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Role of enzymes on the susceptibility of three cucumber varieties to the infestation with whitefly *Bemisia tabaci* Genn in Egypt

Fatma, S. Ismail[1], Wedad A. Atwa[1], Enayat, M. El qady [1] and Mona, I. Ammar[2]

[1] *Zoology and Entomology Department- Faculty of Science (Girls) - Al-Azhar University.*
[2] *Plant Protection Research Institute, ARC. Dokki, Giza, Egypt*

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ABSTRACT

Background: Whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), consists of genetically diverse species known to cause significant destruction in several crops around the world. The vegetable cucumber (*Cucumis sativus*.) is a popular one and has a flavor that is both fresh and unique. Cucumbers, like other fruits and vegetables grown commercially, have long been the subject of research efforts to increase their economic viability. The investigation was carried out to study the influence of enzymes and phyto-chemical components of three varieties of cucumber plant (F1 hybrid Muluki, F1 hybrid Hayal, and F1 hybrid Basha) on the population dynamics of whitefly (*Bemisia tabaci* Genn.). Concerning phenoloxidase enzyme, peroxidase, GST enzyme, α-esterases, β-naph-tholα-naphthol, ions (N, P and K) and total proteins. The highest level of the enzyme was found in Muluki and Hayal, while moderate amount was found in Basha variety. The obtained results showed level of influence pronounced differences in the population dynamics of this pest species on the tested plant varieties. A positive relationship between protein and carbohydrate contents. According to these results, resistant varieties can be recommended in the integrated pest management programs for depressing tested pests on cucumber plants.

Graphical abstract

To study the influence of three varieties of cucumber plant on the population dynamics of whitefly (*Bemisia tabaci Genn*).

Results recorded a positive relationship between total protein, total carbohydrate, free amino acids, total phenols, and total flavonoids content additionally, the plant enzymes namely, GST, Phenol-oxidase, and Peroxidase.

⁎ *Corresponding author*
E-mail address: Fatmaismail1886@gmail.com

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1. Introduction

Around the world, *Bemisia tabaci* is regarded as a damaging insect pest of different crops. This pest actively damages crop while feeding on them, and it indirectly damages crop by spreading viral infections. Despite several disadvantages, the management of *B. tabaci* mainly relies on pesticides. The IPM strategy, which incorporates the use of biological control and is solely based on the efficient use of natural parasites (parasitoids), predators, and entomopathogens, is therefore believed to be a safer and more effective control measure to reduce *B. tabaci*. Studies have clearly shown that using entomopathogenic fungi to manage *B. tabaci* biologically is a successful strategy [1].

The insect fauna on most field vegetable crops has been studied in Egypt [2,3] but further studies are needed to assess the relationship between the chemical constituents of plant varieties and the levels of population densities of the whitefly insects. So, the resistant cucumber varieties played a very important role in suppressing the insect population and should be considered in the integrated pest management programs [4,5]. Therefore, the current study aims to determine the effect of three cucumber varieties on the attraction of whitefly insects.

Cucumber (*Cucumis sativus*.) belongs to the family curcubitaceae and is one of the most important vegetable crops in Egypt. It is infested by many pests which are great economic pests of cucurbitaceous vegetable plants that cause serious damage directly by sucking plant juice and indirect damage by phytopathogenic transmission [6,7,8].

Despite the cucumber's growing importance, several problems, including a lack of proper planting materials, restricted access to financing, climatic conditions, and plant pests and diseases, among others, severely restrict output. The culture and production of C. sativus are severely hampered by more than 40 illnesses brought on by bacterial, fungal, viral, and nematode pathogens. The importance of identifying and treating insect pests before infestation is highlighted by reports that they devour enough crops to feed an additional one billion people on a global scale [9].

2. Materials and Methods

Greenhouse Experiments:

Experiments were conducted in a greenhouse at National Research center, Dokki, Giza, Governorate, Egypt during two successive seasons (October 2021 and October 2022). Cucumber seedlings of three cucumber varieties namely, F1 hybrid Muluki, F1 hybrid Hayal, and F1 hybrid Basha were transplanted on September 2021 and September 2022 in the greenhouse. The area of the greenhouse was 9×40 m². The inspection started on the 8thof October, after sowing for 18 days. A sample of 15 leaves / replicate and three replicates for each cucumber variety were collected randomly in the early morning each week until the harvest to investigate the sensitivity of the tested cucumber varieties depending on the general mean number of whitefly infestation (X) and the standard deviation (SD). The usual field procedures were practiced, and the experimental area was kept without any pesticide application.

The sensitivity of the investigated cucumber varieties was classified according to the overall mean number as follows:

a) Highly susceptible (HS) = more than X +2 SD
b) Susceptible (S) = between X and X +2 SD
c) Low resistant (LR) = between X and X -1 SD
d) Moderately resistant (MR) = between X -1 SD and X -2 SD
e) Highly resistant (HR) = less than X -2 SD.

Samples of cucumber leaves on October for each variety were taken at the fruiting stage for determining the phytochemical components and enzyme contents. The total protein, total carbohydrate, free amino acids, total phenols, and total flavonoid content were determined according to the methods of [10,11,12,13,14,15,16].

Additionally, the plant enzymes namely, glutathione S-transferases, phenoloxidase, and peroxidase were determined according to the methods of [15,16,17,18,19]. The tested plant enzymes and phytochemical leaf components were analyzed and determined at Chemical Analysis Constituent, Insect Physiology Dept., Plant Protection Research Institute. The obtained data were statistically analyzed by using the SAS program including F-test, simple correlation and explained variance. Revised least significant differences (LSD) at 5% levels of probability were used for comparing means.

3. Results and discussion

The common vegetable cucumber (*Cucumis sativus*.) is grown all over the world and frequently consumed raw. Investigated in this study were the phytochemical and proximate components of cucumber. The homogenate of the C. sativus fruit underwent quantitative phytochemical analysis, and the results revealed that reducing sugars were present in the highest concentration compared to other phytochemicals, alkaloids and flavonoids were present in a moderate amount, and cyanogenic glycoside was found in the least amount. According to a preliminary investigation, C. sativus fruit has the following nutrients: fiber, moisture, protein, lipids, carbohydrates, and ash[20]

Bemisia tabaci is considered one of the most important agricultural pests globally. Besides being highly polyphagous, this insect can cause physiological disorders and loss of vigour while facilitating the growth of sooty mould (*Capnodium* spp.) associated with the excretion of honeydew. However, *B. tabaci* stands out by its ability to transmit hundreds of plant viruses, from the genera *Begomovirus, Carlavirus, Crinivirus, Ipomovirus, Torradovirus and Polerovirus*. *Bemisia tabaci* is now considered a complex of distinct cryptic species, in which the Middle East-Asia Minor 1 (MEAM1, former B biotype) and Mediterranean (MED, former Q Biotype) are the most invasive and destructive species worldwide.

The investigation was carried out to study the influence of enzymes and phytochemical components of three varieties of cucumber plant (F1 hybrid Muluki, F1 hybrid Hayal, and F1 hybrid Basha) on the population dynamics of whitefly (*Bemisia tabaci* Genn.). Our study was a novel study.

Sensitivity of cucumber varieties namely**,** Hayal, Muluki, and Basha under field conditions to whitefly (*B. tabaci* Genn.) infestation during two successive winter seasons, October 2021 and October 2022 was studied. Relation of sensitivity with phytochemical components and plant enzymes was also evaluated.

Results showed that the cucumber varieties revealed significant difference of sensitivity throughout the two studied seasons, (Table 1). The deposited egg of *B. tabaci* Genn during season 2021 differed significantly between the variety Hayal, Muluki, and Basha. The mean numbers were 9.8, 6.2, and 7.7egg/ leaf during the season, 2021 respectively. (F value =6.01*, L.S. D=32.42.) On the other hand, the deposited egg was 10.2, 5.4 and 9.0 during season 2022, respectively (F value =7.10**, L.S. D= 20.31)

The sensitivity of the tested cucumber varieties might be classified depending on the overall mean and LSD value of *B. tabaci* Genn egg into three groups (Hayal Muluki, and Basha), the highest mean number was recorded with Hayal variety followed by the other tested cucumber varieties (Muluki and Basha).

The sensitivity was also classified into two degrees. The 1st group was susceptible to degree "S" represented by Hayal variety with a mean of 14.9 egg/leaf of the two seasons, while the other tested varieties (Muluki and Basha) were the second group with low resistant degree (LR) infested with low numbers with a mean 8.9 and 8.35 egg/leaf, (Table 1).In case of the nymph, a significant difference in sensitivity was recorded between the three investigated cucumber varieties during two seasons 2021 and 2022, depending on the overall mean numbers of *B. tabaci* Genn (nymph /leaf). In the first season, the infestation with *B. tabaci* Genn nymphs was recorded for three varieties (Hayal, Basha and Muluki) with means 10.6, 9.8 and 8.2 nymph/ leaf and two groups, respectively. (F value =11.88***, L.S. D= 33.31), whereas, in the second season, the infestation with *B. tabaci* Genn nymph was recorded on the three tested cucumber varieties (Hayal, Basha and Muluki) with mean numbers 8.8, 8.5, and 7.0 nymph/leaf, respectively for three groups (Hayal, Basha and Muluki). (F value =2.70*, L.S.D= 33.58)

The sensitivity was also classified into two degrees. The 1st group was susceptible degree "S" represented by Hayal and Basha varies with a mean of 9.95 and 9.3 nymph/leaf of the two seasons, while the other tested variety Muluki showed the second group low in resistant degree (LR) infested with low numbers with a mean of 8.35 nymph/leaf.

The present results agree with previous findings [23,24] which mentioned that the tomato whitefly, *Bemisia tabaci*, is considered as one of the most serious insect pests of cucumber plants. The obtained results showed pronounced differences in the population dynamics of this insect on the tested plant varieties (F1 hybrid Myadine, F1hybrid Hayal, and F1 hybrid Reda).

[25] showed that the integration of these biopesticides in the management schedule of the whitefly under protected conditions will enhance the quality and market value of parthenocarpic cucumbers.

Table (1): Sensitivity infestation with whitefly: *Bemisia tabaci* Genn. on three different cucumber varieties.

Var.	Mean no. of egg/leaf		Overall mean	Sensitivity degree	Mean no. of nymphs/leaf		Overall mean	Sensitivity degree
	2021	2022			2021	2022		
Hayal	9.8±3.7a	10.2±3.7a	14.9a	S	10.6±2.6a	8.8±2.8a	9.95a	S
Muluki	6.2±1.7b	5.4±2.1b	8.9b	LR	8.2±2.3b	7.0±2.3b	8.35c	LR
Basha	7.7±3.9ab	9.0±3.6a	8.35c	LR	9.8±3.0a	8.5±2.7ab	9.3b	S
F value	6.01*	7.10**	74.10**		11.18***	2.70*	50.3**	
L.S.D. value	32.42	20.31	0.20		33.31	33.58	0.41	

SD = Standard Deviation **LSD =** Least Significant Difference
Susceptible (S) = between $\bar{X}$ and $\bar{X}$ +2SD **Low resistant (LR)** = between $\bar{X}$ and $\bar{X}$ -1SD

Enzymes, phenoloxidase, peroxidase, and glutathione s-transferase (GST) were estimated in the leaves of the three investigated cucumber varieties, and the infestation rates with the whitefly, *B. tabaci* Genn were discussed according to the content of such enzymes.

Results showed that the cucumber varieties were insignificantly different in their levels of the three investigated plant enzymes (Table 2). Concerning phenoloxidase enzyme, the highest level of the enzyme was found in Muluki variety with a mean of 9.51 ΔO.D. units/min/gdw, followed by the moderate enzyme level represented with Hayal variety (9.07 ΔO.D. units /min/gdw). On the other hand, the lowest level was represented in the Basha variety recording 8.73 ΔO.D. units/min/gdw, respectively.

In case of peroxidase enzyme, leaves indicated insignificantly differences in their levels for the three investigated plants Basha variety contained the highest enzyme activity followed by Muluki variety with significant differences from other tested varieties.

Table (2): Levels of plant enzymes in leaves of the three tested cucumber varieties.

VAR.	Plant Enzymes					Plant composition					
	Phenoloxidase (ΔO.D. units/min/gm)	Peroxidase (ΔO.D./min/gm)	Glutathione S-transferase (GST) (mmolsub. Conj./min/gm)	A-esterases (ug α-naph-thol/min/gm)	B-esterases (ugβ-naph-thol/min/gm)	Total pro-teins (mg/g)	Total car-bohy-drates (mg/gm)	Total phenols (mg/gm)	N (mg/gm)	P (mg/gm)	K (u/gm)
Hayal	9.07	2011.67B	10.10B	207.00A	154.33A	7.17 B	17.70AB	2.20	1.13B	0.42A	12.27A
Muluki	9.51	3197.67A	10.90B	201.67A	144.00A	8.73 A	20.03A	2.37	1.55A	0.37B	12.05A
Basha	8.73	3257.00A	19.87A	148.00B	85.00B	8.37 A	15.40B	2.14	1.43A	0.30C	9.53B
F value	2.75INSIG.	171.81***	159.10***	27.63**	20.98**	11.33*	11.33*	3.44 IN-SIG.	25.30**	18.61**	45.76**
L.S.D		210.4	1.68	24.37	32.06	0.95	2.70		0.16	0.05	0.88

Means with different letters were significantly different at p≤0.05. If two means have the same letter, they were not significantly different, at p<0.05. The mean with letter a was the highest. * Represent statistically significant at 0.01. ** represent statistically significant at 0.05. ***represent statistically significant at 0.001.

Table 3: Correlation between the infestation of *B. tabcai* nymphs and studied plant enzymes in leaves of cucumber varieties.

VAR.		Phenoloxidase	Peroxidase	Glutathione s transferase (GST)	A-ester-ases	B esterases	Total pro-teins	Total carbohy-drates	Total phenols	N	P	K
Hayal	r	0.39	-0.31	0.08	0.58*	0.22	-0.34	0.21	0.12	0.16	0.12	0.15
	P	0.19	0.32	0.78	0.04	0.47	0.26	0.49	0.69	0.60	0.69	0.63
Muluki	r	0.286	0.94*	0.42	0.56*	0.227	-0.96 *	0.83*	-0.32	0.25	0.32	0.27
	P	0.36	0.02	0.22	0.05	0.47	0.01	0.003	0.30	0.42	0.30	0.38
Basha	r	0.39	0.91*	0.32	0.64*	0.26	0.95*	-0.77	-0.81*	-0.87	-0.92	- 0.89
	P	0.20	0.03	0.31	0.02	0.40	0.02	0.04	0.01	0.01	0.02	0.04

Means with different letters were significantly different at p≤0.05. If two means have the same letter, they were not significantly different, at p<0.05. The mean with letter a was the highest. * Represent statistically significant at 0.01. ** represent statistically significant at 0.05. ***represent statistically significant at 0.001

Table 4: Effect of enzymes of leaves the developmental stage on the population densities of *B. tabcai* nymphs on cucumber plants.

Var.	Phenoloxidase	Peroxidase	Glutathione stransferase (GST)	A-esterases	B-esterases	Total pro-teins	Total carbohy-drates	Total phe-nols	N	P	K
Hayal	16%	10%	7%	28%	5%	12%	4%	2%	3%	2%	3%
Muluki	8%	5%	18%	27%	5%	4%	2%	10%	6%	10%	8%
Basha	15%	10%	10%	42%	7%	0.4%	0.8%	0.6%	0.9%	0.2%	0.6%

Peroxidase enzyme showed a wide range in tested varieties (3257.00& 3197.67ΔO.D.$_{430}$/min/, respectively). The Hayal variety recorded the lowest activity of peroxidase enzyme (2011.6 ΔO.D.$_{430}$/min/gdw). The reported amount of GST in cucumber leaves was 19.87 mM substrate conjugated/ min/ gdw with Basha variety. The Muluki and Hayal varieties showed a moderate level of GST enzyme (10.90- & 10.10-mM substrate conjugated/ min/ gdw, respectively).

The reported activity of α-esterases (ug α-naphthol in cucumber leaves was 207.00 and 201.6mM substrate conjugated/ min/ gdw with Hayal and Muluki varieties, respectively. The Basha varieties showed the lowest activity of α-esterases (ug α-naphthol enzyme (148 mM substrate conjugated/ min/ gdw). In the case of β-naphtholα-naphthol, cucumber leaves of Hayal variety had the highest enzyme activity followed by Muluki variety with significant differences from other tested varieties. β-naphtholα-naphthol enzyme showed wide range in tested varieties (154.33 & 85.00ΔO.D.$_{430}$/min/gdw). The Basha variety contained the lowest activity of β-naphtholα-naphthol enzyme (85.00ΔO.D.$_{430}$/min/gdw) (Table 2).

The obtained data illustrated significant differences between the leaves content of three plant enzymes (phenoloxidase, peroxidase, GST, A-esterases(ug α-naphthol and β-naphtholα-naphthol) during the two seasons. A low enzyme level was found in Basha variety (phenoloxidase) Moreover, the Basha variety contained the highest amount of peroxidase enzymes (Table 2).

Data in table 3 showed that in Hayal variety, there was a significant positive relationship between A-esterase enzymes and the deposited nymphs of *B. tabaci* whereas r value = 0.58 with EV% equal to 28%. According to the correlation coefficient values, there was a significant positive relationship between the fluctuation of *B. tabaci* nymph and both of peroxidase and A-esterases enzyme leaves contents with r = 0.94, 0.56, 0.91and 0.64, respectively with EV% equal 5, 27,10 and 42%, respectively for varieties Muluki and Basha.

As shown in table 4; Hayal was found to contain high level of phenoloxidase, A-esterases, total proteins and low level of peroxidase, glutathione stransferase (GST), B-esterases, total carbohydrates, total phenols, and ions (N, P and K). Muluki was found to contain high level of glutathione stransferase (GST), A-esterases, ions (N, P and K) and low level of phenoloxidase, peroxidase, B-esterases, total proteins, total carbohydrates, and total phenols. Basha was found to contain high level of phenoloxidase, A-esterases, and low level of peroxidase, glutathione stransferase (GST), B-esterases, total carbohydrates, total proteins, total phenols, and ions (N, P and K).[26] study was carried out to clarify the effects of different cucumber cultivars (Ghazeer, Melouky, and Zeen) secondary metabolites on antioxidant enzymes of whitefly, Bemisia tabaci (Gen.), catalase, CAT, and superoxide dismutase (SOD). Results showed that the antioxidants enzyms, CAT and SOD were found to be increased in the cultivar Melouky, which had a high content of plant secondary metabolites. Also, results showed that increasing levels of plant secondary metabolites tend to increase B tabaci CAT and SOD levels.

According [27] herbivorous insects have developed protective antioxidant systems in response to ingesting plant secondary metabolites. Reactive oxygen species (ROS) toxicity was significantly reduced thanks to antioxidant systems. They also mentioned that these systems are made up of non-enzymatic compounds and enzymatic antioxidants. The enzymatic antioxidant components catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) shielded tissues against ROS.

These results supported our findings. Enzymatic antioxidants that shield tissues from ROS include ascorbate peroxidase (APOX), superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT). The numerous plant defense actions on cucumbers may be one of the major factors influencing *B. tabac* according to [28]. The digestive and defensive enzymes of the whitefly may also play a crucial regulatory role in regulating its ability to settle and oviposit. These outcomes support our discovery that cucumber Melouky, which had the highest concentration of plant secondary metabolites, also had the highest CAT and SOD activity levels.

Alternatively, [29] assessed the growth capabilities and enzymatic responses of the grasshopper *Calliptamus abbreviatus* Ikonn to six plant-derived compounds (rutin, quercetin, nicotine, matrine, azadirachtin, and rotenone). They continued by saying that antioxidant enzymes including superoxide dismutase, catalase, and peroxidase were considerably elevated following exposure to the six compounds. These results demonstrated that C. abbreviatus was harmful to the six plant-derived compounds. Our findings led us to assume that rising plant secondary metabolites were activating the insect antioxidant enzyme in a protective manner.

4. Conclusion

The effect of three plant cultivars' secondary metabolites on the antioxidant system of the whitefly, *B. tabaci*, is examined in this field investigation. Melouky cucumber cultivars stimulate *B. tabaci* antioxidant enzymes, making them potentially effective defenses against *B. tabaci* attack.

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