TOXIC EFFECTS OF SPINOSAD (BIOINSECTICIDE) ON LARVAL INSTARS OF DATE MOTH *ECTOMYELOIS CERATONIAE* (LEPIDOPTERA, PYRALIDAE) UNDER CONTROLLED CONDITIONS

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RESUME

En raison des conséquences catastrophiques sur l'environnement et sur la santé humaine par l'application massive des pesticides, cette suituation a poussé les scientifiques à trouver des méthodes moins nocives. Dans ce contexte Nous avons réalisé notre travail pour explorer l'activité larvicide du Spinosad sur les différents stades larvaires d'*Ectomyelois ceratoniae*, qui est le ravageur le plus dangereux de la production dattiere en Algérie et dans le monde.

L'étude de l'effet toxique du Spinosad sur la mortalité du premier stade larvaire a révélé que les concentrations utilisées étaient significativement et positivement corrélés avec la mortalité corrigée pour les différentes durées d'exposition des larves au bio-pesticide (100% avec une concentration de 100ppm). L'étude des paramètres démographiques pour les individus résultant des larves traitées pour les quatre concentrations du Spinosad a montré que ce bio-pesticide perturbe la fertilité des femelles et diminue la fertilité de la femelle de la pyrale.

MOTS CLES: Control biologique, *Ectomyelois ceratoniae*, Spinosad, mortalité corrigé, toxicité, fertilité.

ABSTRACT

Due to the catastrophic consequences of the massive application of pesticides, scientists have been finding less harmful methods. In this context we conducted our work to explore the larvicidal activity of Spinosad on the instars larvae of *Ectomyelois ceratoniae*, which is the most important and dangerous pest of date palm in the world and particularly in Algeria.

The study of the toxic effect of Spinosad on the mortality of the first larval stage revealed that the concentrations used were significantly and positively correlated with mortality adjusted for different durations of exposure of larvae to the bio-pesticide (100.00% with the concentration 100 ppm). The study of demographic parameters for individuals resulting from the larvae treated by four amounts of Spinosad, showed that this bio-pesticide decreases the female fertility and the fertility of eggs.

KEYWORDS: Biocontrol, Ectomyelois ceratoniae, Spinosad, mortality, toxicity, fertility.

ملخص

بسبب النتائج الكارثية لإستعمال الكميات الهائلة من المبيدات الحشرية والمبيدات العشبية، وجد العلماء أساليب أقل ضررا على المحيط. وفي هذا السياق أجرينا عملنا لاستكشاف النشاط السمي للمبيد Spinosad على أطوار من يرقات سوسة التمر*Ectomyelois ceratoniae* التي تعتبر من أخطر الآفات على النخيل في العالم، وخاصة في الجزائر.

أظهرت دراسة التأثير السام لSpinosad على الوفيات في أول طورين من اليرقات أن الجرعات المستخدمة كانت بشكل ملحوظ وإيجابي مرتبطة مع معدل تصحيح الوفيات لليرقات لفترات مختلفة من التعرض للمبيد الحيوي. كما تبين أن المعاملات الديموغرافية للأفراد الناتجة عن معاملة اليرقات بالجرعات الأربعة لSpinosad أن المبيد الحيوي يقلل من خصوبة الأنثى و عدد البيوض.

الكلمات المفتاحية

تحكم بيولوجي- وفيات معدلة- سمية-خصوبة- Spinosad-*Ectomyelois ceratoniae*

1 INTRODUCTION

In Algeria, the date palm is the mainstay of the oasis ecosystem in Saharan and pre-Saharan regions. It provides, through marketing nationally and internationally for its fruit a regular income for farmers. It plays a screen role in protecting oasis against the desert influences and creates a microclimate favouring development of underlying crops and stabilization of local populations in these ecologically fragile areas (Majourhat et al., 2002). The date production has declined over the years because of the attacks of different pests and diseases such as: Bayoud, Rot inflorescences, mites, mealy bugs and the date moth Ectomyelois ceratoniae Zeller. This latter is a polyphagous pest whose larvae identified as economically damaging pest, it can cause considerable damage that can reach 20 to 30% of the date production (Mehaoua et al., 2013) and this insect poses a serious economic threat to the date industry. During the latest 25 years, the carob moth caused between 10 and 40% damage in the harvestable crop annually (Warner, 1988, Nay et al., 2006). Larvae are polyphagous and attack both stored products and field crops in the Mediterranean basin and countries in the Near East region (Gothilf, 1969). The date moth *E. ceratoniae* (Zeller) (Lepidoptera:Pyralidae) is considered to be one of the key pests on carob, pomegranate, dates, citrus and almond worldwide (Gothilf 1969; Warner, 1988; Ksentini et al., 2010).

During seasonal population growth, the carob moth uses different stages of the date fruit for ovipositionand development, resulting in an increasing density through the growing season (Nay and Perring, 2005).

Many control methods have been used to keep populations below economic threshold levels. Controlling the carob moth with insecticides is not efficient because larvae feed and develop inside the fruit, where they are protected (Dhouibi, 1989). There is therefore a great need to develop alternative control methods, which are both effective and friendly to the environment.

This test was carried out in order to study the toxicity of Spinosad, biopesticides, against the larvae of the carob moth *E. ceratoniae* and its effect on female fertility and eggs under controlled conditions to reduce the harmful effects of its products on the environment and beneficial fauna useful in our palm groves.

2 MATERIALS AND METHODS

2.1 Mass-Rearing of date moth

Our breeding was conducted with a strain of *E. ceratoniae* from the palm grove of Biskra of the year (2012).We have put infested dates in the breeding cage in a controlled growth chamber at a temperature of 30 ± 1 °C, relative humidity (RH) of 70± 5% and a photoperiod of 16h:8h (L:D) (Al-Izzy et al., 1987; Mediouni and Dhouibi, 2007).At the emergence, the adults of *E. ceratoniae* are captured by using a test tube. Then they are put inside the plastic boxes for coupling. After mating, females will lay eggs inside the jars. Eggs are discharged through fine mesh tulle (1mm of diameter) in the plastic boxes (20cm x40cm x10cm). Those were reared on an artificial diet containing flour dates (50g), wheat bran (50g) and distilled water (20ml). After some days, the eggs hatch and we get larvae's for bio-pesticide treatment *E. ceratoniae* passed through five instars larvae (28-30days),the pupal period (6-8 days)and adults (3-4 days), three generations in succession during the year and a fourth-generation there sometimes,(Hadjeb et al., 2014).

2.2 Study of the toxicity of Spinosad on larvae and eggs

A sample of the commercial formulation of Spinosad concentrated liquid suspension formulation(Tracer 480SC) was obtained as a gift from Dow AgroSciences, Algeria, containing480 g of Spinosad active ingredient (a.i.) per litre.

In sterile plastic Petri dishes (9 cm in diameter) containing the artificial diet sprayed with the bio-pesticide. We applied a treatment of four concentrations of the Spinosad, (25 ppm, 50 ppm, 100 ppm and 200 ppm) with a control concentration; the all with three repetitions, and we've filed 20 of the first instars larvae per test. The observations were every 24 h for counting dead larvae.

Another test was conducted with the same concentrations but this time we sprayed the four concentrations of Spinosad on 20 eggs directly with a control concentration all in three repetitions. The ratings of hatched eggs are performed every 24 hours using a binocular microscope.

Experimental dilutions were not exposed to sunlight, and excess volumes of each dilution were discarded within 24 h of having been prepared.

2.3 Statistical Analysis

In order to characterize the power of insecticidal molecule used, we determined the 50% lethal concentration (LC 50). We determine all concentrations according the mathematical process of Finney (1971).

The mortality rate is corrected by Abbot's formula (1925). Different rates of mortality undergo an angular transformation according to the tables established by Bliss (Fisher and Yates, 1975). The transformed data are subject of analysis variance (ANOVA) with a single classification

criterion. The calculations were realized by the program XLSTAT 2013.The method of Swaroop and al. (1966), allows the calculation of the confidence interval for the LC50.

3 RESULTS AND DISCUSSION

3.1 Study of mortality of E. ceratoniae larvae exposed to Spinosad

Our results showed that the application of four concentrations of Spinosad between 25 ppm and 200 ppm on the first stage larvae of the date moth caused mortality which varied between 10.0 % and 100.00 % during treatment monitoring. The insecticide effects of Spinosad, increases more we increase the time exposure.

The variance analysis of the corrected mortality of first stage larvae after 24h, 48h, 72h, 96h,120h and 144h of exposure duration to Spinosad shows very highly significant differences between the four concentrations used with respectively p: 0,1063; p: 0,0004; p: < 0,0001; p: < 0,0001, p: 0, 0003 and p: < 0,0001 (Table. 1).

The two highest concentrations of Spinosad (100 ppm and 200 ppm) resulted in the most significant mortality of larvae *E. ceratoniae* with 100.00 % in lethal time long enough (120 h). While the lowest concentration used (25 ppm) induced in 24 h and 48h the lowest mortality rate with 15.00 and 30.56 % respectively (Tab. 1).

Table0 1: Corrected mortality rates of first stage larvae of E. ceratoniae treated with the Spinosad

Time of exposure	Concentrations				F_{obs}	p
	25 ppm	50 ppm	100 ppm	200 ppm		
After24 h	10,09±4,87	15,26±5,02	18,68±3,23	22,02±2,64	2,831	0,1063
After48 h	20,70±0,61	32,72±1,98	36,14±4,25	41,32±4,14	21,221	0,0004
After 72 h	35,05±4,99	51,86±1,62	55,46±2,48	64,95±4,99	34,288	< 0,0001
After96 h	47,05±5,12	66,82±5,03	72,60±2,23	72,60±0,00	194,938	< 0,0001
After120 h	61,27±6,60	75,61±5,36	85,91±8,75	100,00±0,00	23,495	0, 0003
After 144 h	68,10±4,69	84,13±3,61	100,00±0,00	100,00±0,00	167,062	< 0,0001

From the results shown in table 2, we note that the corrected mortality of L1 larvae *E. ceratoniae* exposed to Spinosad are significantly correlated to the different concentrations used for lethal time 24h, 48h, 72h, 96h, 120h and 144h with respectively $R^2 = 0.903$; $R^2 = 0.878$; $R^2 = 0.926$; $R^2 = 0.747$; $R^2 = 0.789$; $R^2 = 0.895$ (Table. 2).

The results analysis of this bioassay provides an estimated value LC 50%at24 h of 8992.46 ppm. The limits which take the value of LC50% are estimated at 4172.99 ppm for lower limit and 19378.01 ppm for the upper limit.

After 144 hours of treatment, we estimated that value of LC50% is 20.60 ppm. The confidence limits are estimated between 18.86 ppm for the lower limit and 22.50 ppm for upper limit (Tab.2).

Table 02: Toxicological parameters of Spinosad on the first instar larvae L1

Time of exposure	Regression equation	R^2	*p*	$LC_{50\%}$ (ppm)	$LC_{50\%}$ (ppm)	$LC_{84\%}$ (ppm)	S (Slope)	Lower limit of LC50%	Upper limit of LC50%
After24 h	Y = 3,11+0,48 x	0,90	0,04	12,09	8992,46	15843,21	121,59	4172,99	19378,01
After48 h	Y = 3,34+0,66 x	0,88	0,06	10,05	320,66	8626,62	31,79	184,41	557,56
After96 h	Y = -0,95+3,85x	0,75	0,13	3,11	35,17	13455,03	1,81	31,97	38,68
After120 h	Y =-0,146+3,50x	0,79	0,11	2,85	29,45	4186,15	1,92	26,52	32,69
After144 h	Y = -0,45+4,15x	0,86	0,07	5,46	20,60	1081,59	1,74	18,86	22,50

3.2 Study of female fertility and eggs of *E. ceratoniae*

The statistic analysis (ANOVA) of the average number of eggs laid per female and the average number of eggs hatched from *E. ceratoniae* issue from lots handled by four concentrations (25 ppm, 50 ppm, 100 ppm, 200 ppm) showed very highly significant difference with p:<0,0001 (Tab. 3).

The result of table 3 show that the highest number of eggs was recorded at the control female's (without treatment),

followed by females issue from treated larvae's with concentrations of 25 ppm, 50 ppm, 100 ppm and 200 ppm. The results showed that the application of various concentrations of Spinosad on the first instar larval of *E. ceratoniae* inhibits the development and the growth of larvae and causes their death. From the observations recorded we note that mortality rates are positively correlated to the different concentrations used, regardless of the exposure time of eggs to Spinosad.

Table 03: Average number of eggs laid per female and percentage of hatched eggs

Control	Control	25ppm	50ppm	100ppm	200ppm	d.f.	F	*p*
Average number of eggs laid per female (%)	159.67±28 .27	107.33±27,48	95.5±53,87	66.67±19.22	54.67±19.4	5	7.96	0.0001
Average rate of hatched eggs (%)	95.67±2,89	2.33±0.44	1.33±0.44	1.00±0.00	0.67±0.44	5	5.412	0.0001

According to Jacquet et al. (2002), spinosad basically swallowed by attacking the cells of the digestive tract, but also in contact deform the larval cuticle. It was also observed malformations emerged imagines that die after two days of their outputs pupae. The same author states that death can occur up to two days after treatment.

Our results show that the average rate of mortality corrected are proportional to the dose levels used, regardless of larval exposure time Spinosad. So the observed mortality rate is positively correlated with the dose and duration of exposure of larvae Spinosad, with a low mortality rate for a short time and a lethal high death for a long exposure time, and the lowest dose causes the lowest mortality.

Spinosad was clearly the most efficient larvicide tested with absolute or near absolute control of developing *Aedes spp*. and *Culex spp*. larvae for periods of 6–8 weeks depending on season and concentration (Marina et al,2012).Our results confirm the biological control test carried out in Tunisia in 2000 on the date palm and show that Spinosad is very effective against date moth even at allow concentration (Khoualdia et al., 2002).

According to (Kirst, 2010) and (Thompson et al., 2000) Spinosad is an insecticide (larvicide) relatively broad spectrum registered for many cultures. It is deemed effective against larval Lepidoptera and Diptera. The effect of Spinosad through ingestion requires a longer lethal time, even with low concentrations. It can cause total mortality of larvae. Arlaet al. (1998) state that Spinosad is 5 to10 times more effective through ingestion than by contact.

Larval mortality by different doses used is positively correlated with the duration of exposure of carob moth to the product. This is probably the result of the combined effect produced by contact and ingestion. Our results confirm the result of Robert et al. (2006), Spinosad is effective on larvae, this biopesticide inhibits the development and growth of the larvae.

Spinosad has been active demonstrated in various species, including pests of Hymenoptera (Penagos et al., 2005). Spinosad is also effective against Aedes albopictus (Skuse) with an LC50 of 0.3 ppm (Bond et al., 2004). Another study showed that Spinosad is highly toxic against *Helicoverpa armigera* (Hübner) with an LC50 of 0.41 ppm (Wang et al., 2009). Other results demonstrated that Spinosad with sub-lethal doses affects the brain *Oreochromis niloticus* (Piner et al., 2012). According Williams et al. (2003) Spinosad is effective on young lepidopteran larvae.

4 CONCLUSION

The female survivors of exposure to an LC50 concentration of Spinosad showed increased reproductive capacity, probably owing to the elimination of the smaller and more susceptible fraction of the date moth population. This result underlines the need to maintain concentrations of Spinosad at lethal levels in larval habitats. A requirement that is particularly challenging in situations where exposure to strong sunlight or continuous water flow, degrade or dilute the toxicant (Kirst, 2010). Sustained release formulations of Spinosad may greatly assist in overcoming this problem but are not yet widely available for testing.

Initial studies have indicated that Spinosad may be a promising new bio rational insecticide, it provided effective lasting control of *E. ceratoniae* in experimental conditions. It might be a good alternative to chemical pesticides, while preserving human health and the environment.

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