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PRELIMINARY BIOASSAY OF BIOLOGICAL EFFECTS OF OILS FROM OREGANO AND CLOVES ON CALLOSOBRUCHUS MACULATUS

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Abstract:-

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Callosobruchus maculatus (Coleoptera: Bruchidae), this beetle infests mainly Vigna unguiculata. There are several strategies to control of CW, by synthetic pesticides and physical means. The chemical applications are cheaper and easier to be applied but cause obvious consequences, which lead to a number of problems, The Essential oils (EOs) are widely applied in the traditional practices and in organic plant protection as insecticides repellents and antifeedant compounds. This study was carried out on 3 bioassay. The bioassay 1, carried out on artificial seeds, showed that the oily solutions could affect the oviposition. The average number of eggs laid by CW,was the highest on the oregano oil treatment at the dose of 2.0% followed by the control and the oregano oil treatment at the dose of 0.5%. The clove oil treatments induced the lowest egg laying. The bioassay 2, carried out on chickpea seeds in a completely randomized pattern, demonstrated that the average number of eggs laid on the chickpeas , was the highest on the control treatment followed by the oregano oil treatments at the doses of 0.5% and of 2.0%. The clove oil treatments induced the lowest egg- laying. The bioassay 3 treating directly the chickpea seeds. The average number of eggs laid on the chickpeas by the females within their life span was the highest on the oregano oil treatment at the dose of 2.0% followed by the oregano oil treatment at the dose of 0.5% and control. The clove oil treatments induced the lowest egg laying.

Keywords: - *Cowpea Weevil (CW), chickpea seeds (Cicer arietinum), Oregano oil, Cloves oil (EOs)* **.**

INTRODUCTION

Callosobruchus maculatus (F.) (Coleoptera: Bruchidae), known as the Cowpea Weevil (CW),. This beetle infests mainly *Vigna unguiculata* (L.), this beetle species invades pulses and causes heavy economic losses which are lower in the field and larger in stores (Southgate et al., 1979). Male of cowpea beetles are easily distinguished from and female by their general appearance. The most distinguishing characteristic is the coloration on the plate covering the end of the abdomen. The plate is enlarged and is darkly colored on both sides in the females. The plate is smaller and lacks stripes in the males. Generally, females are larger in size than males, but there is much variation. (Beck et al., 2006; Ricci, 2006). Apart the control strategy applied in the field (genetic resistance and chemical control) or before the storage (solar radiation in polyethylene bags), the control of CW in storehouses is made mainly by synthetic pesticides and physical means. The application of the atmosphere at very low concentration of oxygen (<1%) is an efficient control method (100% of mortality for adults, eggs, larvae and pupae) (Storey, 1978). More commonly, dried heat at 57°C applied for about 1 hour in storehouse causes the devitalization of all life stages (Zewar, 1993). Similarly, all life stages are killed by temperature of about 0°C (Yus Ramos, 1976). Also microwave effects are well documented (Watters, 1976; D'Ambrosio, 1982; Locatelli&Traversa, 1989; Zaied et al., 2002). The chemical applications cause obvious consequences which lead to a number of problems, including genetic resistance by the insects, failures in seed germinability, pest resurgence, residual toxicity, human hazards and increasing costs of applications (Rahman &Talukder, 2006).A certain literature is available on the applications of the bio-pesticides extracted by plants which have antifeedant, repellent or insecticide activity (Boeke et al., 2001) also against CW. The use of oils to protect grains from damage caused by storage insects is a traditional practice in certain countries. It should be observed that these chemicals do not modify the organoleptic, physical and cooking properties of the seeds (Van huis, 1991). However, the application parameters (doses, method of supply, effect duration, etc.) as well as the efficiency of these bio-pesticides in storehouses are still under investigation.Essential oils (EOs) have gained a great research and practical interest because of their potential use in food preservatives, pharmaceuticals and cosmetics. Such substances derive from aromatic plants and exhibite various antibacterial (Moghimi et al., 2016), antifungal (Basak and Guha, 2015) and antioxidant biological activities (Scopel et al., 2014). EOs are widely applied in the traditional practices and in organic plant protection as insecticides (Kumar et al., 2011), repellents (Zhang et al., 2011; You et al., 2015) and antifeedant compounds (Baskar and Ignacimuthu, 2012, Julio et al., 2015). These biological activities are determined mainly by two or three major components at high concentrations (20–70%) compared to other 20-60 components present in trace amounts (Pavela, 2015).The use of 262 EOs has been the new alternative for the synthetic pesticides in plant protection since they are volatile, easy to decompose and are consisting of terpenes, phenolics and alcohols (Li et al., 2015).Considering the need of increasing knowledge on the bio-pesticide applications, the current study is aimed at evaluating the efficiency of two types of oils (oregano and cloves) applied in no-choice and multi-choice bioassays at two doses against CW on chickpeas (*Cicer arietinum*) L. or artificial seeds

Material and methods

Culturing of Insect Species

A population of *C. maculatus* was reared in laboratory on commercial chickpeas ("ceciGiganti" produced in Molise, by Select). The rearing was synchronized in order to avoid the simultaneous presence of all stages of the beetle. Usually, air-ventilated plastic containers of about 50 ml were used. They contained about 100 chickpeas previously sterilised in a freezer at 18ºC for 24 hours and warmed in a desiccator. About 30 adults of *C. maculatus* were passed from old seeds to new seeds for a maximum of 4 containers contemporary used.

Extraction of essential oils

The clove essential oil was provided by Elcaptain Company (Cairo, Egypt), while the oregano oil was obtained from 20g were submitted to water distillation in a Clevenger typeapparatus with a flask of 500 mL for 4 h, following the standards of the European Pharmacopeia (Council of Europe, 2007).

Bioassays

Two different doses for each plant extract were applied: 0.1 (0.5%) and 0.4 (2.0%) ml in 20 ml of distilled water at which 20 ml of Tween 20 were added for favoring the dispersion of the oily plant extracts. Control treatment was made by a water solution of 20 ml of Tween 20. Therefore, a total of six treatments were set per each bioassay and each bioassay was replicated 3 times. All the bioassays on the chickpeas were carried out on a commercial product "ceciGiganti" produced in Molise (by Select) previously frozen for 24 hours and left in desiccator.

1 Indirect effects on the oviposition on artificial seeds (Multi choice bioassay)

Plant extracts were assayed for their ability in stimulating/deterring egg laying in *C. maculatus*. Metallic beads (8.5mm diameter) were used as egg laying substrate and are termed artificial seeds hereafter. Group of artificial seeds were coated immerging them into a solution of the plant extracts at the doses above mentioned and immerging them in the control solution. The seeds were washed in the solutions for 2 minutes, then, were dried in a desiccator overnight.Twenty artificial seeds per each treatment were used and placed into 263 The of a 100-well plate in a completely randomized pattern. The plates of this assay were made in polystyrol; before each assay they were washed with Et-OH 60% (for about 30 minutes) and, then, left to dry in a desiccator. The assay was carried out keeping the plate in a container which was covered by a plastic film in order to avoid the escaping of the insects.

Five females and five males, 24-hours old, were released into each plate and could move freely among the wells and the artificial seeds contained in them. This microcosm was kept in an incubator set at 27±1°C, 70±5% RH in completely darkness. The number of eggs laid on each of the artificial seed was recorded after 7 days.

2 Indirect effects on the oviposition on seeds (Multi choice bioassay)
Following the same procedures and purpose of bioassay 1, this bioassay was performed on commercial chickpea seeds.

3 Direct effects on the oviposition (No choice assay)
The test was performed using PVC cylindrical containers (5 cm diameter, 8 cm depth for 0.1 L) with non-woven fabric lids. Each container was filled with 30 chickpea seeds previously treated with the plant extracts (the same concentration used in the previous bioassays). The seeds were washed in the solutions for 2 minutes, then, were dried in a desiccator overnight.
Three females and three males of *C. maculatus* (all emerged within the last 24 hours) were released in each container. The microcosms were kept in the dark at 27C±1°C, 70±5% RH and the adults were removed after the death of all of them for counting the eggs laid on the seeds.

Results

1 Indirect effects on the oviposition on artificial seeds (Multi choice bioassay)
The average number of eggs laid on the artificial seed after 7 days of the bioassay was the highest on the oregano treatment at the dose of 2.0% followed by the control treatment and the oregano treatment at the dose of 0.5% (Tab. 1). The clove treatments induced the lowest egg-laying. Statistical differences were found between the means of the treatments with oregano 2.0% and clove 0.5%.

Table 1 – Oviposition rate (± standard error of the averages) of *Callosobruchus maculatus* on treated artificial seeds (multi choice bioassay 1; 3 replicates of 20 seeds each per treatment), on treated chickpea seeds (multi choice bioassay 2; 3 replicates of 20 seeds each per treatment), on treated chickpeas (no choice bioassay 3; 3 replicates of 30 seeds each per treatment).

	Bioassay 1	Bioassay 2	Bioassay 3
Treatment	n. eggs/ female±SE	n. eggs/ female±SE	n. eggs/ female±SE
untreated	1.17±0.52 ab	1.70±0.42 c	3.66±0.27 ab
Oregano 0.5%	1.13±0.95 ab	0.93±0.31 b	3.94±0.28 a
Oregano 2.0%	1.83±0.96 b	0.68±0.42 ab	5.94±0.37 d
Clove 0.5%	0.33±0.41 a	0.55±0.26 ab	3.11±0.22 bc
Clove 2.0%	0.78±0.78 ab	0.38±0.19 a	2.51±0.24 c

Means within columns followed by different letters are significantly different (Duncan's test: $P<0.05$)

2 Indirect effects on the oviposition on seeds (Multi choice bioassay)
The average number of eggs laid on the chickpeas after 7 days of the bioassay was the highest on the control treatment followed by the oregano treatment at the doses of 0.5% and of 2.0% (Tab. 1). The clove treatments induced the lowest egg-laying. Significant differences were found among the averages clove at 2.0%, oregano at 0.5% and untreated. A dose dependent effect could be observed, as expected.

3 Direct effects on the oviposition (No choice assay)
The average number of eggs laid on the chickpeas at the death of all adults was the highest on the oregano treatment at the dose of 2.0% followed by the oregano treatment at the dose of 0.5% and control treatment (Tab. 1). The clove treatments induced the lowest egg-laying. Statistical differences were found among the averages of the different treatments.
The higher rate of egg-laying per female recorded for this bioassay for almost all treatments is dependent on the longer period of this bioassay (up to the death of the adults) in respect to the previous one (1 week).

Discussion

The implemented bioassays showed results that apparently are not related each to the other. These preliminary bioassays could have been affected by some practical problems. Bioassay 1 was performed on artificial seeds consisting in iron balls with a smooth and glossy surface. Even though the balls were carefully mixed with the plant extract mixture, the oily nature of the extracts could have made difficult their adhesion on the ball surface, even though a surfactant was used, and this difficulty could have been more relevant at the highest concentrations of the plant extracts due to the oil drop coalescence. This could explain why the rate of oviposition increased with the concentration of the plant extracts. Therefore, the results of this bioassay may be uncertain. Considering the complete randomization of the seeds and the multi choice approach, the number of females cannot have had negative influences on the results.

Bioassay 2 was performed on chickpeas provided with an irregular surface and the seed coat should have taken plant extracts. Also in this case, the multi choice approach could have avoided the effects of a variable number of females. Therefore, the results of this bioassay can be considered reliable. Whereas in bioassay 3 we used to treat the chickpea seeds. In both cases the distribution of the plant extracts is not affected by the mode of treatment and the particularity of the treated surfaces. On the contrary, the no-choice approach could have been affected by the introduction of a variable number of females into the containers of the replications and treatments. The sex distinction is not always easy for this beetle species and the irregularity of the data obtained during these two bioassays induces some doubts about the results.

Conclusion

The bioassays carried out during the current experiment have shown a potential role applied by the plant extracts from clove and oregano. Oregano extracts seem to be more repellent than clove extracts whereas clove extracts seem to reduce the egg-laying. However, both extracts were not able to contrast completely the bruchid beetle.

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