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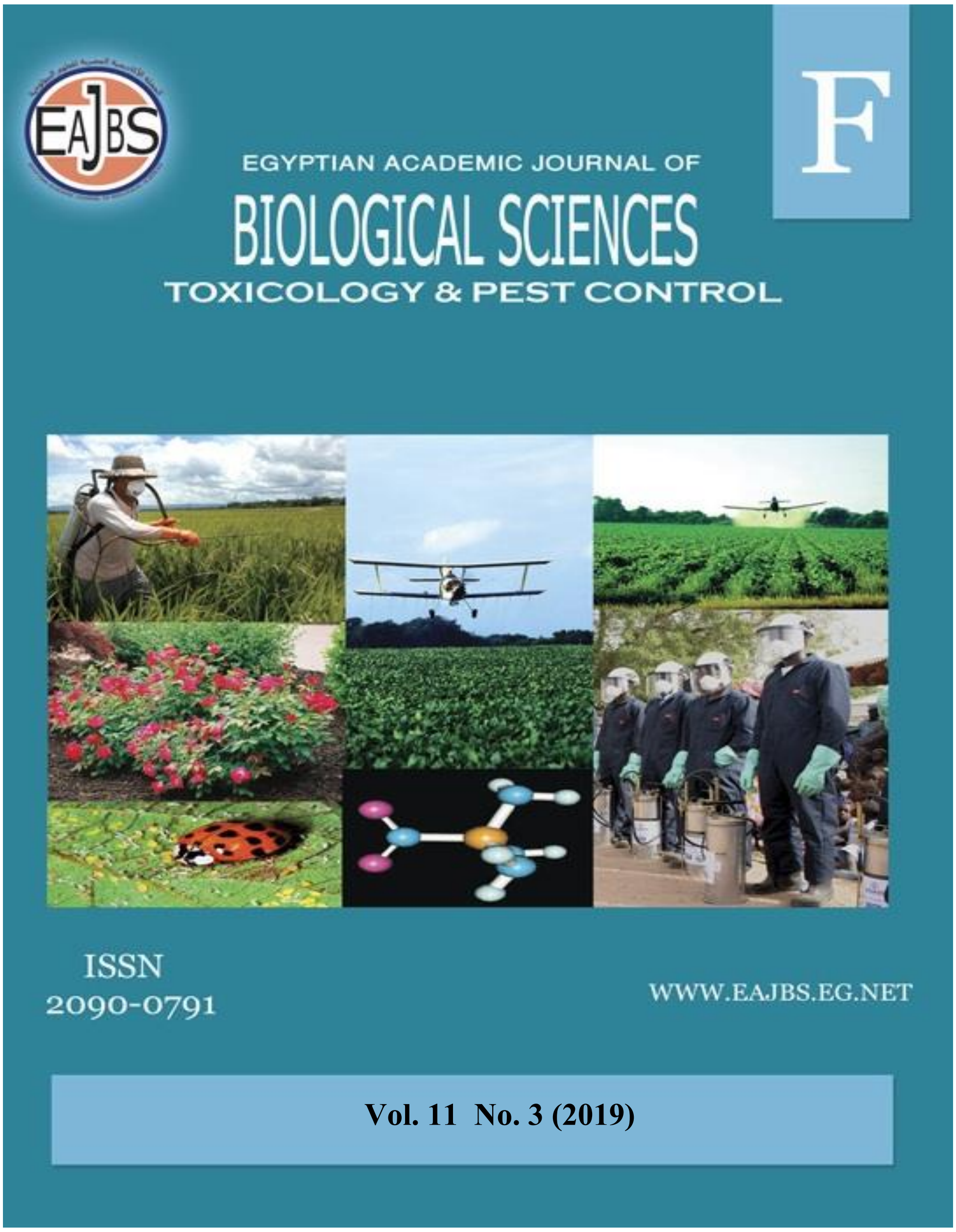


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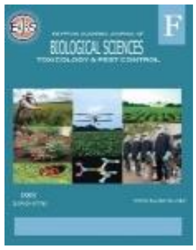


# Toxicity Effects and Biochemical Changes of Insecticide Alternatives on Cowpea Aphid, (*Aphis craccivora*) (Homoptera: Aphididae)

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## ABSTRACT

The cowpea aphid; *Aphis craccivora* is one of the harmful pests, which infest cowpea bean, *Faba bean* plants and many crops leading to a decrease of crop production. Plants including; *Citrullus colocynthis*, *Peganum harmala*, *Senna alexandrina*, and *Apium graveolens* were extracted by methanol 70%, in order to evaluate its toxicity effect on *Aphis craccivora* in laboratory and field, as well as Alkaline phosphatase, non-specific esterase and mixed-function oxidase were evaluated after treatment with $LC_{50}$ of different plant extracts. The results showed that $LC_{50}$ was 4890.079, 5537.761, 6118.4856 and 6118.4856 ppm respectively, while it was 74.1996 ppm for Pirimicarb. Moreover, the combination of the different plant extracts showed or exhibit such a dramatic reduction in *A. craccivora* in the field and minimize the concentration of the pirimicarb required to reduce the aphid population in the field. Also, *P. harmala*, *S. alexandrina* showed an increase in Alkaline phosphatase, non-specific esterase and mixed-function oxidase, while it was moderate for C. *colocynthis* and low for *A. graveolens* compared with the control. These results should be helpful in the rational applications of insect pest management.

## INTRODUCTION

Aphids in genus *Aphis* are highly distributed worldwide, The cowpea aphid; *Aphis craccivora* is one of the harmful pests, which infest cowpea bean, *Faba bean* plants and many crops leading to decrease of crop production, honeydew excretion, sucking of plant sap and viruses transport ( Shehawy and Qari, 2019). One of the important insects in *Medicago sativa* L. (alfalfa) growing areas of the world (Lilian *et al*., 2015). The cowpea aphid is an important insect pest on *Phaseolus lunatus* L. (lima bean) (Luiz *et al*., 2019). In Egypt, the cowpea aphid is the most important insect attacking leguminous crops (El-Ghareeb *et al*., 2002). Also, the adverse effects of conventional chemical pesticides on domestic animals and humans are caused by residues of pesticide left on plant crops. These different residues can affect human health negatively through different chronic diseases (Bale *et al*., 2008).

Moreover, the resistance mechanisms involved in the *A. craccivora* resistant strain to pirimicarb may be non-target site resistance due to higher activity of both Esterases and MFO monooxygenase (Kandil *et al*., 2016). However, the application of chemical pesticides has many adverse effects on humans as well as the environment.

Nowadays, there are interesting in the use of different natural botanical pesticides as an alternative to chemical pesticides and Plant extracts provide suitable source of natural active compounds such as different terpenoids, alkaloids, cucurbitacin, flavonoids, glycosides that have been used as toxic materials against different economic insect, which affect crop plants (Koul and Walia, 2009).

Also, the secondary metabolites which were produced by the plants are very important for the plant survival, and to protect the plant from different environmental changes, moreover, these compounds have fixed role in the defense of the plant against herbivorous insects and may act as nutritional deterrent, repellent or toxic compounds (Mollashahi *et al.,* 2017). Meanwhile, botanical insecticides could be one of the alternatives to chemical synthetic insecticides due to many physical and chemical features such as minimal effect on different natural enemies, maybe nontoxic to another vertebrate, no phytotoxicity and highly degradable in the environment (Isman, 1997). *C. colocynthis* extracts as a natural botanical insecticide have been investigated against many insect pests (Soam *et al.*, 2013).

*P. harmala* L. is a widely distributed plant in the eastern Mediterranean region and It is used commonly in traditional medicine worldwide as sedative and abortifacient but when it used in overdose leads to poisoning (Mahmoudian *et al.*, 2002; Moloudizargari *et al*., 2013; Berdai *et al*., 2014). Cytotoxic effects of *P. harmala* extract were reported by Sobhani *et al*., (2002) who concluded that $\beta$-carboline like harmaline and harmine are the major alkaloids present in the seeds of the *P. harmala*.

Moreover, using botanical insecticides origin play significant role in insect control because lack of environmental pollution as well as the protection of human health (Mollashahi *et al.,* 2017). Herbivores insects feeds on different plants may ingest some proteins with toxic effects (Sanchis and Bourguet, 2008). These proteins may be toxic compounds extracted from plants or plant lectins and ribosome inactivating proteins (Ramzi *et al.,* 2013). Since synthetic insecticides cause dangerous concerns regarding non-target organism, environment, and food biosafety, general trends nowadays have been directed to investigate derived materials from plants and microbes in pest control. Also, the plant gene encode responsible for production of toxic proteins which enable plants to overcome herbivores attacks (de Oliveira *et al*., 2011; Saadati and Bandani, 2011).

This study aimed to investigate the toxic effect of methanolic extract of *Citrullus colocynthis, Peganum harmala, Senna alexandrina,* and *Apium graveolens* against *Aphis craccivora* and enzyme activity in the *Aphis craccivora* after treatment with $LC_{50}$ of plant extracts as well synergistic and/or antagonist effect of combination of these plant extracts with conventional insecticide pirimicarb on *Aphis craccivora* in the field.

## MATERIALS AND METHODS

### Preparation of Plant Extracts:

The commercial methanol 70% was used as a solvent. the dried parts of *Citrullus colocynthis, Peganum harmala, Senna alexandrina,* and *Apium graveolens* were transformed to powder. Then, 100 grams of each powder was soaked in 500 ml of the commercial methanol 70% for 24 hours in dark containers to prevent direct sunlight, then shake on the shaker (150 rpm). Then, filtration using Whatman filter paper No. 1 was performed. Finally, the solvent was evaporated using a rotary evaporator, moreover, the final extract was stored in a dark container of glass until use.

**Insect Breeding in the Laboratory:**

The insects Breeding was carried out under laboratory conditions in cages of wood with dimensions of 35×35×35 cm, in sucking insect's department, plant protection, research institute, Agriculture Research Center, Dokki, Egypt. 16 hours Photoperiod and 8 hours of darkness. To breed adult insects, wooden cages with dimensions of 35×35×35 cm were recruited. These cages were covered with a net of wire, the temperature of 22±3°C, a relative humidity of 35±5%.

**Laboratory Bioassay:**

In the laboratory the LC values of different plant extracts, as well as pirimicarb insecticide, evaluated, serial concentration (1000, 2000, 3000, 4000, 5000 and 6000 ppm) of plant extracts; *Citrullus colocynthis, Peganum harmala, Senna alexandrina,* and *Apium graveolens* performed, and the toxicity against *A. Craccivora* were evaluated by leaf-dip technique, in which, *Faba bean* leaf discs dipped in the toxic solution for 10 seconds, then dried in the laboratory condition, after that 10 of Apterous aphid put on each plant disc, the toxicity effect evaluated after 24 hours for each compound, five replicates were performed in each concentration.

**Table 1:** Name of plants used in the study

| Scientific name | Family name | Part extracted |
|---|---|---|
| *Citrullus colocynthis* | Pyrgomorphidae | Fruits and leaves |
| *Peganum harmala* | Zygophyllaceae | Seed |
| *Senna alexandrina* | Fabaceae | Leaves |
| *Apium graveolens* | Apiaceae | Seeds |

**Field Bioassay:**

This study was carried out at in Kaha agricultural research Station (2018) in order to evaluate the combined effect of Pirimicarb and *Citrullus colocynthis, Peganum harmala, Senna alexandrina,* and *Apium graveolens against A. craccivora* in *Faba bean* field. Each treatment and untreated one was replicated four times in 4x4 meters in complete randomized block design. All the normal agricultural experiments were carried out with a foliar spray of $LC_{50}$ of each plant extract with $LC_{25}$, $LC_{15}$ and $LC_{5}$ of Pirimicarb insecticide which equals 40.0, 29.0 and 16.5 ppm respectively, during the growing season. To determine the synergistic and/or antagonist effect of these combinations on the reduction percentages of Cowpea aphid *A. craccivora* (all stages) in the field.

**Sample Preparation for Biochemical Assay:**

For biochemical analysis, 50 individuals of alive aphid species treated with $LC_{50}$ of each plant oil after 24 hours from treatment were collected. These different samples were preserved in a laboratory refrigerator until analysis performed, then the specimens were homogenized in distilled water using the homogenizer which was surrounded by ice for 3 minutes. Homogenates were centrifuged at 8000 rpm. for 15 minutes at 5°C, pellet excluded while the supernatants were used directly to determine the activity of the mixed-function oxidase (MFO), alkaline phosphates, and Alpha and Beta-esterase.

**(a)- Alkaline Phosphatase Enzyme:**

Alkaline phosphatase was determined according to the method prescribed by Powell and Smith (1954). The enzymatic activity is expressed as µg phenol released/gm body weight/ minute. Alkaline phosphatase was measured, immediately, by spectrophotometer at 510 nm.

**(b)- Non- Specific Esterase:**

According to the method of Van Asperen (1962) α- esterases and β- esterases were determined using alpha naphthyl acetate and beta naphthyl acetate as substrate, respectively. The activity of Non- specific esterase was read at 600 or 555 nm for alpha and beta-naphthol, respectively.

**C – Oxidase Activity:**

To determine the mixed-function oxidase activity according to the method of Hansen and Hodgson (1971) P-niroanisole o-demethylation was assayed. Oxidase activity was measured at 405 nm.

**Statistical Analysis:**

Analysis of variance performed by SPSS software. Also, toxicity lines, chi-square, correlation co-efficient performed by Ldp-line -software.

## RESULTS AND DISCUSSION

**Mortality Percentages of Plant Extracts Against Aphis Craccivora:**

Data depleted in table (2) and figure (1) showed the toxicity of *Citrullus colocynthis, Peganum harmala, Senna alexandrina,* and *Apium graveolens extracts* were ranged from 8.4- 75.6, 7.2-67.4, 9.6-81 and 5.6-62.6% respectively. Moreover, the coefficient of variation is 8.6, 7.6, 7.4 and 8.5 for each extract respectively, these values of coefficient of variation in the different plant extracts are the indicator for the homogeneity of *Aphis craccivora* under the study.

**Table 2**: Mortality percentages of plant extracts against *Aphis craccivora*

| Plant extract | Concentration in ppm | | | | | | c.v. |
|---|---|---|---|---|---|---|---|
| | 1000 | 2000 | 3000 | 4000 | 5000 | 6000 | |
| *Citrullus colocynthis* | 8.4 | 16.4 | 20.8 | 29.4 | 48.6 | 75.6 | 8.4% |
| *Peganum harmala* | 7.2 | 13.8 | 18.6 | 27 | 43.6 | 67.4 | 7.6% |
| *Senna alexandrina* | 9.6 | 18.2 | 26 | 33.8 | 51.8 | 81 | 7.4% |
| *Apium graveolens* | 5.6 | 11.2 | 16.4 | 24.2 | 37.8 | 62.6 | 8.5% |

c.v.= coefficient of variation

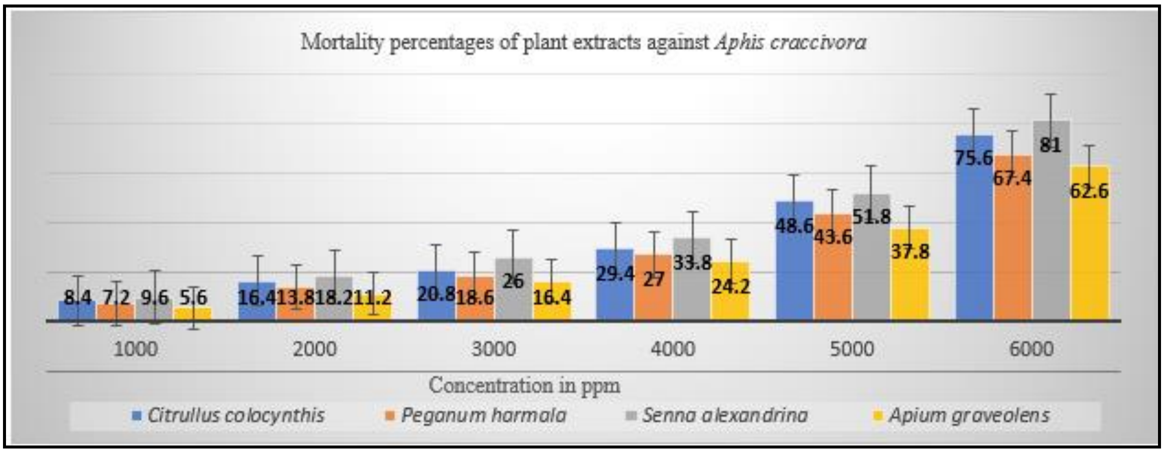


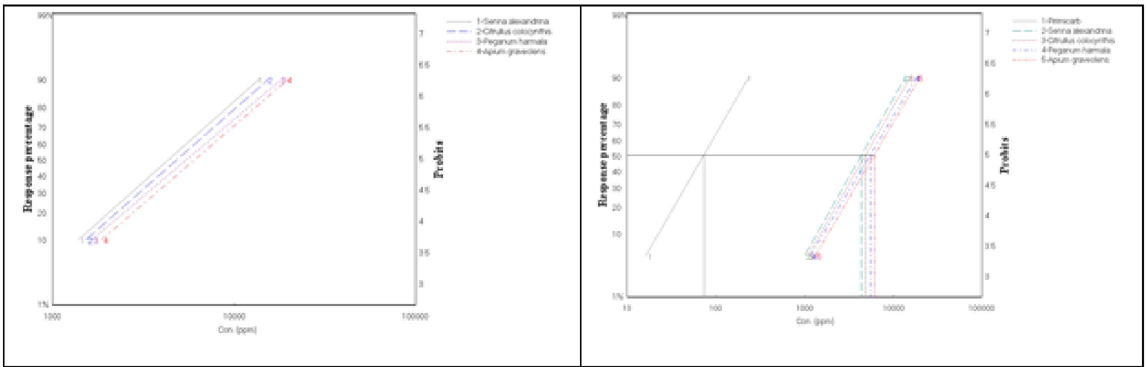

**Fig. 1**: Mortality percentages of plant extracts against *Aphis craccivora*

Also, data presented in table (3) and Fig. (2) showed the LC values of *C. colocynthis, P. harmala, S. alexandrina,* and *A. graveolens* as well as pirimicarb insecticide. It was found that $LC_{50}$ was 4890.079, 5537.761, 6118.4856, 6118.4856 and 74.1996 ppm respectively for *C. colocynthis, P. harmala, S. alexandrina, A. graveolens,* and pirimicarb. While $LC_{90}$ was 15619.0158, 18509.6748, 20022.3887, 13846.5233 and 238.0284 ppm respectively for *C. colocynthis, P. harmala, S. alexandrina, A. graveolens,* and pirimicarb. On the other hand, the correlation coefficient (r) in all treatments is near 1.0 it means that there is a direct relationship between concentration used and mortality percentages in all treatments. Moreover, chi-square in all treatments is highly significant at α< 0.01, which indicated that unique independence of concentration with in the same compound used in this study.

**Table 3**: LC values of plant extracts against *Aphis craccivora*

| Plant extract | $LC_{50}$ | $LC_{90}$ | $Chi^2{}_{(9.5)}$ | (r) | P. value |
|---|---|---|---|---|---|
| *Citrullus colocynthis* | 4890.079 | 15619.0158 | 26.2734 | 0.9018 | 0.0000 |
| *Peganum harmala* | 5537.761 | 18509.6748 | 18.8672 | 0.9197 | 0.0008 |
| *Apium graveolens* | 6118.4856 | 20022.3887 | 16.5850 | 0.9280 | 0.0023 |
| *Senna alexandrina* | 4400.3485 | 13846.5233 | 26.8912 | 0.9044 | 0.0000 |
| Pirimicarb | 74.1996 | 238.0284 | 13.9808 | 0.9534 | 0.0074 |

r= Correlation coefficient, P. value= probability values and Chi2 = chi-square, Chi2 (00, 0.05) = 9.5




A. LC values of different plant extract against *Aphis craccivora*

B. LC values of different plant extracts vs. pirimicarb insecticide against *Aphis craccivora*

**Fig.** 2: LC values of different plant extracts and Pirimicarb insecticide against *Aphis craccivora*

On the other hand, when comparing among the different plant extracts based on the toxicity index, it was found that the extract is *S. alexandrina*, thus, it gave arbitrary value 100. The other extracts compared with *S. alexandrina* and can be arranged ascendingly as following *A. graveolens* (71.91), *P. harmala* (79.46) and *C. colocynthis* (89.98) as toxic as *S. alexandrina* (Table 4).

**Table 4:** Toxicity index, Resistance ration and Potency level of plant extracts against a laboratory strain of *Aphis craccivora*

| Plant extract | Toxicity index | Potency level | Slope |
|---|---|---|---|
| *Citrullus colocynthis* | 89.98 | 1.25 | 2.5412 |
| *Peganum harmala* | 79.46 | 1.10 | 2.4455 |
| *Apium graveolens* | 71.91 | 1.00 | 2.4892 |
| *Senna alexandrina* | 100 | 1.39 | 2.5743 |

Effect of aqueous extracts bitter apple *Citrullus colocynthis* with different concentrations against the bird cherry-oat aphid, *Rhopalosiphum padi* under laboratory conditions was investigated, results showed that the significant reduction in *R. padi* numbers in the laboratory, due to a higher concentration of different secondary metabolites in the plant such as glycosides, terpenoids, cucurbitacin, flavonoids and others which may have a toxic effect against aphid pests (Asiry, 2015). *C. colocynthis* extracts as a natural botanical insecticide have been investigated against many insect pests (Soam *et al.*, 2013). Pesticidal activity of natural product which called lectin of *C. colocynthis* against *Ectomyelois ceratoniae* (Lepidoptera: Pyralidae) (Ramzi *et al.,* 2013).

**Reduction Effect of the Combination of Pirimicarb and Different Plant Oils:**

Data presented in table (5) showed that the reduction percentages of *Aphis craccivora* in the case of application of combination *Citrullus colocynthis* ($LC_{50}$) and pirimicarb ($LC_{25}$, $LC_{15}$and $LC_{50}$) the reduction% were ranged between 91.00-70.0, 85. 66- 64.33, 68.33- 47.33 respectively. Meanwhile, in the case of application of combination *Peganum harmala* ($LC_{50}$) and pirimicarb ($LC_{25}$, $LC_{15,}$ and $LC_{50}$) the reduction % were ranged between 81.33-60.66, 71.66- 50.66, 58. 66- 37.33 respectively. While, in the case of application of combination *Senna alexandrina* ($LC_{50}$) and pirimicarb ($LC_{25}$, $LC_{15,}$ and $LC_5$) the reduction % were ranged between 95.33-76.33, 91.66-67.66, 71.66- 50.33 respectively. Whereas, in the case of application of combination *Apium graveolens* ($LC_{50}$) and pirimicarb ($LC_{25}$, $LC_{15,}$ and $LC_5$) the reduction % were ranged between 78.00-57.00, 68.00-47.00, 55.33- 34.66 respectively.

**Table 5**. Reduction percentages of cowpea aphid, *Aphis craccivora* population infesting *Faba bean* plants after application of $LC_{50}$ of plant extracts and $LC_{25}$, $LC_{15}$ and $LC_5$ of the pirimicarb

| Time | Rate of application of pirimicarb | *Citrullus colocynthis* | *Peganum harmala* | *Senna alexandrina* | *Apium graveolens* |
|---|---|---|---|---|---|
| after 24 hours | 40.0ppm ($LC_{25}$) | 91.00 | 81.33 | 95.33 | 78.00 |
| | 29.0ppm ($LC_{15}$) | 85.66 | 71.66 | 91.66 | 68.00 |
| | 16.5ppm ($LC_5$) | 68.33 | 58.66 | 71.66 | 55.33 |
| After 3 days | 40.0ppm ($LC_{25}$) | 96.00 | 85.00 | 100.00 | 80.33 |
| | 29.0ppm ($LC_{15}$) | 89.33 | 76.33 | 94.00 | 70.00 |
| | 16.5ppm ($LC_5$) | 72.00 | 64.33 | 75.33 | 58.00 |
| After 7 days | 40.0ppm ($LC_{25}$) | 81.00 | 71.33 | 85.33 | 68.00 |
| | 29.0ppm ($LC_{15}$) | 75.66 | 61.66 | 81.66 | 58.00 |
| | 16.5ppm ($LC_5$) | 58.33 | 48.66 | 61.66 | 45.33 |
| After 10 days | 40.0ppm ($LC_{25}$) | 70.00 | 60.66 | 76.33 | 57.00 |
| | 29.0ppm ($LC_{15}$) | 64.33 | 50.66 | 67.66 | 46.00 |
| | 16.5ppm ($LC_5$) | 47.33 | 37.33 | 50.33 | 34.66 |

On the other hand, the initial kill in case of *C. colocynthis* combined with $LC_{25}$, $LC_{15}$and $LC_5$ was 91, 85.66 and 68.33 respectively, while it was 81.33, 71.66 and 58.66 in case of *P. harmala* respectively, also in case of *S. alexandrina* it was 95.33, 91.66 and 71.66 respectively. Moreover, *A. graveolens* was 78, 68 and 55.33 respectively.

Also, the persistence reduction in case of combination between pirimicarb ($LC_{25}$, $LC_{15,}$ and $LC_5$) and *C. colocynthis, P. harmala, S. alexandrina and A. graveolens* was 82.33, 76.44 and 59.22 - 72.33, 62.88 and 50.1- 87.22, 81.10 and 62.44 - 68.44, 58 and 45.99 respectively, (Table 6).

**Table 6**: Initial kill and persistent reduction of $LC_{50}$ of different plant extracts combined with $LC_{25}$, $LC_{15}$, $LC_5$ of pirimicarb against *Aphis craccivora* in the field.

| Concentration $LC_{50}$ of plant extract | Concentration of pirimicarb in ppm | | | Concentration of pirimicarb in ppm | | |
|---|---|---|---|---|---|---|
| | $LC_{25}$ | $LC_{15}$ | $LC_5$ | $LC_{25}$ | $LC_{15}$ | $LC_5$ |
| | Initial reduction % | | | Persistent reduction% | | |
| *Citrullus colocynthis* | 91 | 85.66 | 68.33 | 82.33 | 76.44 | 59.22 |
| *Peganum harmala* | 81.33 | 71.66 | 58. 66 | 72.33 | 62.88 | 50.1 |
| *Senna alexandrina* | 95. 33 | 91.66 | 71.66 | 87.22 | 81.10 | 62.44 |
| *Apium graveolens* | 78 | 68 | 55. 33 | 68.44 | 58 | 45.99 |

Moreover, susceptibility index and Potency level of combination of $LC_{50}$ of plant extracts and $LC_{25}$ of pirimicarb against *Aphis craccivora* in the field based on Initial and Persistent reduction presented in table (7). In case of the initial reduction, it was found that the Susceptibility index was 95, 85 and 81% for *C. colocynthis, P. harmala* and *A. graveolens* compared with *S. alexandrina* respectively, while the Potency level was 1.22, 1.16 and 1.04 folds of *S. alexandrina, C colocynthis and P. harmala* more effective than *A. graveolens* table (7). It may be concluded that the increase in the population reduction in the combination of different plant extracts and pirimicarb may due to the synergistic effect.

**Table 7:** Susceptibility index and Potency level of combination of $LC_{50}$ of plant extracts and $LC_{25}$ of pirimicarb against *Aphis craccivora* in the field based on Initial and Persistent reduction

| Plant extract | Susceptibility index | Potency level | Susceptibility index | Potency level |
|---|---|---|---|---|
| | Initial reduction/$LC_{25}$ | | Persistent reduction/$LC_{25}$ | |
| *Citrullus colocynthis* | 95 | 1.16 | 94 | 1.20 |
| *Peganum harmala* | 85 | 1.04 | 82 | 1.05 |
| *Apium graveolens* | 81 | 1 | 78 | 1 |
| *Senna alexandrina* | 100 | 1.22 | 100 | 1.27 |

Generally, the combination of the different plant extracts showed or exhibit such a dramatic reduction in *A. craccivora* in the field and minimize the concentration of the pirimicarb required to reduce the aphid population, which leads to reduce the consumption of chemical pesticides in plant protection and decrease the residues in the environment.

Moreover, *C. colocynthis* extract was potent toxic on the cowpea aphid (*Aphis craccivora* Koch.) (Sayeda *et al.,* 2009). Similarly, Soliman *et al.* (2005) recorded the toxicity of *C. colocynthis* extracts using different solvents; diethyl ether, hexane, acetone and ethanol, ethyl acetate against *Aphis gossypii* (Torkey *et al.,* 2009). ethanolic extract of *C. colocynthis* fruit showed potent toxicity ($LC_{50}$ 11003ppm) against *A. craccivora* compared with different organic solvents (Soam *et al.,* 2013). Temporal synergism can effectively improve the activity of carbamates and plant extracts against resistant insect pests (Bingham *et al.,* 2008). Potential utilization of Essential Oils which were tested combined with diatomaceous earths (DEs) showing synergistic effects, for the protection of the stored grain in the organic agriculture chain and integrated pest management programs (Pierattini *et.al.,* 2019).

**Enzyme activity in the *Aphis craccivora* after treatment with $LC_{50}$ of plant extracts**

The efficacy of $LC_{50}$ of different plant extracts namely; *C. colocynthis, P. harmala, S. alexandrina* and *A. graveolens* on detoxification enzymes; alkaline phosphatase, Beta-esterase, Alpha- esterase and Mixed-function oxidase in *A. craccivora* was estimated. In the case of *P. harmala*, it was found that alkaline phosphatase, Beta-esterase, Alpha-esterase and Mixed-function oxidase highly increased than other extracts as well as the different plant extracts, except Alpha- esterase which was looks like control.

The biochemical activity of alkaline phosphatase which was evaluated in *A. craccivora* after treatment with $LC_{50}$ *C. colocynthis, P. harmala, S. alexandrina* and *A. graveolens* was 1.84±0.06, 1.93±0.11, 1.75±0.06 and 1.34±0.11 respectively, with activity ratio 1.10, 1,16, 1.05 and 0.73 compared with the control. While, the biochemical activity of Beta-esterase was 31.63±2.07, 47.59±4.44, 34.02±2.20 and 23.51±2.57 for *C.*

*colocynthis, P. harmala, S. alexandrina* and *A. graveolens* respectively, with activity ratio 0.98, 1.48, 1.06 and 0.73 compared with the control, (Table 8).

On the other hand, Alpha-esterase activity recorded 5.57±0.25, 7.65±0.51, 5.98±0.10, 4.03±0.16 in case of $LC_{50}$ *C. colocynthis, P. harmala, S. alexandrina* and *A. graveolens* and the activity ratio was 0.76, 1.05, 0.82 and 0.55 respectively compared with the control. Also, Mixed function oxidase activity registered 1123.33±50.3, 1228.00±22.1, 1020.00±78.1 and 1164.66±47.1 and activity ratio 0.95, 1.04, 0.86 and 0.99, compared with untreated control after treatment with $LC_{50}$ *C. colocynthis, P. harmala, S. alexandrina* and *A. graveolens*, respectively.

**Table 8:** The efficacy of $LC_{50}$ of different plant extracts namely; *C. colocynthis, P. harmala, S. alexandrina* and *A. graveolens* on alkaline phosphatase, Beta-esterase, Alpha- esterase and Mixed-function oxidase.

| Plant extract | Enzyme activity | | | | Activity ratio | | | |
|---|---|---|---|---|---|---|---|---|
| | alkaline phosphatase **(µmole conjugated /min / *mg protein*)** | Beta-esterase **(µg β - *naphthyl etate released /min/mg protein*)** | Alpha- esterase **(µg α - *naphthyl cetate released min/mg protein*)** | Mixed function oxidase **(µ *mole substrate oxidized/min/ mg protein*)** | alkaline phosphatase | Beta-esterase | Alpha-esterase | Mixed function oxidase |
| Control | 1.66±0.12$^{c}$ | 31.96±1.23$^{bc}$ | 7.28±0.23$^{a}$ | 1174.33±84.4$^{ab}$ | - | - | - | - |
| *Citrullus colocynthis* | 1.84±0.06$^{ab}$ | 31.63±2.07$^{bc}$ | 5.57±0.25$^{c}$ | 1123.33±50.3$^{ab}$ | 1.10 | 0.98 | 0.76 | 0.95 |
| *Peganum harmala* | 1.93±0.11$^{a}$ | 47.59±4.44$^{a}$ | 7.65±0.51$^{a}$ | 1228.00±22.1$^{a}$ | 1.16 | 1.48 | 1.05 | 1.04 |
| *Senna alexandrina* | 1.75±0.06$^{bc}$ | 34.02±2.20$^{b}$ | 5.98±0.10$^{c}$ | 1020.00±78.1$^{b}$ | 1.05 | 1.06 | 0.82 | 0.86 |
| *Apium graveolens* | 1.34±0.11$^{d}$ | 23.51±2.57$^{d}$ | 4.03±0.16$^{d}$ | 1164.66±47.1$^{ab}$ | 0.80 | 0.73 | 0.55 | 0.99 |
| LSD | 0.181 | 4.93 | 0.53 | 110.2 | | | | |
| F. value | 15.99* | 30.76* | 73.06* | 4.9* | | | | |
| P. value | P<0.000 | P<0.000 | P<0.000 | P<0.019 | | | | |

*. The mean difference is significant at the 0.05 level, Mean±SD with the same litter in the same column is not significantly different

Generally, *P. harmala, S. alexandrina* showed an increase in Alkaline phosphatase, non-specific esterase and mixed function oxidase, while it was moderate for *C. colocynthis* and low for *A. graveolens* compared with the control. The increase of enzyme activity may be due to the increase of the defense mechanism in the insect against the potent toxic plant extracts (Table, 8).

Involvement of the detoxification enzymes (an esterase inhibitor, a cytochrome P450 monooxygenase, and a glutathione *S*-transferase inhibitor) in the differential susceptibility to insecticide between *Sitobion avenae* and *Rhopalosiphum padi*. (Lu and Gao, 2009). The susceptible insects, showed a low level (baseline level) of metabolic enzyme systems, whereas in the resistant insects, they are elevated levels (Wheelock *et al.* 2001; Young *et al*., 2006).

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## ARABIC SAMMARY

# التأثيرات السامه والتغيرات الكيميائية الحيوية لبدائل المبيدات على حشرة من اللوبيا

**أيمن علي شهاوي 1 ,2 وعبد الحليم خليل1 وأمنه مقلد 1 وسمير حسن قاري2**
1- معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الجيزه – جمهورية مصر العربيه
2- الكلية الجامعية بالجموم – جامعة ام القري – مكة المكرمه – المملكة العربيه السعوديه

يعتبرحشرة من اللوبيا *Aphis craccivora* من اهم الآفات الضارة التي تصيب العديد من المحاصيل ومنها الفول والتي تؤدي الإصابة به إلى انخفاض نسبة إنتاج المحصول. في هذه الدراسه تم عمل استخلاص لبعض النباتات منها الحنظل و الحرمل و سينامكي، و الكرفس بواسطة الميثانول 70 ٪ ، تم تقييم التأثير السام لهذه المستخلصات علي حشرة من اللوبيا معمليا لتقييم التركيز النصف المميت ثم تقييمه في الحقل مع بعض التركيزات المنخفضه من مركب البريميكارب، كما تم تقدير الفوسفاتيز القلوي (Alkaline phosphatase) ، و الفا , بيتا استيريز (α and β esterase's) والأكسيداز المختلط (MFO) في الحشره بعد المعاملة بالتركيز النصف المميت.

أظهرت النتائج ان التركيز النصف المميت $LC_{50}$ لهذه النباتا على الحشرة كانت 4890.079، 5537.761، 6118.4856 و6118.4856 جزء في المليون على التوالي، في حين كان 74.1996 جزء في المليون للبريميكارب. علاوة على ذلك، أظهر خليط المستخلصات النباتية المختلفة مع التركيزات المنخفضة من البريميكارب خفضا معنويا في تعداد الحشرة في الحقل بمقارنته مع الكنترول على الرغم من خفض تركيز المبيد المستخدم.

كما أظهر المستخلص الميثانولي للحرمل و السينامكي زيادة في نسبة الفوسفاتيز القلوي ( Alkaline phosphatase)، و الفا , بيتا استيريز (α and β esterase's) والأكسيداز المختلط (MFO) في حين كانت الزيادة معتدًلة في حال استخدام المستخلص ل الميثانولي لنيات الحنظل. على الصعيد الاخر كان هناك انخفاضا في هذه الانزيمات في حالة استخدام المستخلص الميثانولي لنبات الكرفس مقارنة بالكونترول. من خلال هذه النتائج يتضح الدور الهام لهذه المستخلصات في مكافحة حشرة من اللوبيا وذلك لتقليل الاستخدام الغير أمن للمبيدات الكيميائية كما يمكن استخدام هذه المستخلصات في برامج المكافحه المتكامله للافات.