BIOLOGICAL AND TOXICOLOGICAL EFFECTS OF FOUR MINOR NUTRIENTS ELEMENTS ON THE FOURTH INSTAR LARVAE OF THE BLACK CUTWORM , *AGROTIS IPSILON* (HÜFNAGEL) : LEPIDOPTERA: NOCTUIDAE

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Abstract

The toxic effect of four micro- nutrient elements, zinc (Zn), Iron (Fe) Copper (Cu) and Manganese (Mn) against the fourth instar larvae of *Agrotis ipsilon* was evaluated in laboratory tests. The fourth instar larvae were fed for 48h on castor leaves *Ricinus communis* dipped in a solution of each mineral element of the four elements at different concentrations (1st test) ,hence on a mixture consists of the four elements at the LC_{50} values (2nd test). Manganese (Mn) was the most toxic element against the fourth instar larvae (LC_{50} value was 250 ppm). While, zinc (Zn) was the least toxic one (LC_{50} value was 1400 ppm). The biological activities of the treated larvae with each of the four elements (1st test) were affected. Only Mangenese or Mangenese and copper treatments showed a higher effect than other treatments. However, the larval and pupal duration were prolonged, the pupation and adult emergence percentages were depressed, the pupal weight was decreased ,and pupal malformations percentage were produced. .Also, the sex ratio of the emerged adult was more affected as compared to control. Whereas, Iron treatment had a stronger effect on some biological parameters .This led to an inhibition in both fecundity and fertility, shorten in the adult longevity and caused adult malformations. While the larval treatment with a mixture of the four elements at the LC_{50} values had the most suppressive effect on the above mentioned biological activities.

INTRODUCTION

The black cutworm, *Agrotis ipsilon* is a serious pest of several important vegetable and field crops in Egypt, such as cotton, soybean, corn, potatoes and tomatos, 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 massive 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 an trend for pest management. Plant and insects have a long time coexisted. Plants possess defense mechanisms to maintain the plant integrity in their communities against such preditors, 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. These modes were categorized as chemical and morphological plant defenses. There are two defense pathways but with a special emphasis on morphological plant surface characteristic associated to plant resistance. As examples of theses characteristics, plants present special epidermal characters (glandular trichomes, hairs...) and layers of surface waxes which prevent insects from feeding on them. Resistance to oviposition may come from plant characteristics that either fail to provide appropriate oviposition-inducing stimuli or to provide oviposition-inhibiting stimuli . The chemical inhibition of feeding can be through plant emition of allelochemicals such as repellents, deterrents or inhibitors. Each type of chemical defense engages the plant to different mechanisms to disturb insect behavior. (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 showen 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 metabolities. 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, mangnesium and sulfure 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, manganes, 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 aim of the present study was to evaluate the biological and toxicological activities of four micro- nutrient elements against *A. ipsilon* fourth instar larvae.

MATERIALS AND METHODS

1-Insect rearing:

The fourth instar larvae *A. ipsilon* used in this experiment were reared in the laboratory on castor leaves, *Ricinus communis* inside glass jars (11x16cm.) covered with muslin and provided with a tissue paper over a saw dust layer in the bottom to absorb humidity according to Abdel –Rahim (2002) and incubated at 25Ć, and 70-75% R.H. until the pupation . The newly formed pupae were maintained in the saw dust inside clean jars until the moths emergence. The newly emerged moths were mated within the large glass jars provided with a cotton piece soaked in 10% sugar – solution as a feeding source and muslins as oviposition substrate for moths .The deposited eggs were put into small jars until the egg hatching.

2-Tested materials:

Four micronutrients elements were tested, Manganese (Mn), Copper (Cu), Iron (Fe) and zinc (Zn). They were obtained in a powder form of each element from Insecticides department, Plant Protection Res. Institute, ARC, Giza .

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 chemical element of the four tested elements in water as solvent. 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 the first test. A mixture of the four elements, Mn, Zn, Fe and Cu was added at the LC_{50} values soluble in water in the second test. The castor leaves dipped in only water solution and used as control. The exposure of fourth instar larvae to the elements depended upon the larval feeding for 48h on treated leaves in the first or second tests. After 48h., the treated leaves was replaced by another untreated one and the larvae fed on it until the pupation. Three replicates consists of sixty larvae for each concentration of the seven concentrations of each of the four tested elements were utilized in the two tests and control. 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 (Abbott ,1925).The data were then analyzed using probit analysis (Finney, 1971) , and the LC_{50} values were estimated for each of tested element. The different biological effects such larval and pupal duration, pupation and adults emergence percentage, pupal weight ,Adult fecundity, fertility, longevity, sex ratio was studied at the LC_{50} values, the data of the biology statically calculated through SPSS 10 for windows computer program to determined the F-value, P-value and L.S.D (least significant difference at0.05 or0.01freedom degrees)

RESULTS AND DISCUSSION

1-Toxic effect:

Data presented in Table (1) showed that the four tested micronutrient elements , zinc(Zn) ,Iron(Fe) ,Copper (Cu) and Manganese(Mn) were effective against the fourth instar of *A. ipsilon.* Managenese(Mn) was the most toxic one against the fourth instar (LC_{50}=250 ppm), while Zinc (Zn) was the least toxic one(LC_{50}=1400 ppm).

Table 1. Insecticidal activity of four micronutrient elements against the fourth instar larvae of *Agrotis. ipsilon.*

Treatments	4th instar			
	LC_{50} values	95% Confidence limit		Slope
	ppm	Lower	Upper	function
Zinc	1400	1273	1540	2.63
Iron	1200	1091	1320	2.61
Copper	660	600	726	3.6
Mangenese	250	147	425	3.7

The obtained results were similar to those obtained by El-Sisi and Farrag (1989) proved that copper carbonate was an effective as antifeedant against 4th 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 gossyipella, Prodenia litura , Spodoptera Spp. ,Earias insulana, Agrotis Spp.* and *Heliothis Spp.* . Jermy (1961)found a reduced feeding of *Leptinotarsa decemlineata* adult on potato leaf discs soaked in copper sulphate solution at concentration of 6×10-6 gm/cm giving almost total protection against the adults attack over 20h but it appeared less effective against the larvae. This agree with those obtained by Verma *et al.* (1956) who explained a quick and accurate method for application of copper arsenic as insecticides e .g. Paris Green and Scheeles Green. Likewise, Friend (1945) showed insecticidal activity for Copper, Manganese, Iron and Zinc. Brinley (1926) recorded that the feeding of *Malacosoma americanum* (F.) on bean seedlings is reduced if these are sprayed with copper stearate or copper resinate

Table 2. Latent effect of the larval treatment of 4th instar of *A. ipsilon* with the four micronutrient elements 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
Untreated	14.5±3.3	100±0	0±0	11.0±1.6	471±31.8	100 ±0	0
Zinc	15.8±3 n.s.	62.5±3**	14.3±1.1**	11.5±1n.s	265±33.4**	62.4 ±0.9**	12.7±1.6**
Iron	17±5 n.s.	60±10**	11.6±0.2**	12.3±0.8*	255±66.5**	59.4 ±0.6**	25.5±0.5**
Copper	22.3±4**	55±15**	15.1±1.2**	12.8±0.4**	234±59.9**	60.3±0.8**	10.2±1.9**
Mangenese	22.5±4**	50±10**	21.9±0.3**	13.5±1.1**	151±45.8**	56.2±0.3**	10.9±2.2**
Mixture	26.3±6**	30±10**	25±5**	14.5±0.5**	112±36.1**	50±10**	30± 10**
F value	5.764	17.8	49.960	8.163	10.1	56.3	34.19
P value	. 001	.000	.000	.000	.000	.000	.000
L.S.D at 5% level	5.6	16.7	3.8	1.3	72.3	7.3	4.35
1% level	7.6	23.5	5.4	1.7	99	10.3	6.1

** = Highly Significant ($p<0.01$)   * Significant ($p<0.05$)
S.D.=Standard deviation   Malfo.= Malformatiom%
L.S.D.= Least significant difference

2. Latent effect:

2.1. Larval and pupal periods:

Data in Table(2) demonstrated that the larval treated of the fourth instar of *A. ipsilon* with each of the four tested elements (1st test) alone at LC_{50} % values increased the larval durations . The larval duration was highly significant ($p<0.01$) increased with the larval treated with each Copper or Manganese elements ,it ranged from 22.3-22.5 days, respectively, as compared to 14.5d. of the check .Whereas, the larval treatment with each Zinc or Iron elements induced none significant increase ranged from 15.8to17d., respectively. Likewise, a mixture of the four elements, Mn, Cu, Fe, and Zn (2nd test) at LC_{50} values had the highest effect on the larval duration, it was highly significant ($p<0.01$), the larval period increased to average of 26.3 days.

Also, Table (2) showed that treatment of the fourth larval instar of *A. ipsilon* with each of the four tested elements (1st. test) alone at the LC_{50} values extended the pupal duration . The pupal duration was highly significantly ($p<0.01$) increased in cases of treatments with each Copper or Manganese elements, it ranged from 12.8 to 13.5 days, respectively, as compared to 11 days of control. Whereas, the larval treatment with Iron element alone induced significant increase ($p<0.05$) averaged 12.3 days. While the Zinc treatment induced none significant increase (11.5 days) Also, a mixture of the four elements had the greatest effect on the pupal duration ,it induced highly significant ($p<0.01$) extend in the pupal duration (14.5 days).

These results are similar to those obtained by El-Sisi and Farrag (1989) mentioned that the larval feeding of 4th instar of *S. littoralis* on castor oil leaves sprayed with copper carbonate solution at different concentration(from0.2 to 5% w/v) retard the larval growth and prolonged the larval span .

2.2. Pupation and Pupal weight:

Table (2) showed the effect of the larval treatment of 4th instar larvae with each of the four tested elements (Mn, Zn, Fe and Cu) alone at LC_{50} values (1st test). Highly significant ($p<0.01$) reductions in the pupation percent were recorded. Manganese and Copper treatments showed a higher effect on the pupation, it reduced the pupation by 50 and 55 %, respectively, as compared to 100% of the check. While Iron and Zinc treatments reduced the pupation by 60 and 62.5%, respectively. Whereas, the larval treatment with a mixture of the four elements had the highest effect on the pupation, it highly significantly ($p<0.01$) reduced the pupation by 30%.

On the other hand, the larval treatment (4^{th} instar larvae) with each of the four tested elements (1^{st} test) alone at LC_{50} % values led to highly significant ($p<0.01$) decrease in the pupal weight . Manganese treatment had a pronounced effect, it reduced the pupal weight to 151 mg, as compared to 471 mg of the check. Also, the larval treatment with each of Copper, Iron and Zinc alone induced significant decrease in the pupal weight ; 234,255 and 265 mg., respectively . While the larval treatment with a mixture of the four elements (2^{nd} test) had the strongest effect on the pupal weight, it highly significantly ($p<0.01$) reduced the pupal weight to average 112 mg .

These results are in agreement with those obtained by El-Sisi and Farrag (1989) mentioned that the larval feeding of 4^{th} instar of *S. littoralis* on castor oil leaves sprayed with copper carbonate solution at different concentrations(from 0.2 to 5% w/v) prevented pupation or development to adults.

2.3. Moths emergence:

Data in Table(2) demonstrated that the larval treatment of 4^{th} instar larvae of *A .ipsilon* with each of the four tested elements(1^{st} test) alone at the LC_{50} values induced a highly significant ($p<0.01$) reduction in the moths emergence . The effect was more pronounced with manganese treatment ,it reduced the adults emergence to average 56.2%, as compared to 100% of the check. While, the Iron, Copper and Zinc decreased the adult emergence to 59.4,60.3 and 62.4%,respectively. Whereas, the larval treatment with a mixture of the four elements(2^{nd} test) had the greatest effect on the moths emergence, it highly significant ($p<0.01$) decreased the adult emergence to reach 50%.

2.4. Morphogenetic effects:

Data presented in Table(2) showed that the larval treatment of 4^{th} instar larvae of *A. ipsilon* with each of the four tested elements (1^{st} test) alone at the LC_{50} values induced highly significantly ($p<0.01$) increase in the pupal malformations .The effect was more pronounced with Manganese treatment, it caused 21.9 pupal malformation %, as compared to 0% of the check. While, the larval treatments with iron, Zinc and copper induced pupal malformations by 11.6,14.3 and 15.1 %, respectively. Whereas, the larval treatment with a mixture of the four elements (2^{nd} test) gave the highest significant effect . It increased the pupal malformations to 25%.

With regard to adults malformation (Table2), it was found that the larval treatment of 4^{th} instar larvae with each of the four elements (1^{st}test) alone at the LC_{50} values induced highly significant ($p<0.01$) increase in adult malformations. Iron treatment induced a higher percent of malformation (25.5) as compared to 0% of the check. Whereas, the larval treatment with Zinc, Manganese and copper induced adult malformations by 12.7, 10.9 and 10.2%, respectively. Whereas, the larval treatment with a mixture of the four elements (2^{nd} test) had the strongest effect, it highly significant ($p<0.01$) increased the adults malformations to reach 30%.

fig.1. pupal malformation produced from treated larvae with a mixture of the four element showed as larval pupal intermediates

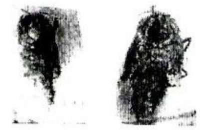

fig.2,3. : Adult malformation result from larvae treated with a mixture of the four element appeared as poorly developed moths or a dult had weakly formed wings.

fig.4,5. pupal malformation gave from treated larvae with manganese showed as pupa with vestiture of larval skin or larval-pupal intermediate failed to cast the old skin.

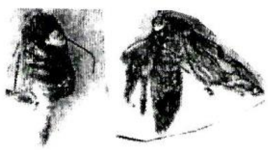

fig.6,7. Adult malformation prouduced from treated larvae with manganese appeared as poorly developed moth or adult had deformed wings.

fig.8. : Pupal malformation result from treated larvae with copper showed malformed pre pupa failed to cast the old cuticle

fig.9. Adult malfomation produced from treated larvae with copper gave adult with deformed twisted wings

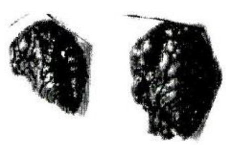

fig.10,11. Pupal malformation resulted from treated larvae with iron showed as abnormal pupa with body shrinkage or undersized pupa with larval skin

fig.12. Adult malformation produced from treated larvae with iron appeared as adults had twisted wings

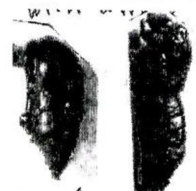

fig.13,14. pupal malformation produced from treated larvae with zinc appeared as pupa with vestiture of larval skin or larval-pupal intermediates.

fig.15. Adult malformation resulted from treated larvae with zinc showed as adult had deformd body and wings

fig.16.normal pupa

fig.17. normal adult

Malformations of pupae and adult of *Agrotis ipsilon* treated as larvae with manganese, copper , iron and zinc elements.

A. ipsilon pupae malformations resulting from of 4th instar larvae treated with a mixture of the four tested elements (2nd test) in the present work mostly appeared as larval-pupal intermediates (Fig.1) ,while adult malformations showed as poorly developed moths or adults had weakly formed wings (Fig.2,3) .On the other hand, Managenese treatment alone(1st test), pupal malformations appeared as pupa with vestiture of larval skin or larval-pupal intermediates failed to cast the old skin(Fig.4,5) however, adult malformations showed as poorly developed moths or it had deformed wings(Fig,6,7). With regard to Copper treatment alone (1st test), pupal malformations appeared as malformed pre-pupa failed to cast the old cuticle (Fig.8) and adult malformations showed as adults with deformed twisted wings (Fig.9).In respect to Iron treatment, pupal malformations appeared as abnormal pupa showing body shrinkage or undersized pupa with larval skin (Fig.10,11) and adult malformations showed as adults had twisted wings (Fig.12). In case of Zinc treatment, pupal malformations appeared as pupa with vestiture of larval skin or larval –pupal intermediates (Figs.13,14) and adult malformations showed as adults had deformed body and wings (Fig.15) as compared to normal pupa (Fig.16) and normal adult (Fig.17).

Table 3. Latent effect of the larval treatment of 4th instar of *A. ipsilon* with the micronutrient elements at the LC_{50} values.

Treatments	Fecundity	Fertility	Longevity	Adult sex ratio (%)	
	Mean_+S.D. (eggs/f)	Mean_+S.D (eggs/f)	Mean_+S.D (days)	Male	Female
Untreated	815.8±269.4	737.7±216	10±2.1	50	50
Zinc	52.5±2.5**	46.5±1.5**	8.3±2.5 n.s	49.2	50.8
Iron	21.3±3.8**	17±4.5**	6.8±2.7*	51.7	48.3
Copper	34.5±13.5**	28.2±18.7**	7.5 ± 2.3 n.s	51.6	48.4
Mangenese	39.2±5.9**	33.2± 4.9**	8±1.0 n.s.	55.4	44.6
Mixture	6.9±1.9**	5.5±1.5**	5.3±1.1**	58.8	41.2
F value	25.445	32.5	2.985		
P value	.000	.000	.031		
L.S.D at 5% level	196.0	157.1	2.68		
1% level	274.8	220.3	3.634		

** = Highly Significant (p<0.01)
* Significant (p<0.05)
S.D.=Standard deviation
Malfo.= Malformation%
L.S.D.= Least significant difference

2.5. Adult fecundity and fertility

Treatment of *A. ipsilon* 4th instar larvae with each of the four tested elements alone (1st test) lead to highly significant ($p<0.01$) reduced in the fecundity of adults females (Table 3). The least number of eggs (21.3 eggs/f) laid by adult females treated as larvae with Iron element, as compared to 815.8 eggs/f of the check. Whereas, the larval treatment with Zinc, Copper and Manganese reduced the fecundity to range from 34.5-52.5 eggs/f. While the larval treatment with a mixture of the four elements (2nd test)had the most potent on the fecundity ,it reduced the fecundity to average 6.9 eggs/f.

Likewise, the larval feeding of 4th instar larvae of *A. ipsilon* on the four tested elements alone (1st test) at the LC_{50} values indicated a highly significant ($p< 0.01$) reduction in the fertility of eggs (Table 3). Also ,the least number of viable eggs(17 eggs/f.) laid by adults treated as larvae with Iron, as compared to 737.7 eggs/f of the check. While, the larval treatment with Zinc, Manganse and Copper inhibited the fertility to range from28.2-46.5 eggs/f. Whereas, the larval treatment with a mixture of the four elements (2nd test)had the strongest effect in reducing the number of viable eggs (or fertility)to average 5.5 eggs/f.

The same results was 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 4th instar larvae on artifical 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 antifeedant factor).

2.6. Adult longevity:

Data presented in Table (3) indicated that treatment of 4th instar larvae of *A .ipsilon* with each of the four elements (1st test)alone decreased the longevity of the emerged adults. Only Iron treatment recorded a significant decrease ($p< 0.05$) in the adults longevity reached to 6.8 days, as compared to 10d. of the check. While the larval treatment with Copper, Manganese and Zinc induced none significant decrease in the adults longevity ranged from 7.5-8.3d. .Whereas ,the larval treatment with a mixture of the four elements (2nd test) induced highly significant decrease($p<0.01$) in the adults longevity (average 5.3d.)

2.7. Adult sex ratio:

Data in Table(3) showed that the larval treatment of 4th instar of *A. ipsilon* with each of the four tested elements (1st test)alone at LC_{50} values shifted the sex ratio ,it increased the males and decreased the females percent, as compared with the check.

The effect was more pronounced with Managenese treatment, where it increased the adult males emergence to reach 55.4 %, and decreased the adult females to reach 44.6 %, as compared to 50 % of both adult males and females of the check.. While the Zinc treatments had adverse effect on the sex ratio, it decreased the adult males to reach 49.2 %and increased the females to reach 51% .Whereas, the larval treatment with a mixture of the four elements (2^{nd} test) had the strongest effect on sex ratio, it increased the males to reach 58.8% and decreased the females to reach 41.2%.

CONCLUSION

The results of the present work demonstrated that the four micro-nutrient elements were effective against the survival and biology of *Agrotis ipsilon ,* especially Managenese or 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 treatments .Whereas a solution of the four elements at LC_{50}% values had the highest effect on the various biological activities of this insect . Thus, the elements solution may added at the obtained LC_{50} values around the aerial parts(roots) will improve the plant growth because of it acts as plant inducers and avoid 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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التأثيرات البيولوجية والسمية لاربعة من عناصرالتغذية الصغرى على العمراليرقى الرابع للدودة القارضة ، اجروتس ابسيلون-(حرشفية الأجنحة:الليلية)

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معهد وقاية النباتات . محطة بحوث سدس . مركز البحوث الزراعية. دقى . جيزة

اجريت هذه الدراسات بغرض تقيم التاثير البيولوجي و السمى لاربعة عناصر تغذيــة صــغرى وهى الزنك، الحديد، النحاس و المنجنيز اختبرت معمليا ضد يرقات العمر اليرقى الرابــع للــدودة القارضة. غذيت يرقات العمر الرابع لمدة٤٨ ساعة على ورق خروع مغطس فــى محلــول احــد العناصر المعدنية الاربعة المشار اليها اختبرت عند تركيزات مختلفة (اختبار اول) وبناء على قــيم التركيز النصفى المحددة لكل عنصر من العناصر الاربعة خلطت العناصر وغذيت عليها اليرقــات لمدة٤٨ ساعة (اختبار ثانى) . وجد ان العنصر الاول وهو المنجنيز كان له التأثير الأقوى والغالب حيث بلغت قيمة التركيز النصفى له pp m ٢٥٠ . بينما كان العنصر الرابع وهو الزنك لــه التأثير الآقل حيث بلغت قيمة التركيز النصفى لــه ppm ١٤٠٠. تــأثرت المعــايير البيولوجيــة لليرقات المعاملة بأى من العناصر الاربعة (اختبار اول) بدرجة كبيرة. وكان لمعاملة يرقات العمــر الرابع بالمنجنيز او المعاملة بالمنجنيز او النحاس كانت لها تأثير اعلى عــن المعاملــة بالعناصــر الاخرى. حيث أدى ذلك الى طول فى العمر اليرقى والعذرى و نقص فى نسب التعذير والآختــراق والوزن العذرى وادى الى ظهور نسب من التشوهات العذرية والنسب الجنسية للذكور والانــاث ايضا تأثرت كثيرا بالنسبة للحشرات الغير معاملة بينما كان لمعاملة الحديد التأثير الاقــوى علــى بعض المعايير البيولوجية . حيث ادى ذلك الى تثبيط فى معدل وضع البيض ودرجــة الخصــوبة و وقصر فى عمر الفراش المختزق واحدث نسب من التشوهات للحشرة الكاملة . بينما المعاملة اليرقية بخليط من الاربعة عناصر عند قيم التركيز النصفى اعطى التــأثير الاعلــى علــى النشــاطات البيولوجية السابق ذكرها. وبالتالى محاليل هذه العناصر الاربعة تكون فعالة لو اضيفت كمخصــبات حول جذور النبات تعطى حماية ذاتية آمنة للنبات ضد الافة و تقلل من استخدام المبيدات المصــنعة لتأثيرها الضار على البيئة.