2-METHYL-5-(1-METHYLETHENYL)-2 CYCLOHEXENONE INSECTICIDAL EFFECT ON *TRIBOLIUM CONFUSUM*

EFEITO INSECTICIDA DE 2-METHYL-5-(1-METHYLETHENYL)-2-CYCLOHEXENONE SOBRE TRIBOLIUM CONFUSUM

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ABSTRACT

The insect *Tribolium confusum* is considered one of the most important storage pests due to the significant reductions either in quantity or quality of cereal grains. Alternatives to chemical synthesis insecticides should be found due to their obvious disadvantages. Those alternatives should be specific to target plant enemies, with no risks to the ecosystem. Hence, the aim of this work was to study the insecticidal effect of a monoterpene, 2-methyl-5-(1-methylethenyl)-2-cyclohexenone, on *Tribolium confusum*. Acute essays were performed with the following monoterpene concentrations: pure 2-methyl-5-(1--methylethenyl)-2-cyclohexenone, 10, 1, 0.1 and 0.01%. To compare the monoterpene insecticidal activity, tests with a standard insecticide, pirimiphos-methyl, were also conducted. Mortality was observed at one, two, three, six, 12, 24, 36, 48 and 96 hours. The obtained results showed that 2-methyl-5-(1--methylethenyl)-2cyclohexenone behaviour was comparable to that of the pirimiphos--methyl. Therefore, further studies should be conducted to confirm the bioinsecticidal potencial of this monoterpene.

Keywords: Bioinsecticide, 2-Methyl-5-(1--methylethenyl)-2-cyclohexenone, monoterpene, storage pest, *Tribolium confusum*.

RESUMO

O insecto *Tribolium confusum* é uma das mais importantes pragas de produtos armazenados, causando prejuízos consideráveis na quantidade e qualidade dos cereais. Perante as desvantagens do uso de insecticidas químicos de síntese, procuram-se alternativas específicas para os organismos - alvo, que causem o mínimo de prejuízos no ecossistema. O objectivo deste trabalho foi estudar, em laboratório, o efeito insecticida de um monoterpeno, 2-methyl-5-(1-methylethenyl)-2--cyclohexenone, sobre *Tribolium confusum*. Foram realizados ensaios de toxicidade aguda com as seguintes concentrações: 2-methyl-5-(1-methylethenyl)-2-cyclohexenone puro, 10, 1, 0,1 e 0,01%. Foi ainda estudada a actividade do insecticida padrão, pirimifos--metilo, puro. A mortalidade foi registada uma, duas, três, seis, 12, 24, 36, 48 e 96 horas após o início da experiência. Os resultados

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mostraram que o monoterpeno, na sua forma pura, teve um comportamento comparável ao do pirimifos-metilo, justificando-se, por isso, a realização de mais estudos, tendo em conta as características bioinsecticidas reveladas neste estudo.

Palavras-chave: Bioinsecticida, 2-Metyl-5--(1-metylethenyl)-2-ciclohexanona, monoterpeno, praga de armazém, *Tribolium confusum.*

INTRODUCTION

Tribolium confusum Jacquelin du Val (Coleoptera: Tenebrionidae), the confused flour beetle, is considered a major pest of stored grains. Besides its classification as a secondary pest, it can infest grain kernels being often found in raw grain. This obvious grain decay and adverse changes in flavour, taste, appearance and nutrition value are responsible to post-harvest deterioration, leading to considerable economic losses (Aitken, 1975 in Athanassiou *et al.*, 2008; Arlian *et al.*, 1996 *in* Kumar *et al.*, 2008).

In order to control these insects promoting a yield increase, protection of stored products is essentially based on the use of broad-spectrum synthetic insecticides. Even though their effectiveness, their continued applications and repeated use for several decades, has disrupted the natural enemy biological control system, thus leading to outbreaks of insect pests, widespread development of resistance, undesirable effects on non-target organisms, environmental concerns, and serious human health hazards (Pemonge *et al.*, 1997; Zettler and Arthur, 1997; Lamiri *et al.*, 2001; Ribeiro *et al.*, 2003; Bughio and Wilkins, 2004; Jbilou *et al.*, 2006; Çalmasur *et al.*, 2006). Therefore, there is a prompted demand for developing safer, non-polluting and biologically specific alternatives to synthetic insecticides for an integrated approach to pest management (Pemonge *et al.*, 1997; Mewis and Ulrichs, 2001; Mohan and Fields, 2002; Ramos-Rodríguez *et al.*, 2006).

During recent years, there is a renewed interest in using natural products including plant derived materials to control insects in many agricultural environments, including storage facilities (Arthur *et al.*, 2004; Jbilou *et al.*, 2006; Kumar *et al.*, 2008). In fact, higher plants are a rich source of novel natural substances that can be used to develop safe methods for insect control including stored-product control (Park *et al.*, 2003; Jbilou *et al.*, 2006) (b). Several formulations have been prepared as botanical pesticides for plant protection since they prevent the environment from the impact of toxic residues and have lower toxicity to organisms. Many of those secondary metabolites are also used as flavours (Duke, 1985 *in* Negahban *et al.*, 2007). Lately, research on essential oils have attracted particular attention as a new alternative for pest control due to their specificity to pests, biodegradable nature and potential for commercialisation (Liu *et al.*, 2006).

2-Methyl-5-(1-methylethenyl)-2--cyclohexenone, a monoterpene, which is the main compound of the essential oil of caraway (*Carum carvi* L.) fruits, was reported as an antimicrobial agent, as insecticide and as biochemical environmental indicator (Carvalho and Fonseca, 2006), as well as an antisprouting agent (Hartmans *et al.*, 1995; Oosterhaven *et al.*, 1995; Costa e Silva *et al.*, 2007, 2010). This compound was already registered by the European Commission for use as flavourings in foodstuffs, meaning that presents no risk to consumer's health (OJEU, 2002). It also appears on the Everything Added to Food in the US' (EAFUS) list as it was classified as Generally Recognised As Safe (GRAS) and approved food Additives by the United States Food and Drug Administration (FDA, 2011).

In this paper, we evaluated the insecticide effect of 2-methyl-5-(1-methylethenyl) -2-cyclohexenone on the confused flour b eetle *Tribolium confusum*.

MATERIAL AND METHODS

Insects

Insects were obtained from laboratory cultures maintained in the dark in incubators at 30 ± 1ºC and 70 ± 5% r. h. This culture was reared on wheat flour mixed with yeast (10:1, w/w) for at least two years. Two-week old adults were used for the study of 2-methyl-5-(1-methylethenyl)-2-cyclohexenone insecticidal effect.

Preparation of solutions

Pure 2-methyl-5-(1-methylethenyl)-2-cyclohexenone was obtained from the commercial Fluka®. The different concentrations solutions, 10000, 1000, 100 and 10ppm were prepared by sequential dilution using distilled water. Tween 20® at 5000 ppm was added to each solution.

Insecticidal activity

One millilitre of either test solution was transferred separately to pre-sterilised 9 cm diameter Petri dishes containing sterilised filter paper (Whatman nº1). A standard insecticide, pirimiphos-methyl, was used as a standard. Petri dishes containing either filter paper, with distilled water or distilled water with Tween 20®, were used as controls. Ten insects were introduced in each Petri dish and placed in an incubator at with 30 ± 1ºC and 70 ± 5% r.h. All treatments were replicated four times and data were presented in terms of the mean ± S.D. Mortality of exposed insects was observed after one, two, three, six, 12, 24, 36, 48 and 96 hours in all solutions and control.

Data analysis

Control mortality was corrected by using Abbott's formula (1925). Within each exposure interval, the data were submitted to one-way analysis of variance (ANOVA) using Statistica software, to determine differences among the monoterpene concentrations. Post hoc testing was carried out using Tukey test. A significance level of 0.05% was used for all statistical tests (Zar, 1996).

RESULTS AND DISCUSSION

The results showed that the 2-methyl-5-(1-methylethenyl)-2-cyclohexenone has insecticidal activity on *Tribolium confusum* (Table 1). On the whole, it was verified a dose-responsive mortality being firstly registered 100% mortality one hour after exposure in pure monoterpene. The same was observed in pure pirimiphos-methyl. In 10 and 1% 2-methyl-5-(1-methylethenyl)-2-cyclohexenone, 100% mortality was verified after 12 and 24 hours exposure in 10 and 1%, respectively. At 0.01 % and 0.1% exposure neither mortality nor mobility were affected.

Throughout experiment mortality was observed in any of the controls assuming that both filter paper and Tween 20® (5000ppm) are nontoxic to *T. confusum*. Based on these facts the percentage of cumulative mortality corresponds to the percentage of the corrected cumulative mortality.

According to ANOVA, at two hours, the behaviour of pure monoterpene and the standard insecticide was significantly different from the other treatments ($F=1022.625$; $df=8$; $p<0.05$). At six hours, 10% and 1% monoterpene concentrations shown significantly different behaviour of pure monoterpene and standard insecticide as well as from the other treatments ($F=210.977$;$df=8$;$p<0.05$). At 12 hours exposure, 10% concentration showed the same behavior of pure monoterpene and standard insecticide ($F=2928.000$; $df=8$; $p<0.05$), and after 24 hours, monoterpene concentrations higher than 1% had the same behaviour, 100% mortality with no observed alterations until the end of the experiment.

These results showed that the 2-methyl-5-(1-methylethenyl)-2-cyclohexenone had similar insecticidal activity to that observed on the pirimiphos-methyl. This monoterpene effect was dose-responsive as in

Table 1 – Effect of 2-methyl-5-(1-methylethenyl)-2-cyclohexenone on *Tribolium confusum* mortality.

	Exposure time (hours)							
Treatment	1	2	3	6	12	24	48	96
	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD
Filter paper	0.0±0.00	0.0±0.00a	0.0±0.00a	0.0±0.00a	0.0±0.00a	0.0±0.00	0.0±0.00	0.0±0.00
Destilled water	0.0±0.00	0.0±0.00a	0.0±0.00a	0.0±0.00a	0.0±0.00a	0.0±0.00	0.0±0.00	0.0±0.00
Destilled water +Tween 20®	0.0±0.00	0.0±0.00a	0.0±0.00a	0.0±0.00a	0.0±0.00a	0.0±0.00	0.0±0.00	0.0±0.00
10 ppm	0.0±0.00	0.0±0.00a	0.0±0.00a	0.0±0.00a	0.0±0.00a	0.0±0.00	0.0±0.00	0.0±0.00
100 ppm	0.0±0.00	0.0±0.00a	0.0±0.00a	0.0±0.00a	0.0±0.00a	0.0±0.00	0.0±0.00	0.0±0.00
1000 ppm	0.0±0.00	0.5±0.58a	2.0±1.83b	6.8±1.26b	9.5±0.58b	10.0±0.00	10.0±0.00	10.0±0.00
10 000 ppm	0.0±0.00	0.5±0.58a	0.8±0.96ab	7.0±1.41b	10.0±0.00c	10.0±0.00	10.0±0.00	10.0±0.00
Pure	10.0±0.00	10.0±0.00b	10.0±0.00c	10.0±0.00c	10.0±0.00c	10.0±0.00	10.0±0.00	10.0±0.00
pirimiphos-methyl	10.0±0.00	10.0±0.00c	10.0±0.00c	10.0±0.00c	10.0±0.00c	10.0±0.00	10.0±0.00	10.0±0.00

Results represent the mean of four replications ± standard deviation.
Numbers with different letters are statistically different (p£0.05), according to Tukey test

the case of residual insecticides used as grain protectants (Vayias and Athanassiou, 2004). In future bioassays, control can be carried out only with water, as mortality was observed in none of the controls; on the other hand, the acute bioassays could be conducted for 96 hours exposure as no mortality and no abnormal behaviour was observed in controls.

In our work the probit analysis could not be performed and hence, the LC_{50} value could not be determined for the different exposure times, since mortality values were either extreme, or similar, for the tested concentrations. Although, we can assume that LC_{50} value relies between 1% (1000 ppm) and 0.01% (100 ppm) concentrations. Somehow these values could be comparable to those pointed out by Stamopoulos *et al.*, 2007) who studied the effect of other caraway monoterpenes on *T. confusum*. The LC_{50} intervals for limonene and linalool varied between 4 and 278 ppm, and between 8.6 and 183.5 ppm, respectively.

The studied monoterpene was already referred as a potential insecticide against other species (Carvalho and Fonseca, 2006; López *et al.*, 2008). In fact, several studies conducted on plant derived materials as extracts, essential oils, and metabolites, have demonstrated their effectiveness against several insect species with varying potencies (García *et al.*, 2007).

CONCLUSIONS

This study showed that this monoterpene, 2-methyl-5-(1-methylethenyl)-2-cyclohexenone, has insecticidal effect against *T. confusum*. Although, further studies should be conducted either in vitro to accurate the LC_{50} value, and in house storage conditions in order to confirm the viability of the monoterpene insecticidal effect. In fact, 2-methyl-5-(1-methylethenyl)-2-cyclohexenone could become an alternative to synthetic insecticides which could be an enormous advantage as higher plant volatiles could be less toxic to treatments of stored-products.

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