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GROWTH-INHIBITING EFFECTS OF A NEEM-BASED INSECTICIDE (MARGOSAN-O) AGAINST *SPODOPTERA LITTORALIS* (LEPIDOPTERA: NOCTUIDAE)

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Abstract— Margosan-O®, a commercial neem-based insecticide, incorporated into an artificial diet prolonged the development of larvae of *Spodoptera littoralis* Boisduval. It also affected the rate of adult emergence at the end of pupation. Larvae treated during one stadium continued to show the effects of their previous exposure. The susceptibility to Margosan-O was more pronounced when larvae ate the diets containing Margosan-O for the first time during the fifth or sixth instar. The results indicate that Margosan-O has an activity similar to that of other neem-seed extracts. In the course of this work morphological anomalies were also recorded. The most striking was the appearance of black stripes on the abdominal tergites of the fifth and sixth instar larvae fed on diets containing 0.01, 0.05 or 0.1% of Margosan-O.

Key Words: growth inhibitor, Margosan-O®, neem extract, *Spodoptera littoralis*

Résumé—Un insecticide commercial à base d'azadirachtine (Margosan-O®), incorporé à la nourriture artificielle, a prolongé la durée de développement des larves de *Spodoptera littoralis* Boisduval. Il a également affecté le nombre d'émergence d'adulte à la fin du stade nymphale. Les larves traitées pendant un stade donnée continuaient à manifester les effets de leur exposition au substrat. La sensibilité du Margosan-O était plus importante lorsque les larves se nourrissaient d'un substrat contenant Margosan-O au cours du cinquième et sixième stade. Les résultats montrent que le Margosan-O a la même activité que d'autres extraits à base de neem. Cet insecticide provoque également chez *Spodoptera littoralis* des anomalies morphologiques au cours des stades de développement; des bandes noires apparaissent sur les tergites abdominales lorsque les larves du cinquième et du sixième stade se nourrissent d'un substrat contenant 0.01, 0.05, ou 0.1% de Margosan-O.

INTRODUCTION

The neem tree (*Azadirachta indica* A. Juss.) produces numerous chemicals that have biological activities against insects. Neem seed extracts (NSE) are potent antifeedants (Volkonsky, 1937; Pradhan et al., 1962; Butterworth and Morgan, 1968), antioviposants (Ruscoe, 1972; Saxena et al., 1981a) and have also an impact on the regulation of insect growth (Leuschner, 1972; Redfern et al., 1981). The extracts reduce the rate of development of insect larvae (Redfern et al., 1981; Larew et al., 1985; Prabhaker et al., 1986) and have a negative impact on the success of their moulting and pupation (Rembold et al., 1982; Saxena et al., 1982b; Sieber and Rembold, 1983; Schlüter et al., 1985).

The Egyptian cotton leafworm (ECL), *Spodoptera littoralis*, is a highly polyphagus pest that causes severe damage to agriculture in Africa, in the Middle East and in the Mediterranean region. Various insecticides are available which control this insect but they sometimes are ineffective against infestations. Investigations are therefore under way to identify alternative methods, such as the use of

NSE. Thus, this study tested the effectiveness of Margosan-O against *Spodoptera littoralis*. Margosan-O is the first registered formulation of NSE.

MATERIALS AND METHODS

The stock culture of the ECL and the experimental insects were grown at 27°C, 70% RH and a photoperiod of 14:10 (L:D). Groups of 30 adults in a sex-ratio of 1:1 were confined in plastic containers (270 x 140 x 80 mm). Adults had access to a 5% solution of honey in distilled water. Females laid eggs on a cylinder of green cardboard put in the middle of the containers. Pieces of this cardboard were subsequently cut up around egg batches that were then transferred to large Petri dishes (120 mm diameter). Immediately after hatching, larvae began to feed on an artifical diet prepared as described by Poitou et al. (1972). The different ingredients of this diet were blended into agar-agar while the mixture was in a liquid state. The mixture was shaken for 1 min to ensure that the ingredients were homogeneously distributed in the medium. Some of the larvae were reserved for experiments while the rest perpetuated the stock culture. Larvae were fed until pupation, which took place in culture containers filled with sawdust.

Margosan-O® (supplied by W. R. Grace & Co.-Conn., Columbia, Md.) is a water-soluble formulation containing 3 mg/ml of azadirachtin. Successive aqueous dilutions were made and 30 ml of each were incorporated into an artificial diet for ECL larvae. Diets containing respectively 0.0005, 0.001, 0.005, 0.01, 0.05 and 0.1% Margosan-O (final wt/wt concentrations) were prepared. For the control, a similar procedure was followed but 30 ml of pure distilled water was added to the diet. The control and the Margosan-O diets (MO-diets) were poured into plastic Petri dishes (140 x 20 mm) and allowed to cool and solidify at room temperature.

The effects of Margosan-O (MO) on larval development of ECL were studied. However, because first instar larvae were easily lost or injured in handling, the total larval developmental time discussed in this paper is the period between the beginning of the second instar and the beginning of pupation.

Experiment 1: Susceptibility of larval instars

Twenty second instars were taken from the stock culture when they had just moulted and were individually placed in Petri dishes 55 mm in diameter. They were fed on the control diet and the different Margosan-O diets until the end of their development. All the Petri dishes were cleaned and fresh diet was presented daily.

The duration of each stadium was determined by measuring the time between two successive observations of a head capsule in Petri dishes. Only larvae that successfully moulted at the end of one instar were used to calculate the average instar durations. The rate of adult emergence was also determined.

The experiment was repeated with four other batches of 20 second instar larvae but the diets were given at the beginning of the third, the fourth or the fifth stadium. In this case, the rate of adult emergence was not measured.

Experiment 2: Persistence of action

Second instar larvae that had just moulted were sorted out as in the first experiment. They were fed on one of the diets for the duration of this stadium. At the beginning of the third stadium, they were offered the control diet until the end of their growth. The duration of the development of these larvae was determined by the method used in the first experiment and compared to the duration of the larvae reared on the control diet. The rate of adult emergence was also determined for larvae on the 0.01% MO-diet.

The experiment was repeated four times but the MO-diets were offered during the third, the fourth, the fifth or the sixth instar. Adult emergence was also recorded as above.

All data were tested for significance by analysis of variance (ANOVA) and the means were compared by Newman-Keuls test adapted to means with unequal numbers of replications (Kramer, 1956).

RESULTS

Experiment 1: Susceptibility of the larval instars

Margosan-O diets introduced at the beginning of the second instar had significant biological effects only from the next stadium onwards (Table 1). The third and the fourth stadia were significantly longer when the larvae were fed the diet containing 0.01% Margosan-O. Higher doses completely inhibited larval development. The effects of MO-diets became more pronounced during the fifth and the sixth instars when they caused extended larval development even at 0.001%. Sixth instar larvae failed to complete their development at 0.005%.

The introduction of MO-diets during the second stadium also affected the rate of adult emergence. A 5% mortality was recorded at 0.005% and reached 100% at 0.01% (Table 2).

Table 1. Stadia duration of *S. littoralis* larvae fed on treated diet from the beginning of a stadium until full development of the control

Treated instar	Margosan-O concentration (%)	Duration of stadium* (days) 2	3	4	5	6	Total development time (days)**
2	Control	3.0 a	3.1 a	2.3 a	2.7 a	6.9 a	18.0 a
	0.001	3.4 b	3.5 a	3.3 a	3.6 b	7.9 b	21.7 b
	0.005	3.0 a	3.5 a	4.1 a	4.8 c	–	–
	0.01	2.7 a	4.0 b	4.6 b	5.4 c	–	–
	0.05	3.3 b	–	–	–	–	–
	0.1	2.6 a	–	–	–	–	–
3	Control		1.8 a	2.2 a	2.5 a	6.4 a	15.5 a
	0.001		2.8 b	3.0 b	3.6 b	7.6 b	19.2 b
	0.005		3.1 b	3.6 b	5.0 c	–	–
	0.01		2.9 b	4.5 c	5.9 c	–	–
	0.05		3.1 b	4.9 d	–	–	–
	0.1		3.1 b	–	–	–	–
4	Control			2.4 a	2.9 a	6.6 a	17.9 a
	0.001			2.8 ab	3.5 b	8.4 b	21.0 b
	0.005			3.2 b	5.4 c	–	–
	0.01			3.1 b	6.0 c	–	–
	0.05			4.0 c	7.0 d	–	–
	0.1			4.1 c	–	–	–
5	Control				2.9 a	7.4 a	18.7 a
	0.001				3.5 b	10.1 b	21.9 b
	0.005				3.3 ab	–	–
	0.01				3.2 a	–	–
	0.05				4.3 c	–	–
	0.1				5.2 c	–	–

*Means within treated instar followed by the same letter are not significantly different ($P \leq 0.05$; Newman-Keuls).
**Total development time is the duration from the beginning of the second stadium to pupation.

The introduction of MO-diets during the third instar affected this stadium (Table 1) compared to the control. Larval development lasted longer at all concentrations. When larvae reached the next instar, however, there was a relation between lengthening of larval instar and concentration of MO-diet. Larvae that were first fed the MO-diet at the fourth, the fifth or the sixth instar behaved in a similar way to those fed MO-diets from the beginning of the third instar (Table 1). Moreover, the highest concentration of 0.1% prolonged developmental time and inhibited larval growth in the following instar larvae. The effects of MO-diets were more marked during the fifth and the sixth instar larvae.

Experiment 2: Persistence of action

Larvae fed MO-diets during the second instar took significantly more time to complete this stadium, although there was no clear relationship between the doses and the effects. At 0.1% concentration the larvae needed more time than at the other concentrations to reach the intermoult (Table 3). The delays induced by MO were still visible during the third instar, even after transferring the larvae to the uncontaminated diet. Moreover, the higher concentration of Margosan-O that had resulted in the elongation of the developmental time during the second instar, had now a larval growth-inhibiting effect. The duration of larval development did not change with treatment during subsequent instars. Nevertheless, the total developmental time was insignificantly affected by the introduction of MO-diets during the second instar (Table 3). These results indicate that Margosan-O ingested during one

Table 2. Mortality at emergence of adult *S. littoralis* when larvae were fed on a treated diet from the beginning of the second instar to pupation

Concentrations (%)	Mortality°* (%)
Control	0.0 a
0.0005	0.0 a
0.001	0.0 a
0.005	5.0 a
0.01	100.0 b

°Mortality corrected by Abbott's (1925) formula.
*Means followed by the same letter are not significantly different ($P \leq 0.05$; Newman-Keuls).

Table 3. Stadia duration (days) for *S. littoralis* larvae fed on diets containing different concentrations of Margosan-O

Treated instar	Margosan-O concentration (%)	Duration of stadium* (days) 2	3	4	5	6	Total development time (days)**
2	Control	3.0 a	2.9 a	3.0 a	3.1 a	7.6 a	19.6 a
	0.0005	3.6 bc	3.1 b	3.0 a	3.6 ab	8.4 a	21.8 b
	0.001	3.6 bc	3.3 b	2.8 a	3.7 ab	7.8 a	21.2 ab
	0.005	2.9 a	3.6 b	3.2 a	3.2 ab	8.6 a	21.4 ab
	0.01	3.4 ab	3.6 b	2.8 a	3.9 b	7.9 a	21.0 ab
	0.1	4.2 c	–	–	–	–	–
3	Control		2.6 b	2.8 a	2.9 a	7.0 a	17.9 a
	0.0005		3.2 bc	2.7 a	2.8 a	7.2 a	18.4 a
	0.001		2.8 b	3.0 a	3.2 a	7.6 a	18.9 a
	0.005		1.8 a	2.8 a	3.3 a	7.3 a	18.5 a
	0.01		3.75 c	4.2 b	4.6 b	10.0 b	24.3 b
	0.1		4.4 d	–	–	–	–
4	Control			3.5 a	3.7 a	7.6 a	20.2 a
	0.0005			3.9 a	4.0 ab	8.6 abc	22.2 bc
	0.001			3.7 a	4.1 ab	8.8 bc	22.7 bc
	0.005			3.7 a	4.5 b	9.8 c	23.6 c
	0.01			3.4 a	4.6 b	8.6 ab	21.0 ab
	0.1			4.7 b	–	–	–
5	Control				3.8 ab	7.8 a	19.9 a
	0.0005				4.0 ab	8.6 ab	21.2 a
	0.001				4.6 b	8.7 ab	22.4 ab
	0.005				4.7 b	10.7 b	24.4 b
	0.01				3.0 a	9.75 ab	19.0 a
	0.1				4.4 ab	–	–
6	Control					8.4 a	22.5 a
	0.0005					9.6 ab	23.0 a
	0.001					11.1 b	24.8 ab
	0.005					11.9 b	26.1 b
	0.01					–	–
	0.1					–	–

*Means within treated instar followed by the same letter are not significantly different ($P \leq 0.05$; Newman-Keuls).
**Total development time is the duration from the beginning of the second stadium to pupation.

Table 4. Mortality at emergence of adult *S. littoralis* when larvae were fed on a 0.01% treated diet during one stadium

Treated stadium	Mortality°* (%)
2	26.3 a
3	52.6 b
4	42.1 ab
5	73.6 c
6	100.0 d

°Mortality corrected by Abbott's (1925) formula.
*Means followed by the same letter are not significantly different ($P \leq 0.05$; Newman-Keuls).

stadium was still active after the larvae were transferred back to the control diet.

Margosan-O delayed larval development during the third instar, and once again the 0.1% MO-diet had the most significant effect. However, unlike in the second instar larvae, the effect of Margosan-O did not persist throughout the next instar except for 0.01 and 0.1% doses (Table 3). The 0.1% concentration had a growth-inhibiting effect while the 0.01% concentration induced a marked lengthening of the development during the fourth, the fifth and the sixth instar, thus lengthening total developmental time (Table 3).

Larvae treated during the fourth or the fifth instar grew similarly to those feeding on MO-diets during the third stadium (Table 3). The larvae seemed more susceptible to Margosan-O at the sixth instar than at earlier instars, as evidenced by the relatively lower level of this diet at which a growth-inhibiting effect was observed. Doses as low as 0.001 and 0.005% induced a significant lengthening of development.

Twenty-six per cent of *Spodoptera littoralis* adults failed to emerge from pupae when larvae were fed diets containing 0.1% MO (Table 4). The proportion became significantly higher (52.6%

mortality) when this diet was offered to larvae during the third instar increasing to 100% with the sixth instar larvae. This confirms the higher susceptibility of these larvae to Margosan-O.

DISCUSSION

The prolonged duration of larval instars and the total developmental time observed even when larvae were fed on low concentrations of Margosan-O is in agreement with results obtained with Margosan-O on nymphal *Blatella germanica* (L.) and *Periplaneta americana* (L.) (Adler and Uebel, 1987). Other NSEs produced the same effects on *Spodoptera frugiperda* (J. E. Smith) (Redfern et al., 1981) and *Spodoptera exigua* (Hübner) (Prabhaker et al., 1986).

The observed decrease in the rate of adult emergence due to MO-diets supports observations by Meisner et al. (1981) on *S. littoralis* larvae treated with another NSE. The tendency for older larvae to respond faster to MO-diets than younger larvae was also observed by Saxena et al. (1981b) on *Cnaphalocrocis medinalis* (Guenée).

Physiological effects of neem extracts on insect larvae and nymphs have not been fully elucidated. Treatment with azadirachtin causes reduction, delay, or absence of ecdysones and juvenile hormones during the last larval instars and nymphal periods (Rembold and Sieber, 1981; Rembold et al., 1987). Azadirachtin probably has more than one site of action, depending on time and stage of treatment (Koul et al., 1987; Koul and Isman, 1991).

During the two experiments, several larvae displayed morphological deformities which impeded their development. Some larvae had a wet aspect, suggesting an incomplete resorption of the exuvial fluid as was the case with *Locusta migratoria* L. treated with azadirachtin (Sieber and Rembold, 1983). Larva-pupa intermediates were also observed. They generally had a pupal cuticle on the posterior part of the abdomen and a fore body with typical larval characters. Larvae treated from the beginning of the fifth or the sixth instar with MO-diets at 0.01, 0.05 or 0.1% exhibited transversal black stripes on the tergites. The higher the concentration, the darker the bands. At first sight, the position of these bands corresponded to the insertion zones of external dorsal muscles (Snodgrass, 1935). Some pigments may be regarded as metabolic waste products the accumulation of which is a kind of storage excretion (Chapman 1969; Hepburn, 1985). According to Chapman (1969) melanin production is a method of disposing of toxic phenols arising from metabolism; this pigment is often produced over metabolically active tissues such as muscles.

Although it is difficult to equate laboratory and field conditions, these preliminary observations with MO-diets suggest that this NSE could be used effectively in integrated pest control against ECL. Two possibilities are available: early treatments at high concentrations (corresponding to 0.05 and 0.1% in the experimental diets) directed at young larvae, or applications at low concentrations (corresponding to 0.005% in the MO-diets) when sixth instar larvae appear in the fields.

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REFERENCES

Abbott W. S. (1925) A method for computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18, 265–267.

Adler V. E. and Uebel E. C. (1987) Effects of Margosan-O on six species of cockroaches (Orthoptera: Blaberidae, Blattidae and Blatellidae). In *Natural Pesticides from the Neem Tree* (Azadirachta indica) *and Other Tropical Plants* (Edited by Schmutterer H. and Ascher K. R. S.), pp. 387–392. Proc. 3rd Int. Neem Conf., Nairobi, 1986.

Butterworth J. H. and Morgan E. D. (1968) Isolation of a substance that suppresses feeding on locust. *Chem. Commun.* 1, 23–24.

Chapman R. F. (1969) *The Insects Structure and Function.* English Universities Press Ltd. pp. 438–439.

Hepburn H. R. (1985) The integument. In *Fundamentals of Insect Physiology* (Edited by Blum M. S.), pp. 139–183. John Wiley & Sons.

Koul O., Amamai K. and Ohtaki T. (1987) Effects of azadirachtin on the endocrine events of *Bombyx mori*. *J. Insect Physiol.* 33, 103–108.

Koul O. and Isman M. O. (1991) Effect of azadirachtin on the dietary utilization and development of the variegated cutworm *Peridroma saucia*. *J. Insect Physiol.* 37, 591–598.

Kramer C. Y. (1956) Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics* 12, 307–310.

Larew H. G., Knodel-Montz J. J., Webb R. E. and Warthen J. D. (1985) *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) control on *Chrysanthemum* by neem seed extract applied to soil. *J. Econ. Entomol.* 78, 80–84.

Leuschner K. (1972) Effect of an unknown plant

substance on a shield bug. *Natuurwizsenschaften* 59, 217–218.

Meisner J., Ascher K. R. S. and Aly P. (1981) The residual effects of some products of neem seeds on larvae of *Spodoptera littoralis* in laboratory and field trials. In *Natural Pesticides from the Neem Tree* (Edited by Schmutterer H. and Ascher K. R. S.), pp. 157–170. Proc. 1st Neem Conf., Rottach-Egern, 1980.

Poitou S., Blues R. and Lerumek C. (1972) Elevage sur milieu artificiel simple de deux noctuelles parasites du coton *Earias insulana* et *Spodoptera littoralis*. *Entomol. Exp. Appl.* 15, 341–350.

Prabhaker N., Coudriet D. L., Kishaba A. N. and Meyerdirk D. E. (1986) Laboratory evaluation of neem-seed extract against larvae of the cabbage looper and beet armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 79, 39–41.

Pradhan S., Johwani M. G. and Rai D. K. (1962) The neem seed deterrent to locusts. *Indian Farming* 12, 7–11.

Redfern R., Warthen Jr. W., Uebel E. C. and Mills G. D., Jr. (1981) The antifeedant and growth disrupting effects of azadirachtin on *Spodoptera frugiperda* and *Oncopeltus fasciatus*. In *Natural Pesticides from the Neem Tree* (Azadirachta indica) (Edited by Schmutterer H. and Ascher K. R. S.), pp. 129–136. Proc. 1st Neem Conf., Rottach-Egern, 1980.

Rembold H. and Sieber K. P. (1981) Inhibition of oogenesis and ecdysteroid synthesis by azadirachtin in *Locusta migratoria migratorioides* (R. F.). *Z. Naturforsch.* 36C, 466–469.

Rembold H., Sharma G. K. and Czoppelt Ch. (1982) Growth regulating activity of azadirachtin on two holometabolous insects. In *Natural Pesticides from the Neem Tree* (Azadirachta indica) (Edited by Schmutterer H. and Ascher K. R. S.), pp. 121–128. Proc. 1st Neem Conf., Rottach-Egern, 1980.

Rembold H., Uhl M. and Miller T. (1987) Effect of azadirachtin A on hormone titers during the gonadotrophic cycle of *Locusta migratoria*. In *Natural Pesticides from the Neem Tree* (Azadirachta indica) *and Other Tropical Plants* (Edited by Schmutterer H. and Ascher K. R. S.), pp. 289–298. Proc. 1st Neem Conf., Eschborn, 1987.

Ruscoe C. N. E. (1972) Growth disruption effects of an insect antifeedant. *Nature, New Biol.* 236, 159–160.

Saxena R. C., Liguido N. J. and Justo H. D. (1981a) Neem seed oil, a potential antifeedant for the control of the rice brown planthopper, *Nilaparvata lugens*. In *Natural Pesticides from the Neem Tree* (Azadirachta indica) (Edited by Schmutterer H. and Ascher K. R. S.). Proc. 1st Neem Conf., Rottach-Egern, 1980, pp. 171–180.

Saxena R. C., Waldbauer G. P., Liquido N. J. and Puma B. C. (1981b) Effects of neem seed oil on the rice leaf folder, *Cnaphalocrocis medinales*. In *Natural Pesticides from the Neem Tree* (Azadirachta indica) (Edited by Schmutterer H. and Ascher K. R. S.), pp. 189–204. Proc., 1st Neem Conf., Rottach-Egern, 1980.

Schlüter U., Bidmon H. J. and Grewe S. (1985) Azadirachtin affects growth and endocrine events in larvae of the tobacco hornworm, *Manduca sexta*. *J. Insect Physiol.* 31, 773–777.

Sieber K. P. and Rembold H. (1983) The effects of azadirachtin on the endocrine control of moulting in *Locusta migratoria*. *J. Insect Physiol.* 29, 523–527.

Snodgrass R. E. (1935) *Principles of Insect Morphology*. McGraw-Hill, New York.

Volkonsky M. (1937) Sur l'action acridifuge des extraits de feuilles de *Mela azadirachta*. *Arch. Inst. Pasteur d'Algérie*. XV, No. 3.