EFFECT OF PROPOLIS ON DAMPING- OFF DISEASE OF CUCUMBER IN PROTECTED CULTIVATION

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Abstract

Different concentrations of the proplis ethanol extract (PEE) reduced the radial growth of *Fusarium solani, Pythium ultimum* and *Sclerotinia sclerotiorum* and the higher concentration (5000 ppm) was more effective against all the tested fungi . Seedling treatment with the highest concentrations 5 and 4 g/l gave the maximum protection and reduced the disease incidence followed by other the treatments . This treatment improved plant height, increased the percentage of flowering, fruit setting and the fruit yield was also increased. The highest concentration (5g/l), was nearly as effective as the fungicide Topsin M 70 . The seed treatment with the proplis extract PEE was less effective than seedling treatment in both examined seasons.

INTRODUCTION

Cucumber [*Cucumis sativus L.*] is one of the most important vegetable crops cultivated under plastic houses in Egypt. It is affected by various plant pathogens causing damping off and root rot which result in great losses in cucumber fruit yield. In the last few years, many efforts were done to reduce the environmental pollution resulting from the application of pesticides.. Propolis is a natural byproduct of honey bees which could be applied safely to the cultivated plants to control phytopathogenic fungi. Bioactivity of propolis against phytopathogenic fungi had been reported by many investigators. Ghisalberti (1979), Peoplinijak *et al* (1982) and La-Torre *et al*(1990) found that the alcoholic solutions of propolis exhibited fungicidal activity against *Botrytis cinerea in vitro* and the effect was proportional to the concentration of propolis . *In Vivo* tests on infected strawberry plants, propolis solution (4000ppm) reduced infection compared with the control. Abdulsalam *et al* (1995) studied the bioactivity of propolis ethanol extract against ten soil fungi. He indicated that the growth diameter of the tested fungi was decreased significantly with each of the tested propolis extract concentrations. The highest decrease in linear growth was observed in the fungi *Fusarium solani, Botrytis* sp. and *Helminthosporium* sp. D'Aulerio *et al* (1996) observed that the activities of propolis was low against *Rhizoctonia solani* and *Pythium ultimum* and *F.solani in vitro.* Hassanein (1997) found that propolis extract had no antifungal activity against *F.moniliforme, Helminthosporium* sp,

Macrophomina phaseolina and *R.solani* .The aim of the present study was to evaluate effectiveness of propolis on some cucumber damping –off fungi *in vitro* & *in vivo* as well as the growth and fruit yield of treated plants.

MATERIALS AND METHODS

Effect of propolis on the causal organisms of root-rot disease

Local prepration of raw materials of propolis was collected from honey bee colonies , *Apis mellifera* L. in EL. Gharbia Governorate . Collected samples were mixed together and the active ingredients were extracted by ethyl alcohol following the method of Vechet (1978) . Propolis ethanol mixture (1:15) was prepared and shaken for 15 hours, with an average of three hours daily during five successive days at room temperature . Then the mixture was filtered and ethanol was evaporated under low pressure at 60 °C. The resulting dry matter was dissolved in 80% aqueous ethanol to obtain different concentrations. Different dilutions of crude propolis ethanol extract (PEE) were used to evaluate its antifungal activity on *Fusarium solani, Pythium ultimum* and *Sclerotinia sclerotiorum*

In Vitro experiments

Propolis ethanol extract (PEE) was added to hot potato dextrose agar (PDA) to obtain concentrations of 2000,3000,4000,5000 ppm. Four plates (9cm in diameter) from each concentration as well as plain PDA only, as control, were inoculated with fungal discs of *F.solani, P.ultimum* and *S.sclerotiorum* and incubated at 28 °C. The radial growth was measured in cm and the inhibition percentage of the pathogen was calculated .

In Vivo experiments

Assessment of propolis effect was carried out during 2004-2005 seasons in a greenhouse at Tokh , Kalubia Governorate , which has a disease history of damping-off and stem rot. Six treatments included the 4 tested proplis treatments(2,3,4and5 g/l), the fungicide Topsin M 70 (3g/l) and untreated control were distributed in a completely randomized block design .

Seed and seedling treatment

Delta Star cucumber cultivar seeds or seedlings were dipped in the 4 concentrations of propolis (2,3,4 and 5 g/l) for 2 hours before sowing or transplanting in the greenhouse.

The fungicide treatment

The cucumber seeds or seedlings were soaked in Topsin M70 praparation at the rate of 3 g/l. They were sown or transplanted into the soil in rows (each of 7m length and 1.5m in width) on two ridges. Distance between holes or transplants was 50 cm . Each treatment was replicated in 4 randomized 10.5 m^2 plots. Cultural practices were applied as usual. Data were recorded as percentage of disease incidence plant heights and percentage of flowering as well as fruit setting and the yield.

RESULTS AND DISCUSSION

Data presented in table(1) and figures (1,2 and 3) show the effect of different concentrations of propolis ethanol extracts (PEE) on the radial growth of *F.solani* , *P.ultimum* and *S.sclerotiorum*. The data indicate that the radial growth of the tested fungi was greatly decreased by the propolis treatments. The higher concentration of PEE (5000 ppm)was more effective than the lower concentrations against all tested fungi . It inhibited the radial growth of *F.solani*, *P.ultimum* and *S.sclerotiorum* by 88.9, 86.7 and 83.3% respectively .

Table1. Effect of different concentration of propolis on radial growth (cm)of *F.solani* , *P.ultimum and S.sclerotiorum In Vitro.*

Concentration of propolis ppm	*F.solani*		*P.ultimum*		*S.sclerotiorum*	
	Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)	Radial growth (cm)	Inhibition (%)
2000	4.3	52.2	5.5	38.9	4.1	54.4
3000	3.2	64.4	4.0	55.6	3.5	61.1
4000	1.5	83.3	2.6	71.1	2.5	72.2
5000	1.0	88.9	1.2	86.7	1.5	83.3
Control	9.0	0.0	9.0	0.0	8.6	0.0
L.S.D at 5%	0.25	----	0.31	----	0.35	----

The lowest concentration of PEE(2000 ppm) inhibited the radial growth of the tested fungi by 52.2 , 38.9 and 54.4% respectively. The other treatments 3000 and 4000 ppm fall in between . These results are in line with those reported by Abdul salam and Mohamed(1989) , who stated that the effective concentrations to inhibit 50% (lc50) of growth of *Fusarium tabacinum*, *F.heterosporium* and *F.solani* were 250 , 320 and 420 ppm, respectively . Fungal growth was significantly decreased by each of the tested PEE concentrations with the higher concentration of PEE being more effective than the lower ones against the tested fungi *F.solani*, *Alternaria alternata* and *Helminthosporium* sp. (Abdul salam, 1995).

Data in table (2) show that seed and transplant treatments with the preparations of propolis significantly decreased the percentage of disease incidence on cucumber in the two growing seasons 2004 and 2005 comparing to the untreated control; however, the fungicide Topsin M70 was superior in this respect during the two seasons. The results prove that transplant treatment with propolis generally gave higher protection against cucumber damping off than seed treatment in both seasons at any concentration used. Consequenstly, the percentage of survival plants was increased by increasing the propolis concentration. Concentration of propolis 5 g/l gave the lowest percentage of disease incidence (12.95%), while 2g/l. was less efficient in reducing the disease incidence (23.15%) compared with the control (30.92%). The obtained results in 2004 were more or less similar to those obtained in 2005 . These results are in accordance with those reported by Fahmy & Omar (1989), who found that soaking onion seeds in 1 and 2.5% propolis alcoholic extract (PAE) for 4 or 8 h decreased the percentage of infection or dipping onion seedling for 4 h in (PAE) minimized the number of sclerotia produced and reduced white rot incidence caused by *sclerotium cepivorum* under greenhouse condition. La-Torre *et al.* (1990) found that propolis solution (4000 ppm) reduced infection of strawberry plants by *Botrytis cinerea* compared with control *in vivo* . El-Asiuty *et al* (2000) noted that propolis extract could successfully decrease the incidence of downy mildew on sorghum .

Table. 2. Effect of propolis concentrations on damping-off incidence of cucumber caused by *F.solani, P.ultimum* and *S.sclerotiorum* under greenhouse condition during 2004-2005 seasons.

Propolis treatments g/l	Seed treatment				Seedling treatment			
	2004		2005		2004		2005	
	Disease incidence (%)	Survival (%)	Disease incidence (%)	Survival (%)	Disease Incidence (%)	Survival (%)	Disease Incidence (%)	Survival (%)
2	29.02	70.98	24.94	75.06	23.15	76.85	20.35	79.65
3	25.24	74.76	22.18	77.82	20.85	79.15	18.94	81.06
4	23.93	76.07	20.79	79.21	19.98	80.02	16.64	83.36
5	18.65	81.35	14.53	85.47	12.95	87.05	8.88	91.12
Topsi M 70	12.32	87.68	9.45	90.55	7.33	92.67	6.25	93.75
Control	39.81	60.19	36.94	63.06	30.92	69.08	29.54	70.46
L.S.D. at 5%	4.89	5.12	5.94	6.14	6.54	7.01	6.99	7.62

Data in table (3)show that seedling treatment with the propolis preporation was more effective in increasing the plant height and yield of cucumber plants more than seed treatment in both two seasons . Data also revealed that treating the seedlings with propolis at 5 and 4 g/l were the most effective in improving the plant height from 1.91m to 3.42 and 3.21m, respectively and the yield from 40.14 to 69.95 and 66.45 kg/plot. respectively . The concentration 3 and 2 g/l. were the least effective giving plant heights of 3.12 and 3.02 m, respectively, and a yield of 59.74 and 56.98 kg/plot , respectively. The results obtained were more or less similar in both seasons and are in agreement with those obtained by EL.Assiuty *et al* (2000) on downy mildew of maize , who noted that propolis extract could significantly increase the grain yield per plant significantly.

Table 3. Effect of propolis concentrations on plant height and yield of cucumber under greenhouse conditions during 2004,2005 seasons

Propolis Treatments g/l	Seed treatment				Seedling treatment			
	2004		2005		2004		2005	
	Plant heights (m)	Yield (kg/plot)	Plant heights (m)	Yield (kg/plot)	Plant Heights (m)	Yield (kg/plot)	Plant Heights (m)	Yield (kg/plot)
2	2.82	49.73	2.96	51.21	3.02	56.98	3.10	60.58
3	2.91	50.98	3.04	54.65	3.12	59.74	3.21	62.36
4	3.06	54.69	3.10	60.19	3.21	66.45	3.39	68.48
5	3.12	59.93	3.19	64.28	3.42	69.95	3.54	71.89
Topsin M 70	2.98	58.28	3.08	62.28	3.12	68.12	3.21	69.42
Control	1.79	36.29	1.77	38.83	1.91	40.14	2.13	42.28
L.S.D at 5%	0.20	5.14	0.08	5.89	0.19	7.15	0.22	5.21

Plot = 1 /400 feddan

Data presented in Table (4) indicate that seedling treatment with the propolis extract gave higher percentage of flowering and fruit setting compared with seed treatment in both season . Treatments of seedling with propolis of concentrations 5 and 4 g/l increased the percentage of flowering from 66.14 to 94.92 and 92.45%, respectively, and fruit-setting from 61.48 to 92.86 and 90.14%, respectively in 2004. Propolis concentrations of 3 and 2 g/l gave lower percentage of flowering of 89.64 and 83.45% and fruit-setting of 86.92 and 80.79%, respectively . This is probably a reflection of the better plant growth parameters as a result of disease control and possible direct effect of metabolites and chemical constituents on the plant. Similar trends were observed in the 2005 experimebts. These results are in accordance with those obtained by EL.Assiuty *et a.* (2000) who found that propolis extract stimulated plant growth of maize .

The result reported herein indicate the possibility of using proplis preparation to reduce the incidence of damping-off in cucumber caused by different fungi, as a component of an integrated disease control program.

Table 4. Effect of propolis concentrations on flowering and fruit-setting of cucumber under greenhousecondition.

Propolis Treatment g/L.	Seed treatment				Seedling treatment			
	2004		2005		2004		2005	
	Flowering (%)	Fruit-setting (%)	Flowering (%)	Fruit-setting (%)	Flowering (%)	Fruit-setting (%)	Flowering (%)	Fruit-setting (%)
2	80.26	76.77	81.82	78.18	83.45	80.79	82.16	80.23
3	85.95	81.09	87.15	85.49	89.64	86.92	90.09	89.43
4	89.87	86.79	91.46	89.76	92.45	90.14	92.65	90.72
5	91.96	90.18	92.15	91.85	94.92	92.86	95.59	94.09
Topsin M70	90.42	89.08	92.38	90.43	93.08	91.18	94.18	94.98
Control	60.72	54.74	61.49	56.21	66.14	61.48	67.54	62.81
L.S.D at 50%	6.82	5.10	4.98	4.11	5.08	3.18	6.98	5.62

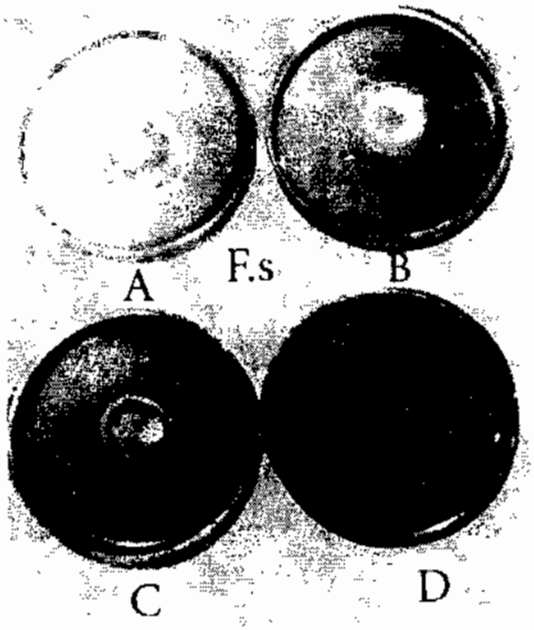

Fig.1. Effect of four concentrations propolis ethanol extract (PEE) against *Fusarium soloni(F.s) in vitro*. (A) 2000 ppm (B) 3000 ppm (C) 4000 ppm (D) 5000 ppm

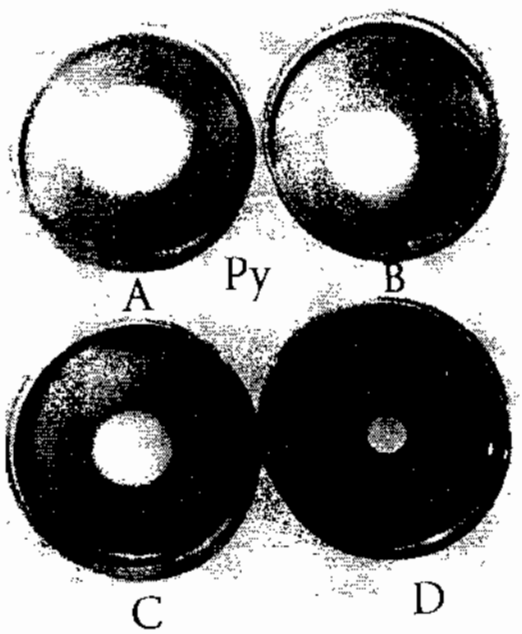

Fig. 2. Effect of four concentrations propolis ethanol extract(PEE)against *pythium ultimum (Py) In vitro* (A) 2000 ppm (B) 3000 ppm (C) 4000 ppm (D)5000 ppm

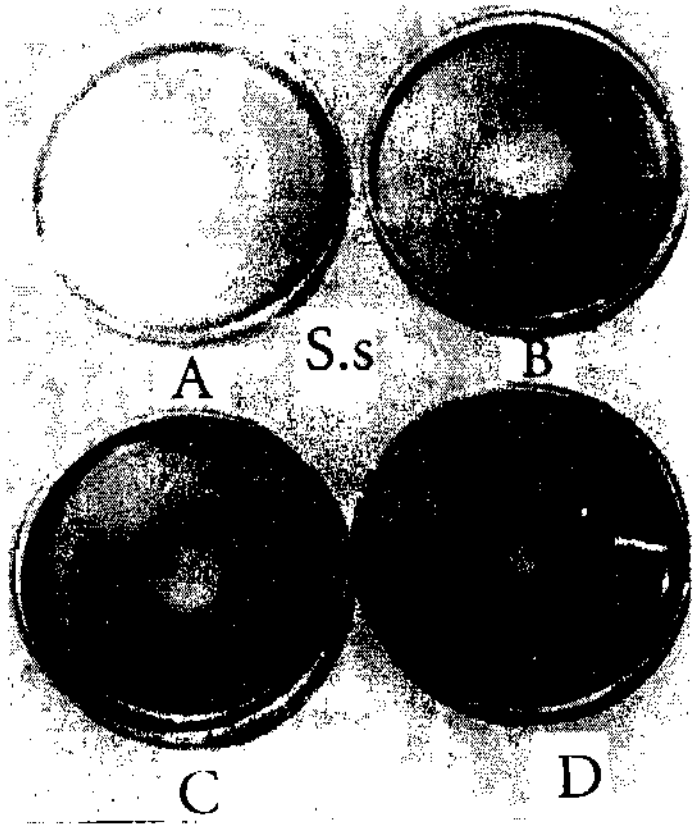

Fig. 3. Effect of four concentrations propolis ethanol extract (pee) against *Sclerotinia sclerotiorum*(S.s)*In vitro* (A) 2000 ppm (B) 3000 ppm (C) 4000 ppm (D) 5000ppm

REFERENCES

1. Abdul salam, K. S. and M. I Mohamed. 1989. Effect of propolis on some *Fusarium* spp. Egypt. J. Phytopathol . , 21(2):149-155
2. Abdulsalam , K. S. 1995. Bioactivity of propolis extract against certain soil borne fungi . Alex . J. Agric. Res . 40(3):305-313 .
3. D'Aulerio , A. Z., A. Zambonelli and A. Bianchi. 1996. Natural products in the protection of medicinal plants . Colture-protette . 25(11):99-102
4. EL-Assiuty, E. M. and Zeinab M. Fahmy. 2000. Propolis in controlling sorghum downy mildew and stimulating plant growth of maize. Egypt J. Appl . Sci , 15(12):45-54 .
5. Fahmy, F. G. and M. O. M. Omar. 1989. Potential use of proplis to control white rot disease of onion . Assiut . J. Agric , Sci., 20(1):265-275 .
6. Ghisalberti, E. L. 1979. Propolis . A Review . Bee World , 60(2):59-84
7. Hassanein, F. M. 1997. Evaluation of antimicrobial activity of some bee products against certain phytopathogenic bacteria and fungi . Alex . J. of Agric . Res., 42(3):239-250
8. La-Torre, A., M. Guccinone and G. Imbroglini. 1990. Effect of proplis reparations on Botrytis cinerea on strawberry . Aplicoltura ,(6):169-177 .
9. Peoplinijak, S., D. Maysinger and I. jalsenjak . 1982. Effect of propolis extracts on some fungi . Scientia Pharmaceutic ., 50(2):165 .
10. Vechet, L. 1978. Effect of propolis on some species of microorganisms and moulds . In a remarkable hive product propolis . 535 PP.

تاثير البروبليس على أمراض موت البادرات في الخيارتحت ظروف الصوبه

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تم استخدام التركيزات المختلفة من المستخلص الايثانولى للبروبوليس والتى ادت الي خفض النمو الفطرى لفطريات فيوزاريوم سولانى والبيثيم اولتيمم وسكيلورتينيا سكليروشيورم . وقد وجد ان التركيز العالى من المستخلص الايثانولى(٥٠٠٠ جزء في المليون) ذو تاثيراكبر ضد هذة الفطريات المختبرة عن التركيزات الاقل.

واعطت معاملة النباتات بالتركيزات ٤ ، ٥ جم/لتر اعلى حماية وأدت الى خفض الشدة المرضية للفطريات المختبرة تحت ظروف الصوبة. مقارنة بالمعاملات الاخرى . وأدت هذه المعاملات أيضا إلى تحسين ارتفاع النباتات وزيادة نسبة التزهير والعقد وبالتالي زيادة المحصول .

ووجد أن التركيز الاعلي من المستخلص الايثانولى للبروبوليس كان أقرب في تأثيرة لمبيد توبسين ام ٧٠ فى مكافحة موت بادرات الخيار . وكانت معاملة البذور بالمستخلص الايثانولي أقل تأثيرا من معاملة الشتلات في كلا الموسمين في هذا الخصوص .