EFFECT OF THE NEEM SEEDS OIL EXTRACT ON SOME IMMATURE STAGES OF THE WHITEFLY *BEMISIA TABACI* (GENNADIUS)

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ABSTRACT

The neem seeds oil extract was tested against the immature stages(third instar larvae and pupal stage) of the insect species, Bemisia tabaci (Gennadius). The concentrations of 0.1, 0.125, 0.25, 0.3, 0.5, 1.0 and 1.5 ml / 100 ml of distilled water were used. The concentration that inhibited 50% of pupal formation (IC_{50}) from the larvae was 0.22 %. When the third instar larvae and pupal stage were treated, the concentrations that inhibited 50 % of adult formation (IC_{50}) were 0.17 % and 0.21 % , respectively. The larvae of the whitefly were affected severely by neem seeds oil extract and died within 3 to 8 days after treatment with 1.0% and 1.5 % concentrations. The tested concentrations of neem seeds oil extract generally produced malformations or morphological abnormalities in all the treated immature stages of B.tabaci. The adult was produced either by failure or partial emergence. Few of the adults emerged with distorted crippled wings. The female adults deposited few eggs which failed to hatch, and some of females failed to lay any eggs. This is mainly due to the failure of the rupture of longitudinal and transverse molting sutures to different stages. The neem seeds oil extract apparently acted as a growth regulator, and suppressed the build up of the whitefly B. tabaci.

INTRODUCTION

Sweet potato whitefly Bemisia *tabaci* (Gennadius) is known as a major agricultural pest all over the world especially in tropical and subtropical regions (Toscano *et al.*, 1994). It spreads on many plants including agricultural crops, ornamental plants and weeds. It cause serious damage and loss up to 60% of the crop in greenhouses (Ioannou, 1995). *B. tabaci* destroys the plants by using its mouth parts in penetrating and sucking the plant sap which includes very important nutrients. As a result, weakening and stunting of the plant, deformation of the leaves, flowers and fruits. *B. tabaci* also secretes the honey dew, which is covered with black sooty fungi and dust, causing reduction in the plant breath and photosynthesis rate. In addition, *B.tabaci* transmits TYLCV which is a very dangerous virus causing serious disease to tomato plant (Czosnek & Laterrot, 1997). Therefore *B. tabaci* must be controlled but without using chemical insecticides which are very dangerous on human health and plant crops (Pimentel, 1977). The continuous use of concentrated insecticides kills useful insects such as predators and parasitoids (Pimental, 1981).

Increasing numbers of *B. tabaci* (Forer, 1988 and Dimetry & Schmidt, 1992) also cause insects' resistance to chemical insecticides (Waiss *et al.*, 1981). On the contrary the plant extracts such as neem seeds extract were used for controlling *B. tabaci*, and are safe for humans, animals, plants and its biological enemies. In addition they do not cause environmental pollution. The neem tree grows in tropical and subtropical regions. It was brought in Saudi Arabia and planted in western region as an ornamental plant (Al-Qahtani, 1999). The neem seeds contain some complicated substances which belong to triterpenoid group such as azadirachtin that concentrates more in kernel seeds than any other parts of the tree. This azadirachtin forms about 40% of the seeds. Azadirachtin also works as a repellent substance and antifeedent (Ladd *et al.*, 1978 and Redfern *et al.*, 1979). It also prevents eggs laying and works as a growth and reproduction regulator in many insects (Fagoonee, 1981; Koul *et al.*, 1987 and Pener *et al.*, 1988). It was found that neem extracts are effective on 200 species of insects belonging to 7 orders (Saxena, 1989) and they are safe for humans, hot blooded animals and natural enemies of insects (Schmutterer, 1988). In Saudi Arabia *B. tabaci* is noticed on growing vegetables that planted in both greenhouses and openfields throughout the year. The whitefly was first recorded in Saudi Arabia by the British Museum Natural History. They were also recorded by Abu-Yaman (1971) on eggplant in Riyadh and Al- Abdel Mohsen (1992) on some varieties of tomato. The purpose of this work is to determine the effectiveness of the neem seeds oil extract in inhibiting the growth of immature stages of *B. tabaci* and its role in long effects on the morphological characters of the insect.

MATERIAL AND METHODS

Extraction of neem seeds oil: The neem seeds were collected from the trees growing in the gardens in Jeddah city. The extraction was prepared with petroleum ether using Soxhelt apparatus according to Islam (1983). The kernels were removed from the seeds and dried at 37°□ for two days. Then they were crushed and put in Soxhelt apparatus with the solvent until the extraction was formed.

Chemical structure of the neem seeds oil extract : The scientific name of neem plant is *Azadirachta indica* A.Juss . It belongs to family Meliceae. The effective substance is azadirachtin which is one of the components of the chemical compound triterpenoid. The molecular weight of azadirachtin is 720, and its chemical formula is C_{35} H_{44} O_{16} (Schmutterer,1990) (fig.1). The extract is an emulsified oil which dissolves in organic solutions.

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Host plant : Tomato (Super Marmand variety) was used as a host plant for the whitefly *B.tabaci*. The seeds were planted in plastic pot measured 17×18×33 cm^3 in fine sand and petmose. The seeds were transplanted after 25-30 days in plastic pots of 10 cm diameter. They were used in laboratory experiments after one month.

Rearing of the whitefly *B. tabaci* in laboratory: Many pairs of *B. tabaci* were obtained from Hada-Alsham research station. Healthy tomato plants in 20-25 cm in length were exposed to these insects and were placed in wood cages measured 2×1.5×1 m^3. The cage's sides were covered with muslin while the roof and the frontal side were made from glass. Four florescent lights of 120 cm length and 40 watt power were present inside the cage at a height of 70 cm. The insects were left to complete their life cycle under laboratory conditions where the temperature was 24-26 °□, the relative humidity was 50-60 % , 16 hours light and 8 hours dark.

Tested concentrations: The tested concentrations were prepared by solving certain amount of the extraction in a certain amount of distilled water. Tween 80 substance was added to help emulsifying the extract in water. The prepared concentrations were 1.5%, 1%, 0.5%, 0.3%, 0.25%, 0.125%, and 0.1%.

Experimental cages: Experimental cages measuring 70×40×60 cm^3 were used. The sides of the cages were covered with muslin. The roof and frontal side were made from glass. Inside the cage, there were 4 florecent lights of 60 cm length and 20 watt power. The door had an opening for easy handling of the plants. The healthy plants were exposed to the natural infestation of the whitefly *B. tabaci* in the culture cages until the insects deposited their eggs on the leaves. The adult whiteflies were removed by the aspirator. Then, the plants were transferred to the cages under the experimental conditions. Egg hatching and larval emergence were observed until obtaining the stages required for the experiments. The numbers of the third larval instar and pupae found on the plant leaves were recorded. These numbers were then sprayed with 0.1 ml of the extract emulsion and returned back to the experimental cages. These plants were daily investigated to count the number of insects that failed reach to the adult stage. Both percentages of inhibition of pupal and adult stage were recorded and compared with those in the control group which were in the same stage and the same age but treated only with distilled water with Tween 80 .

Statistical analysis: All data obtained in the study were analyzed following the probit analysis technique of Litchfield and Wilcoxon (1949).

RESULTS AND DISCUSSION

Tables (1&2) show the percentages of inhibition of pupal and adult formation from the third instar larvae of the whitefly *B. tabaci* exposed to several concentrations of neem seeds oil extract (N.S.O.E.). These concentrations were 0.1%, 0.125%, 0.25%, 0.3%, 0.5%, 1.0% and 1.5%. The percentages of inhibition of pupal and adult formation caused by these concentrations were 23%, 33.3%, 47%, 61.1%, 75.2%, 88.2%, 92.2% and 29%, 44%, 67%, 73% 86.5% 96.4%, respectively. When these percents ages were plotted against their considered concentrations on a semilogarethemic-probit, and a regression line was drawn, the statistical analysis proved the goodness of fit of the obtained line (Figs.2&3), and the insignificant heterogeneity of the data as the experimental calculated chi^2 value were 4.04 and 4.51, respectively for inhibition of pupal and adult formation. These were less than tabulated chi^2 11.1 and 9.49 at 5 and 4 degrees of freedom and 5% probability level. Also, when the concentration inhibited 50% of the treated third larval instar to develop to the pupal and adult stage, the IC_{50} values were found to be 0.22% and 0.17%, respectively with 95% confidence level (Tables 1&2). The slope of IC_{50}-pl obtained for inhibition of both pupal and adult formation was 1.77 and 2.32, respectively. Table (3) and Figure (4) show the data obtained after treating the third instar larvae of *B. tabaci* with N.S.O.E. in order to facilitate the comparison between the effect on the inhibition of pupal and adult formation. In this table it could be easily noticed that there were no differences between all the data obtained for inhibition of pupal and adult formation. The value of IC_{50} and slopes obtained in regression lines were nearly the same as they were approximated to the nearest figures (o.22, 1.77 and o.17, 2.32, respectively for inhibition of pupal and adult formation). The percentages of inhibition of pupal formation were 23, 33.3, 47, 61.1, 75.2, 88.2, and 92.2%. These figures were close to those obtained in case of inhibition of adult formation which were 29, 44, 67, 73, 86.5 96.4 and 100% , respectively (Table 4 and Fig.5). In addition, the neem seeds oil extracts were compared on the basis of their effects on the treated third larval instar and the pupal stage. The results showed that the third instar larvae proved to be more affected or potent. The two IC_{50}-pl showed marked parallelism, which was reflected by the mode of action of the N.S.O.E. compound on the third larval instar and the pupal stage.

As another criterion for the determination of the effect of N.S.O.E. when the pupae of *B. tabaci* were exposed to the same concentrations, the percent inhibition of adult formation were 23, 33, 58, 67, 88 and 96% , respectively (Table5). The regression line proved to be good fit (Fig.6) , and the data obtained were insignificantly heterogenous as the experimental chi^2 value (3.56) was less than the tabulated chi^2 (9.49) at the degrees of freedom 3 and 5% probability level (Table 5), and the IC_{50} was (0.21%). The slope of the obtained line was 2.46 (Table5 & Fig.6), which may show slight heterogeneity in the ages of selected and tested pupae. Table (6) and Fig.(7) show that the percentages of inhibited pupae which were 23, 33, 58,

67, 88, 96, and 100% increased with increasing the concentrations of N.S.O.E.

The present findings proved that at 0.5% and 0.3% concentrations, the adult stage was affected severely by neem seeds oil. The treated larvae produced a few pupae and some of them died. The percentage of the adults formed from these larvae was low, and the adult females could not lay any eggs. These results agree with Serra & Schmutterer (1993) when they used N.S.O.E. against *B. tabaci* on tomato leaves. Also, at 0.3% concentration some of the formed pupae had atrophy in their eyes dye. After treating the 3rd larval instar by concentration of o.25% and 0.125%, the larval ages were prolonged. They lived up to 6 days without moulting then died, opposite of 2-4 days in untreated larvae. Also, after treating the 3rd larval instar with the concentration of 0.125% , they did not moult to pupae, and became swollen, and the fluids passed out from them and finally died. Similar findings were reported by Natarajan & Sundarmurthy (1990). This happened because the effective substance in N.S.O.E. which was azadirachtin inhibited new cuticle formation, which was confirmed by Schmutterer (1990). After treating the pupae with 0.25% and 0.125% concentrations, some of them showed prolongation in their life, as they lived up to 5 days and then died. Some of the formed adult could not emerge or only the anterior side of the body emerged, and others emerged with distorted crippled wings. These results agree with those of Dorn *et al.* (1987). This may be due to the failure of rupture of the longitudinal and transferred moulting suture to different stages. The moulting phenomenon is controlled by endocrine system of insect, and the neem seeds oil hindered the normal function of the endocrine system of insects (Karnwar, 1987). Similar findings were reported by Coudriet *et al.* (1985b) on the whitefly *B. tabaci* , and also confirmed by Rao & Rembold (1983) and Sieber & Rembold (1983) on the same insect. The formed females could not lay any eggs, or laid a few eggs which didn not hatch. Steets (1976) and Schmutterer (1990) found that neem oil extract caused completely or partly insect sterilization.

In the present investation, the high concentrations of N.S.O.E. such as 1.5% or 1.0% tested against the third instar larvae or pupae, they died in 1-3 days or lived to 8 days without moulting and then died. These results agree with those of Schmutterer (1990) who found that the effective substance in natural neem oil had long effect from 4-8 days according to environmental conditions and the kind of plant used. The present study indicated that the larval stages of *B. tabaci* were more susceptible to N.S.O.E. extracts than the pupae. Similarly Prabhaker *et al.* (1989) reported that the pupae were more tolerant to neem than the larvae. This could be attributed to the fact that late stages in the insect development such as the pupae are usually highly protected by fat bodies (Schluter, 1995) that may either detoxify or sequester toxins better than the third instar larvae. The present findings revealed that neem seeds oil act as a growth regulator, and thereby suppress the population build-up of the whitefly. In conclusion it appears that neem seeds oil can control insects by killing with safe concentrations or by interfering life cycle as potent insecticides inhibiting adult formation and prolonging its duration.

Fig. (1): Chemical structure of neem seeds oil extract (after Schumutterer, 1990).

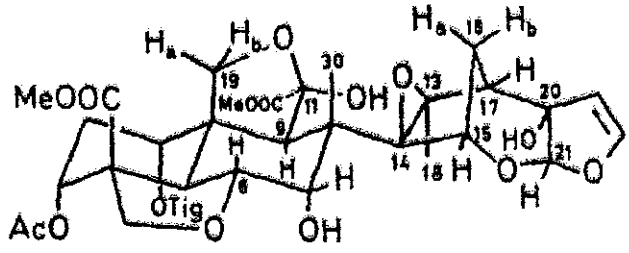


Table (1): Percentage inhibition of pupal formation after treating the 3rd instar larvae of the whitefly *B.tabaci* with different concentrations of N. S.O.E. at 26.5±1°□ and 61.79±4.2% . R.H.

Concentrations	No. of insects	Percentage of inhibited observed larvae	Percentage of inhibited expected larvae	(obs. - exp.)	Chi²
0.10	82	23	28	-5	0.0125 (1.025)
0.125	96	33.3	33.3	0	0.001 (0.096)
0.25	85	47	56	-9	0.030 (2.55)
0.30	98	61.1	61.1	0	0.001 (0.098)
0.50	89	75.2	75.2	0	0.001 (0.089)
1.0	85	88.2	88.2	0	0.001 (0.085)
1.5	90	92.2	93	- 0.8	0.001 (0.09)

IC_{50} = 0.22 Calculated Chi² = 4.04 Tabulated Chi² at freedom degree 5 = 11.1

Slope function = 3.67 Slope = 1.77

Fig. (2): Percentage inhibition of pupal formation after treating the 3rd instar larvae of the whitefly *B.tabaci* with different concentrations of N.S.O.E.

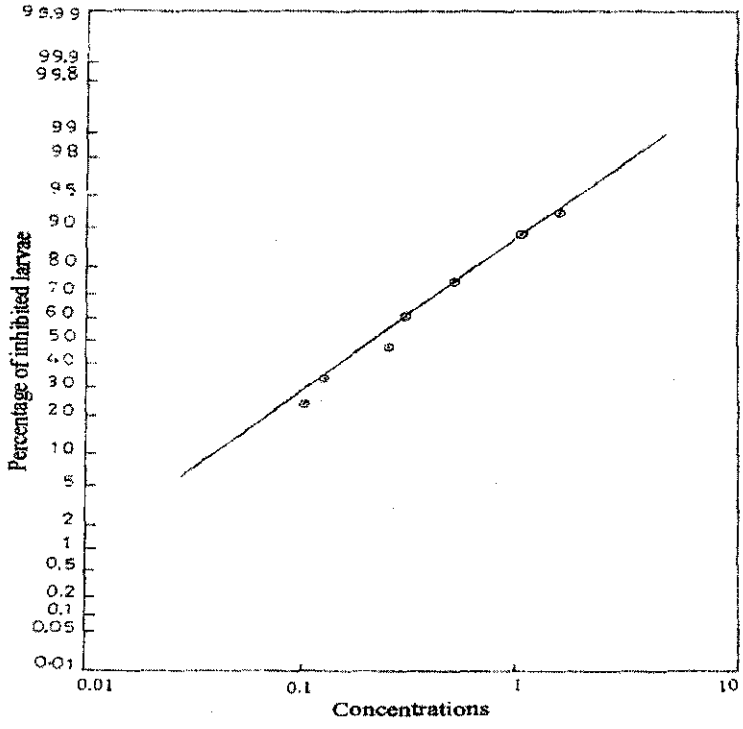


Table (2): Percentage inhibition of adult formation after treating the 3rd instar larvae of the whitefly *B.tabaci* with different concentrations of N. S.O.E. at 26.5±1°□ and 61.79±4.2% R.H.

Concentrations	No. of insects	Percentage of inhibited observed pupae	Percentage of inhibited expected pupae	(obs. - exp.)	Chi²
0.10	82	29	29	0	0.001 (0.082)
0.125	96	44	38	6	0.0150 (1.44)
0.25	85	67	58	9	0.032 (2.72)
0.30	98	73	73	0	0.001 (0.098)
0.50	89	86.5	86.5	0	0.001 (0.089)
1.0	85	96.4	96.4	0	0.001 (0.085)

IC_{50} = 0.17 Calculated Chi² = 4.51 Tabulated Chi² at freedom degree 4 = 9.49
Slope function= 2.69 Slope = 2.32

Fig. (3): Percentage inhibition of adult formation after treating the 3rd instar larvae of whitefly *B.tabaci* with different concentrations of N.S.O.E.

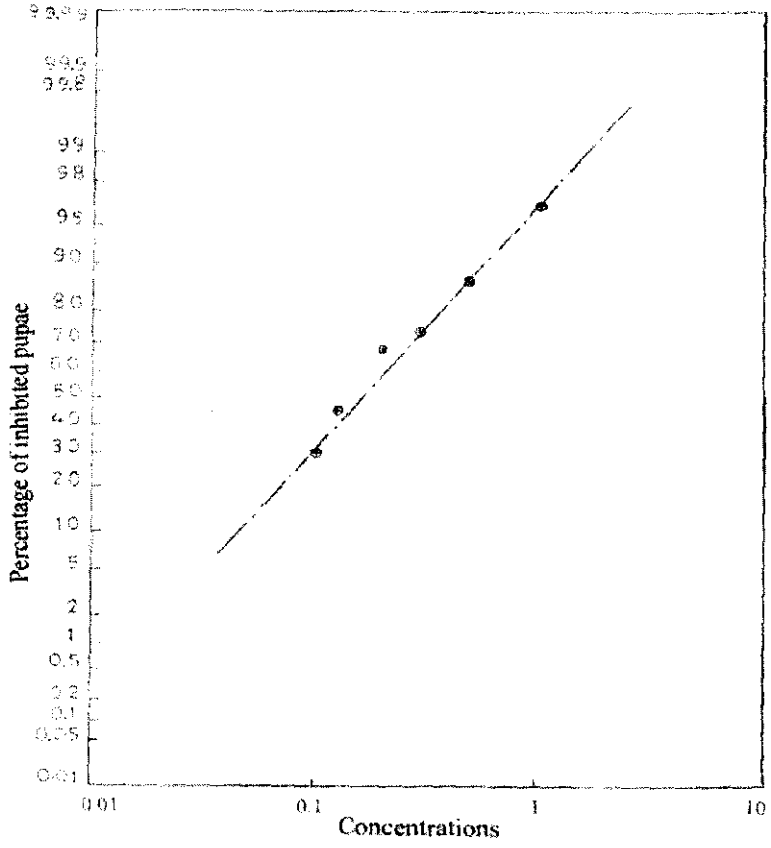


Table (3): Comparison of inhibition effect on pupal and adult formation after treating the 3rd instar larvae of the whitefly *B.tabaci* with different concentrations of N.S.O.E.

The stage	3rd larval instaar	Pupal stage
IC_{50}	0.22	0.17
Calculated Chi^2	4.035	4.514
Tabulated Chi^2	11.1	9.49
Random degree	5	4
Slope function	3.67	2.69
Slope	1.77	2.32

Fig. (4): Percentage inhibition of pupal and adult formation after treating the 3rd instar larvae of the whitefly *B.tabaci* with different concentrations of N.S.O.E.

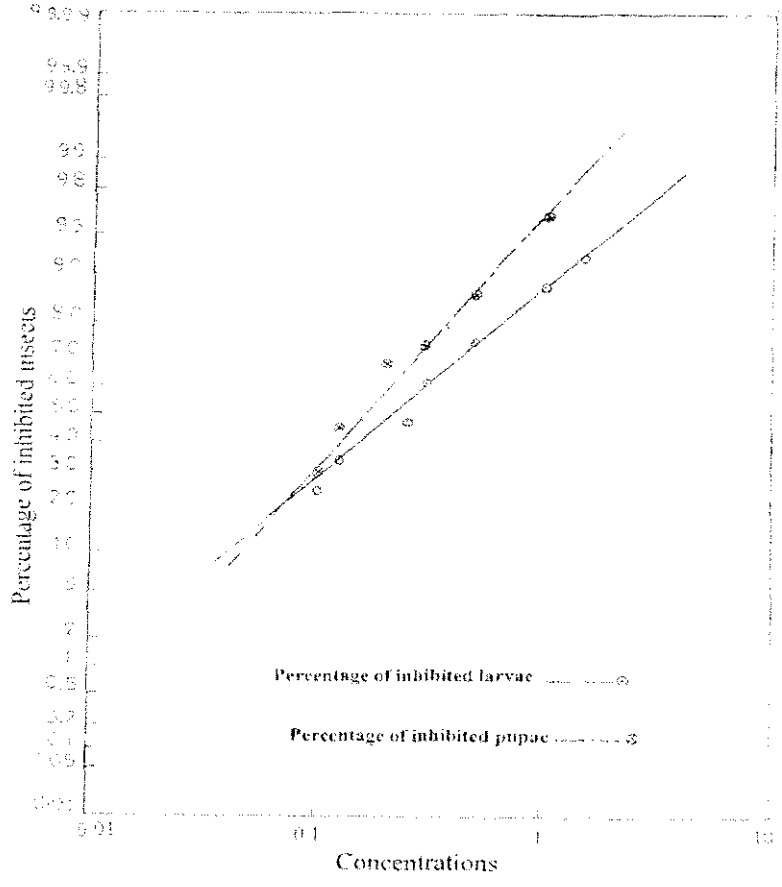


Table (4): Effect of N.S.O.E. on the whitefly *B.tabaci* after treating the 3rd instar larvae.

Concentrations	No. of insects	Percentage of inhibited insects	
		3rd larval instar	Pupa
0.10	82	23	29
0.125	96	33.3	44
0.25	85	47	67
0.30	98	61.1	73
0.50	89	75.2	86.5
1.00	85	88.2	96.4
1.50	90	92.2	100

Fig. (5): Effect of N.S.O.E. on the whitefly *B.tabaci* after treating the 3rd instar larvae\.

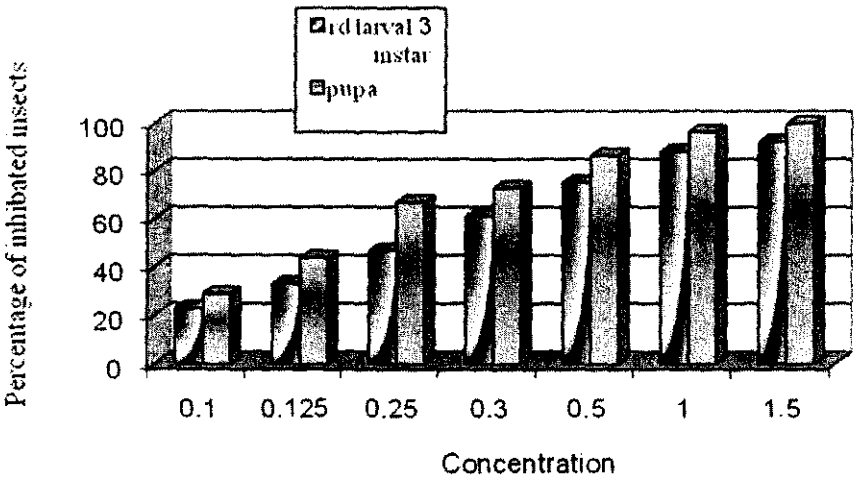


Table 5: Percentage inhibition of adult formation after treating the pupae of the whitefly *B.tabacil* with different concentrations of N.S.O.E. at 26.5±1°□ and 61.79±4.2% R.H.

Concentrations	No. of insects	Percentage of inhibited observed pupae	Percentage of inhibited expected pupae	(obs. - exp.)	Chi²
0.10	103	23	23	0	0.001 (0.103)
0.125	128	33	30	3	0.004 (0.512)
0.25	128	58	58	0	0.001 (0.128)
0.30	120	67	67	0	0.001 (0.120)
0.50	153	88	83	5	0.017 (2.601)
1.0	99	96	96	0	0.001 (0.099)

IC_{50} = 0.21 Calculated Chi² = 3.56 Tabulated Chi² at freedom degree 3 = 9.94
Slope function = 2.55 Slope = 2.46

Fig. (6): Percentage inhibition of adult formation after treating the pupae of the whitefly *B.tabaci* with different concentrations of N.S.O.E.

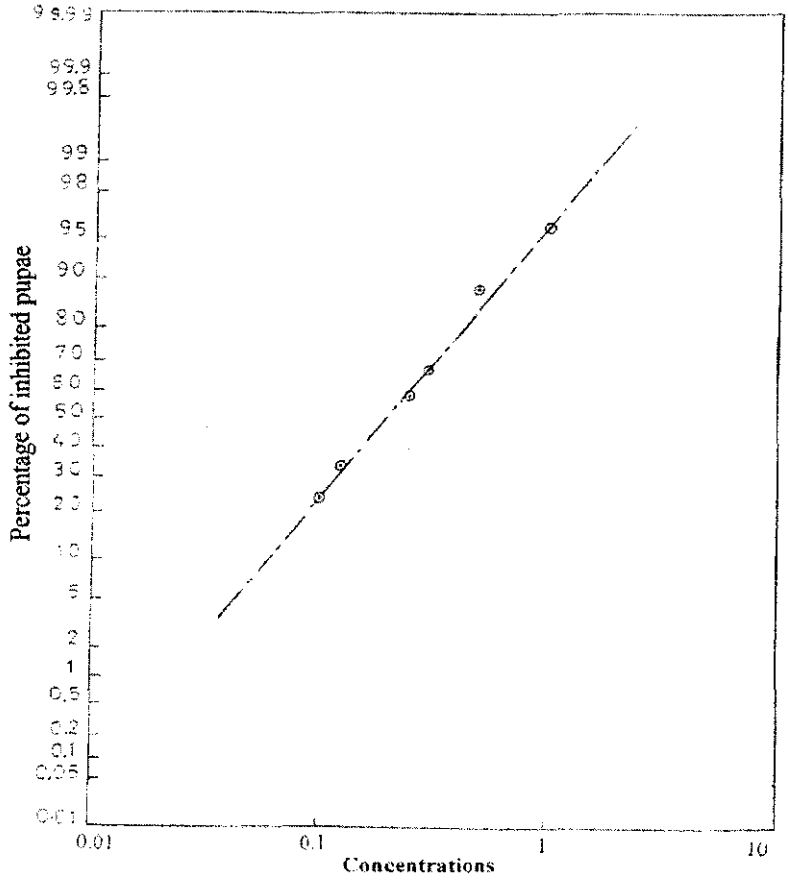
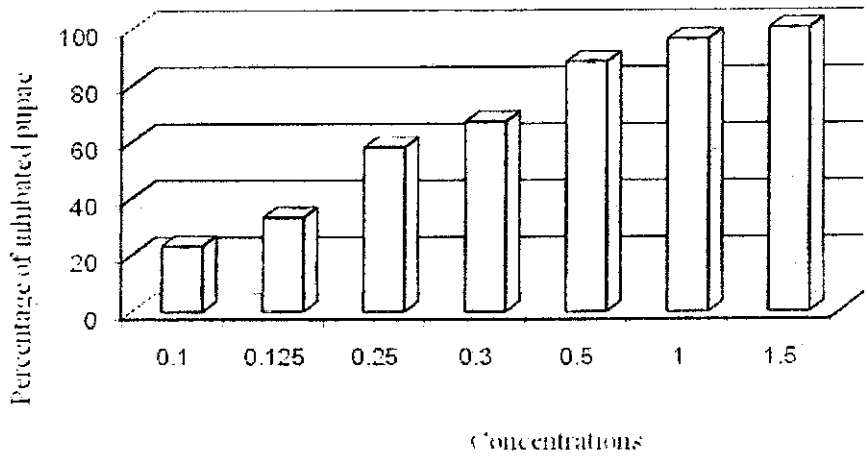


Table (6) : Effect of N.S.O.E. on the whitefly *B.tabaci* after treating the pupal stages .

Concentrations	No. of insects	Percentage of inhibited pupae
0.10	103	23
0.125	128	33
0.25	128	58
0.30	120	67
0.50	153	88
1.00	99	96
1.50	83	100

Fig (7): Effect of N.S.O.E. on the whitefly *B.tabaci* after treating the pupal stages.



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الملخص العربى

تأثير مستخلص زيت بذور النيـــم الطبيعي على بعض الأطوار غير الكاملة للذبابة البيضاء *Bemisia tabaci* (Gennadius)

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استخدم مستخلص زيت بذور النيـم الطبيـــعي في مـكافحـــة حشرة الذبابـــة البيـضـــاء *Bemisia tabaci* (Gennadius) التي تصيب أوراق نبات الطماطم. حيث تمت معاملة بعض الأطوار غير الكاملة للحشرة (العمر اليرقي الثالث وطور العذراء) باستخدام سلسلة تركيزات مختلفة لهذا المستخلص وهي ٠,١ ، ٠,١٢٥ ، ٠,٢٥ ، ٠,٣ ، ٠,٥ ، ١,٠ و ١,٥ مل / ١٠٠ مل من الماء المقطر. وقد اتـضح أن التركيز الذي ثبط ٥٠% من يرقات العمر الثالث عن التحول إلى عذارى (IC_{50}) هو ٠,٢٢ %. أما التركيز الذي ثبط ٥٠% من العذارى عـن التحول لحشرات كاملة (IC_{50}) فقد كانا ٠,١٧ و ٠,٢١% بعد معاملة كلٍ من يرقات العمر الثالث وطور العـذراء علـى الترتيـب. ونتيجـة للمعاملة بالتركيز ١,٠ و ١,٥ % حدثت إطالة في الأطوار غير الكاملة المعاملة وانتهت بموتهـا خـلال ٣-٨ أيـام. كمـا أدت التركيـزات المستخدمة إلى حدوث تشوهات مورفولوجية مختلفة للأطوار غير الكاملة المعاملة أو للحشرات الكاملة الناتجة تمثلت في فشل انسلاخ الأطـوار غير الكاملة أو فشل خروج الحشرات الكاملة جزئياً أو كلياً أو خروجها بأجنحة مشوهة ومشلولة. كما لم تتمكن الإناث الناتجة من وضع البيض إطلاقا أو تضع عدداً قليلاً جداً من البيض الذي لا يفقس. ويرجع هذا إلى تثبيط عملية تكوين ميزاب الانـسلاخ الطـولي أو العرضـي عنـد مستويات مختلفة. ويتم التحكم في عملية الانسلاخ بواسطة جهاز الغدد الصماء للحشرة، ويعمل مستخلص زيت بذور النيم على اضـطراب أو توقف الوظيفة الطبيعية لهذا الجهاز. وتوضح هذه النتائج أن مستخلص زيت بذور النيم الطبيعي يعمل كمنظم نمو وبالتالي يلعب دوراً مهماً في خفض أعداد هذه الحشرة.