

**INSECTICIDAL ACTIVITIES OF CERTAIN MINOR NUTRIENTS  
AGAINST THE LARVAE OF THE EGYPTIAN COTTON LEAFWORM,  
*SPODOPTERA LITTORALIS* (BOISD.)  
NOCTUIDAE: LEPIDOPTERA**

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**Abstract**

The insecticidal effects of four micro-nutrients, Iron (Fe<sup>+++</sup>), Copper (Cu<sup>++</sup>), Manganese (Mn<sup>++</sup>) and Zinc (Zn<sup>++</sup>) against the second and fourth instar larvae of *S. littoralis* was evaluated in laboratory tests. Larvae were fed for 48h on castor leaves *Ricinus communis* dipped in a solution of each element at different concentrations. Copper (Cu) was the most toxic nutrient against the second and fourth instar larvae (LC<sub>50</sub> values were 330 and 500 ppm, respectively). While, Manganese (Mn) came next (LC<sub>50</sub> values were 350 and 660 ppm, respectively). While, Iron (Fe<sup>+++</sup>) was the least one (LC<sub>50</sub> values were 560 and 1500 ppm, respectively). The biological activities of the treated larvae with each of the four nutrients, were affected. Copper and Manganese treatments showed as higher effects than other treatments. However, the larval and pupal duration were prolonged, the pupation and adult emergence percent were decreased, the pupal weight was decreased and pupal and adult malformation percent were noticed. Also, the fecundity and eggs hatching% were inhibited. The sex ratio of the emerged adults was more affected as compared to control. Whereas, Iron treatment had a stronger effect on some biological parameters. This led to an increase in the pupal duration and malformations and decrease in adult emergence. While the larval treatment with Zinc had the strongest effects on adult longevity and sex ratios shifting of both males and females in respect to control.

**INTRODUCTION**

The cotton leafworm, *Spodoptera littoralis* (Boisd) is one of the major pests. In addition, this insect causes a considerable damage to many of the important vegetables and field crops in Egypt. Control of this pest, usually depends exclusively on conventional insecticides. In the developing countries, pesticides have played an extremely important role to maintain the high agricultural productivity. But the extensive application of pesticides has created serious problems such as the build-up of pest resistance, the upsetting of natural balance, acute and chronic hazards to man and animals. The use of resistant varieties is a trend for pest management. Plants and insects have a long time coexisted. Plants possess defense mechanisms to maintain the plant integrity in their communities against such predators, competitors,

pathogens. Three mechanisms to account for plant's resistance to insect damage were proposed by Al-Ayedh (1997). These are known as (i) Antixenosis describes the inability of a plant to serve as a host to insect herbivore. It has a particularity of modifying insect behavior once in contact with the plant host, without effect on metabolism of both plant and insect. There are several modes of resistance used by plant to deter insects. (ii) Tolerance, this is the ability of certain plants to withstand insect attack without appreciable loss in vigor or crop yield. (iii) Antibiosis, this comprises the defensive mechanism of plants against their pests through adverse influence on growth, survival or reproduction of insects by means of chemical or morphological factors. Phytochemicals play an important role in plant defences.

A number of compounds have been shown to induce resistance in plants when applied to the foliage. Among the compounds demonstrated to have this effect are salicylic acid, potassium phosphate, a water solution of NPK fertilizer, certain plant extracts, and extracts of microbial metabolites. Also, among these chemicals are plant nutrients elements. There are 17 elements considered essential for plant growth. Three of them, carbon, hydrogen and oxygen are supplied by the air and water around us and are generally not thought of as fertilizers. Of the remaining 14 elements, nitrogen, phosphorus, potassium, calcium, magnesium and sulfur are considered macronutrients were present in most of plants at high concentrations (*e.g. Brassica juncea*, Mark *et. al.* 2000). While, Iron, manganese, zinc, copper, boron, molybdenum, chlorine and nickel are recognized as micronutrients, often, were found at low concentrations in the plants.

The most common approach is to add micronutrients to these plants as a foliar fertilizer is essential for plant growth and as inducers for plant resistance against insect attack (Ruter, 2006), such as Micromax, generally contain iron, manganese, zinc, copper, boron and molybdenum. These elements serve many roles in plant metabolism and growth. Chlorine is an essential element for functioning of electron transport and water splitting in the photosynthetic process, whereas iron is important for numerous oxidation / reduction reactions. Boron is important for cell differentiation, division, and elongation, and copper is a key component of several enzyme complexes. Manganese and boron are essential for the proper germination and growth of pollen tubes. In Egypt efforts should be directed to test the mineral elements and their salts as pesticides for decreasing pest control costs.

The present study was to evaluate the biological and toxicological activities of four micro-nutrient elements against of second and fourth instars larvae of *S. littoralis*.

## MATERIALS AND METHODS

### 1- Insect rearing

The cotton leafworm, *S. littoralis* was reared in the laboratory for several generations at room temp. ranged between 25 - 28 C° and 60 -65% R.H. Larvae were fed on castor bean leaves, *Ricinus communis* (L.) in a wide glass jars until pupation period and adults emergence. The newly emerged adults were mated inside glass jars supplied with a piece of cotton wetted 10% sugar solution as feeding source for the emerged moths and branches of Tafla (*Nerium oleander* L.) as an oviposition site (El- Defrawi *et al.*, 1964). Egg masses were kept in plastic jars until hatching.

### 2-Tested micronutrients

Four micronutrients elements were tested Iron(Fe<sup>+++</sup>) ,Copper (Cu<sup>++</sup>) , Manganese(Mn<sup>++</sup>) , and zinc (Zn<sup>++</sup>).They were obtained in a powder form of each element from Syngenta Co.

### 3- Test procedures

Series of different concentrations were prepared on the active ingredient basis (p.p.m) by diluting the weighted amounts of powder of each element in water. Seven concentrations , i. e .3000, 2000, 1000,500,250,125 and 62.5 ppm were used for each of the four tested elements were prepared in this test. The castor leaves were dipped in water solution and used as control . The exposure of the second and fourth instar larvae to the elements depended upon the larval feeding for 48h on treated leaves. After 48h., the treated leaves were replaced by another untreated ones and the larvae fed on it until the pupation. Three replicates consists of sixty larvae for each concentration of the four tested elements and control were utilized .Also , the observed malformations were recorded and photographed

### 4- Statistical analysis

The total percent of the larval mortality after 48h were recorded and corrected according to the check by using Abbott formula (1925).The data were then analyzed by using probit analysis (Finney, 1971) , and the LC50 values were estimated for each of tested element. The different biological effects such larval and pupal duration, pupation and adult emergence %, pupal weight, Adult fecundity, eggs hatching %, longevity, and sex ratio were studied at the LC50 values. The obtained data of the biology were statically calculated through Excel for windows computer program to determine the F-value, P-value and L.S.D ( least significant difference) at 0.05 or 0.01 freedom degrees.

## RESULTS AND DISCUSSION

### 1- Insecticidal activities

Data in Table (1) showed that the four tested micronutrients, Iron (Fe) , Copper (Cu), Manganese (Mn) and Zinc (Zn) were effective against the second and fourth instar larvae of *S. littoralis*. Copper(Cu) was the most effective one against the second and fourth instar larvae , its LC50values were330 and 500ppm for the two instars, respectively. Mn had came next, its LC50 values were350and 660ppm for the two instars, respectively. The LC50values of Zinc were 400 and 720ppm for the two instars, respectively, while Fe was the least toxic one, its LC50values were 560 and 1500ppm for the two instars, respectively.

Table 1. Insecticidal activities of tested micronutrients against the 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *Spodoptera littoralis* (Boisd).

Compounds Tested	2 <sup>nd</sup> instar				4 <sup>th</sup> instar			
	LC <sub>50</sub> values ppm	Slop function	95% confidence limit		LC <sub>50</sub> values ppm	Slop function	95% confidence limit	
			Lower	Upper			Lower	Upper
Iron	560	3.56	233	1344	1500	2.34	1000	2250
Copper	330	3.5	107	1023	500	8.25	263	950
Manganese	350	3.712	184	665	660	3.48	254	1716
Zinc	400	2.55	182	880	720	3.51	288	1800

The obtained results were similar to those obtained by El-Sisi and Farrag (1989); they stated that copper carbonate was an effective as antifeedant against 4<sup>th</sup> instar larvae of *Spodoptera littoralis* and produced a high mortality, but none of the concentrations used caused apparent phytotoxicity .Also, Salama *et al*(1988) reported that the incorporation of chemical additives such some inorganic salts (copper and zinc sulphate) increased the potency of D-endotoxic of *Bacillus thuringiensis* Var kurstaki (Dipel 2x) against *S. littoralis* .Also, Wada and Munakata (1968) demonstrated a feeding inhibition of *S .littoralis* larvae that fed on castor oil leaves soaked in copper carbonate solution at different concentrations ranged 0.2- 5%. Also Kabbe *et al.* (1977) reported that the use of trace nutrients ( such as salts of Iron , Managenese ,Boron ,Copper , Cabalt , Molybdenum and Zinc ) mixed with certain chromenes compounds for example precocene 1 and precocene 11 were active compounds well tolerated by plants and used for combating arthropod pests , especially insects of order Lepidoptera ;*Pectinophora gossypipella* , *Prodenia litura* , *Spodoptera Spp.* ,*Earias insulana* , *Agrotis Spp.* and *Heliothis Spp.*

## 2. Latent effect

### 2.1. Larval and pupal periods

Data in Table (2 and 3) indicated that the larval treatment of both 2<sup>nd</sup> and 4<sup>th</sup> instars of *S. littoralis* with each of the four tested elements at LC<sub>50</sub> values, significantly ( $p < 0.01$ ) increased the larval period. The effect was more pronounced with the copper and manganese treatments, where the larval periods averaged 15.3, 14.5 and 12, 13 days for the larvae treated at both instars, respectively, as compared with 11 and 8.5 d for the two instars, respectively, of the check. While with both Iron and Zinc treatments the larval period averaged 13 d for the 2<sup>nd</sup> instar and 9.5 and 11 d for both treatments, respectively, for the 4<sup>th</sup> instar, as compared to control.

Tables (2 and 3) showed that the larval treatment of both 2<sup>nd</sup> and 4<sup>th</sup> instars with the four tested elements at LC<sub>50</sub> values highly significantly ( $p < 0.01$ ) increased the pupal periods of the pupae. The effect was more pronounced with the Iron, Copper and Manganese treatments for the second instar and Copper treatment for the 4<sup>th</sup> instar, where the prolonged to 13, 15, 12.8 and 13 d, respectively, as compared to 8 and 7.3 d of the 2<sup>nd</sup> and 4<sup>th</sup> instar larvae, respectively, of control. Whereas, the Zinc treatment of 2<sup>nd</sup> instar larvae, or Iron and Manganese treatments of 4<sup>th</sup> instar larvae induced significant increase ( $p < 0.05$ ) averaged 11 and 11, 12 d, respectively, as compared to control (8 and 7.3 d for the two instar, respectively). While Zinc treatment of 4<sup>th</sup> instar larvae gave none significant increase in the pupal period averaged 8.5 d.

These results are similar to those obtained by El-Sisi and Farrag (1989) mentioned that the larval feeding of 4<sup>th</sup> instar of *S. littoralis* on castor oil leaves sprayed with copper carbonate solution at different concentration (from 0.2 to 5% w/v) retard the larval growth and prolonged the larval span.

Table 2. Latent effects of the larval treatment of 2<sup>nd</sup> instar with the four tested micronutrients at LC50 values

Treatments	Larval duration (days)±S.D	% Pupation ±S.D.		Pupal duration (days) ±S.D	Pupal weight (mg) ±S.D	% Adult emergence±S.D	
		Normal	Malfo			Normal	Malfo
Iron	13±0.9*	61±5.4**	38**	13±1.9**	239±25**	50±9.9**	9.1*
Copper	15.3±1.3**	47±16**	28**	15±0.8**	186±40**	38±6.2**	35**
Manganese	14.5±0.7**	52±4**	15*	12.8±2.2**	204±37**	45±11**	20**
Zinc	12.8±0.8*	63±5.2**	13*	11±2*	252±15**	90±8n.s	3.7*
Control	11±0.4	100	0	8±0.9	362±20	100	0
F value	24.0	139.125	738.8	449.9	88.283	99.322	401.5
P value	0.0122	0.0165	0.0209	0.00023	0.00016	0.0145	0.00098
L.S.D at 5% level	1.98	23.3	10.3	1.6	47.5	27.3	6.1
1% level	3.632	53.7	18.95	2.95	61.6	63.125	11.1

\*\* = Highly Significant (p<0.01)

\* Significant (p<0.05)

S.D.=Standard deviation

Malfo.= Malformation%

L.S.D.= Least significant difference

Table 3. Latent effects of the larval treatment of 4<sup>th</sup> instar with the four tested micronutrients at LC50 values

Treatments	Larval duration (days)±S.D	% Pupation ±S.D.		Pupal duration (days) ±S.D	Pupal weight (mg) ±S.D	% Adult emergence ±S.D	
		Normal	Malfo			Normal	Malfo
Iron	9.5±0.5*	68±1.7**	35**	11±1.5*	255±30**	80±7.1*	5.3*
Copper	12±0.8**	50±19**	20**	13±0.8**	234±10**	65±15*	23**
Manganese	13±1**	57±8.2**	8.8*	12±1.8*	233±25**	73±11*	17.5**
Zinc	11±0.7*	65±12**	7*	8.5±1.1n.s	273±22**	97±2n.s	2.9*
Control	8.5±0.9	100	0	7.3±0.4	370±15	99.8±0.1	0
F value	34.4	847.97	113.54	13.1	84.21	15.088	401.5
P value	0.0177	0.00096	0.022	0.0216	0.00186	0.02183	0.000978
L.S.D at 5% level	1.9	20.4	9.22	2.59	40.9	15.97	6.1
1% level	3.4	27.7	16.9	4.8	67.8	29.325	11.1

\*\* = Highly Significant (p<0.01)

\* Significant (p<0.05)

S.D.=Standard deviation

Malfo.= Malformation%

L.S.D.= Least significant difference

## 2.2. Pupation and Pupal weight

Table (2 and 3) demonstrated that of the larval treatment of 2<sup>nd</sup> and 4<sup>th</sup> instars with each of the four tested elements at LC50 values induced highly significant ( $p < 0.01$ ) reductions in the pupation percent. Copper and Manganese treatments showed a higher effect on the pupation, it reduced the pupation by 47,52 and 50,57 % for the two instars, respectively, as compared to 100% of the check. While Iron and Zinc treatments reduced the pupation by 61, 63 and 68, 65% for the two instars, respectively, as compared to control.

On the other hand, the larval treatments resulted in highly significant ( $p < 0.01$ ) decrease in the pupal weight. Copper and Manganese treatments had a pronounced effect, it reduced the pupal weight to 186, 204 and 234, 233mg for the two instars, respectively, as compared to 362 and 370mg mg for the two instars, respectively, of the check. While, Iron and Zinc treatments decreased the pupal weight to 239, 252 and 255, 273 mg as compared to control (362 and 370mg mg for the two instars, respectively).

These results are in agreement with those obtained by El-Sisi and Farrag (1989)

## 2.3. Moths emergence

Data in Table (2 and 3) showed that the larval treatment with each of the four tested elements at the LC50 values induced a highly significant ( $p < 0.01$ ) reduction in the moths emergence. The effect was more pronounced with Iron, copper and manganese treatments, reached 50, 38, 45 %, respectively, for adults produced from treated 2<sup>nd</sup> instar, as compared to 100% of the check. Whereas, these treatments induced significant ( $p < 0.05$ ) decrease in the adult emergence to 80, 65 and 73%, respectively for adults produced from treated 4<sup>th</sup>. While, Zinc treatment of both 2<sup>nd</sup> and 4<sup>th</sup> instars induced none significant decrease in the adult emergence averaged 90 and 97, respectively, as compared to control (100%).

## 2.4. Morphogenetic effects

Data presented in Table (2 and 3) indicated that the larval treatment of 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *S. littoralis* with each of the four tested elements at the LC50 values had highly significant ( $p < 0.01$ ) increase on the pupal malformations. Iron and Copper treatments induced the greatest percent of pupal malformation reached 38, 28 and 35, 20% for the two instar larvae, respectively, as compared to 0% of the check. Whereas, the larval treatment with Manganese and Zinc treatments induced significant ( $p < 0.05$ ) increase in pupal malformation reached 15, 13 and 8.8, 7% for the two instars, respectively, as compared to the check (0%).



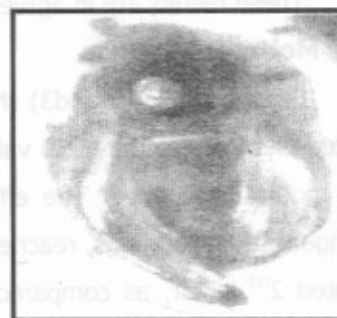
(Fig. 1) or larval-pupal intermediates with larval cuticle patches, head capsule and thoracic legs, posterior half of the body has the pupal properties



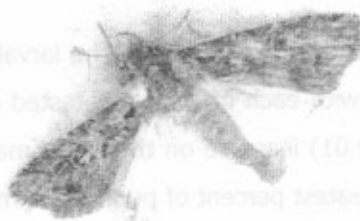
(Fig.2) Normal pupae



(Fig. 3) Adult malformations often appeared as poorly moth failed to emerge from the pupal cast



(Fig. 4) Malformed adults showed as abnormal bodies or wings.



(Fig. 5) Normal adult

Pupal and adult malformations produced from the treatment of the 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *S. littoralis* with the four tested elements ,Iron(fe),Copper(Cu),Manganese(Mn)and Zinc(Zn).



With regard to adults malformation, it was found that the larval treatments induced highly significant ( $p < 0.01$ ) increase in adult malformations. Copper and Manganese treatments induced the highest percent of adult malformations reached to 35, 20 and 23, 17.5% for the two instars, respectively, as compared to 0% of the check. Whereas, the larval treatment with Iron and Zinc treatments induced significant ( $p < 0.05$ ) increase in pupal malformations reached 9.1, 3.7 and 5.3, 2.9% for the two instars, respectively, as compared to the check (0%).

*S. littoralis* pupae malformations resulting from 2<sup>nd</sup> and 4<sup>th</sup> instar larvae treated with the four tested elements in the present work mostly appeared as larval-pupal intermediates with larval cuticle patches, head capsule and thoracic legs; posterior half of the body has the pupal properties (fig.1) as compared to normal pupae (fig.2).

Adult malformations often appeared as a moth appeared as poorly moth failed to emerge from the pupal cast (fig.3) or a moth showing deformed twisted wings (Fig.4), as compared to normal adult (fig.5).

### 2.5. Adult fecundity and fertility

Treatment of 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *S. littoralis* with the four tested elements resulted in highly significant ( $p < 0.01$ ) reduction in the fecundity of adults females (Tables 4 and 5). The least number of eggs (62 and 68 eggs/f) laid by adult females treated as 2<sup>nd</sup> and 4<sup>th</sup> instars, respectively, with Manganese, as compared to 561 and 612 eggs/f respectively, of the check. Whereas, the larval treatment with Zinc, Copper and Iron reduced the fecundity to average 73, 121, 124 and 150, 75, 134 eggs/f, respectively, for the two instars, respectively,

Likewise, the larval feeding of 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *S. littoralis* on the four tested elements at the LC50 values indicated a highly significant ( $p < 0.01$ ) reduction in the eggs hatching % (Table 3). The least eggs hatching percent recorded by Copper and Manganese treatments were 47, 58 and 60, 68% for eggs laid by adults emerged from the two instars, respectively, as compared to 95 and 100% of eggs of adults produced from 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of the check, respectively. While, the larval treatment with Iron and Zinc inhibited the eggs hatching to 71, 76 and 74, 78%, as compared to that of the check (100%).

The same results were obtained by Hashem *et al.* (1994) who demonstrated a reduction in both fecundity and fertility as a result of abnormalities in the ovaries of *S. littoralis* adults fed as 4<sup>th</sup> instar larvae on artificial diet mixed with 2% of fruit extract of *M. azedarach* for 72h. Likewise, Jermy (1961) recorded an oviposition inhibition of *Leptinotarsa decemlineata* adults that fed on potato leaf discs treated with copper sulphate due to the adult females are deprived of food (because the copper acts as an antifeedant factor).

Table 4. Latent effect of the larval treatment of 2<sup>nd</sup> instar of *S. littoralis* with the micronutrient elements at the LC50 values

Treatments	Longevity	Eggs laid no. Mean $\pm$ S.D (eggs/f)	Eggs hatching (%) (eggs/f)	Adult sex ratio (%)	
	Mean $\pm$ S.D (days)			Male	Female
Iron	6+1.5*	124+17*	71	46	55
Copper	4.6+1.2**	121+16*	47	60	40
Manganese	3.6+1.1**	62+6.5*	58	60	40
Zinc	3+0.9**	73+7.4*	76	63	37
Control	8.2+1.6	561+126	95	55	45
F value	23.3	28.633			
P value	0.00122	0.03409			
L.S.D at 5% level	1.5	377.3			
1% level	2.2	869.8			

\*\* = Highly Significant (p<0.01)  
S.D.=Standard deviation

\* Significant (p<0.05)  
Malfo.= Malformation%

Table 5. Latent effect of the larval treatment of 4th instar of *S. littoralis* with the micronutrient elements at the LC50 values.

Treatments	Longevity	Eggs laid no. Mean $\pm$ S.D (eggs/f)	Eggs hatching (%) (eggs/f)	Adult sex ratio (%)	
	Mean $\pm$ S.D (days)			Male	Female
Iron	6.5+1.5*	134+5*	74	53	47
Copper	4.7+1.2**	75+2.9*	60	55	45
Manganese	3.9+1.4**	68+1.6*	68	65	35
Zinc	6.8+1.9*	150+8.2*	78	59	41
Control	8.4+0.6	612+97	100	55	45
F value	27.4	61.672			
P value	0.0121	0.01609			
L.S.D at 5% level	1.4	289			
1% level	2.0	665.3			

\*\* = Highly Significant (p<0.01)  
S.D.=Standard deviation

\* Significant (p<0.05)  
Malfo.= Malformation%

## 2.6. Adult longevity

Data in Table (4 and 5) indicated that treatment of 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *S. littoralis* with the four elements significantly ( $p < 0.01$ ) decreased the longevity of the emerged adults. The effect was more pronounced with Zinc, Copper and Manganese treatments, where they decreased adult longevity to reach 3, 4.6 and 3.6d for adults emerged from the treated 2<sup>nd</sup> instar with the three treatments, respectively, as compared to 8.2 d adult longevity of the check. Also, Copper and Manganese treatments of 4<sup>th</sup> instar decreased the adult longevity to reach 4.7 and 3.9d, respectively. While the larval treatment with Iron treatments of the two instars significant ( $p < 0.05$ ) decreased the longevity of the emerged adults to reach 6 and 6.5d, respectively.

## 2.7. Adult sex ratio

Data in Tables (4 and 5) showed that the larval treatment resulted in shifting the sex ratio. It increased the males and decreased the females percent, as compared with the check. The effect was more pronounced with Zinc, Manganese and Copper treatments, where it increased the adult males emergence to reach 63, 60 and 60% of the 2<sup>nd</sup> instar and also, 59 and 65% for 4<sup>th</sup> instar treated with Manganese and Zinc treatments, respectively, as compared to 55% of the check. While those treatments (Zn, Mn and Cu) decreased the adult females to reach 37, 40 and 40%, respectively, of the 2<sup>nd</sup> instar and also, 41 and 35% for 4<sup>th</sup> instar treated with Manganese and Zinc treatments, respectively, as compared to 45% of the check. Whereas, the larval treatment with Iron treatment had an adverse effect on the sex ratios, it decreased the male to reach 46 and 53% for adults produced from the two instars, respectively, as compared to 55% of the check. And increased the female% to reach 55 and 47% for adults produced from the two instars, respectively, as compared to 45% of the check.

## CONCLUSION

The results of the present work demonstrated that the four micro-nutrient elements were effective against the survival and biology of *S. littoralis*. Manganese and copper treatments had more toxic effect and also, affect most of the tested biological activities of the insect. But some biological activities were more affected with Iron and Zinc treatments. Whereas a solution of the four elements at LC50% values had the highest effect on the various biological activities of this insect. Thus, the elements solution may add at the obtained LC50 values via the leaf spraying and around the infested regions for improvement the plant growth because of it acts as plant inducers and avoids the pest infestation due to its toxic action on the pest.

Therefore, the four elements were effective if applied as fertilizers give safe self – protection of the plant and use as replacement means for the synthetic insecticides that caused serious effects on the environment.

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## الأنشطة الأباديه لبعض العناصر الصغرى ضد العمر اليرقى لدودة ورق القطن الكبرى

الهام فاروق محمود عبد الرحيم و على مختار مطر

معهد وقاية النباتات - مركز البحوث الزراعية - الدقى - الجيزة

أجريت هذه الدراسة بغرض تقييم التأثير البيولوجي والأبادى لأربعة عناصر تغذية صغرى وهى الحديد، النحاس و المنجنيز و الزنك التي اختبرت معمليا ضد يرقات العمر الثاني و الرابع لدودة ورق القطن. غذيت يرقات العمر الرابع لمدة ٤٨ ساعة على ورق خروج مغطس في محلول احد العناصر المعدنية الأربعة المشار إليها مع تركيزات مختلفة وبناء على قيم التركيز النصفى المحددة لكل عنصر من العناصر الأربعة. وجد أن عنصر النحاس كان له التأثير الأقوى والغالب حيث بلغت ٥٠٠،٣٣٠ . بينما كان عنصر الحديد له التأثير الأقل حيث بلغت قيمة التركيز النصفى ٥٦٠،١٥٠٠ و تأثرت المعايير البيولوجية لليرقات المعاملة بأى من العناصر الأربعة بدرجة كبيرة. وكان لمعاملة يرقات العمر الثاني و الرابع بالنحاس أو المنجنيز التأثير الأقوى على مختلف الأنشطة البيولوجية حيث أدى ذلك إلى طول في العمر اليرقى والعذري و نقص في نسب التعذير والخروج والوزن العذري وعدد البيض ونسبة الفقس للبيض الموضوع لكل أنثى وادي إلى ظهور نسب من التشوهات العذرية والحشرية والنسب الجنسية للذكور والإناث أيضا تأثرت كثيرا بالنسبة للحشرات الغير معاملة. كان لمعاملة الحديد التأثير الأقوى على بعض المعايير البيولوجية حيث أدى إلى زيادة في مدة البقاء العذري وخفض في نسب خروج الفراش وحدثت نسب من التشوهات العذرية. بينما المعاملة اليرقية بالزنك كان لها تأثير في نقص عمر الفراشة و في تغير النسب الجنسية للذكور والإناث حيث أدى إلى زيادة نسب الذكور نقص في نسب الإناث بالمقارنة بالغير معاملة. محاليل هذه العناصر الأربعة تكون فعالة لو استخدمت كرش ورقى و حول المناطق المصابة تعطى حماية ذاتية أمنة للنبات ضد الآفة و تقلل من استخدام المبيدات المصنعة لتأثيرها الضار على البيئة.