

Insecticidal Activity of an Indian Botanical Insecticide ULTRA ACT[®] against the Olive Pest *Bactrocera oleae* (Diptera: Tephritidae) in Tunisia

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Abstract

Bactrocera oleae is the most economically damaging insect of olive in the Mediterranean. As a reliable alternative to synthetic chemical insecticides, botanical pesticides from plant extracts are also considered natural control methods safe for the environment and human health. In practical applications up to date, studies demonstrating toxic effects of biological control agents on the insect have been carried out in organic farming systems based on some industrial microbial formulations. But much less attention has been given to botanical insecticides against *B. oleae*. Thus, a certified botanical insecticide from various plants and approved per Indian and International organic standards was tested against larvae and pupae of the olive pest *Bactrocera oleae* in the east of Tunisia under laboratory conditions. The experiments were conducted with 3 normal treatments and 1 control. Two techniques were used for larvae and pupae: direct contact using concentration from 0.5; 0.750; 1 and 1.5 mg/l and fumigation treatment using 2 mg/l conducted in rectangular boxes (25°C - 27°C, 75% - 85% RH and 14:10 LD photo period). For larval stages, LC50 and LC90 values for Ultra-Act were 0.45 and 1.22 mg/L in direct contact and 1.5 - 2 mg/l with fumigation, respectively. For pupal stage, the ID25 and ID50 values were estimated to be 0.71 mg and 1.26 mg respectively corresponding to the inhibition of emergence of 25% and 50% of *B. oleae* adults from pupa. Our results indicate that sprayed Ultra-Act product has the potential to control the three olive fly instar larvae. Pupal emergence was decreased significantly with fumigation treatment. In conclusion, Ultra-Act is a promising botanical insecticide against *B. oleae* that could

be used as a successful alternative for chemicals in integrated control methods of this pest in Tunisia.

Keywords

Olive Insect Pest, *Bactrocera oleae*, Indian Botanical Insecticide Ultra-Act, Insecticidal Activity, Contact, Fumigation

1. Introduction

The intensive use of pesticides has been the major practice in crop protection for decades to control pests and plant pathogens to avoid yield losses. *Bactrocera oleae* Gmel. (Dipt., Tephritidae) is the major pest of commercial olives worldwide (Daane and Johnson, 2010) [1]. The insect accounts for approximately 30% - 40% of loss of olive production and the affected olive fruit is unsuitable for processing a good quality of olive oil. It is estimated that in the Mediterranean region (Fimiani, 1989) [2], currently, the most common control practices against this pest are based on the use of synthetic insecticides (Katsoyannos, 1992 [3]; Nardi *et al.*, 2006 [4]; Daane and Johnson, 2010 [1]). But this has created problems related to adverse environmental effects, resistance of the insect pest and negative impact on non-target organisms and natural enemies in addition to high operational costs. Besides, common insecticides such as dimethoate failed to protect olives for as long a period after the last spray. Research for biological control alternatives against this pest is based essentially on mass trapping methods and the use of natural product-based pesticides such as the *Bacillus thuringiensis* (Bt), spinosad that offer a more sustainable solution to pest control than synthetic alternatives (Lambert and Peferoen, 1992 [5]; Federici and Sakanoo, 2006 [6]; Sameh *et al.*, 2009 [7]). Strategies involving semiochemical-based products have been pursued in the development of integrated pest management strategies for *B. oleae* (Mazomenos and Haniotakis, 1981 [8]; Gil-Ortiz, 2012 [9]; Canale *et al.*, 2014 [10]). However, still heavy *B. oleae* infestations were recorded in case of enormous populations so that further applications are required for efficient control (Saour, 2004) [11].

Insecticides of botanical origin are considered eco-friendly products for control of crop pests and have been reported as easily biodegradable, effective safe control, not toxic to human and domestic animals and useful for control. In this regard, interest in plant botanical derivatives has been extensively used for insect control (Balandrin, 1985 [12]; Perich *et al.*, 1994 [13]; Giatropoulos *et al.*, 2018 [14]). More than 1200 plant species are already known to have insecticidal properties (Sukumar *et al.* 1991) [15] and the current state of knowledge on larvicidal plant species has been reviewed (Shallan *et al.*, 2005) [16]. The Neem (*Azadirachta indica* A. Juss) belonging to the Meliaceae family is one of the most recognized plant biopesticide in progress replacing toxic insecticides (Chaudhary *et al.*, 2017) [17] with highly larvicidal toxic effects on insects tephritidae (Vi-

jayalakshmi *et al.*, 2016). Manufactured products from plant based biopesticide offer today an important perspective of being widely used according to their share in the global market. Numerous products are produced today by relatively small companies of local value and developed on the basis of active substances in plant species for the protection of harmful insects.

The product ULTRA ACT[®] is an organic certified botanical pesticide considered as an eco-friendly alternative to unsafe chemicals. Various plant species, seeds and animal wastes used in organic farming systems are applied in manufacturing formulation (ISO9001 certified company Vivekon, 2015) [18]. Regarding the importance of botanical plant insecticides for the insect pest control, ULTRA ACT[®] is an organic certified botanical pesticide developed by Vivekon Company, India that provide a new opportunities for plant protection with new Insecticides of botanical origin. In the ecological context adopting the organic farming system sustainable and environmentally, this product is a biologically active compound mentioned as an eco-friendly plant-derived insecticide. ULTRA-ACT[®] is a combination of various plants, seeds and animal extracts. The pesticidal efficacy, environmental safety and farmers acceptability of ULTRA-ACT for control of common crop insect pests such as whitefly, mealy bug, mites, jassid thirps, armyworms, round worm, thread worm caterpillar, borer larva, paddy larva and all other similar sucking insect pests., has led to its adoption into various crops for insect pest control programmes in many countries. The present paper aims to evaluate the effectiveness of ULTRA ACT based biopesticide as a sustainable means to control the olive fly *B. oleae* under laboratory conditions. The research study intends to examine the larvicidal activity of the product and to discuss its fumigant insecticidal potential on the pupal growth of the insect.

2. Materials and Methods

2.1. Insect Rearing

Larvae of second and third instar of *B. oleae* were obtained from laboratory-established colony from infested olive fruits collected in a Tunisian olive farming grove in Sousse Government (Bouficha). Fruits were put in aerated plastic boxes and supplemented with distilled water and examined each day separately under binocular to isolate larvae with available stage and vivacity.

2.2. Preparation of ULTRA ACT[®] Tests

The commercial formulation of ULTRA ACT[®] product was dissolved in distilled water and used in all experiments. Two techniques were used: direct contact for larvae and fumigation treatment for pupae. Four concentration were used for each technique 0.5; 0.750; 1 and 1.5 mg/l. Each concentration preparation was kept in a small glass bottle (50 ml) and stored at room temperature 25°C until experimental use.

2.3. Bioassays

Effects of ULTRA ACT[®] on larval mortality

For direct contact experiment, ten fruits with larvae were put in a separate set of petri dishes. A total of 5 dishes were used for each concentration treatment as well as 5 dishes taken up as control without insecticide. As method of treatment, the liquid insecticide product is applied directly to larvae using a small jet sprayer. The bioassay was conducted with 3 replicates. Dishes were covered with a tissue permeable to provide aeration and to avoid the escape of the larvae. The data of the larval mortality was assembled after 24, 48 and 72 hours for 2nd and 3rd instar of *B. oleae*. The dead larvae were removed after every adding up. The percent corrected mortality was calculated using Abbott's formula (Abbott, 1925) [19] and Log probit analysis was used to determine the median lethal concentration LC50 and 90% lethal concentration LC90 of the product.

Effects of ULTRA ACT[®] on pupal emergency

Fumigation treatment of a box rearing habitat aims to test effect of insecticide residues on pupa before they can mature into adult and emerge in nearby area with fruits. For fumigation treatment test, a total of twenty infested fruits with their nearby pupa (olive fruit and pupae) were put into an aerated box that was previously pulverized with the insecticide. Five replicates of each concentration were run under the same conditions along with untreated control.

Pupal emergency was numbered by counting emerged adults from pupa after one week of pupation. Emerged adults were removed after each count. Pupa missing emergency were considered as inhibited. The inhibition percentages of adult emergence were also corrected in accordance with Abbott (Abbott, 1925) [19]. The inhibition doses (ID) ID25 and ID50 (doses causing 25% and 50% inhibition of adult emergency) were determined with corresponding 95% fiducially limits (95% FL) by means of a non linear regression.

Effects of ULTRA ACT[®] on pupal weight

ULTRA ACT[®] was administered by fumigant application at its ID25 and ID50 on 1-day newly ecdysed pupae (<24 h). Three replicates with 20 pupa were used in each ID treatment and 20 pupa used as control. The pupal weight was registered each 2 days after treatment until the emergence of adults.

All Experiments were conducted in laboratory conditions (25°C - 27°C, 70% - 75% RH and 16:8 LD photo period).

2.4. Statistical Analysis

Results were presented as means and standard deviation (S.D). The homogeneity of variances was checked by a Levent's test. The significance between different series was tested using Student's t-test at 5% level. Data were subjected to one-way or two-way analysis of variance (ANOVA) followed by a posthoc Tukey test HSD. All analyses were carried out using the SAS package (SAS Institute, 2013).

3. Results

3.1. Toxicity Effect on Larvae

Trials with ULTRA ACT[®] on *B. oleae* larvae showed highly significant results

(Tukey HSD) at 1 mg/l and 1.5 mg/l, showing 69.3 ± 1.8 (%) and 88.2 ± 3.36 (%) of the 2nd larval mortality respectively ($p < 0.001$), and 58.2 ± 3.2 (%) and 72.4 ± 1.4 (%) of the 3th larval mortality respectively ($p < 0.001$) (Table 1). The second and third instars of *B. oleae* are both affected by the insecticidal product, in comparison with controls where there is no difference between arbitrary mortality due to the experiment whether; 3.25 ± 2.2 (%) and 2.66 ± 2.3 (%) of the 2nd and the 3th larval stage respectively ($p > 0.05$). Therefore, results revealed that earlier larval instars (L2) of *B. oleae* are more susceptible to ULTRA ACT[®] than older instars (L3) ($p < 0.001$) (Table 1).

The concentrations resulting in 50% and 90% mortality of the larvae were determined with 95% confidence limits (95% FL, $R^2 = 0.99$) using an exponential regression equation (Figure 1); Mean LC50 and LC90 values are respectively: 1.21 mg/l (0.22 - 0.73) and 2.01 mg/l (0.52 - 0.86).

Table 1. Dose mortality relationship of ULTRA ACT[®] against 2nd and 3th instar larvae of *B. oleae*.

	Larval mortality of <i>B. oleae</i> (%)	
	2nd instar	3th instar
	(Mean \pm sd)	(Mean \pm sd)
0.5 mg/l	26.6 ± 3.3 a*	17.2 ± 2.6 a'
0.75 mg/l	29.2 ± 1.3 a	22.3 ± 4.1 a'
1 mg/l	69.3 ± 1.8 c	58.2 ± 3.2 c'
1.5 mg/l	88.2 ± 3.36 d	72.4 ± 1.4 d'
Controls	3.25 ± 2.2 e	2.66 ± 2.3 e'

95% confidence limits; Number of replicates: 3. *Mean values (\pm SE) within a column followed by different letter (a, b, c or a', b', c') are significantly different by Tukey's HSD test ($p < 0.001$).

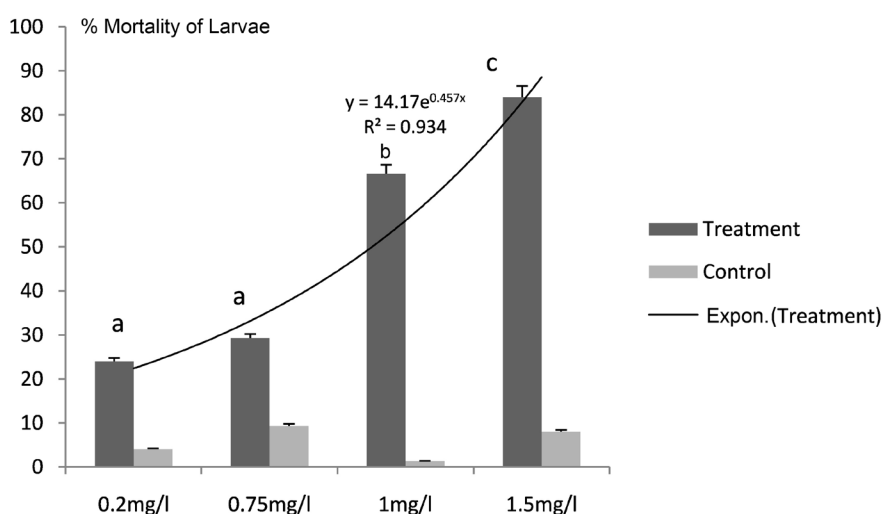


Figure 1. Effect of ULTRA ACT[®] on *B. oleae* larvae (% larval mortality), Mean \pm standard deviation, $n = 3$ repetitions at 25 larvae each, values followed by different letters are significantly different (Tukey test HSD, $p < 0.001$).

3.2. Fumigant Toxicity on Pupa

ULTRA ACT[®] product applied on newly ecdysed pupae causes an inhibition of adult emergence with a dose-response effect (Figure 2). Indeed, our data revealed that the inhibitory effect of adult's emergency from treated pupae, vary between $20.19\% \pm 2.35\%$ for the lowest concentration (0.5 mg/l) to $68.56\% \pm 3.15\%$ for the higher concentration (1.5 mg/l). The Tukey HSD test indicated that there was a significant relationship between the different tested doses ($p < 0.001$). In addition, the inhibition recorded in controls was $4.86\% \pm 1.19\%$ (Figure 2). The ID values with 95% fiducially limits (95% FL, $R^2 = 0.99$) were recorded of 0.71 (0.42 - 0.79) mg/l and 1.26 (0.88 - 1.48) mg/l, respectively for ID25 and ID50.

3.3. Weight of Pupae

The weight of pupae was significantly affected by ULTRA ACT[®] fumigation. Data showed that respective ID25 and ID50 caused a significant reduction in the weight of pupa compared to controls during the pupal stage at 6 ($p < 0.0001$) and 2 days ($p < 0.0001$) respectively. Pupae obtained from fumigation treatment were significantly heavier than those in control box ($F_{2,19} = 3.42$; $P < 0.0025$). Furthermore, there was no significant difference between the weight of pupa for the two tested doses ID25 and ID50 after 6 days of pupal stage ($p > 0.05$) (Table 2).

4. Discussion

The olive pulp was fed to the larva of *Bactrocera oleae* by incorporation into ULTRA ACT[®] at various concentrations under controlled conditions. 1 mg/L ULTRA ACT[®] caused a significant dose-dependent increase in mortality of

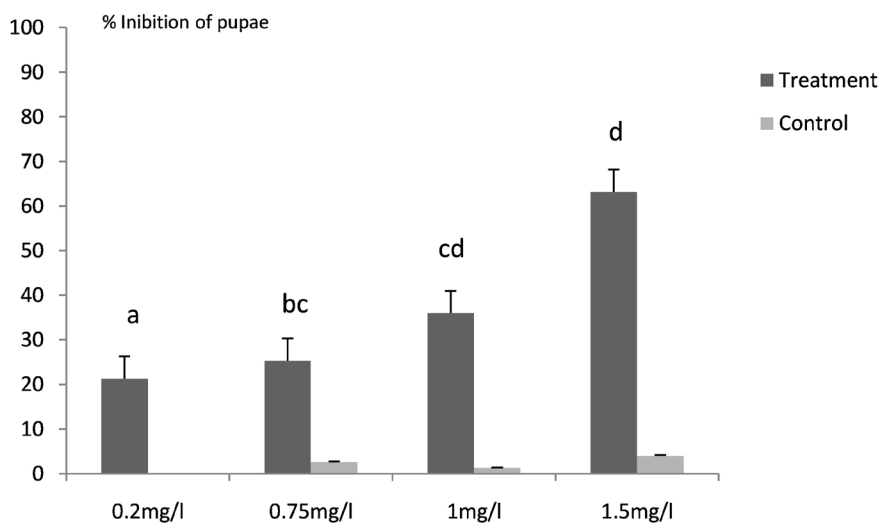


Figure 2. Effect of ULTRA ACT[®] on *B. oleae* pupae (% inhibition of pupae emergence), Mean \pm standard deviation, $n = 3$ repetitions at 25 pupae each, values followed by different letters are significantly different (Tukey HSD test at $p < 0.001$).

Table 2. Effect of inhibitory doses ID25 and ID50 of ULTRA ACT[®] on the pupal weight (mg) of newly ecdysed pupae of *B. oleae* (Mean \pm SD; n = 20 repeats).

Time (Days)	Control	ID25	ID50
2	8.24 \pm 0.04 a	7.23 \pm 0.02 a	7.08 \pm 0.03 b
4	8.13 \pm 0.02 a	7.26 \pm 0.10 a	7.05 \pm 0.02 b
6	8.25 \pm 0.01 a	6.01 \pm 0.03 b	6.05 \pm 0.05 b
8	8.15 \pm 0.02 a	5.73 \pm 0.02 b	5.75 \pm 0.09 b

Mean values (\pm SE) within a column followed by the same letter (a or b) under different days are not significantly different by Tukey's HSD test (p, 0.05).

second and third instars in comparison to controls (3.25% and 2.66% respectively). Moreover, Trials with ULTRA ACT[®] on *B. oleae* larvae at 1.5 mg/l, showed the highest larval mortality on the second (88.2%) and on the third (72.4%) larval stage of *B. oleae*.

Recent studies have confirmed the lethal effect of extracts and essential oils from some plants on immature stages of fruit flies of the family Tephritidae (Ruiz *et al.*, 2014 [20]; Ilyas *et al.*, 2017 [21]; Ghabbari *et al.*, 2018 [22]). Other studies also demonstrated a potential insecticidal effect of extracts and seeds from plants and seeds on *Bactrocera* species (Singh, 2003 [23]; Khan *et al.*, 2007 [24]; Khan *et al.*, 2016 [25]). For *B. oleae*, prospects for biological control using plant extracts are very limited to bioassays with very few plant species. Actual studies indicate that neem oil formulations from the Indian neem tree, *Azadirachta indica* (Schmutterer, 1990; National Research Council, 1992; Schmutterer and Singh, 2002) [26] [27] [28] are the only products with insecticidal compounds used in the control of *B. oleae* (Abd El-Salam *et al.*, 2018) [29]. Our results indicate that the formulation ULTRA ACT[®] have significant larvicidal activity on the second (88.2%) and the third larval stage (72.4%) of *B. oleae*. Similar results are seen for fused Neem based pesticides formulations showing 90% on the second larval stage with the recommended rate of 1 ml/L up to day 7 of post application in the field (Abd El-Salam *et al.*, 2018) [29]. In the present investigation, ULTRA ACT[®] based bio-insecticide product was found effective to control *B. oleae* larvae at 88.2% of mortality in second larval stage under laboratory conditions and more than 65% of inhibition of pupae emergency up to 8 days of post application. Moreover, there was a significant reduction in pupa weight when treated with ULTRA ACT[®] fumigant at inhibitory doses ID25 and ID50 after 4 and 2 days respectively. This result indicates that the sizes of treated pupa were small and the rates of emergency were decreased. This suggests that fumigant toxicity of volatile compounds in ULTRA ACT[®] could influence pupal development of *B. oleae* as well as its emergency. Indeed, using pupal weight as a measure of size of the insect, it was found that the heaviest flies (5.75 - 6 mg) were most affected by the insecticidal fumigant in comparison to *B. oleae* in its natural rearing conditions where pupal weight is almost about 7.5 mg (Genç and Nation, 2008) [30]. ULTRA ACT[®] most likely caused lethal effects on newly

emerged pupae of *B. oleae* during the pupal stage leading to inhibition of adult formation. These results are in agreement with Virendra *et al.* (2009) [31] reporting that insecticidal active volatile compounds in Azadirachtin formulations affect larvae by growth inhibition effect which degenerates in freshly ecdysed pupae. Also, other findings suggest that botanical extracts containing active insecticidal phytochemicals function as toxics which inhibit reproduction and other processes of growth of insects such as partial ecdysed and moults blocked (Rattan, 2010) [32].

Essential oils and organic solvent extracts from plants are used in plant based insecticides formulations because they contain bioactive toxic substances against a variety of insects (Pavela 2016) [33] and considered as enviro-friendly ingredients (Chemat *et al.*, 2012) [34]. In addition, Pavela (2014) [35] as well as several studies mentioned in Pavela review (2016) [33] have been carried out to investigate the role of volatile compounds in commercially produced plant based insecticides on Tephritid flies that could influence larval development survival, weight of pupae, fecundity and fertility. In general, with reference to botanical insecticide research, many publications have been listed but useful data are limited for producers (Isman and Grieneisen 2014) [36]. In fact, the commercialisation and adoption of new botanical insecticides in the marketplace are only based on the data requirements for low risky pesticide products (Murray, 2015) [37]. Indeed, data related to the efficacy of specific active compounds towards insect pest would promote and manage the use of botanical products into the commercial field. For instance, Campolo *et al.* (2018) [38] had reviewed the insecticidal activity of botanical products (contact toxicity, fumigation and mode of action) and discussed the toxically potential of fumigant essential oils extracted from various plants towards the most studied insect pests influencing the reduction in pupation and adult emergence. This fact could extend the effectiveness of ULTRA ACT[®] insecticide and lead our future research study to define high volatility and persistent compounds affecting the pupation and the emergency of *B. oleae* adults that could be used in sustainable biological control. In fact, Research on this topic is still very limited to some *Bactrocera* species only (Dhillon *et al.*, 2005; Kaur *et al.*, 2014; Sharma and Sohal, 2016) [39] [40] [41] where selective substances including phenolic compounds, polyphenols, terpenoid compounds, alkaloids as well as several plant proteins and enzymes are acting as inhibitors of insect's nutrition and retarding their development (Rattan, 2010 [32]; Bai *et al.*, 2014 [42]).

Commonly, bioactive compound products can be found in several parts of the plant than isolated using diverse extraction methods and techniques of manufacture. Moreover, a grounded review research suggests that it would be important to identify practices that value manufactured plant products used as biopesticides in Insect Pest Management (Khater, 2012) [43]. In fact, the competitiveness of the pesticide market and the environment protection require technological innovations by botanical pesticide industry in cost-effective production of

high quality extracts based on selective bioactive compounds of great potential in crop protection against targeted insect pest.

5. Conclusion

This study demonstrated significant improvement in the biological control program of *Bactrocera oleae*, the major pest of olives worldwide, using a new based biopesticide from botanical origin; ULTRA ACT[®]. Important steps for conducting experimental method have been provided. Insect sampling, rearing material and conditions are provided as well as the experimental design for laboratory bioassay focused on lethal and sublethal effects of the product where two techniques are used: direct contact for larvae and fumigation treatment for pupae. Interesting results show larvicidal activity from inhibiting doses I25 and I50. Indeed, statistical data revealed a significant fumigant inhibitory effect of adult's emergency from newly treated pupae. Further discussions on ULTRA ACT[®] potential on the pupal growth of the insect are provided. Manufacturing process of the botanical product ULTRA-ACT[®] may consequently standardize ratios of selective bioactive toxic substances that can significantly increase the biological insecticidal efficacy and enhance the potential fumigant insecticidal effect. Prospects for biotechnological production of insecticides from botanical plants in India grant a new opportunity for biological control against the olive fly insect pest. In this study, it appears that the pesticidal efficacy of the manufactured product ULTRA-ACT[®] proposes multiple undoubted benefits not only due to its environmental safety and manageable use against common Indian crop insect pests but particularly due to its importance likely related to the presence of bioactive metabolites with insecticidal potential against larvae and pupa of *B. oleae* insect pest in organic olive crops.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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