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CHEMOTHERAPEUTIC AND BIOCHEMICAL ACTIVITIES OF SOME FUNGICIDES AGAINST SQUASH DOWNY MILDEW DISEASE

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ABSTRACT

The potency of five selected fungicides as chemotherapeutic agents against squash downy mildew was studied as well as their effect on biochemical changes of the host plant as chlorophyll, β-carotene and sugar contents, in addition to polyphenol oxidase activity. The fungicides testecd were propamocarb hydrochloride (Previcur-N), mikal, mancozeb (Diathane M-45), Ridomil-Plus (metalaxyl-plus) and tolclofos-methyl (Rizolex). The results revealed that: 1) Propamocarb hydrochloride and mikal (systemic fungicides) were highly effective in reducing *Pseudoperonospora cubensis* infection indicated by low disease index and high recovery of infected squash leaves. In contrast, non-systematic fungicides as tolclofos-methyl and mancozeb were lowest affective in this respect. 2) *P. cubensis* infection caused significant decrease in chlorophyll content, β-carotene content and polyphenol oxidase activity of squash leaves. Sugar content was insensitive toward downy mildew causal pathogen infection. 3) Fungicide treatments alone showed a feeble effect against chlorophyll content, β-carotene contents and polyphenol oxidase activity of squash leaves, while sugar content was increased significantly especially due to mikal treatments. 4) Combinations of downy mildew infection and fungicide treatments was in favor of producing healthy plants and increasing some biochemical components related to the resistance phenomenon as sugar content and polypenol oxidase activity of squash leaves. The pigment contents represented as chlorophyll and β-carotene contents, were increased slightly due to therapeutic effect of fungicides, however, the increasing value was not able to restore the values of non-infected plants especially with β-carotene content.

INTRODUCTION

caused by *Pseudoperonospoa cubensis* (Berkely & Curits) Clinton is one of the most serious diseases of cucurbitace crops grown in either greenhouse or in outdoor in Egypt (Shama *et al.*, 1998; Mahrous *et al.*, 1985 and Fadi *et al.*, 1996). Use of fungicides as has always been the most practical and economic method in preventing and controlling downy mildew plant diseases (Rondomanski and Woaniak, 1989 ; Chaban *et al.*, 1990; Apaydin, 1994; Gupta and Shyam, 1996 and Asif, 1999).

The present study was performed to evaluate the efficacy of some systemic and non-systemic fungicides in reducing the cucurbitace disease incidence of squash downy mildew caused by *P. cubensis*. In addition, the biochemical impact of tested fungizcides alone and in combination with the pathogen infection in relation to squash plant host that associated with the resistance of the host plant as chlorophyll, β-carotene content and sugar content as well as polyphenol oxidase activity were also investigated.

MATERIALS AND METHODS

I. Fungicides Used:

1.Propamocarb hydrochloride (Previcur-N)72.2% S.L(systemicfungicide)

Propyl [3-(dimethylamine) propyl] carbamate monohydrochloride.

2. Mikal (50% fosetyl-AL + 25% folpet) 75% W.P (systemic fungicide)

Fosetyl-AL: tris-O-ethyl phosphonate.

Folpet: N-(trichloromethyl thio) phtalimide.

3. Mancozeb (Diathane M-45) 80% W.P (contact fungicide)

Contains 16% manganese, 2% zinc and 62% ethylene-bis-dithiocarbamate ion / manganese ethylene -bis dithiocarbamate plus zinc ion.

4. Ridomil plus (15% metalaxyl + 35% copper oxychloride) 50% W.P (mixture of systemic and non-systemic fungicides)

Metalaxyl: N-(2-6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester.

5. Tolclofos-methyl (Rizolex) 50% W.P (contact fungicide)

O,O-dimethyl-O-(2,6-dichloro-4-methylphenyl)-phosphorothioate.

II. Pathogen:

Pseudoperonospora cubensis, the causal pathogen of downy mildew of squash plant, was obtained from the naturally infected squash.

III. Assay of Eradicate Activity of the Fungicides:

Plant seedlings of squash were sprayed with the fungicide solution after 6 days of inoculation that caused about 20% infection of the host plant. Four replicates for each treatment and control were made. The fungicide concentrations 0.25, 0.5, 0.75 and 2 fold of the field application rates were used.

The plants were maintained under the glass house condition till the real three-four leaves stage. Four pots for each treatment were made as well as the control disease incidence was assessed as follows:

1. percentage of colonized leaves/plant (even when one colony appeared on the leaf).
2. Disease development was expressed as a percentage of infection using the assessment key (disease index) proposed by James (1971) and applying Wenzl equation (Wenzl, 1948):

$$\text{Disease index} = [\ (nr/4N) \times 100\ ]$$

where;

n: number of leaves in each category of the key (5 categories).

r: numerical values given to tested sample.

N: total number of examined leaves.

4: degree of freedom.

IV. The Effects on Squash Plant:

A) Determination of photosynthetic pigments:

1. **Chlorophyll content:** was measured spectrophotometrically at 645 and 663 nm wavelength, according to Grodzinsky and Grodzinsky (1973) with slight modification (Sabra, 1988).

2. **β-Carotene content:** Extraction and purification of carotenes from squash leaves were carried out and measured according to the procedures of Canal Villanueva *et al.* (1985) and Sabra (1993).

3. **Sugar content:** Total sugar content was determined using picric acid method according to Thomas and Dutcher (1924).

B) Determination of polyphenol oxidase activity:

For determination of polyphenol oxidase activity, the method described by Broesch (1954) was used. Enzyme activity was measured as optical density (absorbance) after one hour incubation in a shaking water bath at 45°C.

RESULTS AND DISCUSSION

1. Effect of tested fungicides on disease incidence:

The therapeutic efficiency of selected fungicides against squash downy mildew disease after well established *Pseudoperonospora cubensis* infection indicated the ability of systemic fungicides propamocarb hydrochloride (Previcur-N) and mikal to reduce the infection of pathogen indicated by lowering both parameters of disease index and infected leaves/plant. Tolclofos-methyl (Rizolex) was the weakest fungicide used against squash downy mildew infection that caused lowest recovery (disease index) and least chemotherapeutic ability against P. cubensis infection (Table 1). Contrary, mikal (systemic fungicide) caused the highest chemotherapeutic activity revealing lowest infected leavers/plant and disease index values (31.6 and 21.3, respectively) at the field application rate. The protective contact fungicide mancozeb (Dithane M-45) caused moderate effect against squash downy mildew infection, however, the disease index was decreased by about 45% only at field application rate when applied after established infection. These phenomena of the high efficiency of systemic fungicides as therapeutic agents come in complete agreement with the mode of action of the five selected fungicides (Apaydin, 1994 and Asif, 1999).

Table (1): *In vivo* effect of tested fungicides against squash downy mildew infected plants after 6 days of *Pseudoperonospora cubensis* infection.

Fungicides	Treatments					
	0.0	0.25 F	0.50 F	0.75 F	1.00 F	2.00 F
			Propamocarb hydrochloride			
Infected leaves/plant (%)	81.7 ± 6.1	69.3 ± 2.1	35.6 ± 5.1	50.4 ± 3.1	45.6 ± 5.1	32.5 ± 4.1
Disease index	66.4 ± 3.1	51.6 ± 5.1	39.8 ± 4.1	32.8 ± 7.1	25.6 ± 5.1	22.4 ± 2.1
			Mikal			
Infected leaves/plant (%)	8.17 ± 6.1	65.6 ± 8.4	53.4 ± 2.1	50.3 ± 2.1	31.6 ± 5.1	30.0 ± 4.3
Disease index	66.4 ± 3.1	52.3 ± 2.1	35.4 ± 3.1	30.1 ± 2.9	21.3 ± 2.1	18.4 ± 3.1
			Mancozeb			
Infected leaves/plant (%)	81.7 ± 6.1	75.3 ± 2.1	65.6 ± 4.1	51.3 ± 2.1	50.2 ± 1.1	34.3 ± 4.1
Disease index	66.4 ± 3.1	59.3 ± 2.1	50.4 ± 3.1	42.3 ± 2.1	36.5 ± 4.1	30.2 ± 1.1
			Ridomil-Plus			
Infected leaves/plant (%)	81.7 ± 6.1	69.9 ± 8.1	54.7 ± 6.1	51.4 ± 3.1	46.3 ± 2.1	34.6 ± 4.1
Disease index	66.4 ± 3.1	62.3 ± 2.1	59.3 ± 1.1	42.3 ± 2.1	32.3 ± 3.1	25.4 ± 3.1
			Tolclofos-methyl			
Infected leaves/plant (%)	81.7 ± 6.1	77.8 ± 7.1	69.9 ± 8.1	61.2 ± 5.9	57.2 ± 6.1	43.2 ± 4.5
Disease index	66.4 ± 3.1	59.3 ± 2.1	50.4 ± 3.1	44.3 ± 2.1	40.3 ± 3.3	39.8 ± 4.1

Values are means ± S.E (n = 4). **F = Field rate.**

2. Effect of tested fungicides alone and in combination with pathogen on the squash biochemical parameters:

a) Chlorophyll content:

Four fungicides (propamocarb hydrochloride, Mikal, Mancozeb and Ridomil-Plus) that proved to possesses highest effectiveness against *Pseudoperonospora cubensis* infection, shown as low values of infected leaves/plants as well as disease index, were tested alone and in combination with *P. cubensis* infection on some biochemical changes of squash host plant that related to the resistance phenomenon as chlorophyll, β-carotene, sugar contents and polyphenol oxidase activity.

The results in (Table 2) indicated the significant decrease of chlorophyll content due to *P. cubensis* infection with about 18%. Fungicide treatments alone caused a very weak effect on the chlorophyll content, except propamocarb hydrochloride at high doses of application. The chlorophyll content was 0.83 and 0.67 mg/g fresh weight for control and 2 field rate of propamocarb hydrochloride treatment, respectively. Fungicide treatments in the presence of downy mildew pathogen increased the amount of chlorophyll content by increasing fungicides concentration, this phenomenon was observed for all four tested fungicides. This might be due to some degree of recovery by fungicide treatment. Although infection with *P. cubensis* pathogen alone decreased chlorophyll content as found by (Yarina *et al.*1993), it was noticed that fungicide treatments enhanced the activity of squash leaves to maintain a reasonable photosynthetic activity and these fungicide treatments had reduced the inhibitory effect of *P. cubensis* against leaves chlorophyll content (El-Sherief and Salem, 1998).

Table (2): *In vivo* effect of tested fungicides on chlorophyll content before and after 6 days of *Pseudoperonospora cubensis* infection, expressed as mg/g fresh weight of squash leaves.

Fungicides	Treatments					
	0.0	0.25 F	0.50 F	0.75 F	1.00 F	2.00 F
	Before infection					
Propamocarb hydrochloride	0.83 ± 0.19	0.84 ± 0.16	0.71 ± 0.13	0.87 ± 0.16	0.80 ± 0.14	0.67 ± 0.10
Mikal		0.82 ± 0.15	0.79 ± 0.11	0.81 ± 0.13	0.78 ± 0.13	0.82 ± 0.15
Mancozeb		0.75 ± 0.14	0.79 ± 0.14	0.82 ± 0.12	0.85 ± 0.15	0.87 ± 0.16
Ridomil-Plus		0.79 ± 0.15	0.80 ± 0.15	0.85 ± 0.15	0.89 ± 0.16	0.91 ± 0.17
	Post infection (after 6 days of *Pseudoperonospora cubensis* infection)					
Propamocarb hydrochloride	0.68 ± 0.16	0.64 ± 0.13	0.67 ± 0.09	0.70 ± 0.13	0.78 ± 0.15	0.81 ± 0.15
Mikal		0.69 ± 0.12	0.71 ± 0.12	0.73 ± 0.14	0.75 ± 0.14	0.76 ± 0.13
Mancozeb		0.59 ± 0.11	0.67 ± 0.09	0.69 ± 0.11	0.85 ± 0.16	0.93 ± 0.16
Ridomil –Plus		0.61 ± 0.12	0.63 ± 0.10	0.69 ± 0.10	0.77 ± 0.15	0.83 ± 0.17

Values are means ± S.E (n = 4). **F = Field rate.**

b) β-carotene content:

β-carotene content was decreased by 32% of the control value due to pathogen infection, it was from 29.3 and 19.9 μg/gm fresh weight for non-infected and infected leaves, respectively. The inhibition of pigment contents due to *P. cubensis* infection comes in agreement with Yurina *et al.* (1993). Mancozeb and Ridomil-Plus treatments decreased the β-carotene content, especially at high rate of application by about 30% of the control value, while both propamocarb hydrochloride and mikal showed insignificant decrease of the pigment content. Combination of the pathogen and fungicides did not decrease the plant pigment content. However, insignificant increase of β-carotene content was noticed especially at higher rate of mikal concentrations (Table 3). Treatments with fungicides alone or in combination caused a noticeable increase of chlorophyll and β-carotene content. These results agreed with those reported by Ahmed (1993) and Marei (2002), who found that fungicide treatments either alone or in combination with pathogen had caused a noticeable increase of chlorophyll and β-carotene contents.

Table (3): *In vivo* effect of tested fungicides on β-carotene content before and after 6 days of *Pseudoperonospora cubensis* infection, expressed as µg/g fresh weight of squash leaves.

Fungicides	Treatments					
	0.0	0.25 F	0.50 F	0.75 F	1.00 F	2.00 F
	Before infection					
Propamocarb hydrochloride	29.3 ± 2.1	25.1 ± 2.8	26.3 ± 2.7	24.6 ± 2.7	27.4 ± 2.5	28.9 ± 3.1
Mikal		29.6 ± 3.1	23.2 ± 3.1	24.9 ± 3.3	26.8 ± 2.7	27.6 ± 2.5
Mancozeb		25.3 ± 2.9	23.6 ± 2.9	20.4 ± 1.9	16.7 ± 2.9	21.3 ± 2.9
Ridomil -Plus		23.5 ± 1.9	21.7 ± 2.5	20.9 ± 2.9	20.2 ± 3.1	19.2 ± 1.8
	Post infection (after 6 days of *Pseudoperonospora cubensis* infection)					
Propamocarb hydrochloride	19.9 ± 1.7	21.5 ± 2.7	18.6 ± 2.4	17.3 ± 2.1	15.9 ± 2.1	20.1 ± 1.9
Mikal		18.8 ± 3.4	21.1 ± 2.1	15.4 ± 1.9	20.8 ± 3.4	22.3 ± 3.5
Mancozeb		19.2 ± 2.6	20.3 ± 1.9	19.7 ± 1.3	18.6 ± 1.5	17.6 ± 1.1
Ridomil -Plus		19.4 ± 1.9	18.8 ± 2.7	20.5 ± 1.8	20.8 ± 1.9	18.8 ± 2.1

Values are means ± S.E (n = 4). F = Field rate.

c) Sugar content

The effect of selected fungicides, pathogen and their combinations on sugar content is recorded in Tab1e (4). Infection of squash plant with *P. cubensis* caused an insignificant decrease of sugar content. The values of sugar content were 22.4 and 21.3 mg glucose/g fresh weight for uninfected and infected leaves, respectively. All fungicide treatments alone in the absence of pathogen caused a remarkable increase of sugar content especially at higher doses, except propamocarb hydrochloride which caused an insignificant decrease of the sugar content at all levels of application. Combination of both *P. cubensis* and fungicides showed the same trend of effect. Treatment with mikal either before or after infection with downy mildew pathogen caused the highest increase of sugar content. These results of increasing sugar content due to pathogen infection or fungicide treatments come in complete agreement with Ahmed (1997) and Marei (2002). However, the quantity soluble carbohydrates in plant tissues confirmly related to plant susceptibility (Cole, 1966 and Hwang *et al.*, 1983).

Table (4): *In vivo* effect of tested fungicides on sugar content before and after 6 days of *Pseudoperonospora cubensis* infection, expressed as mg glucose/g fresh weight of squash leaves.

Fungicides	Treatments					
	0.0	0.25 F	0.50 F	0.75 F	1.00 F	2.00 F
	Before infection					
Propamocarb hydrochloride	22.4 ± 2.9	20.1 ± 2.1	20.3 ± 2.2	20.7 ± 2.3	20.9 ± 2.3	21.1 ± 2.2
Mikal		25.3 ± 2.6	24.7 ± 2.4	26.8 ± 2.9	27.2 ± 3.1	28.4 ± 3.1
Mancozeb		23.2 ± 2.4	23.9 ± 2.6	24.6 ± 2.5	25.5 ± 2.9	26.4 ± 2.9
Ridomil –Plus		22.4 ± 2.2	23.8 ± 2.5	24.1 ± 3.1	24.7 ± 2.7	25.3 ± 3.4
	Post infection (after 6 days of *Pseudoperonospora cubensis* infection)					
Propamocarb hydrochloride	21.3 ± 2.4	21.6 ± 2.0	20.5 ± 2.7	22.9 ± 2.1	23.4 ± 3.4	24.2 ± 2.8
Mikal		25.3 ± 2.7	26.7 ± 3.4	28.1 ± 2.9	29.5 ± 2.8	30.2 ± 3.5
Mancozeb		24.8 ± 3.1	26.3 ± 3.9	27.2 ± 3.4	28.9 ± 2.1	29.5 ± 2.1
Ridomil –Plus		21.1 ± 1.9	22.4 ± 2.4	22.7 ± 2.5	23.9 ± 1.5	23.5 ± 2.3

Values are means ± S.E (n = 4). **F = Field rate.**

d) Polyphenol oxidase activity:

The results in Table (5) indicated the inhibitory effect of pathogen infection against squash polyphenol oxidase activity. Fungicide treatments caused inconsistent effect against this enzyme activity. Propamocarb hydrochloride was the strongest inhibitor against the enzyme activity especially at high doses. Combinations of both pathogen and fungicides were not able to restore the enzyme activity up to the control level. However, fungicide treatments were attenuated the inhibitory effect of pathogen infection, mancozeb was the highest and Ridomil-plus was the lowest one in this respect. Either pathogen infection of fungicide treatments have caused inconsistent effect against plant oxidoreductase enzyme activities. Although our resu

lts indicated some sort of polyphenol oxidase inhibitor, Yurina *et al.*(1990) found that pathogen infection stimulated the activity of these types of enzymes.

Table (5): *In vivo* effect of tested fungicides on polyphenol oxidase activity in squash leaves before and after 6 days of *Pseudoperonospora cubensis* infection, expressed as O.D values.

Fungicides	Treatments					
	0.0	0.25 F	0.50 F	0.75 F	1.00 F	2.00 F
	Before infection					
Propamocarb hydrochloride	0.69 ± 0.03	0.70 ± 0.03	0.68 ± 0.19	0.59 ± 0.17	0.54 ± 0.15	0.51 ± 0.10
Mikal		0.60 ± 0.02	0.57 ± 0.25	0.68 ± 0.20	0.73 ± 0.18	0.69 ± 0.12
Mancozeb		0.59 ± 0.01	0.63 ± 0.13	0.68 ± 0.19	0.67 ± 0.13	0.65 ± 0.11
Ridomil –Plus		0.52 ± 0.02	0.53 ± 0.11	0.57 ± 0.11	0.63 ± 0.16	0.69 ± 0.09
	Post infection (after 6 days of *Pseudoperonospora cubensis* infection)					
Propamocarb hydrochloride	0.47 ± 0.11	0.45 ± 0.02	0.44 ± 0.03	0.58 ± 0.29	0.57 ± 0.56	0.56 ± 0.46
Mikal		0.40 ± 0.17	0.53 ± 0.23	0.56 ± 0.08	0.52 ± 0.02	0.57 ± 0.22
Mancozeb		0.54 ± 0.27	0.58 ± 0.06	0.63 ± 0.04	0.62 ± 0.13	0.66 ± 0.38
Ridomil –Plus		0.47 ± 0.16	0.46 ± 0.54	0.49 ± 0.23	0.49 ± 0.18	0.52 ± 0.12

Values are means ± S.E (n = 4). **F = Field rate.**

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النشاط العلاجى والبيوكيماوى لبعض المبيدات الفطرية ضد مرض البياض الزغبى فى الكوسة

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تم إختبار الكفاءة العلاجية لخمسة مبيدات فطرية مختلفة ضد مرض البياض الزغبى فى نبات الكوسة بالإضافة إلى التغيرات البيوكيمائية للنبات العائل مثل الكلوروفيل والبيتاكاروتين والمحتوى السكرى للأوراق بالإضافة إلى نشاط إنزيم البولى فينول أكسيديز فى عدم وجود الإصابة وبعد حدوث الإصابة أيضاً.

وكانت المبيدات الفطرية المختبرة هى: بروباموكارب هيدروكلوريد (بريفيكور-ن)، ميكال، مانكوزيب (دياثين م-٤٥)، ميتالاكسيل (ريدومول-بلس) بالإضافة إلى تولكلوفوس-ميثايل (ريزولكس). ولقد أظهرت النتائج مايلى:

١- كانت أعلى للمبيدات كفاءة علاجية فى تقليل الإصابة بفطر زيدومونس كابنسيس هى بروباموكارب هيدروكلوريد، ميكال حيث أظهرا أقل معامل مرضى وأعلى علاج للنباتات المصابة وهذا عكس بقية المبيدات الفطرية الغير جهازية.

٢- الإصابة بفطر زيدومونس كابنسيس سبب نقص فى الكلوروفيل والبيتاكاروتين وأيضاً سبب تثبيط نشاط إنزيم البولى فينول أكسيداز بينما لم يتأثر محتوى السكر بالإصابة بالبياض الزغبى.

٣- معاملة المبيدات فقط أظهرت تأثيراً بسيطاً جداً للمحتوى من الصبغات (الكلوروفيل والبيتاكاروتين) والنشاط الإنزيمى البولى فينول أوكيديز بينما سببت زيادة معنوية للمحتوى السكرى خصوصاً مع معاملات مبيد الميكال.

٤- الجمع بين الإصابة والمبيدات الفطرية المختبرة سبب زيادة للمحتوى السكرى وأيضاً نشاط الإنزيم بينما المحتوى من الصبغات كانت زيادتها غير كبيرة ولم تصل إلى قيم النبات السليمة.