Efficacy of Some Essential Oil against The Immature Stages of *Spodoptera littoralis*

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ABSTRACT

The toxicity of neem oil; jojoba oil; peppermint oil; garlic oil and ginger oil essential oil were evaluated against the immature stages of the cotton leafworm, *Spodoptera littoralis,* field strain in comparison with conventional insecticide cyhalothrin. The results showed that cyhalothrin was the most potent compound followed by neem oil, and jojoba oil and the least three materials were peppermint oil; garlic oil, and ginger oil. Tested compounds, cyhalothrin at 15.0 ppm achieved 31.9% mortality of treated egg masses, while neem oil; jojoba oil; peppermint oil; garlic oil and ginger oil at the same concentration caused 89.0; 75.9; 59.6; 53.5, and 51.0% mortality, respectively. The toxicity of tested compounds were evaluated against newly hatched larvae at 24, and 48 hrs, post treatment. The results showed that cyhalothrin; neem oil and jojoba oil were the more potent than peppermint oil; garlic oil and ginger oil. Cyhalothrin and tested essential oils gave not exhibited any toxic action against pupae of *S. littoralis*. The interactions of cyhalothrin with tested essential oils *in vivo* on the inhibition of Na^+,K^+-ATPase was investigated. Results proved that pretreated of neem oil, and jojoba oil with cyhalothrin increased the percentage inhibition for Na^+,K^+-ATPase to 91.4, and 88.3% respectively. Results proved that the five essential oils mixed with pyrethroid insecticide, (cyhalothrin), caused more toxic effect than single treatment, the present results proved that the five essential oils are potent for control of cotton leafworm, the neem oil, and jojoba oil, resulted in more effect than peppermint oil; garlic oil, and ginger oil. Generally, essential oils enhance the toxicity of some compounds against the eggs, and newly hatched larvae of this insect pest and conserve the role of beneficial insects and reduce the cost of pest control. This results of the present study may add some more steps to put the essential oils as an alternative to conventional insecticides, especially against this insect.

Key words: Essential oils, Enzyme activit, Cotton leaf worm.

INTRODUCTION

Massive applications of conventional pesticides result in adverse effects on beneficial organisms, leaves their residues in the food and result in environmental pollution. There is need to search about alternative control agents with safe to non target organisms; environment components; compatible with integrated pest management practices; short half life time and biodegradable. This has necessitated the use of target specific compounds with low persistence and an increase in emphasis on integrated pest management (Sharma and Yadav 2001 and El-Zahi, 2013). Therefore, naturally occurring insecticides have been used in pest control, many of these compounds are secondary plant substances, including alkaloids; quinones and essential oils (Coats, 1994 and Appel *et al*., 2001). The essential oils have received much attention as resources of potentially useful bioactive compounds, particular emphasis has been placed on their antimicrobial; antifungal, and insecticidal action (Gerasimos *et al*., 1997).

The Egyptian cotton leafworm, *Spodoptera littoralis* is among the most serious defoliators threaten the crops (Azab *et al.*, 2001). Massive applications of conventional pesticides to control this pest has led to development of multiple insecticide resistance (Saleem *et al*., 2008). Therefore, the aim of this investigation is to evaluate the toxicity of some essential oils as a natural product alone and their pretreated with cyhalothrin on egg stage and to evaluate their toxicity to the newly hatched larvae as well as pupae of *Spodoptera littoralis*, also, study the impact of essential oils on Na^+,K^+-ATPase activity.

MATERIALS AND METHODS

1. Test pest:

Field strain of cotton leafworm, *Spodoptera littoralis* egg masses were collected from cotton fields at Abeis area, Alex. Province, Egypt. Experiments were carried out using egg masses (24 hrs-old), meonate larvae, were chosen for bioassays and the 2nd larval instar were chosen for biochemical assessments. Pupae (48 hrs-old) were also investigated.

2. Chemicals and tested plant essential oils:

Jojoba oil 96% E.C. (*Simmodsia chinensis*), and Peppermint oil (*Mentha piperita*, Fam.,: Labiattae), were obtained from Egyptian natural Co., Egypt. Neem oil (pure oil 10%) (*Azadirachta indica*, Fam.,: Miliaceae), was obtained from Neemguard, Gharda chemicals, Ahme, India. Garlic oil (*Allium sativum*, Fam.,: Liliaceae), and Ginger oil (*Zingiber officinale*, Fam.,: Zingiberaceae), were obtained from Department of Pharmacology, Faculty of Pharmacy, Alex., University.

Basic stock solution of each tested oil was made in distilled water containing 0.5% Triton X-100 as an emulsifier. Series of at least five concentrations of each tested oil were prepared in distilled water.

Cyhalothrin (pyrethroids) provided as technical grade insecticides from U.S.A. Environmental Protection Agency (EPA). Ouabain is a cardiac glycoside which specifically inhibits the Na^+,K^+-ATPase (Mcllwain, 1963). A pure sample was obtained from Sigma Chem., Co. ST. loius.

3. Toxicity bioassay:

3.1. The determination of the ovicidal activity:

Ovicidal activity of the cyhalothrin; neem oil; jojoba oil; peppermint oil; garlic oil and ginger oil against the field strain of *S. Littoralis*, egg masses (0-24 hrs old) was investigated. The upper layers of eggs with a fine hair brush, the lower layer in each egg mass was counted by the binocular. The counted egg samples rest were dipped (5 sec.) in different concentrations of the tested compounds, while untreated control was dipped in water according to Dittrich (1967). Each treatment was replicated three times. Treatment and control were held in a clean plastic cup (9x4 cm) at 27 ± 2 ^oC and 65-70% RH and observed until hatching. The number of unhatched eggs, dead neonates and alive larvae were counted and the mortality percentages were calculated.

3.2. Toxicity of the tested essential oils against larvae:

Newly hatched larvae of *S. Littoralis* were starved for 6 hrs before test. The selected larvae were bioassayed against essential oils (neem oil; jojoba oil; peppermint oil; garlic oil and ginger oil) using three replicates for each concentration with ten larvae in each replicate. Disk dipping technique was used since it has been proved to be the most common procedure for assessing toxicity to essential oils (Tabashink and Chushing 1987). Each castor leaf disc (2 cm^2) was dipped into the suspension of tested essential oil for 10 sec. Tested concentrations were prepared in glass distilled water (GDW) (Toni and Fred 1996). Discs were held vertically to allow excess solution to drip off and placed on a rack to dry for at last 2 hrs. Treated discs were offered to starved larvae (one disc per cup) and left under constant conditions (27±2 ^oC and 65-70 % RH). Thereafter, survivors were transferred with fresh castor oil plant leaves to clean cups and kept under the same conditions. Control larvae were allowed to feed on castor oil leaf discs treated with distilled water. Mortality percentage was calculated for each concentration daily for 24, and 48 hrs and then corrected according to Abbott' s equation (Abbott, 1925), and subjected to probit-analysis using the computer program (Finney, 1971).

3.3. Toxicity of the tested insecticide against larvae:

Cyhalothrin was bioassayed against the newly hatched larvae of *S. littoralis*. The castor leaves were dipped in different concentrations of the tested insecticide, cyhalothrin concentrations was prepared in pure acetone. Treated and control leaves were air-dried for 3 hrs and placed in clean glass container at the laboratory conditions of 27±2 ^oC and 65-70 % RH, ten larvae (field strain) were used for each test with three replicates at least. Number of alive and dead larvae per each replicate was counted 24, and 48 hrs after treatment, concentrations–mortality percentages were calculated and corrected for natural mortality according to Abbott' s equation (Abbott, 1925). LC_{50} values were calculated by using the of probit-analysis method of Finney (1971).

4. Na^+,K^+-ATPase preparation and activity assay:

Head capsoul from *Spodoptera littoralis* fourth-instar larvae dissected and homogenized in a solution of 0.32 M sucrose, 1 mM EDTA and 40 mM tris-HCl buffer (pH 7.4). The homogenate was filtered through two layers of cheese cloth. Mitochondrial ATPase was prepared according to the method reported by Koch *et al*., (1969), by differential centrifugation of the homogenate at 8000 Xg for 10 min. The supernatant was then centrifuged at 20000 Xg for 30 min. The formed pellets were then suspended in the buffer and stored at (-20 ^oC) for use.

The ATPase activity was measured according to the method reported by koch *et al*., (1969) with slight modification by Morshedy (1980) using tris-HCl buffer instead of imidazole buffer. Absorbancy of inorganic Phosphate (Pi) was measured at λ 750 nm (Taussky and Shorr, 1953). This method was based on the spectrophotometric determination of the inorganic Phosphate (Pi) liberated from the hydrolysis reaction of the ATP, mediated by the enzyme.

The ATPase activity was measured in total volume of 1 ml. The mitochondrial preparation was mixed with a reaction mixture (700 µl) containing 100 mM Na^+; 20 mM K^+; 5 mM Mg^{2+} chlorides; 40 mM tris-HCl buffer (pH 7.4) and 5 mM ATP. The volume was completed to 850 µl with buffer. The mixture was incubated for 15 min, in a shaking water bath at 37 ^oC. The reaction was stopped by adding 150 µl trichloroacetic acid (TCA, 30 %).

Hydrolyzed Pi was determined according to the method, described by Taussky and Shorr, (1953). The activity of Mg^{2+}-ATPase was measured after the addition of 1 mM ouabain, whereas the activity of Na^+,K^+-ATPase was calculated as the difference between the total ATPase and Mg^{2+}-ATPase activities.

The protein content in prepared homogenates of *S. littoralis* was assayed spectrophotometrically by the method of Lowery *et al*., (1951) at λ 750 nm using Bovine Serum Albumin (BSA) as a standard protein.

5. *In vivo* inhibition of Na^+,K^+-ATPase activity:

The inhibition of Na^+,K^+-ATPase activity was determined in the 4th instar larvae using the values of each of the tested compounds. In the inhibition studies, of Na^+,K^+-ATPase activity 10 µl of the enzyme preparation was incubated with the inhibitor for 30 min, the enzyme-inhibitor mixture was used to measure the remaining activity. The percent inhibition was calculated using the following formula:-

$$\% \text{ Inhibition} = \frac{V\text{-}Vi \times 100}{V}$$

Where:

(V) is the specific activity without inhibitor.

(Vi) is the specific activity in presence of inhibitor

6. *In vitro* inhibition Na^+,K^+-ATPase activity:

The inhibition of Na^+,K^+-ATPase activity was determined in the 4th instar larvae using the values of each of the tested compounds. The method of Dixon and Weeb (1964) was adopted to draw the Dixon-plots by ploting 1/V versus concentrations of the inhibitor at two concentrations of the substrate. ATP (the substrate of ATPase) concentrations were 3.0 and 5.0 mM.

Estimation of I_{50} value (the concentration of the inhibitor which inhibits 50 % of the enzyme activity) was carried out by per incubating the enzyme with the inhibitor for 30 min using the following concentrations 0.1; 1; 5; 10, and 50 µM. K_i (the inhibition constant) values for each inhibitor were estimated from Dixon-plot.

RESULTS AND DISCUSSION

Ovicidal activity against egg masses:

Plant leaves containing egg masses were dipped in different concentrations of each tested compound. Data in table (1) show the ovicidal activity of the tested compounds against 24 hrs-old eggs of *S. littoralis*. The results showed that, cyhalothrin has low ovicidal activity compared with tested essenatial oils. A significant differences in precentages of unhatched eggs were found between all tested concentrations of the tested compounds and control eggs. The present results demonstrated that the *S. littoralis* eggs were more susceptible to tested essential oils in comparison to cyhalothrin, in general the susceptibility of eggs to the tested essential oils indicated their ovicidal activity. These results are in agreement with several investigators such as McDonald, 1981; Korkor *et al*., 1995; Ngoh *et al*., 1998; Isman, 2000; Choudhury, 2001; Ketta *et al*., 2001; Momen *et al*., 2001, and Eman *et al*., 2004.

Toxicity to newly hatched larvae:

The toxicity results of the tested compounds expressed in terms of LC_{50} are given in table (2) for the larvae of *S. littoralis*. LC_{50} values after 24 hrs were 0.88; 0.96; 1.04; 1.47; 1.86, and 0.50 ppm for neem oil; jojoba oil; peppermint oil; garlic oil; ginger oil, and cyhalothrin against the field strain of *S. littoralis* respectively, while LC_{50} values after 48 hrs were 0.44; 0.53; 0.79; 0.87; 0.99, and 0.31 ppm, respectively.

The interactions of cyhalothrin with tested essential oils against the field strain of *S. littoralis* larvae were studied. Larvae were allowed to feed on castor oil leaf disc treated with LC_{50} values of the different essential oils. The LC_{50} values of cyhalothrin pretreated with the LC_{50} values of each of neem oil; jojoba oil; peppermint oil; garlic oil, and ginger oil on field strain of *S. littoralis* larvae are presented in table (3). The LC_{50} values of cyhalothrin when pretreated with five essential oils were lower than LC_{50} of tested insecticide alone. The enhancement of toxicity was calculated as a potentiation factor (P.f.). P.f. values for cyhalothrin were 2.50; 1.78; 1.35; 1.25, and 1.11 respectively, when pretreated with neem oil; jojoba oil; peppermint oil; garlic oil, and ginger oil after 24 hrs from treatment, while P.f. values were 3.61; 3.26; 2.07; 1.55, and 1.24 respectively, when pretreated after 48 hrs from treatment. It is clear that the LC_{50} values concentrations of essential oils enhanced the toxicity of the tested insecticide to *S. littoralis* larvae, and neem oil and jojoba oil were the most potent followed by peppermint oil; garlic oil, and ginger oil were the least activity against *S. littoralis* larvae. The mixture with neem oil or jojoba oil was the most toxic treatment than mixtures with peppermint oil; garlic oil, and ginger oil.

In general, the susceptibility of *S. littoralis* larvae to cyhalothrin increased when treated after treatment with LC_{50} of essential oils, the tested insecticide had the lowest effect when applied alone but it was the more efficient when mixed with essential oils. The tested insecticide+essenesial oils caused more toxic effect than the effect of single treatment with tested insecticide. It could be concluded that tested essenesial oils enhanced the toxicity effect of cyhalothrin. Based on P.f. values, the field strain of *S. littoralis* larvae is more susceptible to neem oil and jojoba oil in comparison to the peppermint oil; garlic oil, and ginger oil. Generally, efficacy of essential oils have a very good additive toxicity for tested insecticide with the field strain of *S. littoralis*. These results are in agreement with those found by many authors such as, Choudhury, 2001; Ketta *et al*., 2001; Momen *et al*., 2001; Bhargava and Meena, 2002; Webb and David, 2002; Mesbah *et al*., 2004; Tripathy and Singh, 2005, and Moustafa *et al*., 2006.

Table 1: Ovicidal activity of tested compounds against 48 hrs-old eggs of *S. littoralis*

Conc. (ppm)	Cyhalothrin		Neem oil		Jojoba oil		Peppermint oil		Garlic oil		Ginger oil	
	H* %	U* %	H%	U%	H%	U%	H%	U%	H%	U%	H%	U%
Control	98.5	1.8	92.6	7.4	88.8	11.2	86.7	13.3	85.5	14.5	84.7	15.3
0.5	95.1	4.9	66.4	33.6	75.6	24.4	80.5	19.5	82.0	18.0	83.5	16.5
1	78.8	21.2	41.2	58.8	55.4	44.6	70.8	29.2	75.7	24.5	77.4	22.6
5	74.3	25.7	33.3	66.7	42.2	57.8	63.5	36.5	69.5	30.5	73.6	26.4
10	70.2	29.8	28.1	71.9	37.2	62.8	48.2	51.8	55.6	44.4	60.6	39.4
15	68.1	31.9	11.0	89.0	24.1	75.9	40.4	59.6	46.5	53.5	49.0	51.0

*H%: Hatched eggs. *U%: Unhatched eggs.

Table 2: LC_{50} values of tested compounds against the newly hatched larvae of *S. littoralis.*

Compounds	LC_{50} (ppm)	
	24hr	48hr
Cyhalothrin	0.50	0.31
Neem oil	0.88	0.44
Jojoba oil	0.96	0.53
Peppermint oil	1.04	0.79
Garlic oil	1.47	0.87
Ginger oil	1.86	0.99

Table 3: Comparative toxicities of tested cyhalothrin alone or pretreated with tested essential oils on *S. littoralis* larvae.

Compounds	LC_{50} (ppm)			
	24hr		48hr	
	LC_{50}	P.f.*	LC_{50}	P.f.
Cyhalothrin	0.50	------	0.31	-------
Neem oil+ Cyhalothrin	0.20	2.50	0.086	3.61
Jojoba oil+ Cyhalothrin	0.28	1.78	0.095	3.26
Peppermint oil+ Cyhalothrin	0.37	1.35	0.15	2.07
Garlic oil+ Cyhalothrin	0.40	1.25	0.20	1.55
Ginger oil+ Cyhalothrin	0.45	1.11	0.25	1.24

Potentiation factor (*P.f.) = LC_{50} of insecticide alone / LC_{50} of essential oil+insecticide.

The *in vivo* inhibition of Na^+,K^+-ATPase activity:

The *in vivo* inhibitory effects of the LC_{50} values of tested compounds against to the *S. littoralis* 2nd instar field strain larval Na^+,K^+-ATPase is shown in table (4). The data declared that cyhalothrin; neem oil and jojoba oil exhibited significant reduction in Na^+,K^+-ATPase activity, percentages of Na^+,K^+-ATPase inhibition were 82.5%; 63.1% and 54.2% respectively.

Data in table (4) summarize the interaction of neem oil, and jojoba oil on the inhibitory effect of cyhalothrin on Na^+,K^+-ATPase activity. The results proved that the pretreatment of neem oil, and jojoba oil induce increase the inhibition of enzyme activity. Results indicated that essential oils may make activation of Na^+,K^+-ATPase activity and these may be increased the cyhalothrin effects on Na^+,K^+-ATPase activity. It is clear that the cyhalothrin at LC_{50} concentration acts as potential inhibitors for *S. littoralis* larvae Na^+,K^+-ATPase activity when pretreated with essential oils. It was concluded that the tested pyrethroid was potentially potent for control of *S. littoralis* however, with natural compounds, such as essential oils currently in use. This results are in agreement with some investigators such as, Coats *et al*., 1991; Ragnault-Rogel and Hamraoui 1994; Weinzierl, 2000; Soliman, 2006.

The *in vitro* inhibition of Na^+,K^+-ATPase activity:

Table (5) show the *in vitro* interaction of cyhalothrin and two essential oils on Na^+,K^+-ATPase activity of *S. littoralis* 2nd instar larvae. The I_{50} values for cyhalothrin was 0.51 µM. It was shown that the efficacy of neem oil and jojoba oil has a very good additive toxicity for cyhalothrin to the field *S. littoralis* strain (table 2) because the enhancement toxicity of neem oil and jojoba oil, we studied the *in vitro* biochemical interaction of them with the Na^+,K^+-ATPase activity and compare with the cyhalothrin *in vitro* effects. The I_{50} values for neem oil and jojoba oil were 0.73 and 0.85 µM respectively. It clear that cyhalothrin at I_{50} concentration acts as potential inhibitors for *S. littoralis* larvae Na^+,K^+-ATPase activity.

Table 4: *In vivo* inhibition of *S. littoralis* 2nd instar larvae Na^+,K^+-ATPase activity.

Compounds	%Inhibition
Cyhalothrin	82.5%
Neem oil	63.1%
Jojoba oil	54.2%
Neem oil+ Cyhalothrin	91.4%
Jojoba oil+ Cyhalothrin	88.3%

Table 5: *In vitro* inhibition of *S. littoralis* 2nd instar larvae Na^+,K^+-ATPase activity.

Compounds	I_{50} (µM)	K_i (µM)
Cyhalothrin	0.51	32
Neem oil	0.73	54
Jojoba oil	0.85	65

To characterize more details about the *in vitro* inhibition of Na^+,K^+-ATPase by the inhibitors, the I_{50} and K_i values of each inhibitor were estimated from the graphical method of Dixon and Weeb, (1964) (table 5). The compounds competitive inhibition of Na^+,K^+-ATPase activity and the K_i values were 32; 54, and 65 µM for cyhalothrin; neem oil and jojoba oil respectively. These results are in agreement with Desaiah *et al*., 1975; Saleh *et al*., 1984; Ragnault-Rogel and Hamraoui 1994; Korkor *et al*., 1995; Weinzierl, 2000, and Imai *et al*., 2001.

In conclusion, the present study proved the essential oils are potentially potent for control of *S. Littoralis*, the essential oils when pretreated with pyrethroides will produce a promising trend so as reduce the field dose of the conventional insecticides (pyrethroides). In general, it may suggest that essential oils alternatives to the recommended pyrethroides currently in use *S. Littoralis* could be successfully included in the integrated pest management (IPM) programs, further studied will be useful in this concern.

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الملخص العربى

تأثير الزيوت النباتية ضد الأطوار غير الكاملة لحشرة دودة ورق القطن

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الهدف من البحث هوتقييم التأثير الإبادى لخمسة من الزيوت الطبيعية وهى النيم؛ الجوجوبا؛ الثوم؛ النعناع الفلفلى والزنجبيل مع المبيد الحشرى سيهالوثرين ضد الأطوار غير الكاملة للسلالة الحقلية لحشرة دودة ورق القطن سبدوبترا ليتولارس بهدف تلاشى تأثير المبيدات التقليدية الضار على البيئة. أوضحت النتائج أن الزيوت النباتية المختبرة لها سمية على البيض مقارنة بمبيد سيهالوثرين فلقد أوضحت النتائج أنه فى حالة السيهالوثرين كانت النسبة المئوية للبيض غير الفاقس هى ٣١.٩% عند تركيز ١٥ جزء فى المليون بينما النيم؛ الجوجوبا؛ الثوم؛ النعناع الفلفلى والزنجبيل كانت النسبة المئوية للبيض غير الفاقس هى ٨٩.٠؛ ٧٥.٩؛ ٥٩.٦؛ ٥٣.٥ و٥١.٠% على الترتيب، وقد وجد أيضاً أن النشاط الأبادى المتبقى للزيوت الطبيعية على اليرقات حديثة الفقس كان كبيراً. وقد تم تسجيل قيم التركيزات النصف مميتة (LC_{50}) لكل من الزيوت الطبيعية والمبيد الحشرى تحت الدراسة بصورة فردية، وقد أظهرت قيم الـ LC_{50} أن كلاً من سيهالوثرين؛ النيم والجوجوبا كانوا أكثر سمية على اليرقات حديثة الفقس بالمقارنة بالثوم؛ النعناع الفلفلى والزنجبيل. ثم بعد ذلك تم معاملة يرقات العمر الثانى لدودة ورق القطن بتركيزات مختلفة (LC_{50}) من الزيوت الطبيعية تحت الدراسة ثم معاملة هذه اليرقات بتركيزات منخفضة من المبيد المختبر سيهالوثرين تحت الدراسة بعد 24 و٤٨ ساعة من المعاملة بالزيوت الطبيعية، فأوضحت النتائج أن قيم الـ LC_{50} بعد المعاملة أنخفضت بدرجة ملحوظ خاصة فى حالة النيم والجوجوبا ويتضح ذلك من قيم معامل التنشيط الذى تم حسابها. أيضاً تم تقييم الفعل السام لكل من المبيدات المختبرة على طور العذراء وأظهرت النتائج أنه بعد ٢٤، ٤٨ و٧٢ ساعة من المعاملة بأى من الزيوت الطبيعية وجد فروق معنوية فى النسب المئوية للموت فى العذارى بالمقارنة بالسيهالوثرين. وكذلك تم دراسة المقدرة التثبيطية للزيوت المختبرة على النشاط الإنزيمى لإنزيم من أهم الأهداف البيولوجية والحيوية فى الحشرة وهو إنزيم الصوديوم-بوتاسيوم اداينوسين ترای الفوسفاتيز، ولقد أوضحت النتائج أنه فى حالة النيم والجوجوبا بعد المعاملة بالسيهالوثرين كانت النسبة المئوية للتثبيط هى ٩١.٤ و٨٨.٣% على الترتيب. وكذلك فقد تم تقدير قيم الـ I_{50} ولقد أثبتت النتائج حدوث أنخفاض فى تلك القيم وكانت أعلى نسبة للأنخفاض عند معاملة زيت النيم وزيت الجوجوبا مع السيهالوثرين على الترتيب وقد وجد أن هذه المركبات أظهرت تثبيط تنافسى على نشاط إنزيم الصوديوم-بوتاسيوم اداينوسين ترای الفوسفاتيز. ومن هذه النتائج يمكن أستخدام الزيوت كمبيدات طبيعية بديلة عن المبيدات التقليدية لأنها أكثر أماناً للأنسان والبيئة ولذلك يمكن الأستعانة بها عند وضع برامج المكافحة المتكاملة لدودة ورق القطن حتى نحد من سلبيات المبيدات التقليدية.