	66.7
average			13.8	11.4	7.8	67.1%
800	*Pythium sp. (P.s)*		13.3	6.6	10.0	70.0
	Fusarium solani (F.s)		13.3	10.0	6.6	70.0
	Rhizoctonia solani (R.s)		10.0	13.3	10.0	66.7
	P.s + F.s		13.3	10.0	13.3	63.3
	P.s + R.s		16.7	10.0	10.0	60.0
	F.s + R.s		20.0	10.0	10.0	60.0
	P.s + F.s + R.s		20.0	10.0	16.7	53.3
average			15.2	9.9	10.9	63.3%
L. S. D. (at 5%)						
The Concentrations (ppm)		A	**0.388	**0.364	**0.304	**0.304
infested Soils		B	**0.514	N.S	N.S	N.S
		AXB	N.S.	N.S	N.S	N.S

percentage of pre-emergence damping-off (27.0, 13.3 and 15.2%) for ethephone seed soaking 400, 600 and 800 ppm, respectively, compared with untreated control (48.1%). While the treatment of the tested concentrations of ethephon as seed soaking decreased the percentage of post-emergence damping-off to (13.3, 11.4 and 9.9%) compared with the untreaed control 20.9%. Concerning root-rot disease incidence no significant differences were observed between the treatments. Meanwhile ethephon foliar treatments with 300, 400 and 500 ppm were less effective (Table 2). The healthy survival plants were 39.5, 47.9 and 50.9%, respectively, compared with untreated control (24.8%). While the reduction of the percentage of pre-and post-emergence damping-off were as follows: 31.4, 24.3, 25.7% and 19.1, 15.2, 12.8%, respectively, compared with the untreated control 48.1 and 20.9%

Many reports have been published on the use of ethylene releasing compound ethephon for induction of SAR in plant (Orion and Hoestra, 1974 and Benhamou *et al.*, 1987).

Ethephon treatment revealed efficiency in eliciting SAR in cucumber against downy mildew (Okuno *et al.*, 1991). The effect of ethephon in reducing disease incidence due to its effect in synthesis of PR- proteins (Okuno *et al.*, 1991), lignification and papilla formation (Matsumoto and Asada, 1990), activity of B-1-3 glucanase and chitinase (Bloor *et al.*, 1983).

Induced resistance by acetyl salicylic acid

Three concentrations of acetyl salicylic acid (ASA) i.e, 5.0, 10.0 and 15.0 mM were tested as seed soaking while three concentrations i.e 2.5, 5.0 and 7.5mM were used as foliar treatment. Results in Table 3, indicate that soaking the seeds in 10 and 15 Mm ASA reduced the percentage of infection to 71.4 and 68.6% as compared with 28.7% for the unsoaked (control). ASA 5.0 mM was the lowest effective which reduced the percentage of infection to 50.0%. The percentage of pre-and post-emergence damping off were also reduced when the cucumber seeds soaked in 5, 10 and 15 Mm of ASA (21.9, 12.4, 12.9 and 11.4, 9.5, 9.9%, respectively, as compared with untreated control (46.2 and 20.0%). It is also clear that increasing the concentration of ASA led to decreasing the diseases incidence and increasing the percentage

Table 2. Damping-off incidence in cucumber plants foliarly treated with different concentrations of ethephon

Concentrations ppm	Artificial infested soil	Pre-emergence damping-off %	Post-emergence damping-off %	Root rot %	Healthy/ survival %
0.0	*Pythium sp. (P.s)*	40.0	23.3	3.3	26.7
	Fusarium solani (F.s)	40.0	26.7	3.3	30.0
	Rhizoctonia solani (R.s)	46.7	20.0	3.3	30.0
	P.s + F.s	53.3	20.0	6.6	20.0
	P.s + R.s	53.3	20.0	0.0	20.0
	F.s + R.s	56.7	16.7	6.6	20.0
	P.s + F.s + R.s	46.7	20.0	6.6	26.7
average		48.1	20.9	12.7	24.8%
300	*Pythium sp. (P.s)*	26.7	20.0	6.6	46.7
	Fusarium solani (F.s)	30.0	20.0	10.0	40.0
	Rhizoctonia solani (R.s)	26.7	20.0	10.0	43.3
	P.s + F.s	33.3	16.7	10.0	40.0
	P.s + R.s	33.3	20.0	10.0	36.7
	F.s + R.s	36.7	20.0	13.0	30.0
	P.s + F.s + R.s	33.3	16.7	10.0	40.0
average		31.4	19.1	9.9	39.5
400	*Pythium sp. (P.s)*	23.3	13.3	6.6	56.7
	Fusarium solani (F.s)	20.0	13.3	6.6	60.0
	Rhizoctonia solani (R.s)	23.3	13.3	6.6	56.7
	P.s + F.s	26.7	20.0	10.0	43.3
	P.s + R.s	26.7	16.7	6.6	50.0
	F.s + R.s	26.7	16.7	10.0	46.7
	P.s + F.s + R.s	23.3	13.3	6.6	56.7
average		24.3	15.2	7.6	52.9
500	*Pythium sp. (P.s)*	26.7	13.3	10.0	50.0
	Fusarium solani (F.s)	23.3	13.3	6.6	56.7
	Rhizoctonia solani (R.s)	30.0	13.3	10.0	46.7
	P.s + F.s	26.7	13.3	13.3	46.7
	P.s + R.s	23.3	10.0	10.0	56.7
	F.s + R.s	26.7	13.3	10.0	50.0
	P.s + F.s + R.s	23.3	13.3	13.3	50.0
average		25.7	12.8	10.5	50.9
L. S. D. (at 5%)					
The Concentrations (ppm)	A	**0.434	**0.360	**0.294	**0.584
infested Soils	B	N.S	N.S	N.S	**0.773
	AXB	N.S	N.S	N.S	N.S

Table 3. Damping-off incidence in cucumber plants descended from seeds soaked in different concentrations of Acetyl salicylic acid

Concentrations (mM)	Artificial infested soil	Pre-emergence damping-off %	Post-emergence damping-off %	Root rot %	Healthy/ survival %
0.0	*Pythium sp. (P.s)*	36.7	23.3	10.0	36.7
	Fusarium solani (F.s)	40.0	20.0	10.0	36.7
	Rhizoctonia solani (R.s)	46.7	20.0	13.3	30.0
	P.s + F.s	50.0	20.0	6.6	20.0
	P.s + R.s	50.0	20.0	6.6	26.7
	F.s + R.s	56.7	16.7	6.6	20.0
	P.s + F.s + R.s	43.3	20.0	10.0	30.0
average		46.2	20.0	9.1	28.7
5.0	*Pythium sp. (P.s)*	16.7	10.0	6.6	66.7
	Fusarium solani (F.s)	16.7	13.3	3.3	66.7
	Rhizoctonia solani (R.s)	16.7	10.0	3.3	70.0
	P.s + F.s	20.0	10.0	10.0	60.0
	P.s + R.s	26.7	10.0	10.0	53.3
	F.s + R.s	30.0	13.3	13.3	43.3
	P.s + F.s + R.s	26.7	13.3	10.0	50.0
average		21.9.	11.4	8.1	58.6
10.0	*Pythium sp. (P.s)*	10.0	6.6	6.6	76.7
	Fusarium solani (F.s)	10.0	6.6	6.6	76.7
	Rhizoctonia solani (R.s)	10.0	10.0	6.6	73.3
	P.s + F.s	13.3	10.0	10.0	66.7
	P.s + R.s	16.7	10.0	6.6	70.0
	F.s + R.s	10.0	10.0	10.0	70.0
	P.s + F.s + R.s	16.7	13.3	6.6	70.0
average		12.4	9.5	7.6	71.4
15.0	*Pythium sp. (P.s)*	13.3	6.6	6.6	73.3
	Fusarium solani (F.s)	10.0	10.0	10.0	70.0
	Rhizoctonia solani (R.s)	10.0	13.3	10.0	66.7
	P.s + F.s	13.3	10.0	10.0	66.7
	P.s + R.s	16.7	10.0	10.0	70.0
	F.s + R.s	10.0	10.0	6.6	73.3
	P.s + F.s + R.s	16.7	10.0	13.3	60.0
average		12.9	9.9	7.5	68.6
L. S. D. (at 5%)					
The Concentrations	A	**0.452	**0.357	**0.331	**0.577
infested Soils	B	**0.598	N.S	N.S	**0.763
	AXB	N.S.	N.S	N.S	N.S

of healthy plants. No significant differences were observed between the treatments.

Meanwhile ASA foliar treatments with the three teasted concentrations (2.5, 5.0 and 7.5 Mm) were less effective Table 4. The healthy survival plants were: 52.4, 60.5 and 51.0%, respectively, compared with 28.7% for the untreated control The percentage of pre- and post- emergence damping- off were reduced to 25.7, 19.5, 25.7 and 12.4, 11.4, 12.4, for the three tested concentration respectively, compared with the untreated control 46.2 and 20.0%.In this research the ester of salicylic acid (ASA) which used at the concentration of 10.0 Mm as seed soaking and 5.0 Mm as foliar treatment showed high efficiency in reducing disease incidence causing 71.4% and 60.0% healthy survival plants comparing with the untreated control (28.7%). Also, no significant difference between the treatments were observed in case of root- rot disease. Greenhouses experiments showed that, ASA efficacy reducing the infections with other diseases i.e. powdery mildew, fusarium with and root-knot nematode, in addition to downy mildew. Its efficacy prolonged till 8 weeks after treatments. Salicylic acid synthesizes endogenously as result of pathogen infection (Metraux *et al.,* 1990 and Uknes *et al.,* 1993). It was postulated that ASA might act as transmissible signal for induction of resistance and that have a crucial role in the induction of SAR-gene expression (Metraux *et al.,* 1990 and Neunschwander *et al.,* 1995). It was found that, ASA played an important role in inducing SAR by stimulation of biosynthesis of different families of PR-proteins (Raskin, 1992). Pathogenesis related proteins produced in immunized plants can also induced by exogenously applied ASA (Raskin, 1992). Increase of acid PR-proteins after treating cucumber plants with SA was reported by Okuno *et al.* (1991). Induced systemic resistance in cucumber plants by treatment with SA or ASA was reported by Metraux *et al.* (1990) and Okuno *et al.* (1991). This treatment led to an increase activity of chitinase, B-1, 3-glucanase, polyoxidase (PO), polyphenoloxidase (PPO) and phenylalanine ammonia lyase (PAL). The increases of enzymes activity was correlated with increased formation of papillae in epidermal cells (Schneider and Ullrich, 1994).

Table 4. Damping-off incidence in cucumber plants foliarly treated with different concentrations of Acetyl salicylic acid

Concentrations (mM)	Artificial infested soil		Pre-emergence damping-off %	Post-emergence damping-off %	Root rot %	Healthy/ survival %
0.0	*Pythium sp. (P.s)*		36.7	23.3	10.0	36.7
	Fusarium solani (F.s)		40.0	20.0	10.0	36.7
	Rhizoctonia solani (R.s)		46.7	20.0	13.3	30.0
	P.s + F.s		50.0	20.0	6.6	20.0
	P.s + R.s		50.0	20.0	6.6	26.7
	F.s + R.s		56.7	20.0	6.6	20.0
	P.s + F.s + R.s		43.3	16.7	10.0	30.0
average			46.2	20.0	9.1	28.7
2.5	*Pythium sp. (P.s)*		20.0	13.3	10.0	56.7
	Fusarium solani (F.s)		23.3	10.0	6.6	70.0
	Rhizoctonia solani (R.s)		23.3	10.0	10.0	56.7
	P.s + F.s		23.3	13.3	10.0	46.7
	P.s + R.s		30.0	13.3	10.0	46.7
	F.s + R.s		30.0	13.3	6.6	43.3
	P.s + F.s + R.s		30.0	13.3	10.0	46.7
average			25.7	12.4	9.5	52.4
10.0	*Pythium sp. (P.s)*		16.7	10.0	6.6	66.7
	Fusarium solani (F.s)		20.0	6.6	10.0	63.3
	Rhizoctonia solani (R.s)		16.7	13.3	10.0	60.0
	P.s + F.s		20.0	10.0	13.3	56.7
	P.s + R.s		23.3	13.3	10.0	53.3
	F.s + R.s		20.0	13.3	6.6	63.3
	P.s + F.s + R.s		20.0	13.3	6.6	60.0
average			19.5	11.4	9.0	60.5
15.0	*Pythium sp. (P.s)*		20.0	13.3	10.0	56.7
	Fusarium solani (F.s)		26.7	10.0	13.3	50.0
	Rhizoctonia solani (R.s)		20.0	16.7	10.0	53.3
	P.s + F.s		26.7	13.3	6.6	46.7
	P.s + R.s		30.0	10.0	10.0	50.0
	F.s + R.s		26.7	10.0	10.0	53.3
	P.s + F.s + R.s		30.0	13.3	10.00	46.7
average			25.7	12.4	9.0	51.0
L. S. D. (at 5%)						
The Concentrations		A	**0.397	**0.336	**0.266	**0.548
infested Soils		B	**0.501	N.S	N.S	**0.725
		AXB	N.S.	N.S.	N.S.	N.S.

Inducted Resistance by Potassium Phosphate Mono-Basic (K_2HPO_4)

Three concentrations of K_2HPO_4 i.e, 50, 100 and 150mM were used as seed soaking and foliar treatment for inducing systemic aquired resistance (SAR), against damping off diseases infesting cucumber plants. Results in Table 5 indicate that all treatments significantly reduced the disease incidence. Treatments of 50, 100 and 150mM as seed soaking reduced the disease incidence by 50.0, 62.4 and 61.4%, respectively, as compared with untreated control (22.7%).

Seed soaking treatment with the three tested concentrations reduced the pre-and post- emergence damping- off incidence in cucumber plants by 26.2, 17.6, 16.2 and 14.3, 11.9 and 12.8%, respectively, as compared with untreated control 46.2 and 20.0%.

The foliar treatments with the three tested concentrations of K_2HPO_4 were also less effective Table 6. The healthy survival plants were 43.3, 55.2 and 53.8% for the concentration of 50,100 and 150 ppm, respectively, compared with untreated control (28.7%). While the reduction of the percentage of pre-and post-emergence damping-off were: 30.0, 21.9, 21.9 and 16.7, 13.3, 13.3, respectively, compared with the untreated control 46.2 and 20.0%.

Generally, seed soaking and foliar applications with potassium phosphate mono- basic (K_2HPO_4) elicited SAR in many host-pathogen systems (Gottstein and Kuc, 1989 and Mucharromah and Kuc, 1991). In most cases (K_2HPO_4) was the most effective when applied at the concentrations ranging between 20 to 100 Mm. Results in this research indicated that, K_2HPO_4 at 100 and 150 mM were effective in confering SAR in cucumber against damping-off disease incidence. It was found that, SAR was triggered by phosphate through a process involving the consequestring of calcium from host tissue (Doubravera *et al.,* 1988 and Gottstein and Kuc, 1989). It is possible that, these chemical agents elicit the release of a signal triggers the plants general response, the signal might also affect the expression of the defence genes which then make the plant more responsive after subsequent infection (Kuc, 1990 and Mucharromah and Kuc, 1991).

Table 5. Damping-off incidence in cucumber plants descended from seeds soaked in different concentrations of K_2HPO_4

Concentrations (mM)	Artificial infested soil		Pre-emergence damping-off %	Post-emergence damping-off %	Root rot %	Healthy/ survival %
0.0	*Pythium sp. (P.s)*		36.7	23.3	10.0	36.7
	Fusarium solani (F.s)		40.0	20.0	10.0	36.7
	Rhizoctonia solani (R.s)		46.7	20.0	13.3	30.0
	P.s + F.s		50.0	20.0	6.6	20.0
	P.s + R.s		50.0	20.0	6.6	26.7
	F.s + R.s		56.7	16.7	6.6	20.0
	P.s + F.s + R.s		43.3	20.0	10.0	30.0
average			46.2	20.0	9.1	28.7
50.0	*Pythium sp. (P.s)*		23.3	13.3	6.6	56.7
	Fusarium solani (F.s)		20.0	16.7	10.0	53.3
	Rhizoctonia solani (R.s)		20.0	16.7	6.6	56.7
	P.s + F.s		30.0	13.3	10.0	46.7
	P.s + R.s		26.7	13.3	10.0	50.0
	F.s + R.s		33.3	13.3	13.3	40.0
	P.s + F.s + R.s		30.0	13.3	10.0	46.7
average			26.2	14.3	9.5	50.0
100.0	*Pythium sp. (P.s)*		13.3	10.0	10.0	66.7
	Fusarium solani (F.s)		16.7	13.3	6.6	63.3
	Rhizoctonia solani (R.s)		16.7	13.3	6.6	63.3
	P.s + F.s		20.0	10.0	10.0	60.0
	P.s + R.s		20.0	13.3	6.6	60.0
	F.s + R.s		20.0	10.0	10.0	60.0
	P.s + F.s + R.s		16.7	13.3	6.6	63.3
average			17.6	11.9	8.1	62.4
150.0	*Pythium sp. (P.s)*		10.0	10.0	10.0	70.0
	Fusarium solani (F.s)		16.7	10.0	10.0	63.3
	Rhizoctonia solani (R.s)		16.7	13.3	10.0	63.3
	P.s + F.s		16.7	16.7	10.0	60.0
	P.s + R.s		20.0	13.3	10.0	56.7
	F.s + R.s		16.7	13.3	10.0	60.0
	P.s + F.s + R.s		16.7	13.3	13.3	56.7
average			16.2	12.8	10.5	61.4
L. S. D. (at 5%)						
The Concentrations		A	**0.428	**0.344	**0.287	**0.578
infested Soils		B	**0.567	N.S	N.S	**0.726
		AXB	N.S.	N.S	N.S	N.S

Table 6. Damping-off incidence in cucumber plants foliarly treated with different concentrations of K_2HPO_4

Concentrations (mM)	Artificial infested soil		Pre-emergence damping-off %	Post-emergence damping-off %	Root rot %	Healthy/ survival %
0.0	*Pythium sp. (P.s)*		36.7	23.3	10.0	36.7
	Fusarium solani (F.s)		40.0	20.0	10.0	36.7
	Rhizoctonia solani (R.s)		46.7	20.0	13.3	30.0
	P.s + F.s		50.0	20.0	6.6	20.0
	P.s + R.s		50.0	20.0	6.6	26.7
	F.s + R.s		56.7	16.7	6.6	20.0
	P.s + F.s + R.s		43.3	20.0	10.0	30.0
average			46.2	20.0	9.1	28.7
50.0	*Pythium sp. (P.s)*		26.7	16.7	10.0	46.7
	Fusarium solani (F.s)		26.7	20.0	6.6	46.7
	Rhizoctonia solani (R.s)		30.0	20.0	10.0	40.0
	P.s + F.s		33.3	16.7	13.3	40.0
	P.s + R.s		33.3	13.3	13.3	40.0
	F.s + R.s		30.0	13.3	10.0	46.7
	P.s + F.s + R.s		30.0	16.7	10.0	43.3
average			30.0	16.7	10.5	43.3
100.0	*Pythium sp. (P.s)*		16.7	13.3	13.3	60.0
	Fusarium solani (F.s)		20.0	13.3	10.0	56.7
	Rhizoctonia solani (R.s)		20.0	13.3	10.0	56.7
	P.s + F.s		26.7	10.0	10.0	53.3
	P.s + R.s		23.3	16.7	6.6	53.3
	F.s + R.s		23.3	13.3	10.0	53.3
	P.s + F.s + R.s		23.3	13.3	6.6	53.3
average			21.9	13.3	9.5	55.2
150.0	*Pythium sp. (P.s)*		16.7	13.3	13.3	56.7
	Fusarium solani (F.s)		20.0	16.7	13.3	53.3
	Rhizoctonia solani (R.s)		16.7	13.3	10.0	56.7
	P.s + F.s		23.3	10.0	10.0	53.3
	P.s + R.s		30.0	13.3	10.0	50.0
	F.s + R.s		23.3	13.3	10.0	53.3
	P.s + F.s + R.s		23.3	13.3	10.0	53.3
average			21.9	13.3	10.9	53.8
L. S. D. (at 5%)						
The Concentrations		A	**0.381	**0.367	**0.283	**0.597
infested Soils		B	**0.504	N.S	N.S	N.S
		AXB	N.S.	N.S	N.S	N.S

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استحثاث صفة المقاومة فى نباتات الخيار ضد أمراض الذبول وتعفن الجذور باستخدام بعض الكيماويات

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تعتبر الأمراض التى تصيب المجموع الجذرى لنباتات الخيار خاصة الذبول وتعفن الجذور من أهم العوامل المحددة لإنتاج الخيار فى الصوب الزجاجية لهذا فإن هذه الدراسة أجريت لمعرفة إمكانية استحثاث صفة المقاومة فى نباتات الخيار باستخدام الإيثيفون وحامض الأسيتايل سالسيليك وفوسفات البوتاسيوم الأحادية ثم معرفة نشاط تأثير هذه المواد على انزيمات البيرواكسديز والبولى فينول أكسديز وعلى الفينولات الحرة والكلية فى نباتات الخيار.

استخدام المحفزات الكيماوية:

أ- تم نقع بذور الخيار الصنف بابلون فى ثلاثة تركيزات من محلول مادة الإيثيفون (٤٠٠، ٦٠٠، ٨٠٠جزء فى المليون) لمدة ٢٤ساعة كذلك تم رش محلول هذه المادة على النباتات بعد الإنبات بتركيزات (٣٠٠، ٤٠٠، ٥٠٠جزء فى المليون) فكان النقع أكثر فعالية عن الرش حيث بلغت نسبة النباتات الحية السليمة ٥٣,٣، ٦٧,١ ، ٦٣,٣% عند نقع البذرة فى الثلاث تركيزات على التوالى بينما بلغت هذه النسبة ٣٩,٥، ٥٢,٩، ٥٠,٩%عند رش النباتات بالتركيزات المذكورة على التوالى مقارنة بالنباتات الغير معاملة حيث بلغت هذه النسبة ٢٤,٨%.

ب-أيضاً تم نقع بذور الخيار الصنف بابلون فى ثلاثة تركيزات من حامض الاسيتايل ساليسيليك (٥،١٠،١٥ ملليموز) وكذلك رش النباتات بتركيزات (٢,٥، ٥,٠، ٧,٥ ملليموز) وبلغت نسبة النباتات السليمة: ٧١,٤، ٦٨,٦، ٥٠ % علي التوالى عند نقع

البذور بينما كانت هذه النسبة ٥٢,٤، ٦٠,٥، ٥١,٠% على التوالى عند رش النباتات. مقارنة بـ ٢٨,٦% فى حالة النباتات الغير معاملة.

ج- وجد أيضا أن نقع بذور الخيار فى ثلاثة تركيزات من مادة فوسفات البوتاسيوم الآحادية (١٠٠، ٥٠ ، ١٥٠مليموز) أدى إلى أحداث مقاومة جهازية مكتسبة للنباتات حيث بلغت نسبة النباتات السليمة ٥٠,٠، ٦٢,٤ ثم ٦١,٤% على التوالى مقارنة بنسبة ٢٢,٧% فى حالة النباتات الغير معاملة بينما كان رش النباتات بعد الأنبات مباشرة بهذه التركيزات الثلاثة أقل فعالية حيث بلغت نسبة النباتات الحية السليمة ٥٢,٤، ٦٠,٥ ثم ٥١,٠% على التوالى مقارنة بنسبة ٢٨,٧% فى حالة النباتات الغير معامله.

تقدير النشاط الإنزيمى لإنزيمى البيرواكسديز والبولى فينول اكسيديز والفينولات الحرة والكلية:

كل المعاملات السابقة شجعت على زيادة النشاط الأنزيمى للبيرواكسيديز وكان أكثر المعاملات فعاليه هى معامله النباتات بخلات حمض السليسيليك بتركز ١٠% ملليموز يليها المعاملة بمادة فوسفات البوتاسيوم الأحادية بتركيز ١٠٠ملليموز ثم مادة الإيثيفون بتركيز ٦٠٠جزء فى المليون حيث بلغت قيم النشاط الأنزيمى: ١,٤١٨،٠,٨٧٦ ثم ٠,٧٧٣

أكثر المعاملات فاعلية فى زيادة قيم النشاط الإنزيمى لإنزيم البولى فينول اكسيديز كانت خلات حمض السليسيليك ثم الأيثيفون ثم مادة فوسفات البوتاسيوم الأحادية حيث وصلت قيم النشاط الأنزيمى إلى: ١,٨٤٠، ١,٣٣٨ ثم ١,٢٩٢ على التوالى.

أوضحت قيم الفينولات الحرة / الفينولات الكلية حدوث نسبة عالية من المقاومة حيث بلغت هذه النسبة ٧٢,٣٤% عند المعاملة بخلات حمض السليسيليك يليها المعاملة بفوسفات البوتاسيوم الأحادية ثم الأيثيفون حيث بلغت هذه النسبة ٦٦,٣٩ ثم ٦٣,٥٦% على التوالى.