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Control of Powdery Mildew Disease by some Fungicides of Different Chemical Groups and their Side Effect on Squash

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SEEDS of squash (*Cucurbita pepo* Alexanderial var.) were germinated over two successive growing seasons at Gemmeiza Agricultural Research Station (El-Gharbia, Egypt) and sprayed three times during the vegetative stage by half dose or dose of Afugan, Calixin, Topas 100 and micronized sulfur fungicides for controlling powdery mildew disease. Spraying by the recommended dose of these fungicides did not increase the actual fungicide effectiveness (AFE) over that of Afugan (74%). On the contrary, directed squash metabolism towards synthesis and accumulation of stress metabolites affected its normal growth and yield processes. Chlorophyll pigments of squash leaves were sensitive to the fungicides, especially chlorophyll b. Chlorophyll a and b decreased at the dose treatment, while half dose increased chlorophyll a and decreased chlorophyll b. Sugars and protein contents of squash plants also decreased and Afugan and Sulfur caused the minimum decrease. Proline accumulated by the used fungicides especially at their recommended doses. Proline accumulated in response to water stress caused by the fungal infection and/or fungicides effect on water loss control. Proline also, accumulated in the plant due to inhibition in protein synthesis and to protein proteolysis. This study recommends using new doses of these fungicides suitable for the environment. Their formulations must exhibit the least effect on the host plant.

Keywords: Fungicides, Powdery mildew, Chlorophyll, Protein, Proline .

Cucurbits are the major source of vegetable foods and are cultivated in over than 243000 feddans about 26% of the vegetable crop area. Squash (*Cucurbita pepo* L.) is one of the most popular cucurbit crops in Egypt, which occupies alone about 57000 feddans. In addition to the above grown area by squash there is an additional production from plastic greenhouses spread greatly in Egypt. The causal factor fungi for powdery mildew disease was found to attack their hosts in the open fields and protected agricultures (Curger, 1974). They may cause losses reach to 30 – 80% from fruit yield of squash when the percentage of infection reaches to 50 - 100% (Curger, 1974 and El-Nagar *et al.*, 1991). The disease control can be achieved by using resistant cultivars and/or chemicals those may lose its efficacy if new patho-types of fungicide-tolerant form of fungus appear. The fact of increasing demand of integrated disease control led to production of great number of fungicides.

Fungicides in increased concentrations may exhibit phytotoxicity for host plants in addition to their effect on the disease causal factors (*e.g.*) (Zein *et al.*, 1990). This is because the biochemical processes in higher and lower plants are similar in many aspects. For example, the enzyme systems of fungi and higher plants have many common properties. Sulfur formulations when applied on cucurbit crops cause burns as well as roughening and frangibility of the leaves and some times defoliation (Gruzdyev, 1980). The same author found proper application of Sulfur formulations facilitates an increase in yield of crops and an improvement in their quality. The mode of action of a fungicide depends to a great extent to its formulation and components. For example elementary Sulfur found in different formulations (ground, colloidal and wettable Sulfur powder). Powder formulation is effective for preventing fungal spore formulation and maturation that decreased greatly re-infection of the host. The vapor of Sulfur evolved from the powder on the host leaf surface prevents the spore maturation. On the other hand, wet-able powders are effective on the fungal hyphae as it penetrate easily into the leaf cells. Also, the effective part of the fungicides is critical in its function in disease control. Those fungicides acquiring organic or inorganic or complexes of both differ in their control effectiveness.

The aim of the present study is to elucidate the effect of spraying with half dose or dose of some fungicides differing in their effective site on squash powdery mildew disease severity. It is also to study to what extent the biosynthesis of some metabolites process in the important vegetable crops (Squash) has been affected.

Material and Methods

Seeds of squash (*Cucurbita pepo*, Alexanderial) were surface sterilized by 1% $HgCl_2$, washed many times with sterilized distilled water and germinated in 5 x 2 meter plots which arranged according to split plot design with 5 replicates for treated and control treatments. The plots were subjected to normal irrigation and fertilization recommended systems for the crop. Experiment was conducted over two successive growing seasons 2001 and 2002 at Gemmeiza Agricultural Research Station, El-Gharbia, Egypt. The plants were subjected to spray, during the vegetative stage, by a half dose or a recommended dose of the studied fungicides. The common name, active ingredients and concentration of the recommended dose for each fungicide are presented in Table 1. These fungicides are commonly used for controlling *Sphaerotheca fuliginea* (Schlectex Fr., Poll), the causal fungi for powdery mildew disease of squash. Squash plants were sprayed three times with 15 days in between. Before each spray the severity of disease was determined for the calculation of the fungicide effectiveness (FE) and actual fungicide effectiveness (AFE). The measurements of powdery mildew disease severity and FE were carried out according to

Towsend & Heuberger (1943). Calculation of AFE was according to Elhaak & Ismail (2003). The dried materials were powdered by an electric micro-mill and stored in paper pages for further analysis.

TABLE 1. The used fungicides for controlling the powdery mildew on squash (*Cucurbita pepo*, Alexandrial).

Fungicide	Recommended dose	Active ingredients	Formulation
Afugan	100ml/100L water	30% pyrazophos	EC
Calixin	45ml/100L water	75% tridomorph	EC
Topas 100	25ml/100L water	10% penconazole	EC
Sulfur	250g/100L water	80% sulfur	W.P

W.P: Wettable powder E.C: Emulsifiable concntrate

Shoot samples, five plants each, were collected from sprayed and not sprayed (control) plots. These shoot systems were weighed as fresh and died at 80°C to constant weight for the determination of plant biomass and relative water content. Fresh leaves samples were clipped at random from plants of each plot and extracted by 85% acetone/water (v/v) for their content of the photosynthetic pigments chlorophyll a and b according to Metzner *et al.* (1965)

Dry shoot samples (0.5g) were extracted by using borate buffer (pH 7). The soluble sugars in the borate extracts were determined spectrophotometerically according the method of Naguib (1963). Protein content of the dry powders was extracted and determined according to Bradford (1976). The content of proline was extracted by sulphosalcilic acid and determined according to Bates *et al.* (1973).

The results of the two seasons were considered as replicates because the obtained differences were slight. Then these results were treated statistically by applying correlation coefficient t-test and analysis of the variance (ANOVA). The statistical analysis was according to Snedecor & Cochran (1973).

Results

Disease severity and fungicide effect

The represented results in Fig. 1 showed that the used fungicides decreased powdery mildew diseases severity to about one seventh as compared with the control. The maximum decrease in disease severity was by spraying Afugan, while the minimum was by Sulfur. It was also remarkable that the fungicides half doses caused less effect on the diseases severity as compared to their doses. The maximum and minimum values of disease severity after the half dose application were recorded for the same fungicides (Afugan and Sulfur respectively). Calculated effectiveness of fungicides (FE) did not exceed 80%

after their half doses application, but it slightly exceeded this percentage by their doses except for Sulfur. The maximum value of FE was by dose of Afugan while the minimum was by half dose of Topas 100. In general, the FE differed by increase fungicide concentration, where dose application led to greater FE than half dose by a mean difference of 19% (Table 3). The minimum difference was by Sulfur, while the maximum was by Topas 100. This indicated different sensitivity of causal fungi of the disease to the increase in the different fungicides concentration. The variations were highly significant of each disease severity and fungicides effectiveness by sprayed treatments of the fungicides (Table 2).

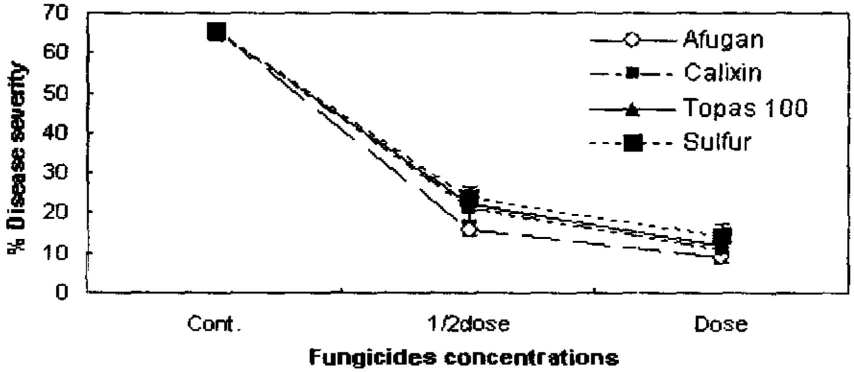

Fig. 1. The percentage of disease severity on *Cucurbita pepo* after application of half or recommended dose of the fungicides.

Plant weight and actual fungicides effect

Squash shoot weights varied significantly by the used fungicides (Table 2 & Fig. 2). The calculated percentages change in biomass (Table 3) showed a decrease ranged between 6.6 – 26.4% with a greatest under the effect of the dose of Calixin and a lowest under the dose of Sulfur. Except, for Topas 100 the plant weight was greater under dose application in comparison with half dose. The dose of Topas 100 decreased the plant biomass than half dose by 14%. The percentage difference between dose and half dose was little by Afugan and high by the other fungicides. The actual fungicide effectiveness (AFE) decreased than FE and by half dose than by dose one of all fungicides. The maximum AFE was by dose of Afugan while the minimum by half dose of Calixin. This resulted in great percentage difference between dose and half dose by Calixin.

Relative water content

The relative water content did not vary significantly by the application of dose or half dose of the used fungicides (Table 2 & Fig. 2). All of the used

TABLE 2. Analysis of variance (ANOVA) for the variations in % of fungicides effectiveness and disease severity, plant weight and the contents of water, chlorophyll a and b, sugars, protein and proline caused by the half dose or dose of the used fungicides on on squash (*Cucurbita pepo*).

Variables	ANOVA -test		T-test propablity levels for the difference between							
	F-value	Propablity level	Control and half dose treatments				Control and dose treatments			
			Afugan	Calixin	Topas100	Sulfur	Afugan	Calixin	Topas100	Sulfur
Fungicide effectiveness	79.57	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Disease sevirity	612.1	0.001	0.001	0.002	0.001	0.001	0.001	0.001	0.001	0.001
Plant weight	10.75	0.004	0.001	0.005	0.001	0.001	0.022	0.001	0.001	0.006
Water content	0.112	0.535	0.406	0.104	0.222	0.504	0.328	0.029	0.013	0.234
Chlorophyll a	4.753	0.039	0.071	0.011	0.283	0.002	0.361	0.173	0.181	0.271
Chlorophyll b	76.21	0.001	0.14	0.027	0.026	0.016	0.392	0.011	0.241	0.104
Chl. a/b ratio	0.081	0.924	0.061	0.011	0.321	0.031	0.254	0.08	0.362	0.291
Sugars content	1.767	0.225	0.001	0.001	0.001	0.501	0.001	0.568	0.022	0.405
Protein content	2.191	0.168	0.001	0.017	0.005	0.001	0.001	0.021	0.023	0.051
Proline content	0.599	0.571	0.001	0.011	0.002	0.001	0.364	0.001	0.002	0.001

P < 0.05 is significan P < 0.01 is high significant fungicides on squash decreased relative water content except dose and half dose of Calixin those increased it by 7 and 28% respectively. In general the relative water content was higher under the half dose than the dose application.

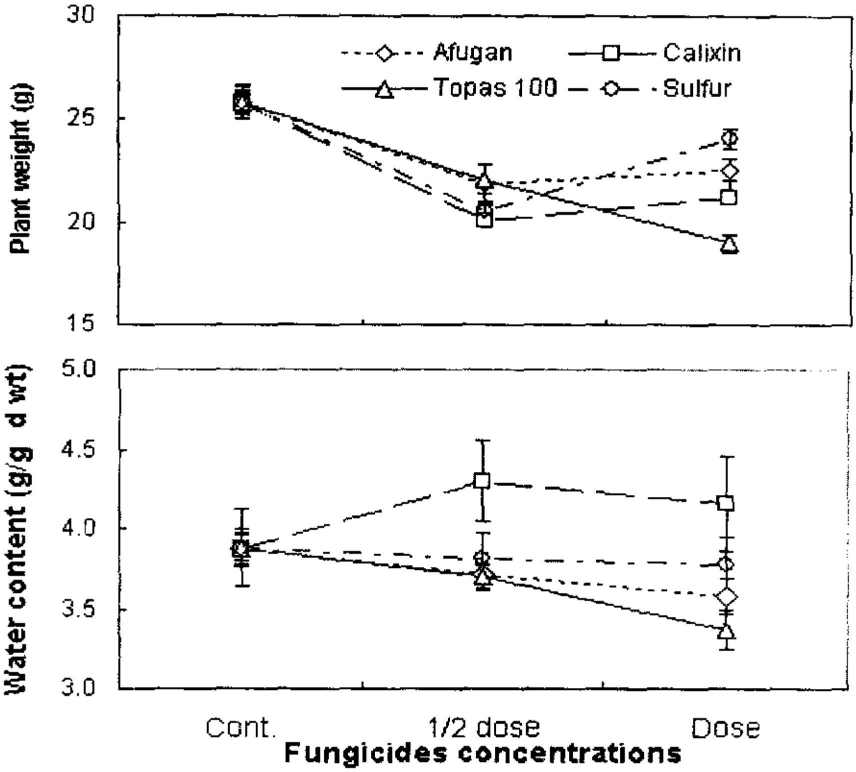

Fig. 2. Plant weight (g) and water content (g/g d. wt) for *Cucurbita pepo* under the effect of half and recommended dose application of the fungicides .

Photosynthetic pigments

The recorded content of photosynthetic pigments (chlorophyll a and b) in squash varied significantly (Table 2 & Fig. 3). The content of the two pigments decreased by the dose of the used fungicides, except for Topas 100. The least content of the two pigments was recorded by spraying Calixin dose. In general, the content of both pigments was also greater under half dose than under dose spraying. Chlorophyll a increased by the half dose of all fungicides except Afugan, the highest chlorophyll a was by that of Sulfur. The greatest increase in chlorophyll a by the half dose in comparison with the control value was by Topas 100. Chlorophyll a was greater than b in squash leaves by all spraying treatments, but their ratio did not vary significantly. Dose spraying of the used treatments, but their ratio did not vary significantly. Dose spraying of the used fungicides decreased the ratio of a/b over that of the control except that of Topas 100. On the opposite, spraying by half dose of the used fungicides increased the a/b ratio than that of the control, except by that of sulfur.

TABLE 3. The used fungicides for controlling powdery mildew on squash *(Cucurbita peppo)*, fungicide effectiveness, change in biomass of host and actual fungicide effectiveness.

Fungicide	Fungicide effectivness (FE)			% Change in biomass (CB)		Actual fungicide effectiveness (AFE)		
	HD	D	%	HD	D	HD	D	% D
Afugan	75.5	86.4	14	-15.0	-12.4	60.5	74.0	22
Calixin	67.9	83.2	23	-22.2	-17.7	45.7	65.5	44
Topas 100	66.3	81.9	24	-14.3	-26.4	52.0	55.5	7
Sulfur	73.5	78.1	6	-20.4	- 6.6	53.1	71.5	35

HD: Half dose D: Dose % D: Percentage difference

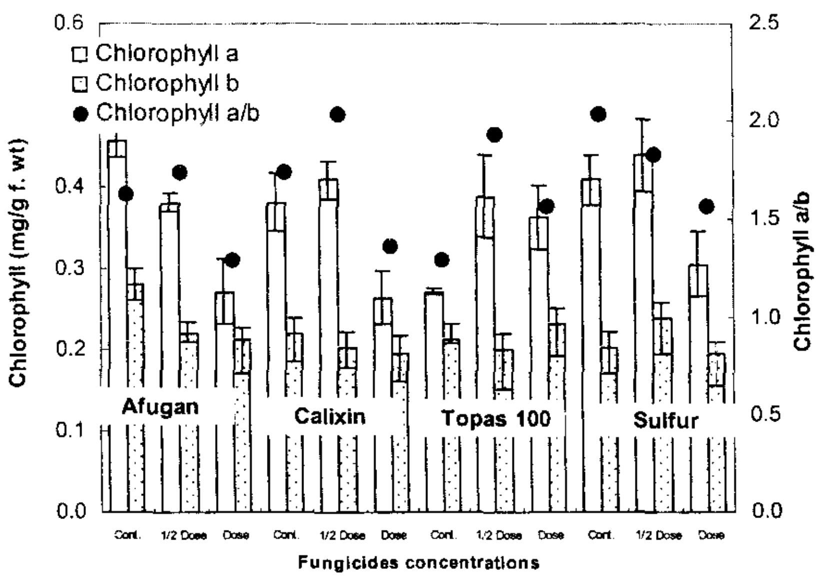

Fig . 3. Variations in chlorophyll a and b and their ratio in *Cucurbita pepo* under the effect of half and recommended dose of the fungicides .

Soluble sugars, protein and proline

The reducing sugars content (Fig. 4) increased by the used fungicides, except spraying by the recommended dose' of Topas 100 and Sulfur. The highest accumulation of sugars was by both half dose and dose of Afugan and it was by 74.4 and 79. 5% as compared with the control for the two treatments respectively. Half doses of the used fungicides increased the content of reducing sugars as compared with their doses especially the half dose of Sulfur that doubled the sugar content. This indicated high sensitivity of the plant to the increase in Sulfur spraying. Statistical analysis (Table 2) indicated that the obtained variations in soluble sugars of squash were significant ($P < 0.05$). The used fungicides did not affect greatly the content of protein in squash, except Afugan which decreased the content to its half or lower. In general, dose application did not decrease the protein contents in the squash leaves as caused by half dose spraying. Proline content increased by all used fungicides treatments, except the half dose and dose of Afugan and Topas 100 which decreased it. The plants subjected to dose application contained greater proline content than those subjected to half dose, except for Topas 100. The greatest accumulation of proline was by spraying the dose of Calixin.

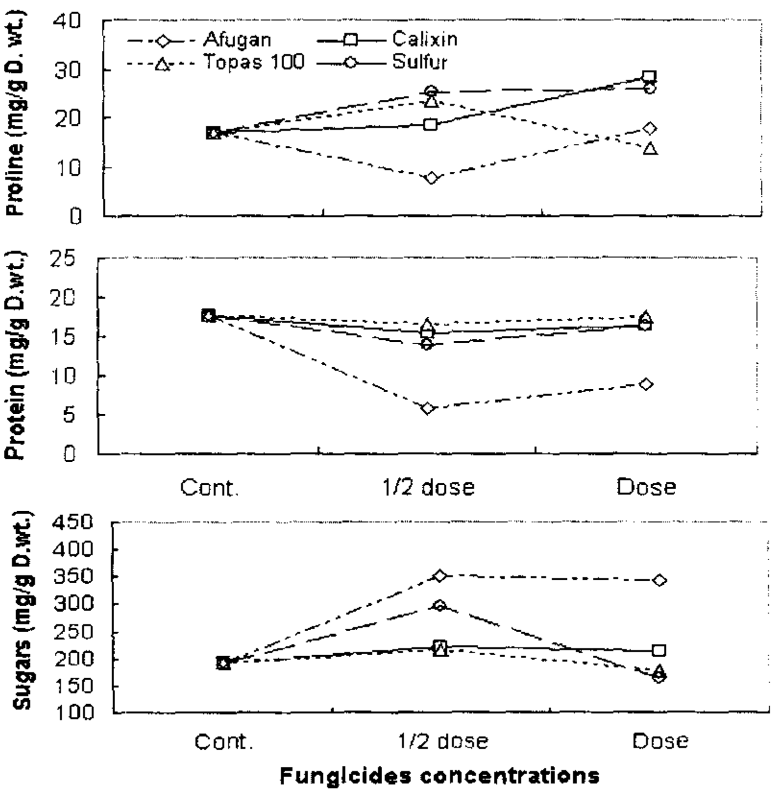

Fig. 4. The variations of proline, proteins and sugars contents in *Cucurbita pepo* under the effect of half or recommended dose of the fungicides.

Relationships between the recorded data under the used fungicides

Table 4 showed that the fungicides effectiveness and disease severity of squash acquired significant positive correlations with plant weight, and its content of photosynthetic pigments (Chlorophyll a and b). Protein content also, correlated significantly with the content of sugars and chlorophyll a. The plant weight was found to correlate with its content of chlorophyll a and b. The two photosynthetic pigments were correlated with each other in squash.

TABLE 4. The correlation matrix of the obtained results for squash (*Cucurbita pepo*)

	FE	DS	PW	WC	Ca	Cb	a/b	Su	Pt	Pl
FE	1.000									
DS	0.770**	1.000								
PW	0.794**	0.781**	1.000							
WC	-0.187	0.053	-0.284	1.000						
Ca	0.584*	0.709**	0.782**	0.188	1.000					
Cb	0.904**	0.921**	0.926**	-0.147	0.751**	1.000				
a/b	-0.217	-0.090	0.009	0.479	0.558	-0.121	1.000			
Su	-0.372	-0.424	-0.283	-0.125	-0.524	-0.388	-0.230	1.000		
Pt	0.397	0.545	0.306	0.199	0.611*	0.442	0.319	-0.934**	1.000	
Pl	-0.309	-0.239	-0.082	0.167	0.324	-0.248	0.774**	-0.293	0.404	1.000

FE: Fungicide effectiveness DS: Disease severity PW: Plant weight WC: Water content Ca: Chlorophyll a Cb: Chlorophyll b a/b: Chl. a/b ratio Su: Sugar Pt: Protein Pl: Proline

Discussion

The used fungicides in the present study decreased the severity of powdery mildew diseases on squash to one seventh. Chemical spraying was found early by El-Helaly *et al.* (1968) to decrease powdery mildew infection and increased squash yield. The greatest inhibition to the disease severity was after spraying Afugan, as also found by Pappas (1985) and Mahrishi & Sirdhana (1988), while the least one was by Sulfur. The decrease in disease severity was a result of the different effects caused by the fungicides used in this study. Gabriele *et al.* (1997) found that penconazole (Topas 100) prevent hyphal development and caused growth distortion with hyphal tip swelling. Gruzdyev (1980) found that sulfur powder prevented fungal spore formulation and maturation that decreased greatly re-infection of the host. The percentages of fungicides effectiveness ranged between 66 and 76 and between 78 and 86 for half dose and dose application respectively. The used fungicides were found to affect both fungus and host plant. The change in squash plants biomass ranged between 14 to 20% decrease under the half dose of the used fungicides. These inhibitions in plant growth activity were improved by increasing the fungicides (except Tops 100) concentration to the dose. The maximum effect was after the dose of sulfur (20.4 to 6.6%). This may indicate that application of the used fungicides, except Topas 100, in the recommended dose under experimental conditions have little effect on the plant and most of the changes in plant biomass were mainly due to the disease severity. El-Shenawy *et al.* (1987) found infection by powdery mildew significantly decreased the growth hormone GA. El-Helaly *et al.* (1968) found also, squash yield was a function of powdery mildew infection that decreases by the chemical spray. Gruzdyev (1980), after writing that sulfur causes burns as well as roughening and frangibilty of squash leaves, recorded that proper application of sulfur formulations facilitated an increase in the yield of the crop and an improvement in the quality. Dose application of Topas 100, on the other hand, inhibited remarkably the squash growth by nearly double the amount caused by the half dose. This indicated high sensitivity of the plant to the increase in fungicide concentration. However, the actual fungicides effectiveness (AFE) must be decreased by their percentage change to the host plant growth (Elhaak & Ismial 2003). The AFE decreased remarkably under the experimental agricultural conditions. The minimum AFE was by the half dose of Calixin (45.7%), while the maximum was by the dose of Afugan (74.0%).

The used treatments of the fungicides decreased the plant shoot water content. This decrease could be due to the mechanical injury resulted from fungal penetration into the leaf surface that increased water loss (El-Shenawy *et al.* 1987). Omar *et al.* (1985) found also infection by powdery mildew significantly increased water deficit signals as the increase in ABA. The recorded lower water content under dose application indicated that increasing concentration of the fungicides on the plant leaves could interfere with their stomatal control to water loss. De Vos *et al.* (1989) referred increased water loss to the uncontrolled redox reactions occurred with the excess supply of fungicides, giving rise to the formation of toxic free radicals that may lead to lipid peroxidation and membrane leakage. Deposition of polymers in cell walls and liginification has also been recorded in various hosts (Elad, 1997).

The used fungicides differently affected the metabolism of Squash photosynthetic pigments. Dose application inhibited the metabolism of each chlorophyll a and b while half dose enhanced that of chlorophyll a and inhibited that of chlorophyll b, except the half dose of Sulfur that enhanced the metabolism of both pigments. These results showed a sensitivity of the two pigments for the used fungicides, chlorophyll b acquired the greatest value. This sensitivity could be one of the reasons for the recorded decrease in squash biomass after the fungicides application (Wood, 1967; Soliman, 1976; Omar, 1977 and Farahat, 1980). The different inhibition in the content of chlorophyll a and b by the different concentrations of the fungicides changed their ratio together (a/b). The fungicides dose decreased chlorophyll a/b ratio giving an indication of greater effect on chlorophyll a. Half dose mostly increased a/b ratio as a result of both decrease in chlorophyll b and increase in chlorophyll a. The highest ratio was by the half dose of Calixin. These obtained ratios confirmed the differential sensitivity of the two chlorophyll pigments.

The sugar content deceased in the infected plants with powdery mildew disease and the disease spread was enhanced in the plant by increasing its sugar content (Nilova & Ksendova, 1966; Omar, 1977; Ghobrial *et al.*, 1982 and El-Shehidi *et al.,* 1985). This was also confirmed in the present study by the resulted significant relationship between the disease severity and the sugars content. The & fungicides enhanced the synthesis and accumulation of sugars in squash, but increasing their doses inhibited the process, especially Topas 100 and Sulfur. The greatest accumulation of sugars was by the application of each half dose or dose of Afugan. The accumulation of sugars may result from inhibition in their utilization. While, increase the fungicides concentration inhibited their synthesis, in addition, partly by Afugan and Calixin or totally by Topas 100 and Sulfur. Vijaya & Lalithakumari (1999) found the decrease in sugars a result of significant variation in the rate of total lipid and ergosterol biosyntheses after penconazole (Topas 100) application.

Protein biosynthesis in squash was more or less reduced by the used fungicides. Their doses spraying slightly inhibited the process as compared with their half doses. The maximum reduction was by Afugan. Proline on the other hand, increased in squash by spraying with most treatments of the used fungicides. Their doses enhanced more proline accumulation in comparison with the half doses. The greatest accumulation of proline was by spraying the dose of Calixin. Proline was found to accumulate in response to water, salinity, temperature and chemical stresses (Hsiao, 1973). However, in the present study, proline may be accumulated accompanying the decrease in water content caused by the fungal or fungicides effect on the surfaces of plant leaves. Proline also, accumulated in the plant on the other side as a result of inhibition in its utilization in protein synthesis and of protein proteolysis by the used fungicides as indicated also by Hsiao (1973).

In conclusion, the study recommended utilization of the fungicides under consideration in modified doses suitable for the plant environment and their formulations must exhibited the least effect on the host plant Results showed application of the used fungicides in the recommended dose did not result in actual fungicide effectiveness over 74%. In addition, the plant directed its metabolism towards stress metabolites that affect normal growth and yield processes. The best results were after spraying Afugan and Sulfur fungicides.

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مقاومة مرض البياض الدقيقى ببعض مبيدات الفطريات واثارها الجانبية على نبات الكوسة

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نميت بذور نبات الكوسة لمدة موسمين زراعيين فى محطة البحوث الزراعية بالجميزة محافظة الغربية بمصر رشت خلالهما ثلاث مرات إثناء مرحلة النمو الخضرى بنصف الجرعة او الجرعة الكاملة الموصى بها من مبيدات افوجان، كالكسين، توباز ١٠٠ و الكبريت الميكرونى وذلك بغرض مقاومة مرض البياض الدقيقى. هذا ولم تزيد كفاءة المبيدات المستخدمة بالجرعة الكاملة عن ٧٤% وهى تلك التى كانت للأفوجان. ولكنها على العكس من ذلك فقد ادت الى توجيه النشاط البنائى للنبات تجاه تراكم المواد المضادة للإجهاد مما اثر على نمو النبات وانتاجيته.

وقد كانت صبغات البناء الضوئى للنبات اكثر حساسية لهذه المبيدات وخاصة كلوروفيل ب. نقص محتوى النبات من السكريات والبروتينات وقد كان ذلك على اقل نطاق مع استخدام الأفوجان والكبريت. تراكم البرولين وكانت اكبر قيمه مع الجرعة الكاملة من المبيدات. وكان هذا التراكم راجعا فى معظمه الى الإجهاد المائى الناتج من العدوى بالفطر او تأثير المبيدات على تحكم الثغور للفقد المائى. وكان ايضا تراكم البرولين راجعا الى عدم استخدامه فى تكوين البروتين وكذلك لتحلله. وتقترح الدراسة استخدام جرعات اخرى مناسبة للظروف السائدة ويكون لها اقل تأثير على النبات المستخدمة عليه.