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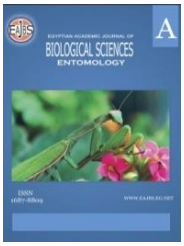

Effect of Secondary Metabolites of Different Cucumber Cultivars on Antioxidant Enzymes of Whitefly, *Bemisia tabaci* (Gen.), Assiut, Egypt

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ABSTRACT

Field and laboratory experiments were 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.

INTRODUCTION

Cucumber, *C. sativus* L. is a popular horticulture crop around the world (Yundaeng *et al.,* 2015). Whitefly, *Bemisia tabaci* (Gen.) (Homoptera: Aleyrodidae) is a polyphagous pest that may adapt to a broad variety of plant hosts (Schoonhoven *et al.,* 2005). Secondary plant metabolites cause toxic effects that can be observed at both lethal and sub-lethal levels, but the most important effect is repellence (Chowanski *et al.,* 2016). As a result, it's an excellent model for investigating the interactions between host plants and polyphagous herbivores. The effect of host plant on *B. tabaci*, however, lacks a comprehensive analysis that includes detoxification, digestion, and antioxidation mechanisms; additionally, the effects of switching to different host species (inter-species) and cultivars (as well as varieties, intra-species) could be varied and hazy (Iida *et al.,* 2009; Mansaray and Sundufu 2009; Yan *et al.,* 2011; Luan *et al.,* 2012).

The present studies were carried out to study the effects of plant secondary metabolites of some cucumber cultivars on the enzymatic defense system of whitefly.

MATERIALS AND METHODS

1- Field Studies:

An area of about a quarter feddan (1100 m^2) was divided into equal plots. Each plot (6 rows/plot) and cultivated with cucumber (Ghazeer, Melouky and Zeen) in a randomized complete block design. Crops were managed using conventional agricultural methods. Weeds were manually cleared by hand. No insecticides were used during the study period. The cultivars were planted on two planting dates (summer and Nile plantations). In summer plantations the seeds were sown on the 31st of April, whereas Nile (fall) plantations were sown on the 14th of August. The selected cultivars were chosen for the study because they are widely available and well-known among farmers in Upper Egypt.

2- Laboratory Studies:

Sample Collection:

Whiteflies (fourth instars) were collected weekly from the beginning of infestation to plant harvest. The collected samples were weighed. Insects were separated and kept in 1.5 ml labeled Eppendorf tubes. The tubes were stored at -80ºC to later use.

Sample Preparation:

The collected samples were homogenized in phosphate buffer saline (PBs) (PH 7). The homogenates were centrifuged at 4,000 r.p.m. for 15 min. The supernatant was pooled and taken in three separated labeled Eppendorf tubes; cucumber (Ghazeer, Melouky and Zeen). Then stored at -20ºC for later use.

Total Protein Assay:

Total protein content was estimated according to Cannon (1974). Chemicals were purchased from Egyptian company for biotechnology (S.A.E).

The amount of protein was calculated from this equation

Protein concentration in tissue (mg/mg fresh body weight) = (ASpecimen/Astandard) ×6× (1/mg tissue used per test**).**

Antioxidant Enzymes Assays:

The activities of CAT and SOD were determined using commercial kites. The enzymatic activities were presented as U/mg protein fresh body weight.

Catalase (CAT) Activity Assay:

Chemicals**:** were purchased from a Biodiagnostic company, (Egypt), and the assay was done according to Aebi (1984).

Calculation: catalase activity in tissue (U/mg) = ($A_{standard} - A_{sample} / A_{standard}$)× (1/mg tissue used per test)

SOD Assay:

Chemicals: were purchased from biodiagnostic company, (Egypt), and the assay was done according to Nishikimi *et al*. (1972)

SOD activity (U/mg tissue) = %inhibition×3.75×(1/mg tissue used)**.**

3- Analysis of Plant Secondary Metabolites:

These analyses were conducted at the analytical chemistry unit (ACAL) of the Chemistry Department at Assiut University, Egypt.

Secondary Metabolite Extraction:

Secondary metabolites were extracted from 5 leaves from each cultivar. All leaves were washed well prior to extraction. One gram of leaves from each cultivar was ground well, then added 2.5 ml of chloroform and 0.5 ml of phosphoric acid were to the ground leaves. The mixture was sonicated (the mixture injected in an ultra-sonic 104x instrument) for 15 min, and then centrifuged at 8,000 r.p.m. for 15 min. The clear chloroform layer was collected and injected in a gas chromatography instrument (GC/Ms) (7890A-5975B) in USA.

Statistical Analysis:

All data obtained for biochemical and secondary metabolite studies were statistically analyzed by using (Excel 2007) computer program calculations at 5% levels.

RESULTS

1- Secondary Metabolites:

The analysis of cucumber leaves revealed that the leaves contain four groups of secondary metabolites: alkaloids, terpenes, phenolic compounds, and flavonoids. Data show that except the cultivar of Zeen, all the cultivars contained alkaloid compounds (Table 1). The highest concentration of alkaloids was detected in Melouky, followed by Ghazeer. The highest levels of terpenes were found in Ghazeer, followed by Zeen, (Table 2). Meanwhile, we detected no phenolic compounds in Zeen, while the highest levels were detected in Melouky, followed by Ghazeer (Table 3). Flavonoid compounds were detected only in Melouky (Table 4).

Table 1: Alkaloid components.

Cultivar	Alkaloids	Value %)
Ghazeer	4-[(1-hydroxy-2-(methylamino)ethyl]1.2-benzenediol	0.67
	Alpha-methyl-beta-oxo-2—pyridinepropanoic acid ethyl ester	1.943
Melouky	Agarine	0.49
	2,6-dimethylmorpholine	1.034
	1,4-diisobutylpiperazine	1.66
	2,6-dichloro-3-phenylpyridine	0.96
	2,6-dimethylpiperazine	0.195
	3',4'-dihydro-2'-(morpholine-4-yl)-5',7'-dinitrospiro[cyclopentane-1,3'-quinazoline	2.92

Table 2: Terpene components.

Cultivar	Terpene	Value (%)
Ghazee	Squalene	2.53
Melouky	Squalene	1.17
Zeen	8-beta-methoxy-5-alpha-hydroxycaryophylla-3-ene	0.27
	Farnesol isomer	0.83
	Squalen	0.69

Table 3:. Phenol compoounds.

Cultivar	Phenol	Value (%)
Ghazeer	2-(2-aminopropyl)phenol	2.36
	4-(-2-Amino-1-hydroxypropyl)phenol	0.66
	5-nitrovanilline-o-acetate	0.27
Melouky	Alpha-(phenylimino)-ortho-cresol	24.6

Table 4: Falvonoid components.

Cultivar	Flavonoids	Value (%)
Melouky	2-(7-dodecynyloxy)tetrahydro-2H-pyran	0.127

2- Antioxidant Enzymes of *B. tabaci*:

Data presented in Table (5) show that antioxidant enzymes CAT and SOD of *B. tabaci* were collected from cucumber cultivars. Data clearly show that the level of CAT was the highest in *B. tabaci* collected from Melouky cultivar (Table 5). On the other hand, the level of CAT was the lowest in *B. tabaci* collected from the Zeen cultivar. Statistical analysis shows that there were significant differences in antioxidant enzymes of CAT and SOD activity of *B. tabaci* that collected from cucumber Zeen and Ghazeer, respectively, while, SOD activity was the highest in *B. tabaci* were collected from Melouky cultivar.

Table 5: Antioxidant enzymes of *B. tabaci* feed on different cultivars of cucumber and total secondary metabolites recovered in the cultivars.

Cultivars	Antioxidants enzymes (U/mg protein) Mean ±SE,		Total secondary metabolites concentration (%)
	CAT	SOD	Total (%)
Ghazeer	0.11±0.039^{b}	0.086±0.015^{b}	8.44±0.54^{b}
Melouky	0.13±0.016^{a}	0.20±0.003^{a}	33.27±6.82^{a}
Zeen	0.10±0.30^{b}	0.077±0.007^{b}	1.79±0.57^{c}

Means followed by the same letters vertically are not significant different at P> 0.05.

DISCUSSION

In the present study, *B. tabaci* was a common pest of cucumber. The results revealed that the enzymatic activity of *B. tabaci* fed on three cultivars of cucumber significantly varied. The highest activity of CAT and SOD were recorded in immature stages of *B. tabaci* collected from Melouky cultivar (which had the highest levels of plant secondary metabolites; alkaloids, terpene and phenolic compounds). While the lowest activity of CAT and SOD were observed in immature stages of *B. tabaci* collected from Zeen cultivar (which had the lowest concentration of Plant secondary metabolite; only terpene was detected). Chen et al., 2015 documented that terpenoids, phenolic chemicals, and alkaloids were only a few of the secondary metabolites in plants created by pests that are particularly harmful to insect growth. The midgut's biochemical and physiological properties contributed to insects' resistance to secondary metabolites. Insects' midguts can secrete a variety of digesting enzymes (e.g., proteases, lipases, amylases, sucrases, and trehalases) as well as defense enzymes (superoxide dismutase, catalase, and peroxidase) that help them fight plant defenses. Lukasik *et al.,* 2011; George and Gatehouse 2013; Jena *et al.,* 2013 said that herbivorous insects have acquired protective antioxidant mechanisms in response to plant secondary metabolites consumption. Antioxidant systems served a critical role in reducing the toxicity of reactive oxygen species (ROS). They added that enzymatic antioxidants and non-enzymatic chemicals make up these systems. Catalase (CAT), Superoxide Dismutase (SOD) and Glutathione Peroxidase (GPX) were enzymatic antioxidant components that protected tissues from ROS. Which agrees with our results. Similarly, (Jena *et al.,* 2013) stated that ascorbate Peroxidase (APOX), superoxide Dismutase (SOD), Glutathione Peroxidase (GPX) and Catalase (CAT) were enzymatic antioxidants that protect tissues from ROS. Lin and colleagues (2019) found that one of the key reasons influencing *B. tabac* predilection could be the many plant defense activities on cucumbers. Furthermore, the whitefly's digestive and defensive enzymes may play an important regulatory function in its capacity to settle and oviposit. These results match our finding, that the highest activity of CAT and SOD were recorded in cucumber Melouky which had the highest content of plant secondary metabolites. Also, (Lukasik *et al.,* 2011; Lukasik *et al.,* 2012; Lukasik and Goawska 2013) analyzed the levels of non-enzymatic and enzymatic antioxidants in the pea

aphid, *Acyrthosiphon pisum.* The results revealed that the levels of non-enzymatic and enzymatic antioxidants were altered by the change in the host plant. As a result, oxidative stress may play a significant role in herbivore insect-host plant interactions. (Kerchev *et al.*, 2012) measured the number of antioxidant enzymes in *M. persicae* fed on anise, coriander and cumin, which varied according to the host plant *M. persicae* demonstrated a predilection for plants that were high in ascorbate. Otherwise, (Wang *et al.,* 2020) evaluated the grasshopper *Calliptamus abbreviatus* Ikonn's growth performance and enzymatic response to six plant-derived chemicals (rutin, quercetin, nicotine, matrine, azadirachtin, and rotenone). They added that after exposure to the six chemicals, antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase all increased significantly. These findings indicated that the six plant-derived chemicals were toxic to *C. abbreviatus*. According to our results, we assumed that the increasing plant secondary metabolites activate the insect antioxidant enzyme as a protective reaction. This field study investigates the impact of three plant cultivars' secondary metabolites on the antioxidant system of the whitefly, *B. tabaci*. Melouky cultivar of cucumber uprouse antioxidant enzymes of *B. tabaci* and therefore it can be suggested as a promising cultivar against *B. tabaci* attack.

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