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Insecticidal activity of 1.8-cineole against *Plodia interpunctella* (Hbn.) (Lepidoptera Pyralidae)

Abstract - A solution of 1.8-cineole (purity ≥ 98%) was tested. Insects were exposed to the active ingredient for 24 hours. The percentage of hatched eggs was detected after 6 days from the treatment; larvae and adults mortality was observed after 24 and 48 hours. 330 µl/l air of 1.8-cineole causes 95% mortality of *Plodia interpunctella* eggs. II and IV instar larvae show different tolerances to the active ingredient. DL_{95} value, recorded after 48 hours, is 23.67 µl/l air for II instar larvae, while it doubles for male and female IV instar larvae. Adult females are more resistant to the active ingredient (DL_{50} adults ♂♂: 0.31 µl/l air and DL_{50} adults ♀♀: 0.38 µl/l air; DL_{95} ♂♂: 7.46 µl/l air and DL_{95} ♀♀: 8.70 µl/l air).

Riassunto - *Attività insetticida di 1,8-cineolo nei confronti di* Plodia interpunctella *(Hbn.) (Lepidoptera Pyralidae).*

1,8-cineolo è stato saggiato in soluzione pura (≥ 98%). Gli individui sono stati esposti al principio attivo per 24 ore. La percentuale di schiusura delle uova è stata rilevata dopo 6 giorni dalla fine del trattamento, mentre la mortalità delle larve e degli adulti è stata osservata dopo 24 e 48 ore. 330 µl/l aria di 1,8-cineolo determinano il 95% di mortalità delle uova di *Plodia interpunctella*. Larve di II e IV età mostrano una diversa tolleranza al principio attivo. Il valore di DL_{95}, calcolato dopo 48 ore, è 23,67 µl/l aria per le larve di II età mentre per le larve di IV età di entrambi i sessi è circa due volte maggiore. Per quanto riguarda lo stadio adulto, le femmine risultano essere più tolleranti al principio attivo (DL_{50} ♂♂ adulti: 0,31µl/l aria e DL_{50} ♀♀ adulte: 0,38 µl/l aria; DL_{95} ♂♂: 7,46 µl/l aria e DL_{95} ♀♀: 8,70 µl/l aria).

Key words: Eucalyptol, Indian Meal Moth, mortality.

INTRODUCTION

Different essential oils, distilled from numerous plant species, could substitute phytosanitary products used in foodstuffs protection (Shaaya *et al.*1991; 1997; Regnault-Roger *et al.* 1993). These extracts are composed of various molecules such as flavonoids, glucosides and several terpenoid compounds that can interfere with the insect

metabolism (Karr & Coats, 1988; Brattsen, 1993; Wantanabe *et al.*, 1993; Tripathi *et al.*, 2000; 2001).

There are several references about 1.8-cineole (eucalyptol) toxicity, present in numerous plant species, mainly in leaves of different *Eucalyptus* spp., against foodstuffs Coleoptera (Morrow & Fox, 1980; Stamopoulos, 1991; Obeng-Ofori *et al.*, 1997; Ngoh *et al.*, 1998; Obeng-Ofori & Reichmuth, 1999; Lee *et al.*, 2000; 2001; Tunç *et al.*, 2000; Neetu *et al.*, 2001; Lee *et al.*, 2003). There are few references to moth species infesting crops or foodstuffs. Gayathri *et al.* (2003) observed that *Spodoptera litura* (F.) (Lepidoptera Noctuidae) larvae, treated with 1.8-cineole, show a shorter larval instar period and smaller dimensions than normal. Tunç *et al.* (2000) proved the efficacy of 1.8-cineole, in different doses, on 24 hour old eggs of *Ephestia kuehniella* (Zell.) (Lepidoptera Pyralidae); a mortality rate of 45% was observed after 96 hours of exposure to 196.9 μl/l air of active ingredient.

The aim of this test was to evaluate the potential inhaling insecticide effect of 1.8-cineole against *Plodia interpunctella* (Hbn.) (Lepidoptera Pyralidae).

MATERIALS AND METHODS

A solution of 1.8-cineole (purity ≥ 98%[1]) was tested on eggs, II and IV instar larvae and adults of *Plodia interpunctella* (Hbn.). The Indian meal moth was reared at the Istituto di Entomologia agraria, dell'Università degli Studi of Milan - at 26±1°C, 60±5% r.h., photoperiod 16:8 (light:dark).

The active ingredient was placed on a filter paper disk (Ø: 6 cm) and then put inside a glass jar (1.7 l). Groups of 100 eggs (24-48 hours old) and 20 individuals (mixed population) of II instar larvae, 20 males and 20 females of IV instar larvae and 20 adults (24-48 hours old) were used for each test. Eggs, larvae and adults were exposed to the active ingredient for 24 hours. Tests were carried out in a thermostatic room [(26±1°C, 60±5% r.h. and photoperiod 16:8 (light:dark)]. At the end of the test, the biological material was transferred in glass jars (Ø: 6 cm) under the same environmental conditions. The percentage of hatched eggs was recorded after 6 days from the treatment; unhatched eggs were counted as dead while larvae and adults mortality percentage was recorded after 24 and 48 hours of exposure. The individuals which, touched with a paint brush, did not show head, legs and pseudopodia or abdomen contractions (Waldstein & Reissig, 2000) and which presented evident signs of intoxication, such as uncoordinated movements or difficulty to move, were considered dead.

Five replications were carried out for each test. Abbot's formula (Abbott, 1925) was used to adjust data when necessary. Results were subjected to Probits analysis in order to determine DL_{50} and DL_{95}.

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[1] Botanical extract produced from Sigma-Aldrich (www.sigma.aldrich.com).

RESULTS

It was observed that 72.8±1.59% of *Plodia interpunctella* eggs, hatches with a dose of 160 μl/l air of active ingredient, while 56.0±1.41% hatches at 180 μl/l air of 1.8-cineole. At 330 μl/l air 5% of eggs survive (Table 1).

DL_{50} and DL_{95} values for II instar larvae, after 24 and 48 hours, are respectively 20.09-13.83 μl/l air and 37.71-23.67 μl/l air (Tables 2, 3). IV instar larvae are less susceptible to the active ingredient compared to the II instar ones; in fact DL_{50} and DL_{95} values are approximately twice or three-times the ones obtained for II instar larvae.

Male and female adults are more susceptible to 1.8-cineole. Male adults tolerate better than females the presence of 1.8-cineole after 24 hours from the treatment (DL_{50} adults ♂♂: 1.67 μl/l air and DL_{50} adults ♀♀: 1.28 μl/l air; DL_{95} ♂♂: 21.23 μl/l air e DL_{95} ♀♀: 16.01 μl/l air). After 48 hours females, on the other hand, are more tolerant to the toxicity of the active ingredient (DL_{50} adults ♂♂: 0.31 μl/l air and DL_{50} adults ♀♀: 0.38 μl/l air; DL_{95} ♂♂: 7.46 μl/l air and DL_{95} ♀♀: 8.70 μl/l air).

Table 1 - Mean values of eggs hatching percentages of Plodia interpunctella *(Hbn.) at different doses.*

Eggs hatching			
Doses (μl/l air)	Mean % (±S.E.)	Doses (μl/l air)	Mean % (μl/l air)
160	72.8±1.59 a	310	25.0±0.84 e
170	65.8±1.68 b	320	12.6±1.36 f
180	56.0±1.41 c	330	4.4±0.93 g
300	32.4±0.51 d	350	3.4±0.51 g

The values followed by a different letter are significantly different for an interval of confidence of 95%.

Table 2 - LD_{50} and LD_{95} (μl/l air) values for the different stages of Plodia interpunctella *(Hbn.) after 24 hrs from the treatment with 1.8-cineole.*

Developmental stage	Slope	Slope S.E.	LD_{50} (Fiducial limits)[a]	LD_{95} (Fiducial limits)[a]
Second instar larvae	6.01	0.51	20.09 (16.65-30.68)	37.71 (26.77-194.11)
Fourth instar larvae				
♂♂	7.73	0.44	40.24 (32.01-47.06)	64.44 (52.52109.89)
♀♀	9.07	0.52	42.79 (41.10-43.80)	64.44 (61.34-68.37)
Adults				
♂♂	1.49	0.09	1.67 (1.43-1.98)	21.23 (15.14 -32.24)
♀♀	1.50	0.10	1.28 (0.93-1.78)	16.01 (8.79-42.25)

[a] Fiducial limits were calculated at P≤0.05 level.

Table 3 - The LD_{50} and LD_{95} (µl/l air) values for the different stages of Plodia interpunctella *(Hbn.) after 48 hrs from the treatment with 1.8-cineole.*

Developmental stage	Slope	Slope S.E.	LD_{50} (Fiducial limits)[a]	LD_{95} (Fiducial limits)[a]
Second instar larvae	7.25	0.51	13.83 (13.26-14.39)	23.67 (21.86-25.32)
Fourth instar larvae				
♂♂	8.36	0.48	37.45 (30.28-44.19)	56.83 (48.12-97.22)
♀♀	10.43	0.64	40.18 (36.99-43.69)	57.97 (51.63-69.97)
Adults				
♂♂	1.19	0.10	0.31 (0.25-0.38)	7.46 (4.88-13.27)
♀♀	1.21	0.10	0.38 (0.32-0.46)	8.70 (5.68-15.42)

[a] Fiducial limits were calculated at P≤0.05 level.

DISCUSSION

High doses of 1.8-cineole are toxic for *Plodia interpunctella* eggs: in fact a dose of 330 µl/l air is required to obtain 95% of eggs mortality. It was observed that, with a dose of 80 µl/l air, the hatched eggs percentage is not statistically different from that one recorded with untreated eggs (Locatelli & Stampini, 2005). It was noticed that unhatched eggs present a brownish colour after 6 days of treatment. It was also observed the presence of partially hatched eggs that showed dead larvae. Triphati *et al.* (2001) observed that an exposure to 250 µl/l air of 1.8-cineole for 24 hours, not only diminishes the hatching percentage of *Acanthoscelides obtectus* eggs (Say) (Coleoptera Bruchidae), but also increases the mortality of newly emerged larvae; this has also been observed in *Tribolium castaneum* (Hbst) (Coleoptera Tenebrionidae). *P. interpunctella* eggs are more susceptible to the active ingredient than the eggs of other insects of foodstuffs such as *Tribolium confusum* (J. du Val) (Coleoptera Tenebrionidae) and *Ephestia kuehniella* (Zell.) (Lepidoptera Pyralidae) (Tunç *et al.*, 2000).

II and IV instar larvae of *P. interpunctella*, show different tolerance to the active ingredient. II instar larvae are more susceptible to the treatment than the IV instar ones. Tripathi *et al.* (2001) didn't find differences among the various larval instars on *T. castaneum* (Hbst). Evident signs of toxicity, such as lack of coordination in the movements and darkening of the cuticle, were observed on larvae even at the lowest doses. In some cases IV instar larvae pupate early, as a reaction to the presence of the toxic active ingredient. It has been noticed that some individuals, after 48 hours from the treatment, start again to move. Lee *et al.* (2000) observed that 1.8-cineole in some species of insects can be detoxified by cytochrome P450-monooxigenase dependent enzymes. As a further mechanism of biochemical detoxification, the action of hydrolasė and esterase seems possible; in fact, the active site of esterase is similar to the one of acetylcholinesterase and consequently can be inhibited by eucalyptol essential oil or by 1.8-cineole.

P. interpunctella adults are more susceptible to 1.8-cineole compared not only with

the other stages but also with the adults of the most common beetles of foodstuffs. In a test carried out by Shaaya *et al.* (1991) 100% mortality was obtained at a dose of 15 µl/l air for all the species considered such as *Rhyzopertha dominica* (F.) (Coleoptera Bostrychidae), *Sitophilus oryzae* (L.) (Coleoptera Dryophthoridae), *T. castaneum* (Hbst), *Oryzaephilus surinamensis* (L.) (Coleoptera Silvanidae). In our tests, however, 9 µl/l air are enough to cause a mean percentage of mortality higher than 95% after 24 hours of exposure. At the concentration of 0.5 and 3 µl/l air evident signs of toxicity were observed on the insects, such as absence of coordination in movements, swelling of the last abdominal segments with consequent abnormal extension of intersegmental membranes and eggs-laying. Results show that DL_{95} values are higher for females than males, after 48 hours from the end of the treatment. In literature it is well-known that females of different species are more tolerant to the toxic substances (Abd-Elghafar *et al.*, 1990).

Although further investigations concerning a practical use of 1.8-cineole to treat foodstuffs premises are necessary, this active ingredient could be considered an interesting alternative to the use of fumigants as it shows low acute toxicity on mammals (DL_{50} 2400 mg/kg on rat) (De Vincenzi *et al.*, 2002) and a high vapour pression (Lee *et al.*, 2003), which allows disinfestations of premises in short times.

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APPUNTI E SEGNALAZIONI