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Effects of two plant oil-based products on the mortality of red palm weevil and morpho-physiological characteristics of palm

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Abstract

The red palm weevil (RPW), *Rhynchophorous ferrugineus* Oliver (Coleoptera: Curculionidae), is an economically important, tissue-boring pest of date palm in many parts of the world. The aim of the work was to investigate the effects of two plant oil-based products (BRK6 and BRK9) on the mortality of RPW (Exp. 1) and morpho-physiological characteristics of palm (Exp. 2). Laboratory experiments showed that the two mixture of oils BRK6 and BRK9 showed larvicide and adulticide properties. These properties were more pronounced on BRK9 than BRK6. The lethal concentration for 50% of individuals (LC_{50}) calculated by probit, of BRK6 was 0.22% (for the 90% of individuals LC_{90} = 0.34) while for BRK9 the calculated LC_{50} was 0.10% (LC_{90} = 0.66). These data show that a smaller amount of BRK9 oil was required to induce the 50% of mortality of RPW larvae. As regards to the adults, BRK9 and BRK6 were effective only at the highest concentration (2 and 5%, respectively). Finally, injection of BRK9 into the palm trunk showed to promote chlorophyll content and photosynthesis of leaves thanks to a good translocation of zinc.

Key words: Biological pest control, *Rhynchophorous ferrugineus*, larvae, endotherapy, chlorophyll, photosynthesis.

Introduction

The red palm weevil (RPW), *Rhynchophorous ferrugineus* Oliver (Coleoptera: Curculionidae), is an economically important, tissue-boring pest of date palm in many parts of the world. The insect is native to South-East Asia and was first described as a serious pest in India by Lefroy [1] on coconut palm and later on date palm [2, 3]. The insect is a major pest of date palm in most of the Arabian Gulf States [4, 5]. During the last two decades, efforts to control RPW in the region have focused primarily on the use of modified cultural practices, biological agents, conventional insecticides and pheromone traps [4]. However, an effective and economic method has yet to be devised for controlling this pest. Since this pest was introduced accidentally in Italy (2004-2005) the insect is creating damage to the "palm heritage" of the Mediterranean, for both biotic reasons such as its life cycle and its biology and abiotic, related to lack of preparation, poor management and underestimation of the problem. RPW is a concealed tissue borer and can spend all of its life stages inside the palm tree.

The insect has a gregarious behavior that determines the survival of the species to adverse environmental conditions: the concentration of many larvae in the trunk of a palm tree determines an increase in internal temperature due to fermentation. For this reason, adults and larvae can survive inside of an attached palm trunk even if the outside temperature is not conducive to the plant.

In Italy, this insect shows a clear preference for the species *Phoenix canariensis*, widespread in Italy for ornamental purposes, but it can attack all other palm species, including endemic to the Mediterranean dwarf fan palm (*Chamaerops humilis*). According to Abraham *et al*. [4], damage on date palms can be recognized by the presence of several symptoms such as tunnels on the trunk, presence of a fermented odor from the fluid inside infested tunnels in the trunk, presence of adults and cocoons in the leaf axils, breaking of the trunk or toppling of the crown when the palm is severely infested.

The life cycle of RPW in the Mediterranean region can reach 300 days. RPW female can lay up to 350 eggs during its life whose fertility is around 75%. On the palm P. *canariensis*, oviposition occurs mainly on the apical shoots.

One of the main problems is the difficulty to visually monitor the symptoms of infestation in the early stages. Since the larva is a miner of the apical bud and the jamb, the macroscopic effects can be seen only in late stages of infestation, just before the death of the plant.

One of the most interesting solutions for the control of RPW infestations is represented by the endotherapy, a technique based on injections of various formulations of biologically active substances which, on entering into the circulation by means of special nozzles connected with pressure pumps, allow a diffusion of the active ingredients within the treated plant. Moreover, endotherapy technique permits to inject in the palm vessel substances that can stimulate plant defense mechanisms, promote plant growth and/or nutritional status. The aim of the work was to investigate the effects of two plant oil-based products on the mortality of red palm weevil (Exp. 1) and morpho-physiological characteristics of palm (Exp. 2).

Materials and Methods

The experiments were conducted at the Tuscia University, Viterbo (Italy) and Green World Consulting Laboratory, Lanuvio (Italy). RPW adults used in Experiment 1 were obtained from field traps and maintained in the laboratory. The maintenance of the adults was carried out using plastic containers of various dimensions on the lid of which have been practiced various holes of about 6 mm in diameter to allow the ventilation of the internal environment. Inside the container was placed a mixture of coconut fiber and fine-grained vermiculite to absorb excess of moisture. Rearing was supplied with apples for adults diet and as a substrate for oviposition. After about 72 hours the apples have been moved to another container prepared in the same manner to allow the development of larvae. The larvae of weight greater than 3 g were used in toxicity tests larvae. For toxicity testing on adults were used individuals collected in the field. Rearing was maintained at constant condition of temperature (T = 29±3°C) and humidity (RH = 60±5%).

The plant oil-based products tested were provided by the Green World Company, Lanuvio (Roma, Italy) and have been identified by the abbreviations Biorynk 6 (BRK6) and Biorynk 9 (BRK9). BRK6 product contains a mixture of 6 essential plant oils (mainly *Cinnamomum camphora*, *Lantana* spp., *Rosmarinus* spp., etc.) while BRK9 was composed by 9 essential plant oils (mainly *Cinnamomum camphora*, *Lantana* spp., *Rosmarinus* spp., *Citrus × bergamia*, *Ocimum basilicum*, etc.) plus 5% of Zn as zinc sulphate. Both products contained 33% of essential oils. These products were diluted in distilled water for the preparation of the stock solutions. Each stock solution was then diluted in a serial manner (ratio 1/5) to obtain solutions at different concentrations to be incorporated in the artificial diet and used in the experimentation.

In the experiments, the oil BRK6 was used at the concentrations 5%, 1% and 0.2%; moreover the test solutions of the oil BRK9 were 2%, 0.4% and 0.08%. The test mixtures were obtained adding the oil solutions of BRK6 and BRK9 in the food artificially prepared in the laboratory in proportion 1/4 w/w (oil solution/artificial food). The artificial food (1 litre) was prepared by mixing tap water (820 ml), sugar (15.9 g) and agar (10 g). The obtained solution was autoclaved at 120°C for 20 min, then the temperature of the solution was lowered to 40°C before adding of cellulose powder (150 g), methyl p-hydroxyl benzoate (1.58 g), and honey (100 g). This mixture was blended with a mixer to obtain a homogeneous suspension.

In Experiment 1, 40 ml of the mixture containing the oil based products were allowed to solidify in the cylindrical containers of 250 ml with screw cap in which they have been practiced 3 holes with a diameter of about 6 mm. Larvae weight greater than 3 g were chosen randomly from the rearing cage and placed inside the containers with the artificial food. The containers thus prepared were kept under constant conditions of temperature and relative humidity (T = 29±3°C; RH = 60±5%).

The oil concentrations in the artificial diet of the different treatments and the general plan of the experiment are summarized in Table 1. As shown in Table 1, two control groups were included. After 72 hours of treatment, the containers were opened and the number of dead larvae in the different treatments counted. Ten replications for each treatments were carried out for statistical analysis of experimental data.

Table 1. Treatments tested in Experiment 1 evaluating the toxicity of BRK6 and BRK9 on large RPW larvae.

Treatment	Product concentration (%)	Diet	
		Food	Oils
Control +	0.00	Yes	No
Control -	0.00	No	No
BRK6-B	1.00	Yes	Yes
BRK6-D	0.20	Yes	Yes
BRK6-E	0.04	Yes	Yes
BRK9-A	0.40	Yes	Yes
BRK9-B	0.08	Yes	Yes
BRK9-C	0.02	Yes	Yes

In the first control called Control +, larvae were fed only with artificial food without oil based products, in the second type of control (Control -), larvae were not fed.

In Experiment 1 with adults, only BRK9 was tested at the same concentrations described for larvae (Table 1). The adults were selected randomly from the rearing set up in laboratory regardless the sex of the individual being investigated. Selected adults were placed individually in containers of 0.5 litre capacity with perforated lids. The artificial food added with the oil BRK9 at various concentrations was offered to individuals of each treatment. Food was supplied in a Petri dish of 5 cm in diameter containing 10 ml of the mixture described previously. The treatments were thus the same as described in the previous paragraph (BRK9-A, BRK9-B, BRK9-C). Two controls have been set up too. In the first control, called C +, adults received only the artificial food with no BRK9; in the second type of control (C-) individuals did not receive any kind of food. After 72 hours of treatment the mortality of the adults in the various treatments was recorded. Ten replications per treatment were carried out statistical analysis of experimental data.

In Experiment 2, ten potted palms (*Phoenix canariensis* Chabaud) of 2-m height were treated by endotherapic injection method [6] with a water solution containing 5% of BRK9. Ten potted palms treated with water were used as control. The injection was done at the base of stem in 4 holes on 14 May 2014. After 2 months, chlorophyll content of leaves was measured with a SPAD instrument (10 measurements per plant). The Photosystem II (PSII) efficiency of leaves, estimated by the variable to maximum chlorophyll fluorescence ratio (Fv/Fm) was measured by chlorophyll fluorometer Handy PEA (Hansatech Instruments Ltd, UK) as described by Colla *et al.* [7]. Mineral analysis of zinc were also made on leaves, previously dried in a ventilated oven at 60°C, using an inductively coupled plasma emission spectrophotometer (ICP Iris; Thermo Optek, Milano, Italy) and the methodology reported by Karla [8].

Data analysis and determination of LC_{50} was accomplished using the Probit analysis of SPSS 15.0 for Windows.

Results and Discussion

Larval toxicity: Fig. 1 shows the mortality of large larvae (weight > 3 g) treated with BRK6 and BRK9. The mortality was presented as the ratio between the total dead larvae and larvae subjected to testing for each treatment group (TG) after 72 h of treatment.

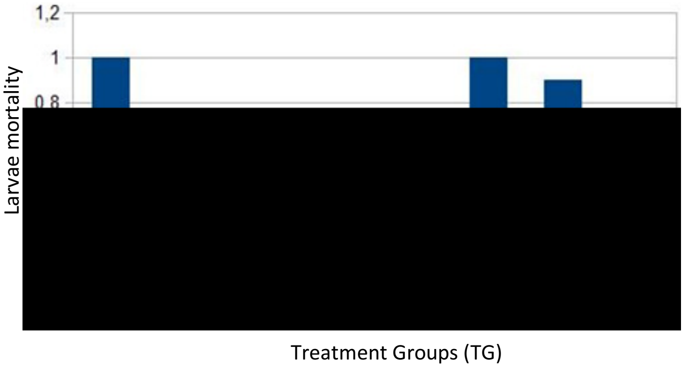

Figure 1. Mortality of the larvae after 72 h of treatment in the different treatment groups (TG). Mortality was expressed as number of dead larvae on number of total larvae.

No mortality has been observed in the two control groups while treatment with BRK6 and BRK9 oils induced various degree of mortality (Fig. 1). This demonstrated that, in order to induce toxic effects leading to death, it is necessary that larvae eat the medium or being in contact with it. Indeed, this kind of test was not able to discriminate between effects induced by feeding and effects induced by contact with the oils. Furthermore, the data showed that the toxicity induced by BRK9 was very high even at the lowest concentration in the range chosen (BRK9-C) that was diluted 25 fold than BRK9-A. No mortality has been observed in the group BRK6-C.

The statistical analysis supported these findings. The one-way ANOVA test demonstrated that there was a highly significant difference among control and treatment groups for both the oil based products (for BRK6 $F = 22.33$; $p<0.01$; for BRK9 $F = 40.66$; $p<0.01$). As regards BRK6 mixture, the Least Significant Difference (LSD) test showed that the mean difference was significant at the $\alpha=0.05$ level between the control group and the BRK6-B (1%) and BRK6-D (0.2%) treatment groups, while no significant difference were detected between the control and the BRK6-E group. LSD test applied to the BRK9 oil showed that there was a significant difference between the control and all the other treatment groups and there was not a significant difference between the two groups treated with the higher concentration (BRK9-A and BRK9-B).

Fig. 2 clearly showed the dose dependent features of both BRK6, and BRK9 products: the mortality rate of larvae after 72 h increased with the increment of the dose of oils in the diet. Exponential trend lines and their equations, displayed in Fig. 2A and 2B, support this observation and also showed that the treatment with BRK9 is more effective than BRK6 since showed a lesser reduction of potency notwithstanding the dilutions of the oil.

The Probit analysis confirmed the conclusion that the mixture of oils BRK9 was more effective than BRK6. The lethal concentration for 50% of individuals (LC_{50}) calculated by probit, of BRK6 was 0.22% (for the 90% of individuals $LC_{90} = 0.34$). For BRK9 the calculated LC_{50} was 0.10% ($LC_{90} = 0.66$). These data show that a smaller amount of BRK9 oil was required to induce the 50% of mortality of RPW larvae.

Adult toxicity: Adults showed similar effects with a partial paralysis after few hours from the beginning of treatment. Adults were completely dead after 24 h especially using BRK9 at 2%. It has been further observed a low level of consumption of the treated food in all treatments. These data taken together indicated that the two oils had a fagodeterrence, repellent properties and neurotoxic properties on adults of RPW.

Morpho-physiological effects on palms: The injection of BRK9 induced after 2 months a significant increasing of chlorophyll content of leaves (SPAD index) from 37.4 (control leaves) to 43.9 (leaves treated with BRK9). Moreover, maximum chlorophyll fluorescence ratio (Fv/Fm) increased from 0.76 to 0.81 in leaves treated with BRK9. Finally, zinc content of leaves increased from 58.2 to 83.7 mg kg^{-1} of dry weight when plant were treated with BRK9. The above findings indicated a positive role of BRK9 in promoting photosynthesis and nutrient status of plants. Injection method was able to increase the accumulation of zinc in leaves. Similarly, zinc sprayed on leaves of *Pinellia ternata* increased the content of photosynthetic pigment and improve the capability of photosynthesis [9].

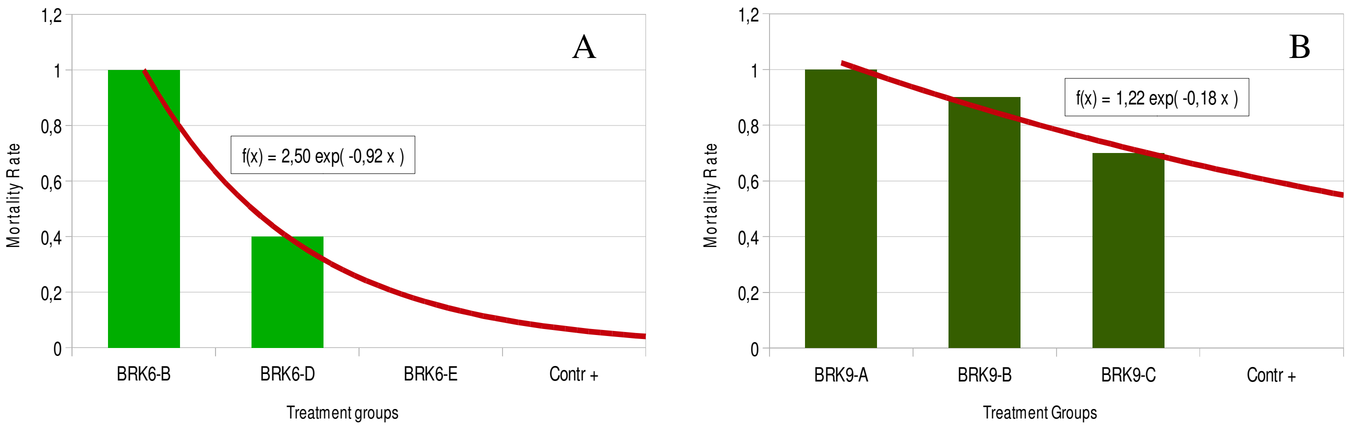

Figure 2. Mortality of the larvae after 72 h of treatment. Mortality rate was expressed as number of death larvae on number of total larvae for each treatment group (TG). Fig. A shows the trend in mortality among the BRK6 TG. Fig. B shows the trend in mortality among the BRK9 TG. Exponential trend lines and their equations are shown.

Conclusions

These data allowed to state that the two mixtures of oils BRK6 and BRK9 showed larvicide properties. These properties were more pronounced on BRK9 than BRK6. As regards to the RPW adults, BRK9 and BRK6 were effective only at the highest concentration (2 and 5%, respectively). Finally, injection of BRK9 into the palm trunk showed to promote chlorophyll content and photosynthesis of leaves thanks to a good translocation of zinc.

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