Romanian Journal for Plant Protection, Vol. IX, 2016
ISSN 2248 – 129X; ISSN-L 2248 – 129X

NEW FORMULATION OF ECOLOGICAL PRODUCTS USED FOR THE BIOLOGICAL CONTROL OF CEREALS WEEVIL, *Sitophilus granarius* L. (COLEOPTERA: CURCULIONIDAE) IN LABORATORY CONDITIONS

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Abstract: Treatments to protect the stored grains with synthetic chemical substances produce the accumulation of chemical residues in the stored product and may result in the occurrence of resistance of pests. The present study conducted by a research team from RDIPP and ICECHIM in 2016 was aimed at developing an ecological method to prevent and biological control of harmful deposits beetles, grain weevils being chosen target species. The project partner ICECHIM had conditioned three biological products (based on Thuja essential oil, Artemisia essential oil and diatomaceous earth) that were tested in three variants and 4 repetitions compared to an untreated control in laboratory conditions (temperature: 25±1°C, relative humidity: 64 ± 5). The insecticidal efficacy of the examined diatomaceous earth against the granary weevil was highly influenced by grain type, exposure time, products granulation and dose. The results showed that the tested products ensures a certain degree of infestation prevention and reduction of the species *S. granarius* population numerical density through the conditioning component of essential oils but with different concentrations of diatomaceous earth had an insecticide effect having a mortality rate with a meaning distinct semnificative value cpmpared with control variant in the case of the concentration of 900 ppm, combined with the two essential oils (3.1 ANOVA, Tukey's test, P> 0.001).

Key words: *Sitophilus granarius, biological control, essential oils, diatomaceous earth, stored products*

INTRODUCTION

Pests of stored products is a serious threat since before the time of harvest and consumption (Dunkel & Sears, 1998) and can produce about 10-30% of crop losses (Ferry et al., 2004). Management of insect pests of stored products is facing a crisis because of several serious drawbacks related to the use of insecticides such as, development of pest resistance to chemicals treatments, toxic waste and increased cost of application (Tapondjou et al., 2002). It is therefore necessary to develop alternative pest control techniques to protect the stored goods (Gunasekaran & Rajendran, 2005). To this end, intensified efforts have led to a growing number of research studies to find alternative safe, effective and viable method (Tapondjou et al., 2002). The essential oils of many plants present a broad spectrum of activity against insect pests and pathogens (fungi) from insecticidal effect, antifeedant, repellent, deterrence, lock oviposition and blocking the activity of growth. The use of carbon dioxide (CO_2) and essential oils from plants have received considerable attention for the monitoring of insect storage food pests, because their safety in relation to non-target organisms (AliNiazee, 1972; Bekele et al., 1996; Juliana et al., 1983; Sudesh et al., 1996; Bouda et al., 2001). Studies related to the response of insect species feeding in the face of certain emissions of volatile plant dates back a long time, with the development of two key disciplines, plant physiology and biochemistry. Phytophagous insects and complex interplay between host species is the result of a long and continuous evolutionary process. If we do not take into account individual cases of mutualism, it seems that any tendency toward parallel development should be limited to the development of defence mechanisms of the plant and to deal with these adjustments by insects. Through this simple vision, interrelations between plants and insects were divided into two directions: the choice of host species of insect or plant resistance against insects. These two classic directions essentially absolutizes each extreme of a highly complicated process, and although essentially proved wrong, allowed to define two essential processes on the physiology and biochemistry interrelation host plant - phytophagous insects. The first direction developed by Thorsteinson (1960) led to defining the mechanism of fagostimulant choice of host insect and the two helped to define by Painter (1951, 1958) the mechanism of plant resistance to insects. After a period of relative stagnation in studies with a more fundamental theory, recent research (Gerber et al., 2005; Turlings & Tone, 2006; Manole, 2008; Bruce et al., 2010) formulated a new concept that unifies two seemingly antagonistic processes that define chemo-olfactory communication system of individuals of both populations. This interest is reflected in numerous studies, interdisciplinary (behavioural, chemical, biochemical, physiological and genetic) whose joint effort enabled the understanding of the mechanism of selective pressure, the environmental consequences as ways of handling and control of important agent pests. Studies on the applicability of this new concept, as many attempts, implementing some experimental eco-chemical research led to a level of development that will allow in the near future settlement innovative food protection of deposits.

As a principle of action known four ways to use green products, non-toxic to non-target organisms (mammals and humans): the use of organic insecticides; the use of pheromonal and hormonal products; products with deterrent effect, repellent and inhibitor of vital functions (feeding, breeding, etc.); the use of attractive product (semio-chemicals emitted by plants).

Using essential oils, "essential" or essences obtained by steam distillation of herbs has a long history of human use as flavouring agents in foods and beverages as in the perfume industry or as agents for aromatherapy. Recently, their use in ecological management of some species of insect pests, in homes and commercial spaces, open up new avenues of use.

Apart from this advantage, commercially in the US, herbal essential oils have other properties that make them suitable for use in insect management. This includes:

1). The widespread use of world production and trade in essential oils from plants for perfume and flavour industry maintain low prices for several of the essential oils;

2). Many of the oils tested on insects so far have multiple modes of action and sites of action in the insect nervous system or other vital organs (Enan, 2001, 2005a, b; Kostyukovsky et al., 2002; Priestley et al., 2003). They can represent a wide range of effects, namely irritability to contact deterrence, repellents, behaviour change through action sublethal, paralysis, etc.;

3). With few exceptions, the essential oils and their major constituents are relatively non-toxic to mammals and humans, with acute oral LD_{50} value in rodents ranging from 800 to 3000mg kg-1 for the pure compounds and 5,000mg kg-1 for formulated products;

4). Because of their volatility, oils and their constituents are organic, non-persistent, with an average duration of life outdoors for less than 24 hours on surfaces, in soil and water.

Based on these attributes and exemption from federal approval of US regulators, the company EcoSmart Technologies has introduced since 1998, a range of agricultural pesticide consumption and professional, based on oils from leaves of cloves *Syzygium aromaticum* L. (Merrill & Perry) (Myrtaceae), rosemary *Rosmarinus officinalis* L. (Lamiaceae), peppermint

Mentha piperita L. (Lamiaceae), cinnamon *Cinnamomum zeylandicum* Blume (Lauraceae), lemongrass *Cymbopogon nardus* (L.) Rendle, (Poaceae) and thyme *Thymus vulgaris* L. (Lamiaceae) (Table 1).

Table 1. Botanical insecticide used in SUA, 2007

Product	Used dose (Kg)	Target field
D-limonene	31,340	89% in pest control
Pyretrum	7,790	51% public health, 24% pest control
Rotenona	3,350	98% in fishery
Azadirachtin (neem)	1,010	Agricultural use, pest control
Sabadilla	95	Agricultural use, pest control

The chemistry of essential oils from plants are characterized often by complex mixtures (50) or sesquiterpene constituents like monoterpene, alcohols, phenols, aldehydes and ketones. Some of the most important commercial sources of essential oils (i.e. *Th. vulgaris, R. officinalis* and Lavendula spp.) are the chemically as distinct races, ecotypes or chemotypes (Lahlou & Berrada, 2003; Kaloustian et al., 2005).

The granary weevil, *Sitophilus granarius,* is a typical cosmopolitan pest (Keszthelyi & Pál-Fám, 2012). Damaged grain has reduced nutritional value, low percentage germination, reduced weight and lowered market value. Environmentally safe, cheap and effective methods for reducing *S. granarius* damage are needed to protect stored grains (Keszthelyi & Pál-Fám, 2012). It can cause significant damage to stored grains, and may drastically decrease yields (Fava & Burlando, 1995). A female lays an average of 125 eggs (Jávor, 1990) and the larvae eat the inside of grain kernels (Stejskal & Kucerova, 1996). Residual insecticides (such as deltamethrine, permethrine, malathion, fenitrothion, pirimiphos-methyl, clorpiriphos-methyl) are the most commonly used protectants in stored grain against stored-product pests in many countries of Europe. They are applied directly to the product and provide protection as long as the insecticidal effect persists. Unfortunately, these pesticides have negative side-effects. They can be toxic to mammals, they can accumulate in the treated products and, unfortunately, pest species could become resistant to the protectants (Arthur, 1996). During recent years, inert dusts have received increased attention as grain protectants, and are considered among the most promising alternatives to residual insecticides (Golob, 1997; Korunic, 1998; Subramanyam & Roesli, 2000). One of these is diatomaceous earth (DE), which consists of the fossilised remains of diatoms (unicellular algae which occurred during the Eocene and Miocene). Although several modes of action have been proposed, it is generally accepted that, as the DE are attached to the bodies of the insects, the silica particles damage the cuticle, and the insects die through water loss and desiccation (Fields & Korunic, 2000; Subramanyam & Roesli, 2000; Mewis & Ulrichs, 2001). In additional, the wounds provide access to insect pathogens. Since the mode of action is physical, it is postulated that physiological resistance will not occur (Korunic, 1998). The insecticidal effect of diatomaceous earth has already been demonstrated (Erb-Brinkmann, 2000; Athanassiou et al., 2003; Vayias & Athanassiou, 2004; Athanassiou et al., 2005; Demissie et al., 2008). However, the insecticidal efficacy of more DE formulations (e.g. Dryacide, Kenite 2-I, PermaGuard, SilicoSec) has already sufficiently ascertained, unfortunately, such experimental data are not yet available concerning of experimental combination of DE formulation including volatile essential oils. Therefore, the aims of our study were, to obtain information about the efficacy of the DE and essential oils formulation against the granary weevil, and to evaluate the influence of the dose rate of the DE formulation on weevil mortality and progeny production.

The present study is a preliminary research based on the concept of the behaviour of the insect *Sitophilus granarius* to emissions of volatile plants, combined with the use of a natural insecticide toxic to humans and animals, namely mineral diatomaceous earth.

MATERIALS AND METHODS

The experiments were carried out in the entomology laboratory from Research Development Institute for Plant Protection Bucharest (RDIPP) in 2016. The insect populations of *S. granarius* used were obtained from mass rearing in the RDIPP facility at 26±2°C, 65% RH and 16:8 light photophase.

The grains used for experiments consist in untreated, clean wheat kernels free from infestation by bacteria, fungi or pests. The ecological products were formulated at ICECHIM Bucharest from a combination mixture of some essential oils with a dose of diatomaceous earth in 3 granulation size: 1mm, 1.6mm and 2mm, respectively. The uniform distribution of products in the grains was secured by 5 minute of thorough mixing. For each dose and size, which meaning a variant, 3 replications of 50g wheat kernels were treated. Each of replication was placed in a small glass vial, 50 healthy *S. granarius* adults of mixed sex were added, after which, the vials were placed in laboratory at 25±1°C and 64%±5RH. Dead adults were counted after 7, 14, 21 days. After the 21 day count, all adult insects (dead and alive) were removed from the vials, after which the vials returned to the laboratory for another 60 days. After this period, the emerged *S. granarius* adults were counted, classified as dead or alive and removed from the vials. Mortality counts were corrected by using Abbott's (1925) formula. The data were analysed by using one-way ANOVA software, with weevil mortality as the response variable and exposure time and dose rate as the main effects. Means were separated by using the Tukey (HSD) test, at $P \leq 0.05$. Insect mortality was compared with Student t-values, using Microsoft Office Excel 2007.

RESULTS AND DISCUSSIONS

The experimental model designed at RDIPP Bucharest in the project PEDIOL is the final step in testing combined formulations of diatomaceous earth with essential oils in certain concentrations. The insecticidal effectiveness of these products has been strongly influenced by the rate of concentration, time of exposure, temperature and the type of formulation, both in terms of particle size and type of essential oil as well. The results obtained are shown in the Table 2 and 3, and are expressed graphically in Figure 1, 2 and 3.

Table 2. Ecological products tested against species *Sitophilus granarius* in laboratory conditions

Variant	Ecological product	Significance compared with control check	
V_1	Thuja essential oil + diatomaceous earth	7 days	*
		14 days	**
		21 days	***
V_2	Artemisia essential oil + diatomaceous earth	7 days	**
		14 days	**
		21 days	**
V_3	Thyme essential oil + diatomaceous earth	7 days	**
		14 days	**
		21 days	***
Control	30 adults of *S. granarius* + 50 g wheat kernels	-	

Table 3. Results obtained on population of *Sitophilus granarius* L control in laboratory conditions using ecological products based on diatomaceous earth

Experimental variant	**Diatomaceous earth dose**	**Essential oil dose**	**Mortality (%)**	**Anova analysis P<0.01**
Diatomaceous earth + linalool	300 ppm	10 µl	96.66	**
Diatomaceous earth + eugenol	500 ppm	15 µl	83.33	ns
Diatomaceous earth + tymol	900 ppm	20 µl	97.24	***
Control	-	-	2.14	-

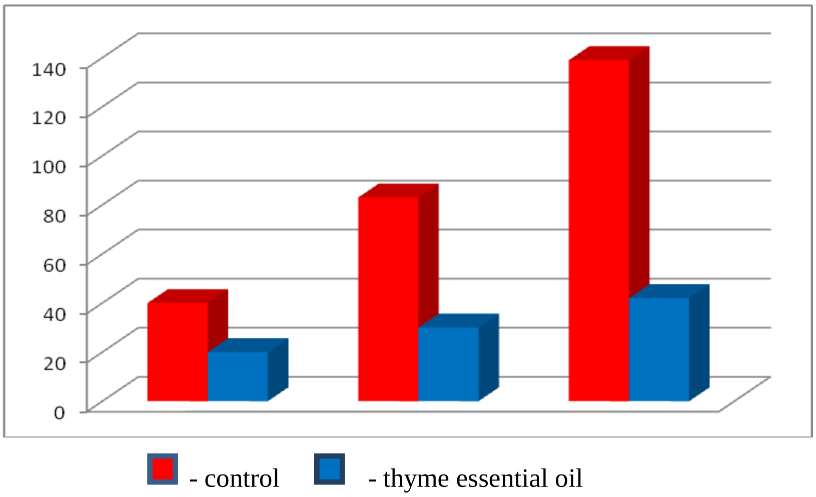

Figure 1. Inhibition of *Sitophilus granarius* oviposition due to wheat seed treatment with essential oil of thyme

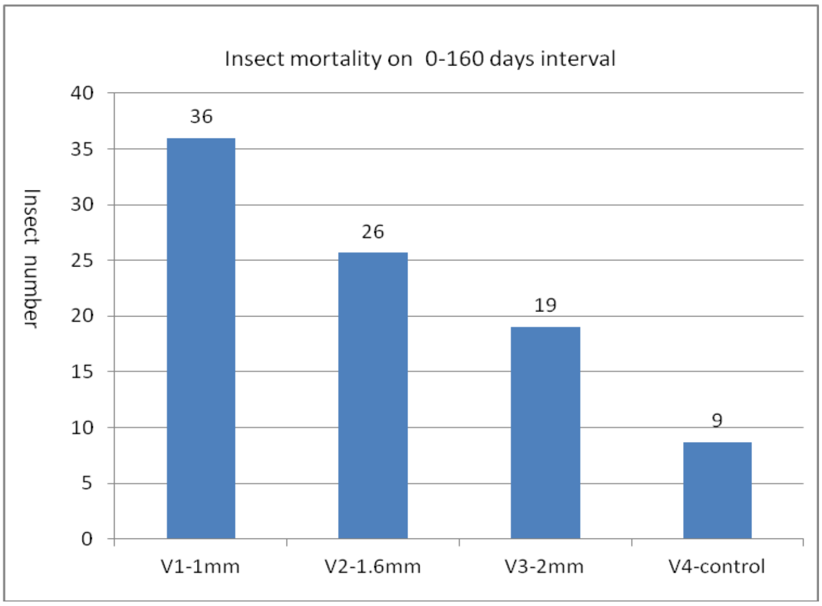

Figure 2. Mortality (total number) of adults of *Sitophilus granarius* including descendents from the next generation at 60 days after treatments

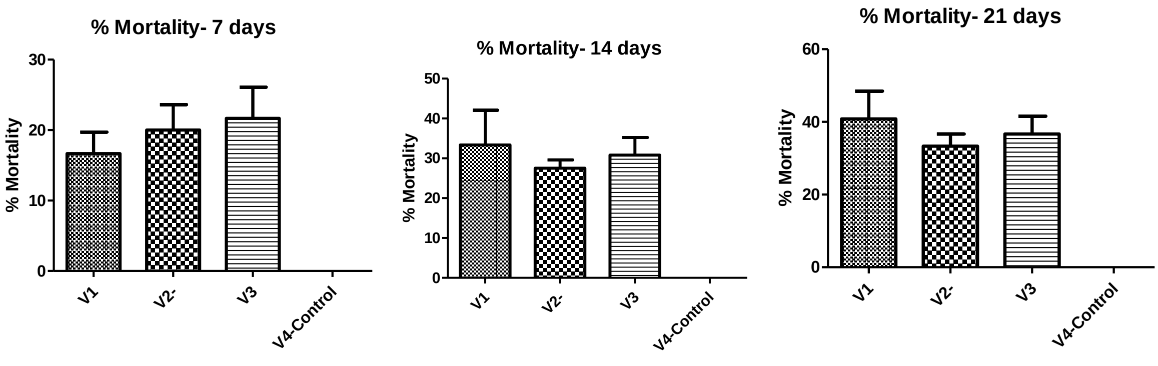

Figure 3. Insect mortality (%) after treatments with ecological products in laboratory conditions

In the case of the product formulated based on Thuja essential oil + diatomaceous earth, a significant mortality occurs after first 7 days following administration, but the maximum effect occurs at 21 days, with statistically assurance. The mixture of Artemisia essential oil and diatomaceous earth shows the percentage of mortality increased to a level significantly distinct but this percentage was maintained after 21 days while the product in the essential oil of thyme + diatomaceous earth was a value highly significant at 21 days from the administration (Fig. 3). Similar results were obtained by previous researches in the literature (Korun, 1997, 2006; Arthur et al., 2001; Arnaud et al., 2005; Asked et al., 2005; Vayias et al., 2006; Kljajis et al., 2006; Koul et al., 2008; Shams et al., 2011; Shakarami, 2013).

In table 3, the results synthesis tests with ecological products based on diatomaceous earth and the insect *S. granarius*, the mortality significance of differences compared to the untreated control were presented. The conclusion was that the diatomaceous earth product + tymol, at a dose of 20ml and the concentration of diatomaceous earth of 900ppm after 21 days recorded a value of 97.24% mortality, very significant compared to the control which is recommended that wording to protect grain in storage.

In the literature (Isman, 2000) the influence of essential oils on other stages of the development of insect pests in storage is less studied. In the study, we tested the action of thyme oil on the egg stage, but we found a strong influence against oviposition process on the female of volatile oil. It is found that, compared to an untreated control at 72 hours after application, reduces the rate of laying eggs in a significant percentage of almost 50% (Fig. 1). The phenomena of blocking the oviposition process is a new aspect of the volatile oils influence of insect behaviour and would represent a new way of research searching for new alternative methods for pest control in storage food deposit.

CONCLUSIONS

The ecological effect of diatomaceous earth combined with some volatile essential oils was tested against pest population of *Sitophilus granarius* in the laboratory conditions, like a new alternative method of pest control to chemical treatments.

The essential oils obtained from *Thymus vulgaris* showed an ecological effect of blocking the oviposition process of *S. granarius* female which is one new aspect of the influence of such chemical products on the behaviour of this species.

The results obtained from the experiments showed that the product of diatomaceous earth + thyme oil had an insecticidal effect on the adults of *S. granarius*, reaching a value of 97.24% mortality after 21 days from administration.

ACKNOWLEDGEMENT

This research work has been performed within Programme Partnerships in Prioritary Areas, Project PN-II-PT-PCCA-2013-4-0425.

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