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The goal of the journal is to advance the scientific understanding of mechanisms of toxicity. Emphasis will be placed on toxic effects observed at relevant exposures, which have direct impact on safety evaluation and risk assessment. The journal therefore welcomes papers on biology ranging from molecular and cell biology, biochemistry and physiology to ecology and environment, also systematics, microbiology, toxicology, hydrobiology, radiobiology and biotechnology.

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Biochemical Markers for Acetamiprid and Imidacloprid Neonicotinoid Insecticides Selectivity in the Cotton White Fly, *Bemisia tabaci*, the Cotton Leafworm, *Spodoptera littoralis* and Honey Bee, *Apis mellifera*

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ARTICLE INFO

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<td>Selective toxicity of acetamiprid (Mospilan 20% SP) and imidacloprid (Imaxi 35% SC) neonicotinoid insecticides to <em>Bemisia tabaci</em>, <em>Spodoptera littoralis</em> and <em>Apis mellifera</em> was studied.</td>
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<td>Biochemical markers <em>Bemisia tabaci</em></td>
<td>Assessment of biochemical alterations total proteins, acetylcholinesterase EC3.1.1.7(AChE) activity, cytochrome P450 monooxygenases EC1.14.14.1 (CP450) activity and glutathione-S-transferases EC2.5.1.18 (GST) activity were recorded and discussed as biomarkers for acetamiprid and imidacloprid selective toxicity in the exposed insects. Imidacloprid treatments caused higher reductions in the total protein levels as compared with acetamiprid treatments. The total protein were decreased by 55, 41, 31 and 14 % in <em>A. mellifera</em> thorax, <em>S. littoralis</em> 2nd larval instar, <em>B. tabaci</em> adult and <em>S. littoralis</em> 4th larval instar, respectively. AChE activity was increased by 29% and decreased by 50% in <em>A. mellifera</em> whole body in acetamiprid and imidacloprid treatments, respectively. Moreover in <em>B. tabaci</em>, the enzyme activity was decreased by 9% and increased by 35% respectively after acetamiprid and imidacloprid treatments. The two tested neonicotinoids elevated CP450 activity in <em>B. tabaci</em> adults and <em>S. littoralis</em> 2nd and 4th larval instars. The highest increase was obtained with imidacloprid treatment in <em>A. mellifera</em> thorax (165%) followed by <em>S. littoralis</em> 2nd larval instar (100%), <em>B. tabaci</em> adults (66%). The obtained results showed that acetamiprid and imidacloprid treatments elevated GST activity respectively by 29 and 1.5 % in <em>B. tabaci</em> adults; 30 and 30% in <em>S. littoralis</em> 4th larval instar ;88 and 59% in <em>A. mellifera</em> thorax . GST activity was decreased by 18% in <em>S. littoralis</em> 2nd larval exposed to acetamiprid and increased by 18% in imidacloprid treatment.</td>
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<tr>
<td><em>Spodoptera littoralis</em></td>
<td>Conclusively, our work suggested that the selective toxicity of the cyanoguanidine neonicotinoid acetamiprid and the nitroguanidine neonicotinoid imidacloprid was based on their chemical structure and metabolic pathways and seemed to be represent a species-specific feature .This was indicated by alterations of the values of total proteins and enzymes specific activity of key enzymes in the exposed target and non target insects .</td>
</tr>
<tr>
<td><em>Apis mellifera</em></td>
<td></td>
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</tbody>
</table>

INTRODUCTION

Sustainable agriculture aims to supply sufficient food for the world population while minimizing environmental impact.
During the last five decades intensive use of organophosphates, carbamates and synthetic pyrethroids resulted in high level of economic insect resistance. High level of resistance resulted in disturbance of the equilibrium of the environmental system beside the increase of pest control costs. This has led to search for and developed of new compound such as neonicotinoides (Kodandaram et al., 2010).

The neonicotinoid insecticides, which include imidacloprid, acetamiprid, clothianidin, thiamethoxam and thiacloprid, are among the most important chemicals in crop protection (Elbert et al., 2008) and they are widely used in seed dressings (Sur and Stork, 2003). Neonicotinoids are neurotoxins that act as agonists of insect nicotinic acetylcholine receptors and are lethal through disruption of the insect nervous system (Matsuda et al., 2001). Imidacloprid (belongs to the nitro-containing neonicotinoids) is the first member of this family and effective against many insects showing resistance to carbamates, organophosphates and pyrethroids (Cox, 2001). Acetamiprid (a cyano-containing neonicotinoids) belongs to second generation of the nicotinoids and has a broad-spectrum insecticide effect against several groups of insects including Lepidopterans, Coleopterans, Hemipterans and Thysanopterans. The insecticide has an ingestion and stomach action and has a strong osmotic and systemic action (Takahashi et al., 1998 and Yamada et al., 1999). The use of neonicotinoid insecticides has grown considerably since their introduction in 1990 (Thany, 2010).

In the last three decades, biochemical biomarkers have been used for the assessment of exposure to environmental contaminants and chemical stress. The aim of the present investigation is assess biochemical alterations (total protein; acetylcholinesterase EC3.1.1.7 (AChE) activity; cytochrome P450 monooxygenases EC1.14.14.1 (CP450) activity; glutathione-S-transferases EC2.5.1.18 (GST) activity) as biomarkers of acetamiprid and imidacloprid selective toxicity in exposed Bemisia tabaci, S. littoralis and Apis mellifera.

MATERIALS AND METHODS
Used Insecticides
Acetamiprid (Mospilan 20% SP)
Chemical name (IUPAC): (E)-N1-[(6-chloro-3-pyridyl)-methyl]-N²-cyano-N¹-methylacetamidine

![Chemical structure of Acetamiprid](image)

Imidacloprid (Imaxi 35%SC)
Chemical name (IUPAC): (E)-1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine

![Chemical structure of Imidacloprid](image)

Tested Insects
The cotton whitefly, Bemisia tabaci (Hemiptera: Aleyrodida):

For rearing, a stock culture of B. tabaci was established from infested tomato fields at the Tenth of Ramadan city, Sharkia Governorate. Tomato leaves
bearing nymphs and puparia were brought to the laboratory and were placed with castor bean plants in pots in wooden cages (60 cm high and 40 cm diameter) covered by fine mesh nylon clothes. Whitefly adults that had emerged from the tomato leaves had been maintained on the castor bean plants for oviposition. The plants were kept under controlled conditions 25±2ºC and 70±5 R.H. for hatching of eggs and development of the nymphs without any exposure to pesticides (Mann et al., 2012 with modification), we used the adult of B. tabaci in our study.

**The Egyptian cotton Leafworm, Spodoptera littoralis** (Boisd.) (Lepidoptera: Noctuidae):

A laboratory susceptible colony of the Egyptian cotton leafworm, S. littoralis (Boisd.) was used in the present study without any exposure to pesticides. The culture was initiated from freshly collected egg masses supplied by the Division of Cotton Pests, Branch of Plant Protection Research Institute at Zagazig, Sharkia Governorate and has been reared for 5 years without any exposure to pesticides under controlled conditions according to El-Defrawy et al. (1964). We used second and fourth instar larvae of S. littoralis in our study.

**Honey bees, Apis mellifera** L. (Hymenoptera: Apidae):

Honey bee workers needed for laboratory tests were collected from the peripheral combs of the colony at the apiary of Plant Protection Research Institute, Zagzig, Sharkia governorate, without any exposure to pesticides.

**Determination of Biochemical markers linked to neonicotinoid insecticides selectivity in insects:**

Total protein (TP), acetylcholinesterase EC3.1.1.7 (AChE), cytochrome P450 monooxygenases EC1.14.14.1 (CP450) and glutathione-S-transferases EC2.5.1.18 (GST) activity were determined in insects exposed to sub lethal concentrations of the tested insecticides.

**Insect treatments:**

Second and fourth instar larvae of S. littoralis were treated with LC25 value of acetamiprid (612.61 and 1057.20 µg a.i./ml, respectively) and imidacloprid (1103.16 and 1142.24 µg a.i./ml, respectively) using leaf dipping technique. Castor bean leaves were dipped for 30 seconds in the concentration then left to dry for one hour and offered to starved larvae. The 2nd and 4th instars larvae of the tested strain were confined with treated leaves in glass jars covered with muslin for 24 hrs. Leaves for the untreated control were dipped in water. Five replicates were made for each concentration, and each replicate contained 20 larvae.

Adults of B. tabaci were treated with LC50 value of acetamiprid and imidacloprid (12.258 and 73.402 µg a.i./ml., respectively) using leaf dipping technique according to Hameed et al. (2010). Castor bean leaf discs of diameter 5 cm were cut and dipped in the test solution for 20 seconds. Leaf discs were then air dried on towel tissue papers and placed in Petri-dishes. Adults (mixed sex population) were immobilized by cooling (after 2 hours of starvation). Thirty to forty adults were placed in Petri-dish with treated leaves and covered. There were five replicates for each concentration. For the untreated control, only distilled water was used.

Workers of A. mellifera were treated with a field rate of acetamiprid (50µg a.i./ml) and the LC50 value of imidacloprid (59.83 µg a.i. /ml) by oral application according to the methods of Szczepanski and Gromiszoa (1979) and Khedr (2002). For each tested compound at each concentration and for the controls 30 honey bees (three cages) were used. Bees were placed in feeding cages of 9×12×20 cm. under room conditions (26±3 ºC) and (65±5 % R.H.). The oral toxicity of the tested compounds against
honeybee workers was evaluated by mixing with food media on 1:1 (W:V) sugar syrup. Containing the tested compound was introduced in piece of wax comb (4×4 cm.) in each cage.

The Survived insects were collected after 24 hours post treatment and stored at −20ºC for biochemical analysis. Samples from the untreated control were collected and served as check.

**Preparation of insects for analysis:**
Insect homogenates and *A. mellifera* haemolymph were prepared according to Amin, T. R. (1998). Sample were homogenized in distilled water (50mg/ml for *S. littoralis* larvae and *A. mellifera* workers) and 5mg/0.5ml for adult of *B. tabaci*. Homogenates were centrifuged at 8000rpm for 15 minutes at 5ºC in a refrigerated centrifuge. The deposits were discarded and the supernatants were kept at -20 ºC till use.

**Biochemical markers determination:**

**Total protein:**
Total proteins were determined by the method of (Bradford, 1976).

**Acetyl cholinesterase (AChE) assay:**
Acetyl cholinesterase (AChE) activity was measured according to the method described by Simpson *et al.* (1964) using acetylcholine bromide (AChBr) as substrate.

**Cytochrome P_{450} monooxygenases (CP_{450}) assay:**
P-Nitroanisole oxidative demethylation was assayed to determine the CP_{450} activity according to the method of Hansen and Hodgson (1971).

**Glutathione-S-transferase (GST) assay:**
Glutathione-S-transferase (GST) catalyzes the conjugation of reduced glutathione (GSH) with 1-chloro 2,4-dinitrobenzene (CDNB) via the-SH group of glutathione. The conjugate, S-(2,4-dinitro-phenyl)-L-glutathione could be detected as described by the method of Habig *et al.* (1974).

**Statistical analysis:**
The differences in biochemical measurements were performed between treatments and control. LSD value was calculated as described by Fisher (1950) and Snedecor (1970) using Costat computer program Cohort Software. P. O. Box.

**RESULTS AND DISCUSSION**

**(TP), (AChE), (CP_{450}) and (GST) activity were determined in insects treated by sublethal concentration of the acetamiprid and imidacloprid.**

**Total protein content:**
Total protein content in body homogenates of *B. tabaci* adult, *S. littoralis* larvae and *A. mellifera* body compartments and haemolymph 24 hours after acetamiprid and imidacloprid exposure, comparing with untreated controls is shown in Table (1). The obtained results revealed that imidacloprid treatments caused higher reductions in total protein content of *B. tabaci* adult, *S. littoralis* 2nd larval instar and *A. mellifera* thorax as compared with acetamiprid treatments. The total protein levels were decreased by 55, 41, 31 and 14 % in *A. mellifera* thorax, *S. littoralis* 2nd larval instar, *B. tabaci* adult and *S. littoralis* 4th larval instar, respectively. Total protein level in *A. mellifera* whole body was decreased by 34% in acetamiprid treatment, whereas, it was increased by 19% in imidacloprid treatment. In *A. mellifera* haemolymph, total protein content was increased by 156 and 47% in acetamiprid and imidacloprid treatments, respectively. Among the treatments, thorax of *A. mellifera* adult exposed to imidacloprid showed the highest reduction in total protein level (55%).
Table 1: Total Protein in the cotton whitefly *B. tabaci*, the Egyptian cotton leafworm, *S. littoralis* and honeybee *A. mellifera* 24 hours after acetamiprid and imidacloprid treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>B. tabaci (adult)</th>
<th>S. littoralis (larvae)</th>
<th>A. mellifera (workers)</th>
<th>Total Protein *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd instar</td>
<td>4th instar</td>
<td>Haemolymph</td>
<td>Whole body</td>
</tr>
<tr>
<td>Untreated Control</td>
<td>85.77 ± 3.91 a</td>
<td>81.33 ± 2.78 a</td>
<td>59.97 ± 2.55 a</td>
<td>4.40 ± 0.27 b</td>
</tr>
<tr>
<td>Acetamiprid**</td>
<td>65.77 ± 4.52 b (0.76)</td>
<td>56.80 ± 0.66 b (0.69)</td>
<td>50.80 ± 2.04 b (0.84)</td>
<td>11.27 ± 1.30 a (2.56)</td>
</tr>
<tr>
<td>Imidacloprid**</td>
<td>59.77 ± 2.06 b (0.69)</td>
<td>48.10 ± 2.72 a (0.59)</td>
<td>51.87 ± 1.91 b (0.86)</td>
<td>6.47 ± 0.50 b (1.47)</td>
</tr>
</tbody>
</table>

Means marketed with the same letter in each column are not significant different (P < 0.05) by Duncan’s test.
* Total Protein expressed as: mg/g.b.wt of insect; mg/ml for insect haemolymph.
** The insecticides were applied at the LC50 for *B. tabaci* (leaf dipping) and *A. mellifera* (oral feeding) and LC25 for 2nd, 4th larval instars of *S. littoralis* (leaf dipping).

Acetylcholinesterase activity:
Data in Table (2) show acetylcholine esterase specific activity in *B. tabaci*, *S. littoralis* and *A. mellifera* 24 hours after acetamiprid and imidacloprid treatments. The obtained results revealed that AChE levels, after acetamiprid and imidacloprid treatments and comparing with untreated control, were elevated, respectively, by 167 and 163% in *A. mellifera* thorax; 140 and 100% in *S. littoralis* 2nd larval instar; 164 and 14% in *S. littoralis* 4th larval instar. At the same time, the enzyme levels were decreased by 70 and 56% in *A. mellifera* haemolymph; and by 39 and 43% in *A. mellifera* head after acetamiprid and imidacloprid treatments, respectively. AChE activity was increased by 29% and decreased by 50% in *A. mellifera* whole body in acetamiprid and imidacloprid treatments, respectively. Moreover in *B. tabaci*, the enzyme activity was decreased by 9% and increased by 35% respectively after acetamiprid and imidacloprid treatments.

Table 2: Acetylcholine esterase activity in the cotton whitefly *B. tabaci*, the Egyptian cotton leafworm, *S. littoralis* and honeybee *A. mellifera* 24 hours after acetamiprid and imidacloprid treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>B. tabaci (adult)</th>
<th>S. littoralis (larvae)</th>
<th>A. mellifera (workers)</th>
<th>AChE activity (µg AChBr /min/ mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd instar</td>
<td>4th instar</td>
<td>Haemolymph</td>
<td>Whole body</td>
</tr>
<tr>
<td>Untreated Control</td>
<td>0.23 ± 0.01 b</td>
<td>0.05 ± 0.01 a</td>
<td>0.14 ± 0.02 b</td>
<td>3.75 ± 0.20 a</td>
</tr>
<tr>
<td>Acetamiprid**</td>
<td>0.21 ± 0.01 a (0.91)</td>
<td>0.12 ± 0.01 a (2.40)</td>
<td>0.37 ± 0.03 a (2.64)</td>
<td>1.13 ± 0.06 a (0.30)</td>
</tr>
<tr>
<td>Imidacloprid**</td>
<td>0.31 ± 0.02 a (1.35)</td>
<td>0.10 ± 0.01 a (2.00)</td>
<td>0.16 ± 0.01 a (1.14)</td>
<td>1.66 ± 0.04 a (0.44)</td>
</tr>
</tbody>
</table>

Means marketed with the same letter in each column are not significant different (P < 0.05) by Duncan’s test.
** The insecticides were applied at the LC50 for *B. tabaci* (leaf dipping) and *A. mellifera* (oral feeding) and LC25 for 2nd, 4th larval instars of *S. littoralis* (leaf dipping).
- Values are the mean ± SD.
- Values in parentheses are ratios of treated to untreated.

Neonicotinoids, targeting insect nicotinic acetylcholine receptors (nAChRs), have veterinary and crop protection applications, with their fast actions providing economic benefits. However, their target-selectivity is important to insure safety and to limit adverse effects on beneficial insects such
as honeybees. They are agonist of the nAChRs (Tomizawa and Casida, 2003 and Tan et al., 2007) and do not exert as a direct inhibition of the AChE activity as shown for other pesticides. AChE is a key enzyme in the nervous system. It plays an important role in neurotransmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine to choline and acetate.

In the present study, the possible indirect inhibitory effects on B. tabaci, S. littoralis and A. mellifera exposed to acetamiprid and imidacloprid were tested. Our results revealed that the enzyme activity was affected in the test organisms exposed to the two compounds and the AChE responses seem to represent a species-specific feature. Similarly, AChE activity was reported to be reduced in many insects like; German cockroach Blattella germanica exposed to the LD50 of acetamiprid (Morakchi et al., 2005); resistant strain of Aphis gossypii treated with acetamiprid (Chen et al., 2013); Apis mellifera, 24 hours post treatment with imidacloprid (Jin et al., 2015). AChE activity was elevated in some insects such as mosquito larvae treated with imidacloprid and its analogues (Rao et al., 2008); S.littoralis, 5th larval instar total homogenate, 24 hours after treatment with LC50 of coragen (Rashwan, 2013); whole body homogenate of moths and 3rd instar larvae of Tuta absoluta treated with imidacloprid (Radwan and Taha, 2012); and A. mellifera exposed to neonicotinoids in field and laboratory (Boily et al., 2013 and Alburaki et al., 2015) . In honey bees, the specific activities of AChE in different tissues of surviving foragers, was varied after 24 hours of acetamiprid treatment (Badawy et al., 2015). Choi et al. (2001) reported that imidacloprid did not inhibit AChE in the resistant and susceptible strain of the green peach aphid, Myzus persicae.

**Cytochrome P450 monoxygenase activity:**

Cytochrome P450 monoxygenase activity in B. tabaci, S. littoralis and A. mellifera 24 hours after acetamiprid and imidacloprid treatments, are presented in Table (3). As compared with the untreated controls, the results indicated that CP450 activity was decreased in A. mellifera heamolymph and head, 24 hours after treatment with acetamiprid and imidacloprid. The enzyme activity in acetamiprid treatment was reduced by 37 and 27% respectively in heamolymph and head while in imidacloprid treatment the activity decreased by 26, 10 and 9% in head, heamolymph and whole body of A. mellifera respectively.

### Table 3: Cytochrome P450 monoxygenases activity in the cotton whitefly B. tabaci, the Egyptian cotton leafworm, S. littoralis and honeybee A. mellifera 24 hours after acetamiprid and imidacloprid treatments.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>B. tabaci (adult)</th>
<th>S. littoralis (larvae)</th>
<th>A. mellifera (workers)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2nd instar</td>
<td>4th instar</td>
</tr>
<tr>
<td>Untreated Control</td>
<td>0.06±0.00&lt;sup&gt;a&lt;/sup&gt; (1.33)</td>
<td>0.09 ± 0.00&lt;sup&gt;b&lt;/sup&gt; (1.33)</td>
<td>0.14 ± 0.01&lt;sup&gt;c&lt;/sup&gt; (1.14)</td>
</tr>
<tr>
<td>acetamiprid**</td>
<td>0.08 ± 0.01&lt;sup&gt;a&lt;/sup&gt; (1.33)</td>
<td>0.12 ± 0.00&lt;sup&gt;b&lt;/sup&gt; (1.33)</td>
<td>0.16 ± 0.00&lt;sup&gt;c&lt;/sup&gt; (1.14)</td>
</tr>
<tr>
<td>imidacloprid**</td>
<td>0.10 ± 0.00&lt;sup&gt;a&lt;/sup&gt; (1.66)</td>
<td>0.18 ± 0.01&lt;sup&gt;b&lt;/sup&gt; (2.00)</td>
<td>0.19 ± 0.00&lt;sup&gt;c&lt;/sup&gt; (1.36)</td>
</tr>
</tbody>
</table>

Means marketed with the same letter in each column are not significant different (P < 0.05) by Duncan’s test.

** The insecticides were applied at the LC50 for B. tabaci (leaf dipping) and A. mellifera (oral feeding) and LC25 for 2<sup>nd</sup>, 4<sup>th</sup> larval instars of S. littoralis (leaf dipping).

-Values are the mean ± SD.

- Values in parentheses are ratios of treated to untreated.
The two tested neonicotinoids elevated CP450 activity in B. tabaci adults and S. littoralis 2nd and 4th larval instars. The highest increase was obtained with imidacloprid treatment in A. mellifera thorax (165%) followed by S. littoralis 2nd larval instar (100%), B. tabaci adults (66%).

The cytochrome P450 enzymes comprise a family of heme proteins, named for the absorption band at 450 nm of their carbon-monoxide-bound form, involved in catabolism and anabolism of endogenous and exogenous compounds such as steroids and pesticides (Feyereisen, 2006). The P450 genes (also called CYP) are found in the genomes of virtually all organisms. In a harmony with the results of the present study, the development of high metabolic resistance to neonicotinoids - including acetamiprid and imidacloprid - has been reported in pests such as B. tabaci is conferred by enhanced oxidative detoxification by overexpression of the cytochrome P450 monooxygenase CYP6CM1 (Karunker et al., 2008 and Nauen et al., 2013); CYP6C and CYP9F (Qiu et al., 2009). The recent association of CYP6CM1 with imidacloprid resistant B. tabaci nymphs and adults, where nymphs are 4-10 times less sensitive to imidacloprid than their adult counterparts, makes this enzyme the most likely candidate underlying this age-specific resistance (Karunker et al., 2008; Karunker et al., 2009).

Oxidative detoxification mediated by P450 monooxygenases is involved in imidacloprid resistance B. tabaci (Wang et al., 2009). Similar observation was recorded by Radwan and Taha (2012) who investigated the effect of imidacloprid in the activity of monooxygenase PCMAN-demethylase and found that imidacloprid increased the activity of the enzyme in moths and 3rd instar larvae of Tuta absoluta treated with LC30, LC50 and LC80 of imidacloprid. In honeybee, acetamiprid and imidacloprid are biotransformed by Phase I enzymes, mainly by mixed function oxidases (Iwasa et al., 2004 and Suchail et al., 2004) and that convert acetamiprid into more polar metabolites (Brunet et al., 2005).

**Glutathione-S-transferase activity:**

Data in Table (4) presented the glutathione-S-transferase activity in B. tabaci, S. littoralis and A. mellifera 24 hours after acetamiprid and imidacloprid treatments as compared with untreated controls. The obtained results showed that acetamiprid and imidacloprid treatments elevated GST activity respectively by 29 and 1.5 % in B. tabaci adults; 30 and 30% in S. littoralis 4th larval instar; 88 and 59% in A. mellifera thorax.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>GST activity (nmole substrate conjugated /min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B. tabaci (adult)</td>
</tr>
<tr>
<td></td>
<td>2nd instar</td>
</tr>
<tr>
<td>Untreated Control</td>
<td>0.14 ± 0.01 c</td>
</tr>
<tr>
<td>acetamiprid**</td>
<td>0.18 ±0.01 b</td>
</tr>
<tr>
<td>(1.29)</td>
<td>(0.82)</td>
</tr>
<tr>
<td>imidacloprid**</td>
<td>0.35 ± 0.03 a</td>
</tr>
<tr>
<td>(2.50)</td>
<td>(1.18)</td>
</tr>
</tbody>
</table>

Means marketed with the same letter in each column are not significant different (P < 0.05) by Duncan’s test.

** The insecticides were applied at the LC50 for B. tabaci (leaf dipping) and A. mellifera (oral feeding) and LC25 for 2nd, 4th larval instars of S. littoralis (leaf dipping).

- Values are the mean ± SD.
- Values in parentheses are ratios of treated to untreated.
The enzyme activity was decreased by 18% in S. littoralis 2\textsuperscript{nd} larval exposed to acetamiprid and increased by 18% in imidacloprid treatment. In A. mellifera whole body, GST activity was increased by 71% in acetamiprid treatment and decreased by 22% 24 hours post treatment with imidacloprid.

Glutathione S-transferases compose an enzyme family of many cytosolic, mitochondrial and microsomal proteins. They are present in eukaryotes and in prokaryotes. GSTs contribute to the phase II biotransformation of xenobiotics as many pesticides; they conjugate these compounds with reduced GSH. Induction of GST activity is an indication of a detoxification process and is associated with pesticide resistance, and in addition, GSTs are well known for their involvement in the mitigation of generalized oxidative stress (Maiza \textit{et al}., 2013). They are also involved in intracellular transport, biosynthesis of hormones, protection against oxidative stress and the regulation of development (Enayati \textit{et al}., 2005; Ranson and Hemingway, 2005 and Kasai \textit{et al}., 2009).

GST is an early marker of induction of the detoxifying system and also appears to contribute to cellular protection against oxidative damage (Barata \textit{et al}., 2005; Babczynska \textit{et al}., 2006). They are active detoxifying enzymes in honeybees but they play a relatively minor role in detoxification as compared to P450s. GSTs inhibitor DEM (diethyl maleate) is shown to increase the toxicity of certain neonicotinoids but this effect is significantly smaller than that for the P450 inhibitor PBO (piperonyl butoxide) (Johnson \textit{et al}., 2009; Iwasa \textit{et al}., 2004). Our results revealed that acetamiprid and imidacloprid treatments affect differently according to type of the test compound and the insect species. Similarly, induction of GSTs activity after imidacloprid treatments has been reported by several investigators in snails \textit{Helix aspersa} (Radwan and Mohamed, 2013); \textit{Aedes aegypti} (Riaz, 2011); \textit{S.littoralis} (Pour and Gurkan, 2013). In honey bees, the specific activities of GST in different tissues of surviving foragers, was varied after 24 hours of acetamiprid treatment. The activity was highly correlated to the toxicity against \textit{A. mellifera} (Badawy \textit{et al}., 2015).

The results indicated that the levels of Protein and specific activity of acetylcholinesterases, glutathione S-transferases and cytochrome P450 monooxygenase in the tested insects, as affected by the tested neonicotinoids differed according to the insect species, stage age, and body compartments as well as the compound tested.

Generally, total proteins, carbohydrates and lipids are major components necessary for an organism to develop, grow and perform its vital activities. The level of protein content in the body of larva is dependent upon the rate of synthesis, the breakdown of proteins and even water movement between tissues. Haemolymph of an insect can also account for change in protein level. The reduction may be due to increased breakdown of proteins to detoxify the active principles. This reduction in the protein content may be due to inhibition of DNA and RNA synthesis. The decrease of the total protein may reflect the decrease in the enzymatic activities of various enzymes (Elbarky \textit{et al}., 2008). Neonicotinoid insecticides act selectively on insect nAChRs as potent agonists. Among ionotropic receptors affected by insecticides, nAChRs are the most abundant excitatory postsynaptic receptors. The central nervous system of insects is rich in nAChRs more so than any other organism (Jones and Sattelle, 2010). They are located postsynaptically and directly activated by ACh, released from presynaptic cholinergic neurons.
facilitating fast excitatory synaptic transmission (Thany et al., 2010). Indeed neonicotinoid bind on the nAChR in competition with ACh. Subsequently, when ACh cannot act because neonicotinoid insecticide is bound on the nAChR, the postsynaptic potential and action potential are absent, and in turn the postsynaptic vesicles cannot liberate the neurotransmitter and indirectly AChE is disrupted. This observation in line with (Maiza et al., 2013) who reported that spinosad and indoxacarb are not AChE inhibitors but cause reduction in AChE in cockroaches Blattella germanica.

In the honeybee brain the highest binding site densities for nAChR are localized in the suboesophageal ganglion, the optic tubercles, optic lobes medulla and lobula antennal lobes, dorsal lobes and the α-lobes of the mushroom bodies (Scheidler et al., 1990). Neonicotinoids cause excitation of the neurons and because of a high concentration of nACh receptors in honeybees the eventual paralysis could be very profound occurring at low concentration of neonicotinoids, leading to death.

Biochemical transformation of insecticides leads to less and/or completely non-toxic metabolites, reducing their capacity to interact with their target proteins. These transformations are mainly carried out by detoxification enzymes, including cytochrome P450 monoxygenases and glutathione S-transferases (Hemingway et al., 2004), although other enzyme families may be involved. At the gene level, elevated insecticide metabolism can be the consequence of gene amplification (increase in gene copy numbers), up-regulation (increased expression without change in the copy number) and nonsynonymous variations (changes in protein sequence) leading to an increased turnover of the insecticide (Li et al., 2007).

Conclusively, the toxicity and selectivity of the cyanoguanidine neonicotinoid, acetamiprid and the nitroguanidine neonicotinoid, imidacloprid are based on their chemical structure and metabolic pathways and seems to represent a species-specific feature. This is indicated by alterations of the values of total proteins and enzymes specific activity of key enzymes in the exposed organisms. Consequently, studies of genomic, proteomic, and metabolomic proﬁles of the exposed organisms as well as development of related biomarkers are of great need and importance in this respect.

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