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Haemocytes and biochemical changes in *Locusta migratoria* (Orthoptera: Acrididae) after treated with some essential oils

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Abstract:

The migratory locust**,** *Locusta migratoria* L. (Orthoptera: Acrididae) is the most widespread species throughout different parts of the world. It feeds on grass and often causes serious damage to agricultural crops. The present study deals with the effect of essential oils of garlic, cumin and basil against the migratory locust, *L. migratoria* in terms of percent mortality. Total Haemocyte Count (THC) and Phenoloxidase (PO), Acid Phosphatase (ACP) and Alkaline Phosphatise (ALP) activity. The effect of different concentrations (0.1, 0.5 and 1%) of essential oils were evaluated on last nymphal instar of L. *migratoria*. The results clearly demonstrated that the tested oils (garlic, cumin and basil) had stomach toxicity through the nymphal feeding on treated diet. the mortality percentages were estimated that reached to 67.86, 48 and 37.93 % in one day old nymph treatment with basil, garlic and cumin oils respectively after ten days of treatment with concentration (1%), The LC_{50} of different oils varied from one to other, the best treatment among all the tested oils was Basil oil with LC_{50}(0.45%), it was more toxic as stomach poison than garlic oil with LC_{50} (1.31%), finally cumin oil with LC_{50} (3.07%). THC increased after 1 and 2 days while decreased after 3 and 4 days after treated with basil essential oil compared to control. Garlic and cumin essential oils showed decreased the (THC) after 2, 3 and 4 days in the same time occurred increase in THC after 1 day compared to control. The insect enzymes PO, ACP and ALP activities were affected fluctuated between increasing and decreasing 2, 4, 6 and 8 days after treatment with garlic, cumin and basil (at LC_{50} value). ACP activity and PO activity were dramatically increased at 2^{nd} and 4^{th}in all treatments and decreased at 6^{th} and 8^{th} days comparing with untreated nymphs. Also, highly significant decline in ALP were recorded in them by garlic and cumin during the study.

Introduction

The migratory locust, *Locusta migratoria* L. (Orthoptera: Acrididae) is greatly distributed in the old world (Uvarov, 1977). It feeds on grass and often raises dangerous damage to agricultural crops (Pener and Simpson, 2009). Population densities increases and nymphs start aggregating (Tanaka and Nishide, 2012). The

main outbreak area in Africa of the *L. migratoria* is existing on flood plain of Niger River in Mali. The huge plague (1928-1934) was started in this region and spread towards the south of the continent. Two other probable outbreak areas are detected north of the Equator: The Blue Nile region in Sudan and the Lake Chad basin. Grangerisation and population in these two areas are less favourable and have never induced a plague (Lecoq, 1991). Around the suitability of the Lake Chad basin area appeared some doubt about the full development of the gregarious phase (Davey and Johnston, 1956). However, sometimes local upsurges occur and are so dangerous that wide areas have to be sprayed for crop protection. The major classes of insecticides in the field of pest control in use today are organophosphates and carbamates (Ware, 1982 and Dorow, 1993). Because of the serious side effects on the environment and human health of these insecticides, alternative agents are being examined for the insect pest control (Franzen, 1993). Botanicals are a hopeful source of pest control compounds. Over 2000 species of plants are known today to possess some insecticidal activity (Jacobson, 1989). Botanicals considered friendly way to environment be used in pest management, but effort has not been made yet to use them as the possible alternatives for the management of pests (Khanikor and Bora, 2012). Essential oils are complex compounds, volatile, natural, distinguished by a strong odour and are formed as secondary metabolites by plants. In nature, essential oils play a necessary role in protection of the plants as antiviral, antifungal, antibacterial, insecticides, and also against herbivorous by reducing their appetite for such plants. (Bakkali *et al*., 2008). Essential oils which are extracted from aromatic plants are used to control insect pests. These essential oils are investigated and will be documented (Isman, 2006; Koul *et al*., 2008 and Rajendran and Sriranjini, 2008). Insect haemocytes are part of immune response in invertebrates in case presence of foreign material, toxin and microorganisms (Gupta, 1991 and Pathak, 1993) and the response is showed in terms of phagocytosis in case of small sized particle and in case of large sized material and pathogens is encapsulation and nodulation (Gillespie and Kanost, 1997). In addition, Acid Phosphatase (ACP) and Phenoloxidase (PO) are two important protein molecules involved in insect immunity (Pathak, 1993). Soltan (2014) showed decreased Total Haemocyte Count (THC) by Neem on the 5thnymphal instar of *Schistocerca gregarea*. PO is required in sequential conversion of dopa into melanin; it is existing in cuticle, haemolymph and oenocyte, and thus helps in the fight against nonself. If phenoloxidase plays paramount role in development of resistance against certain insecticides (Liu *et al*., 2009) or can work as indicator for immune competence in host-parasite model system, as a marker for evaluating toxic action may be used PO activity. Therefore, the possible effect of the plants in question were attempted to be evaluated through studies on effect of the plant's essential oil on percent mortality, THC and PO, ACP and ALP enzymes activity.

Materials and methods

Experimental Insect:

The migratory locust was used as an experimental insect in thiswork. These nymphs obtained from department ofLocust and grasshopper, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza. Insects were reared in wooden formed cages measuring: 60 cm length x 60 cm Width x 70 cm height, with a small door in the front side to facilitate daily routine work. An electric lamp (100 watt) was adjusted to maintain a continuous photoperiod of 12 L: 12 D in each cage as well as in order to maintain an ambient temperature of 32±2 ºC. The insects werehandled andreared under the crowded conditions outlined by Hunter–Jones (1961). The dead locusts, faeces and food remains dailywere removed before introducing the freshly food. Berseem, *Alexandranium*

trifolium fresh leaves, in winter, and the leaves of leguminous plant *Sesbaniaaegyptiaca*, in summer, were used as a food for insects. On the other hand, during the experimental work the Berseemleaves only were introduced as food for insects.

1. Essential oils:

Three essential oils:

Garlic: *Allium sativum L.* (Family: Liliaceae)
Cumin: *Cuminumcyminum L.* (Family: Apiaceae)
Basil: (*Ocimumbasilicum L.*) (Family: Lamiaceae)
Were obtained from EL-Captain Company, elcaptain@elcaptain Co., Al-Obour city (Cairo), Egypt.

2. Nymphal treatments:

Leaves of Berseemwere dippedfor three minutes in three different concentrations of essential oils Garlic, Cumin and Basil (0.1, 0.5, and 1.0 %) andwere allowed to dry before offering to the newly moulted fifth nymphal instar of *L. migratoria*. A day after treatment, treated and untreated nymphs were provided with untreated fresh leaves. Three replicates (ten nymphs for replicate) were used for each concentration. After 24 hrs. from treatment all mortalitieswere recorded to treated and control insects, this data were summarized as estimates of the Median Lethal Concentration (MLC). LC_{25}, LC_{50} and LC_{90} values were calculated by using Lpd line software for calculating and drawing the mortality curve according to Finney Method (1971).

3. Total haemocyte count:

Ninety nymphs treated with our essential oils concentrations samples of the haemolymph were taken at different intervals of 1, 2, 3 and 4 days after treatment. After the flowing of haemolymph, it was quickly drawn up to the "0.5" mark in a Thoma white blood cells diluting pipette. The haemolymph was then diluted to the "II" mark with Tuerks solution (1.5% glacial acetic acid containing few drops of genetian violet) with shaking for 1 min. The diluted haemocytes were counted by a haemocytometer of improved nebauer chamber in four corners squares multiplied by 50 to give the number of cells per cm^3 (Wintrobe, 1974).

4. Sample collection and preparation:

One hundred and Fifty treated nymphs were divided into three replications. Nymphs were kept in cages (25 x 25 x 60cm) with a fluorescent lamp as a light source. The control insects were placed in other cages (Robert *et al.*, 2002). Samples of the haemolymph were taken at different intervals of 2, 4, 6 and 8 days after treatment. The haemolymph was collected through a fine puncture in the hind leg membrane and transferred into clean dry centrifuge tubes. A known volume of the collected haemolymph was centrifuged on 13000 rpm to 15 min. to remove blood cells and pigments. Then the supernatant collected for analyses (El Gawhary, 1997).

5. Phenoloxidase determination:

Determination of phenoloxidase activity was based on a method described by Ishaaya (1972) with some modification. The reaction mixture consisted of 200 µl enzyme solution, 2ml phosphate buffer (0.2 M, pH 7) and 0.5 µl 2 % Catechol. The reaction mixture was incubated at 25 ˚C. The activity was then recorded after 2 min from the beginning of the reaction at absorbency 470 nm.

6. Acid and alkaline phosphatises determinations:

ACP and ALP were determined according to the method described by Powell and Smith (1954). In this method, the phenol released by enzymatic hydrolysis of disodium phenylphosphate, reacts with 4-aminoantipyrine, and by the addition of potassium ferricyanide, the characteristic brown colour is produced. The reaction mixture consists 1 ml citric buffer (pH 4.9), 1 ml of 0.01 M disodium phenyl phosphate (substrate), and 0.1 ml nymphal haemolymph. Mix gently and incubate for exactly 30 minutes at 37 ºC. At the end of incubation period, 0.8 ml of NaOH was added to stop the reaction. Then added 1.2 ml of $NaHCO_3$, followed by 1 ml of 4-

aminoantipyrine solution and 1 ml of potassium ferrricyanide. The produced color was measured, immediately, by spectrophotometer at 510 nm. The enzymatic activity is expresses as mg phenol released/ ml haemolymph. Phenol standard curve was prepared as a stock of phenol was prepared by dissolving 1 gm pure crystalline phenol in 1 literHCl. 10 ml of the stock solution (containing 10 mg) was diluted to 100 ml with distilled water. Aliquots of 0.05, 0.1, 0.2, 0.3 and 0.4 ml of the diluted phenol (equal to 5, 10, 20, 30, and 40 mg phenol) were pipetted into test tubes and the volume was completed to 1 ml with distilled water. 1.1 ml of buffer was added followed by 0.8 ml of NaOH, 1.2 ml of NaHCO3, 1ml of aminoantipyrine and 1ml of potassium ferrricyanide. Each tube was mixed well after each addition. The developedcolour was measured at 510 nm.

7. Statistical analysis:

The percentage of nymphal mortality was corrected according to Abbotts formula (Abbott, 1925)

Corrected % = {1 – (n in T after treatment/ n in Co after treatment)} *100

Where: n = Insect population, T = treated, Co = control.

LC_{25}, LC_{50}, LC_{90} values and slope of regression lines were calculated by using (Lpd line) software for calculating and drawing toxicity lines according to Finney Method (1971).

Other Data were analyzed by analysis of variance (ANOVA) means, within row, bearing different subscripts are significantly different ($P<0.05$) by SPSS Program software (SPSS,2009).

Results and Discussion

1. Effectiveness of basil, cumin and garlic on *Locusta migratoria* by feeding technique:

1.1. Effectiveness of Basil against 5th nymphal instar of *Locusta migratoria*:

Results in Table (1) showed effect three concentrations of basil essential oil (1.0, 0.5 and 0.1%) on the percentages mortality of 5th nymphal instar of *L. migratoria* from one to ten days. Data cleared that the percentages of mortality of the 5th nymphal instar were 33.33,41.38 and 67.86% after 10 days at concentration of 0.1, 0.5 and 1 %, respectively. Figure (1) and Table (2) appeared the lethal concentration values (LC_{25}, LC_{50} and LC_{75}) from basil essential oil were 0.06, 0.45 and 3.21 respectively.

Table (1): Mortality % of 5th nymphal instar of *Locusta migratoria* after treated with different concentrations of basil.

days	1% v/v					0.5%v/v					0.1% v/v				
	r1	r2	r3	mean	corrected %	r1	r2	r3	mean	corrected %	r1	r2	r3	mean	corrected %
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	10	10	10	10	10	10	0	10	6.67	6.67	0	0	0	0	0
3	20	10	20	16.67	16.67	10	10	10	10	10	0	0	0	0	0
4	20	20	20	20	17.24	10	10	10	10	10	0	0	0	0	0
5	30	20	30	26.67	24.14	10	20	20	16.67	16.67	10	10	10	10	10
6	40	30	40	36.67	34.48	20	20	20	20	20	10	10	10	10	10
7	40	40	40	40	37.93	30	30	20	26.67	24.14	20	20	10	16.67	16.67
8	50	50	40	46.67	44.83	30	40	30	33.33	31.03	20	30	20	23.33	23.33
9	60	50	60	56.67	55.17	30	40	40	36.67	34.48	20	30	20	23.33	23.33
10	70	70	70	70	67.86	40	50	40	43.33	41.38	30	40	30	33.33	33.33

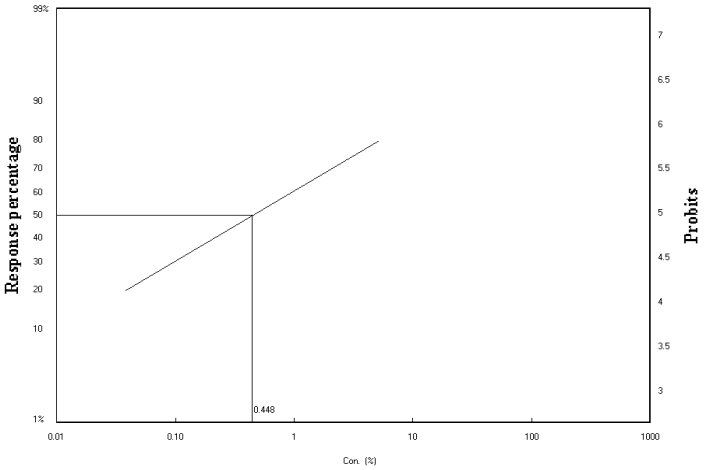

Figure (1): The Lethal Concentration (LC) values of basil by feeding technique on *Locusta migratoria.*

Table (2): The Lethal Concentration (LC) values of basil by feeding technique on *Locusta migratoria*.

LC	Con. %	Lower limit	Upper limit
25	0.06	0.0268	0.1371
50	0.45	0.2974	0.6632
75	3.21	1.3006	8.1479
90	18.92	4.0622	94.1268
95	54.69	7.928	412.313
99	400.36	27.5481	640.197

1.2. Effectiveness of cumin against 5th nymphal instar of *Locusta migratoria*:

Results in Table (3) cleared the percentages of mortality to 5th nymphal instar of *L. migratoria* after treated nymphs with three concentrations of cumin (1.0, 0.5 and 0.1%) from one to ten days were 37.93, 24.14 and 13.79% respectively, on other hand, data in Table (4) and Figure (2) showed the LC values (LC_{25}, LC_{50} and LC_{75}) of cumin against *L. migratoria* after ten days from treatment were 0.39, 3.07 and 23.75%.

Table (3): Mortality % of 5th nymphal instar of *Locusta migratoria* after treated with different concentrations of cumin.

Days	1% v/v					0.5%v/v					0.1% v/v				
	r1	r2	r3	mean	corrected	r1	r2	r3	mean	corrected	r1	r2	r3	mean	corrected
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	10	3.33	3.33	0	0	0	0	0	0	0	0	0	0
3	0	0	10	3.33	3.33	0	0	0	0	0	0	0	0	0	0
4	0	0	10	3.33	3.33	0	0	0	0	0	0	0	0	0	0
5	10	10	10	10	10	0	10	0	3.33	3.33	0	0	0	0	0
6	10	20	10	13.33	13.33	10	10	10	10	10	0	0	0	0	0
7	20	20	20	20	17.24	10	20	10	13.33	13.33	0	0	10	3.33	3.33
8	20	30	20	23.33	20.69	10	20	20	16.67	16.67	0	10	10	6.67	6.67
9	30	40	30	33.33	31.03	20	20	20	20	17.24	10	10	20	13.33	13.33
10	40	40	40	40	37.93	20	30	30	26.67	24.14	10	20	20	16.67	13.79

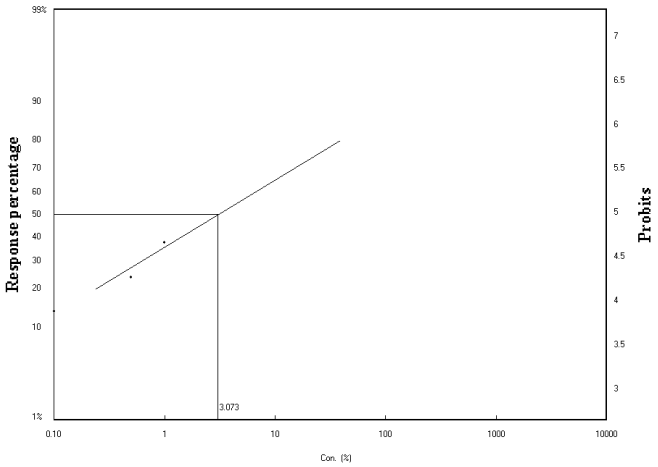

Figure (2): The Lethal Concentration (LC) values of cumin by feeding technique on *Locusta migratoria*.

Table (4): The Lethal Concentration (LC) values of cumin by feeding technique on *Locusta migratoria*.

LC	Con. %	Lower limit %	Upper limit %
25	0.39	0.22	0.67
50	3.07	1.41	29.54
75	23.75	5.68	2067.39
90	149.58	19.33	97619.22
95	449.96	40.04	984400.3
99	3550.37	156.30	75375153

1.3. Effectiveness of Garlic against 5th nymphal instar of *Locusta migratoria*:

Data presented in Table (5) showed the percent reduction of population of *L. migratoria* after ten days from treatment with three concentrations of garlic, this reduction was dose dependent. The percent reduction at 1 % was 48% but at 0.5% was 33.33% finally at 0.1% was 17.86%. Results in Table (6) and Figure (3) showed the LC values and the effect of three concentrations of garlic against *L. migratoria*, where LC_{25} , LC_{50} and LC_{75} were 0.21, 1.31 and 8.16, respectively.

Table (5): Mortality % of 5th nymphal instar of *Locusta migratoria* after treated with different concentrations of garlic.

days	1% v/v					0.5%v/v					0.1% v/v				
	r1	r2	r3	mean	corrected %	r1	r2	r3	mean	corrected %	r1	r2	r3	mean	corrected %
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	10	0	10	6.67	6.67	0	0	10	3.33	3.33	0	0	0	0	0
3	20	10	10	13.33	10.34	10	10	10	10	6.9	0	0	0	0	0
4	20	10	20	16.67	13.79	10	10	10	10	6.9	0	0	0	0	0
5	20	20	20	20	14.29	10	20	20	16.67	13.79	0	10	0	3.33	3.33
6	30	20	30	26.67	21.43	20	20	20	20	14.29	10	10	10	10	6.9
7	40	30	30	33.33	25.93	30	20	20	23.33	17.86	10	10	10	10	6.9
8	40	40	30	36.67	26.92	30	30	30	30	25	20	10	10	13.33	10.34
9	50	40	40	43.33	34.62	30	30	30	30	25	20	10	20	16.67	13.79
10	60	60	50	56.67	48	50	40	30	40	33.33	30	20	20	23.33	17.86

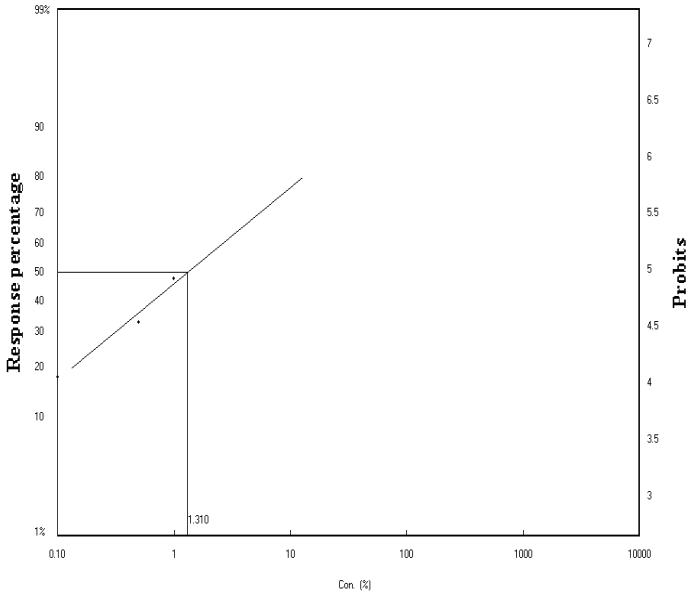

Figure (3):The Lethal Concentration (LC) values of garlic by feeding technique on *Locusta migratoria.*

Table (6): The Lethal Concentration (LC) values of Garlic by feeding technique on *Locusta migratoria*.

LC	Con. %	Lower limit %	Upper limit %
25	0.21	0.098	0.32
50	1.31	0.797	3.76
75	8.16	3.08	92.60
90	42.34	9.85	1748.95
95	113.39	19.63	10210.99
99	719.52	71.16	280839.4

Our results showed that out of the three essential oils of basil, garlic and cumin was the most toxic. The results reflected higher toxicity of basil essential oil against last nymphal instar at the dosages of 1.0, 0.5 and 0.1 % resulted in 67.86, 41.38 and 33.33% mortality of nymphs at 10 days of treatment while LC_{50} was 0.45%, and the order of toxicity of these three oils can be shown as cumin < garlic < basil. The study revealed that last instar nymphs of *L. migratoria* were susceptible to the action of essential oils basil, garlic, where were resistant and could survive after exposure to essential oil cumin treatments. Such resistance of last nymphal instar of *L. migratoria* to the essential oil under consideration may be attributed to age dependent changes in bio constituents. Insecticides upon entering insect body may be acted upon by detoxifying enzyme system for detoxification before they reach the site of action. Age dependent changes in enzyme activity and their subsequent correlation with insecticide toxicity have been reported for several insects with respect to cyto-chrome P450, glutathione S-transferase, malathioncarboxylesterase and microsomal oxidase enzyme (Yu, 1983; Gui *et al*., 2009; Lee *et al*., 1996 and Rajatileka *et al*., 2011). After application of essential oils, values of LC_{50} was considered for study in order to assess response of immune system of *L. migratoria* in terms of total haemocyte count and phenoloxidase, acid phosphatase and alkaline phosphatase enzymes activities.

2. Determination of total haemocyte count on *Locusta migratoria*:

After application of essential oils (Table,7), values of LC_{50} was considered for study in order to assess response of immune system of *L. migratoria* in terms of total haemocyte count and phenoloxidase, acid phosphatase and alkaline phosphatase enzymes activities. On application of essential oil of basil concentration of 0.45% on *L. migratoria* nymphs, THC significantly increased at 1st day (4600±50) which then came down to the level of control (2900±43.3, 2800±90.1 and 2100±25) at 2nd, 3rd and 4th days. On application of 1.31% garlic oil, THC increased significantly at 1st day (5400 ±300), but THC decreased significantly from 2nd day till 4 day (2800 ±25, 1800 ±132.3 and 1380 ±147.3). Application of 3.07% of essential oil of

cumin initially increased THC level at 1st and 2nd days (6800 ±180.3 and 4300 ±86.6) and then significantly decreased at 3rd and 4th days (1750 ±180.3 and 930 ±60.8).

THC is correlated with the rate of phenomena occurring during insect immune response such as phagocytosis, nodule formation, encapsulation, recognition of foreign bodies and wound healing and hence the total number of haemocytes reflect the involvement of immune system to deal with pathogens or chemical molecules (Kraaijeveld *et al*., 2001). In the present study, increase of THC at 1st day of treatment at essential oils, basil, cumin and garlic might reflect activation of immune response. Speedy haemocyte division to facing with the foreign particles may increase THC. The rise and down of THC level which take place on application of essential oil might indicate an active involvement of the defence system to outdo the toxic action in which the haemocytes in the circulation have been used up for the aim of defence. At concentration (LC_{50}) of three essential oils, although at 24 h post treatment THC was high in cases of all the oil, at 48 h in response to action of cumin THC increased. Post treatment of insecticides initial increase followed by reduction at 2nd - 3rd days were reported by several workers (Sharma *et al*., 2008 and El-Aziz and Awad, 2010). Furthermore, dependent THC on ecdyson titre (Ayyangar and Rao, 1990). Abamectin is an insecticide caused the secretion of antidiuretic hormone from neurosecretory cells of the thoracico-abdominal ganglionic mass that slowed the rate of excretion resulting in increase of blood volume and that way decrease of total haemocyte count (Suhail *et al*., 2007). Reduction of haemocytes may also be due to disaster happened in hematopoietic organs which are responsible for production of haemocytes (Tiwari et al., 2002). Also, application of insecticides occurred decrease in THC after 12 h and 1st day of treatment in other insects (Ayyangar and Rao, 1990 and El-Aziz and Awad, 2010).

Table (7): The effect of basil, garlic and cumin on total haemocyte count of the 5th instar nymphs *Locusta migratoria* (the number of cells per cm^3).

Treatment / Days	Control	Basil	Garlic	Cumin
1nd	44a ±86.6	46b ±50	54b ±300	68c ±180.3
2th	34a ± 173.2	29b ±43.3	28b ±25	43c ±86.6
3th	30.5a ± 100	28a ±90.1	18b ±132.3	17.5c ±180.3
4th	33a ± 50	21b ±25	13.8c ±147.3	9.3d ±60.8

3. Effect of essential oils, basil, garlic and cuminon activity of phenoloxidase (O.D. unit x 10^3/min./ml haemolymph) in 5th nymphal instar of *Locusta migratoria*:

The effect of essential oils, basil, garlic and cumin with their LC_{50} values in Table (8). The PO activity measured during four days after treatment with essential oils. In the second day the PO activity highly increased than the control and in the 4th day the activity was still high but lowers than the first day. The PO activity in treatment showed decreasethan control in the 6th and 8th day in all treatment. Phenoloxidase enzyme plays a paramount role in recognition exotic particles and therefore give immunity against external chemicals and different microorganisms in various arthropods (Shelby *et al*., 2000; Shiao *et al*., 2001; Yu *et al*., 2003; Ling *et al*., 2005 and Zhu *et al*., 2009). Black colour nodules formed in the haemolymph, due to melanin which produced by the enzyme PO. studies appeared that PO was existing in major quantities in the serum than in the haemocytes. (Gillespie *et al*., 2000) tyrosine turns into Dopa by PO enzyme which is used in synthesis of melanin for later utilize in immune response and wound healing (Huang *et al*., 2002). Increase of PO activity has long been correlated with increased resistance (Sugumaran, 2002 and Shelby and Popham,

2006) and decrease of PO activity is attributed to weakening of immune system (Hiromori and Nishigaki, 2001). Prevent phenoloxidase production by locust haemocytes may be as a result of destruction of the cells that produce prophenoloxidse (Said, 2014).

Table (8):Effect of essential oils on activity of phenoloxidase (O.D. unit x 10^3/min./ml haemolymph) in 5th nymphal instar of *Locusta migratoria*.

Days	Control	Basil Oil	Garlic Oil	cumin Oil
2days after treatment	3483.33±35.35a	4250.00±132.29c	4133.33±140.47c	3833.33±90.74bb
4days after treatment	3423.33±68.07a	3863.33±47.26c	3616.67±28.87ab	3663.33±40.41bc
6 days after treatment	3460.00±36.05a	2946.67±45.09bc	3040.00±45.83b	2760.00±52.91c
8 days after treatment	3505.00±5.00a	2906.67±15.27bc	3006.67±15.27b	2750.00±62.45c

F: Measurement of distance between individual distributions.
Means with the same letter are not significantly different.
Means with the different letter are significantly different and a=, b=**, c***.*

4-Effect of on activity of Acid andAlkaline phosphatise (IU /ml haemolymph) of the 5th instar nymphs *Locusta migratoria*:

Results presented in Table (9) show that, the ALP activity significant decreased between Garlic oil 13.71±.11, 13.13±0.26, 11.92±0.81 and 9.8633±.08 unit (U) after 2, 4, 6 and 8 days from treatment. Also, in cumin oil significant decreased 10.12±.07, 9.8±.1, 6.27±.15 and 5.11±.08 U after 2, 4, 6 and 8 days after treatment. The ALP activity increased 19.66±.12, 18.98±.1, and 18.02±.01 U by Basil oil after 2, 4 and 6 days while become non-significant 16.9833±.10693a after 8 days compared to control 16.83±.28, 16.82±.11, 16.77±.06 and 16.54±.06 U. Activity of acid phosphatase in haemolymph of *L. migratoria* during treatment with essential oils, basil, cumin and garlic was found in Table (10). Eight days after treatment there was significant differences in ACP activity between Experiment and control. Where, there was a large increase in ACP activityin treated insects on second day and fourth day than become decrease insix and eight day after treatment. Our study showed that the activity of ACP at 2nd and days 4th of treatment fifth nymphal instar of *L. migratoria* treated with 0.45%, 1.31% and 3.07% of Basil, Garlic and cumin essential oils was determined. The tested compounds significantly increased the activity of ACP as compared to the control. The activity of ACP decreased in treated nymphs after day 6 and day 8. All treatments caused increase in ACP activity where, cumin gave the highest increase in ACP activity followed by Garlic than Basil, where these values after 2 day were 13.03±.11, 13.47±.26 and 15.17±.15 IU/l respectively as compared with 10.55±.085 IU/l in the control. ALP and ACP have been shown to be associated with insect development, especially in relation to nutrition and egg maturation (Tsumuki and Kanehisa, 1984). Great interest in ALP and ACP during developmental studies and so, because of its association with histolysis. Whereas, ALP and ACP hydrolyzes a diverse of orthophosphorylation reactions (Hollander, 1971). The number of lysosomes increases as a result for Ecdysone (Radford and Misch, 1971). This indicates that the decreased activity of ALP and ACP in this study may be due to decreased number of lysosomes. Sridhara and Bhat (1963) showed that during development the decrease or increase of phosphatases enzyme reflected an increase or decrease in the acid-soluble phosphorus content.

Table (9): Alkine phosphatase activity (IU /ml haemolymph) of the 5th nymphal instar of *Locusta migratoria* after treated with essential oils.

Days	Control	Basil Oil	Garlic Oil	cumin Oil
2days after treatment	16.83±.28284a	19.6600±.12166b	13.7100±.11000b	10.1200±.07211c
4days after treatment	16.8233±.10786a	18.9833±.10408b	13.1300±.02646b	9.8000±.10000c
6days after treatment	16.7667±.05774a	18.0200±.01000b	11.9233±.08145c	6.2667±.15275d
8days after treatment	16.5367±.06351a	16.9833±.10693a	9.8633±.07506b	5.1133±.07767c

F: Measurement of distance between individual distributions.
Means with the same letter are not significantly different.
Means with the different letter are significantly different and a=, b=**, c***.*

Table (10): Acid phosphatase activity (IU /ml haemolymph) of the 5th nymphal instar of *Locusta migratoria* after treated with essential oils.

Days	Control	Basil Oil	Garlic Oil	Cumin Oil
2 days after treatment	10.55±.08485a	13.0267±.11150b	13.4700±.25632b	15.1667±.15275c
4 days after treatment	11.0833±.10214a	11.9333±.09452b	12.1000±.20000b	13.9333±.25166c
6 days after treatment	11.1267±.06429a	9.4800±.10817b	10.6100±.11533a	12.0000±36056b
8 days after treatment	10.9900±.19672a	8.6200±.08185b	8.6667±.45092b	9.1767±.13650b

F: Measurement of distance between individual distributions.
Means with the same letter are not significantly different.
Means with the different letter are significantly different and a=, b=**, c****

It is concluded that the best treatment among all the tested oils was basil oil then garlic oil finally cumin oil, also all essential oils used effect on immune response in *L. migratoria* nymphs.

Conflict of Interest

The present study was performed in absence of any conflict of interest.

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