

Supporting Information

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Effects of *Olea europaea* L. Leaf Metabolites on the Tilapia (*Oreochromis niloticus*) and Three Stored Pests, *Sitophilus granarius*, *Tribolium confusum* and *Acanthoscelides obtectus*

Ahmet Kısa¹, Mehmet Akyüz¹, Hikmet Yeter Coğun², Şaban Kordali³ Ayşe Usanmaz Bozhüyük⁴, Binnur Tezel¹, Umran Şiltelioğlu¹, Barış Anıl⁵ and Ahmet Çakır^{1*}

¹ Kilis 7 Aralık University, Faculty of Science and Arts, Department of Chemistry, 79000-Kilis, Turkey

² Çukurova University, Ceyhan Veterinary Faculty, Department of Basic Sciences, Ceyhan, Adana, Turkey

³ Atatürk University, Faculty of Agriculture, Department of Plant Protection, 25240-Erzurum, Turkey

⁴ Iğdır University, Faculty of Agriculture, Department of Plant Protection, 76000-Iğdır, Turkey

⁵ Atatürk University, Faculty of Science, Department of Chemistry, 25240-Erzurum, Turkey

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1. Bioassays Procedures

1.1. Bioassays in Nile Tilapia

The study was carried out in the Animal Experimentation Ethics Committee of the Çukurova University (Protocol no: 9-3/2016) (Adana, Turkey). *O. niloticus* were obtained from Çukurova University Fish Farm, Adana, Turkey in the weight range of 12.0 ± 1.4 g and body length of 10.1 ± 0.5 cm (mean \pm SD). They were safely brought to the laboratory and acclimatized for 20 days in large cement tank (containing 1000 L of water) prior to the experiment. During the acclimatization period, fish were fed (Pinar Yem, Turkey) one time a day. Water was renewed (one third of the water) daily and feeding was held 24 h before the commencement of the experiment. The tap water free from chlorine was used and the water had the following physico-chemical characteristics; temperature (25.0 ± 1.2 °C), pH (8.2), dissolved oxygen (7.2 mg L^{-1}) and total hardness (135 mg L^{-1} , as CaCO_3). Before the experiment, fish were randomly divided into five groups which were housed in 100 L aquaria with tap water and continuously aerated. Photoperiod of the study was a 12:12 light-dark cycle.

The nominal concentrations of the olive leaf metabolites including fed mixed of 1 and 4 gram were added in each glass aquaria (40 cm \times 100 cm \times 40 cm) containing 50 L of water. Three replicates were maintained for each concentration groups and 6 fish of equal size and weight were introduced. Feeding was withheld during the bioassay experiment. At the end of 96 h period fish from the control and treated groups were taken for further analysis.

Fish were anaesthetized with MS-222 blood was collected from each fish by cutting the caudal peduncle. Fish blood was collected for hematological parameters. The blood was centrifuged at 4000 rpm over 10 min at 15 °C to obtain the serum. The serum samples were frozen and stored -20 °C until required for assays. Blood samples were sent to Kilis State Hospital Central Laboratory for hematological analysis. The remainder of the blood sample was centrifuged at 5000 g, at 4 °C for 10 min to separate the plasma, which was used for the estimation of biochemical parameters (glucose, ALT, AST and ALP) and electrolytes (Na^+ , K^+ , Ca^{+2} and Cl^-). Those analyses were determined by UV test technique [33]. Biochemical parameters and electrolytes were determined by ROCHE Hitachi E-170 and P 800.

1.2. Insects Materials and Bioassays

Three insects species were collected from the eastern Anatolia storage house. Wheat grains and beans were purchased from local market and stored in a freezer -20 °C. Wheat for *S. granarius* and *T. confusum* and beans for *A. obtectus* washed in tap water, dried and heated at to prevent pre infestation and before using for the experiments. *S. granarius*, *T. confusum* and *A. obtectus* adults were reared in laboratory at 25 ± 1 °C, 64 ± 5 relative humidity and L:D=12:12 in the Department of Plant Protection, at Atatürk University. The adults of obtained from laboratory cultures stored in separate insect cages including wheat and bean. Insecticidal activities of the extracts and pure metabolites of the olive were carried out in under the same condition and the same laboratory.

In order to the cultivation of insects, same age adults of the insects were feed on wheat in the glass jars (5 L) at 27 ± 2 °C, $64 \pm 5\%$ relative humidity and 12h/12h (L:D). The lids of the jars were covered with tulle through rubber band. The solutions of the extracts and the pure metabolites were prepared by suspending and/or dissolving in ethanol/sterile water (1:10 v/v). Twenty insects without distinguishing between male and female were separately placed treated with the each dose of the extracts and pure metabolites into the Petri dishes (9 cm x 1.5 cm) containing 10 g of feed. The solutions of the extracts and the olive pure metabolites were applied topically to the dorsal surface of one insect by a micro applicator (Hamilton, Bonaduz, GR, Switzerland) as 2.5, 5.0 and 7.5 mg/Petri dishes concentrations. Thus, the applications were correspondence to 125, 250 and 375 μg of the extracts or the pure compounds per insect [34]. Then, Petri dishes were closed with adhesive tape and put an incubator. After treatment, number of dead insects was counted after 24, 48, 72 and 96 h. Three replicates were used for each dose. The 2.5, 5.0 and 7.5 mg/Petri dishes concentrations of DDVP (dichlorvos; 2,2-dicholorovinyl dimethyl phosphite) solutions (correspondence to 125, 250 and 375 μg per insect) were used as positive controls. The insects in the Petri dishes applied with only ethanol/sterile water (1:10, v/v) were used as negative control groups. The toxicities of the extracts, the pure metabolites and DDVP were expressed as % mean mortality of the adults.

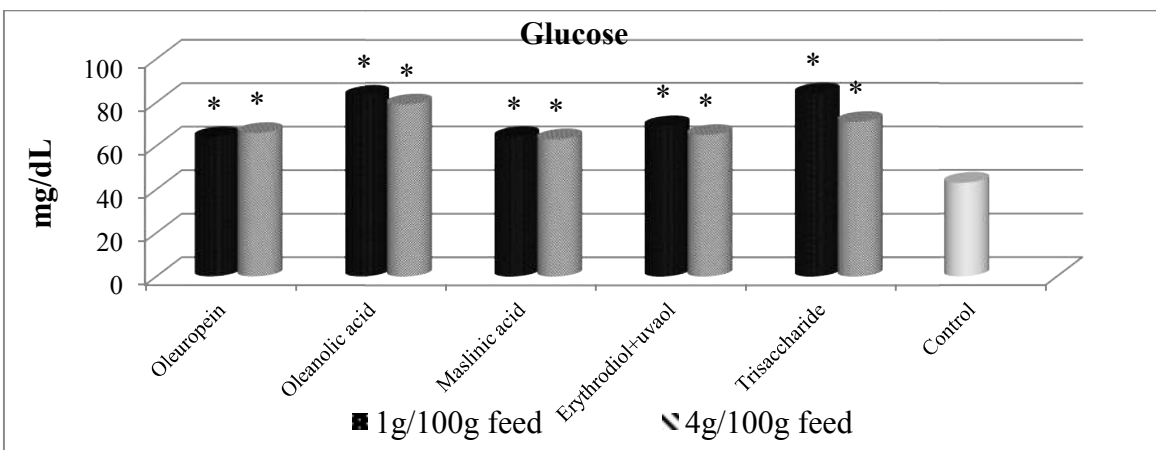
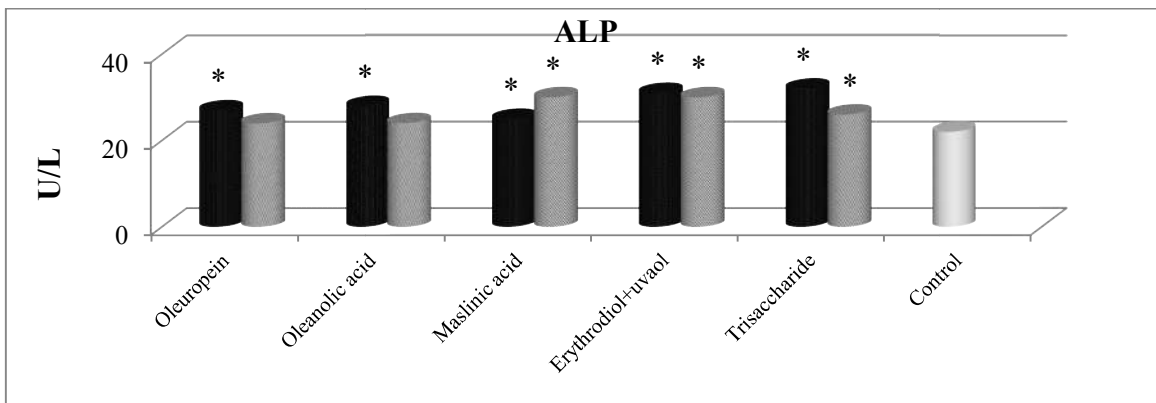
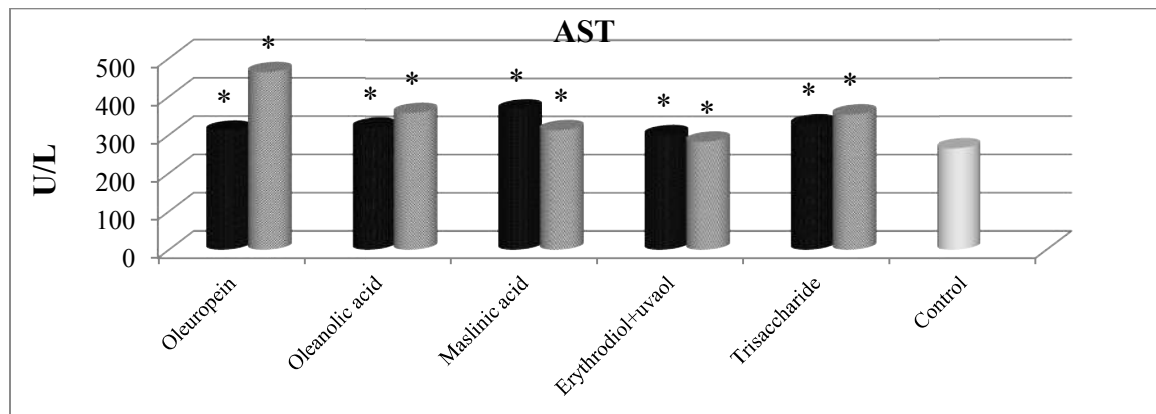
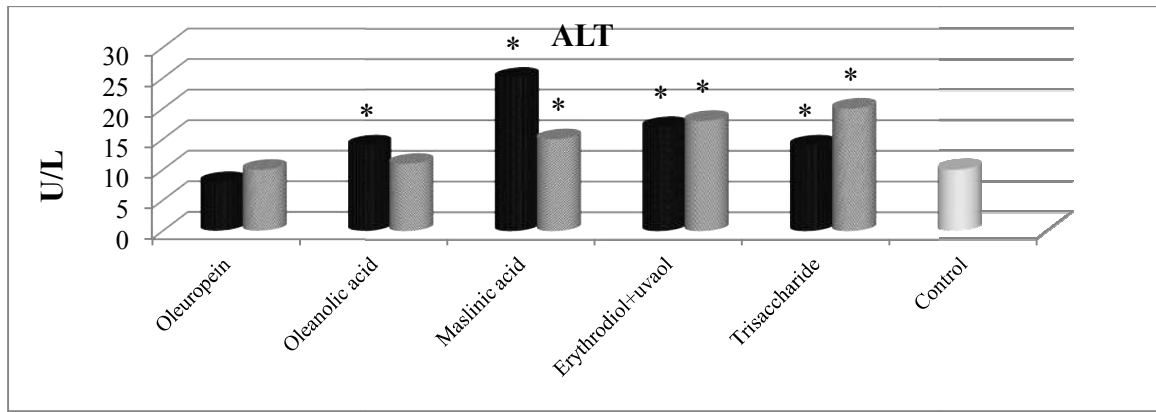


Figure S1: ALT, AST, ALP and glucose levels in the serums of Nile tilapia treated with leaf olive metabolites

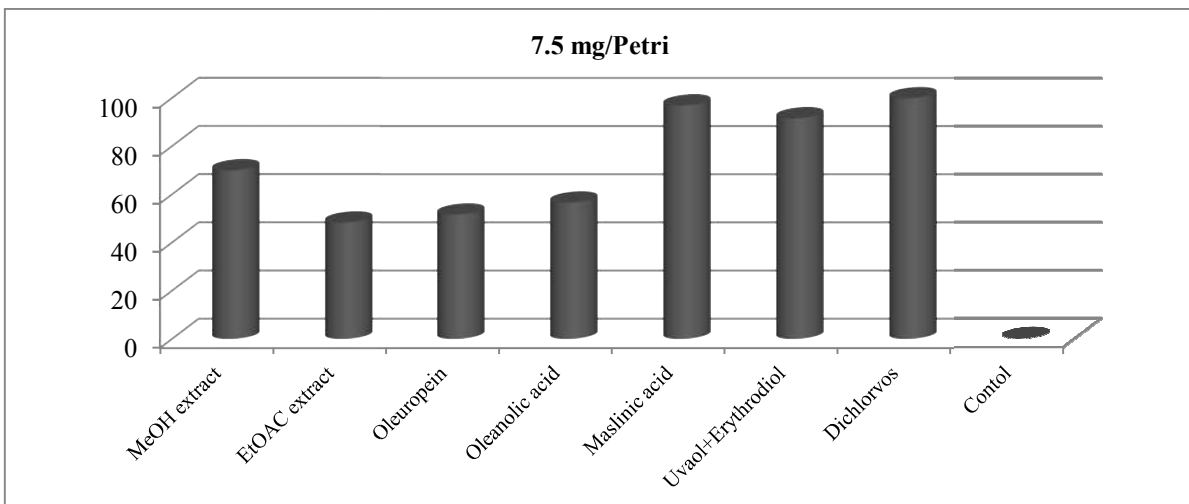
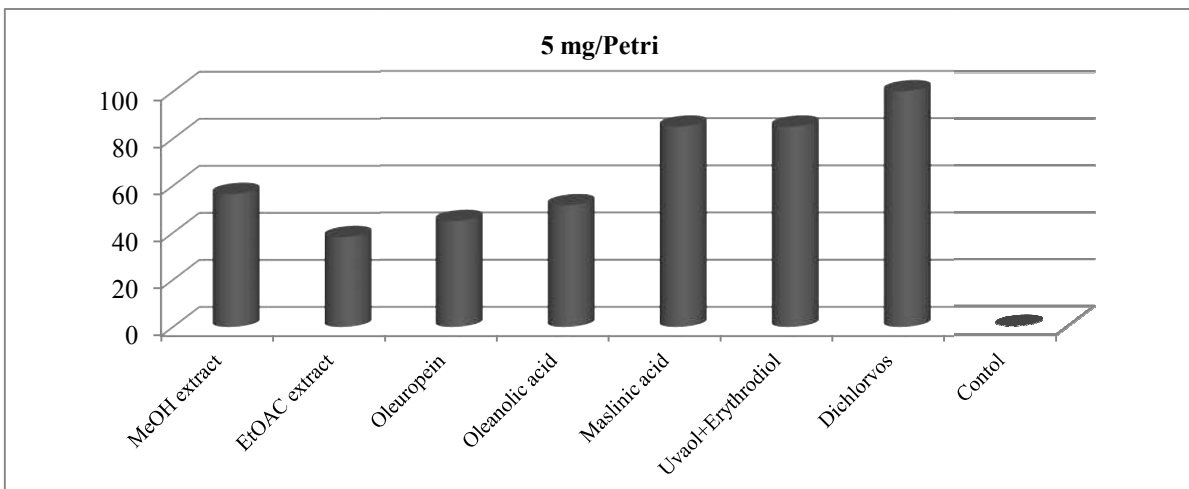
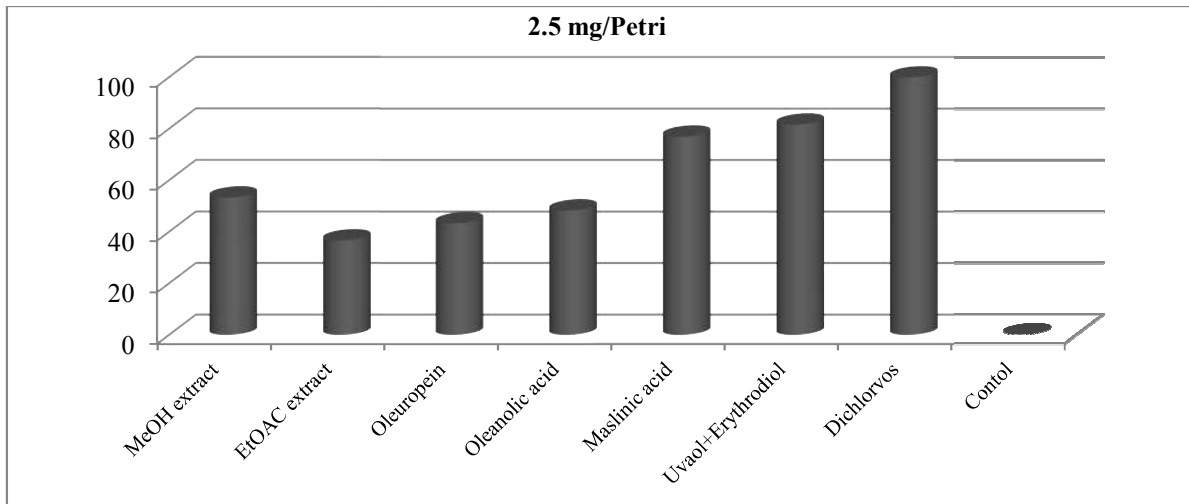


Figure S2: Toxic effects of the extracts and the pure metabolites of the olive leaf against *S. granarius* adults after 96h of treatments

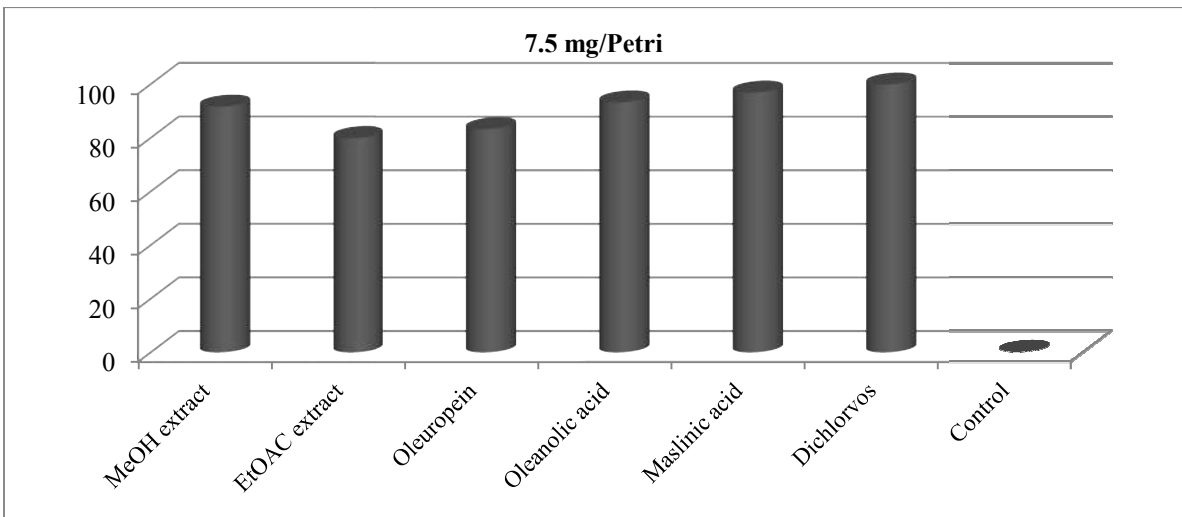
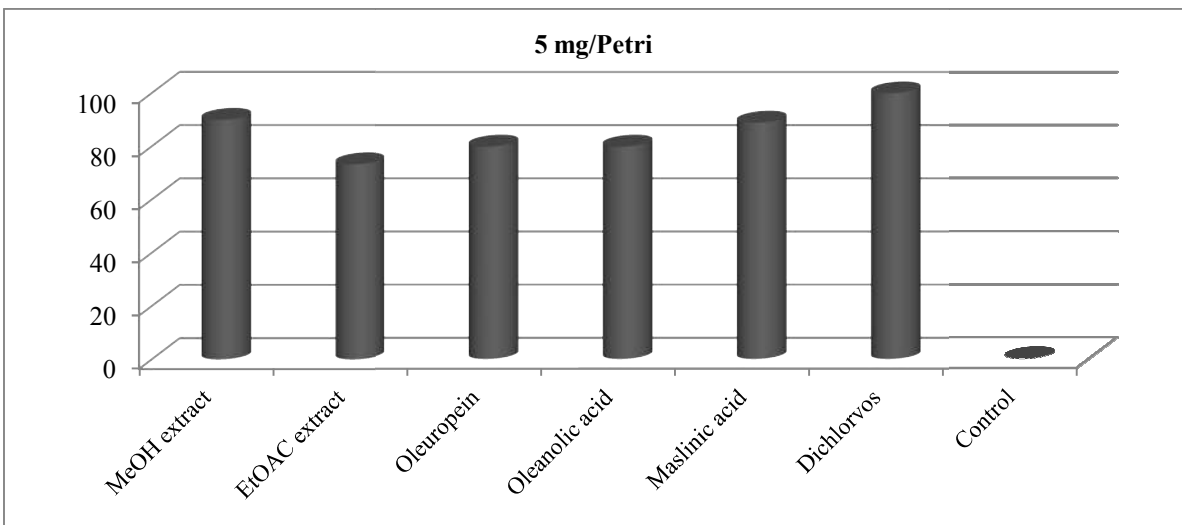
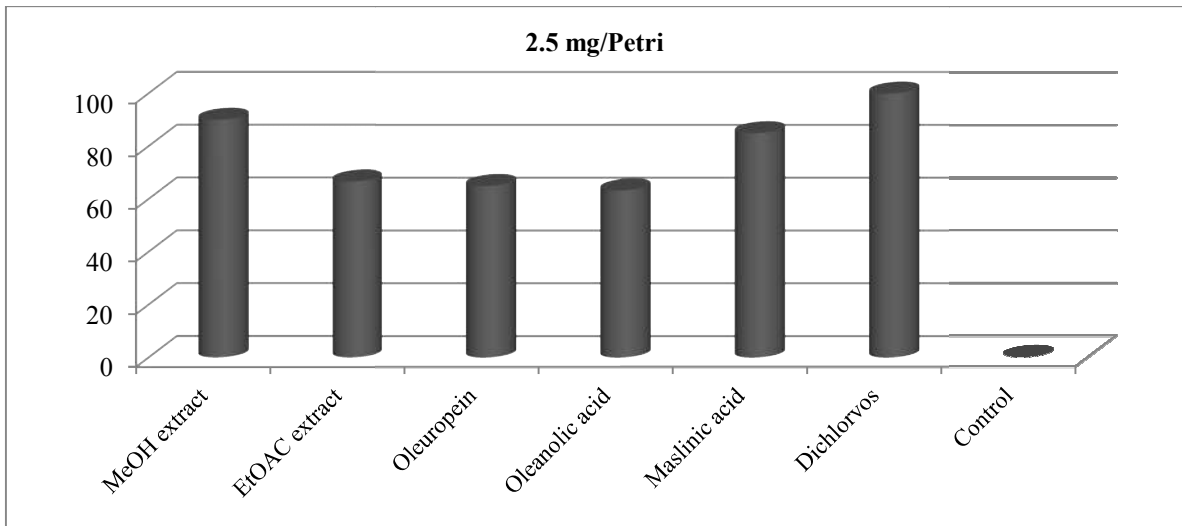


Figure S3: Toxic effects of the extracts and the pure metabolites of the olive leaf against *T. confusum* adults 96h of treatments

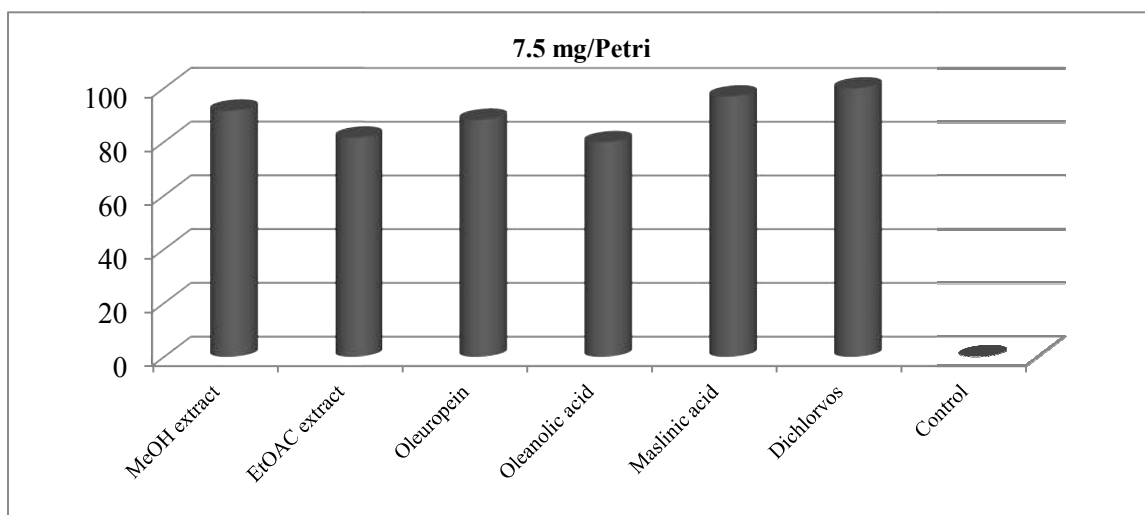
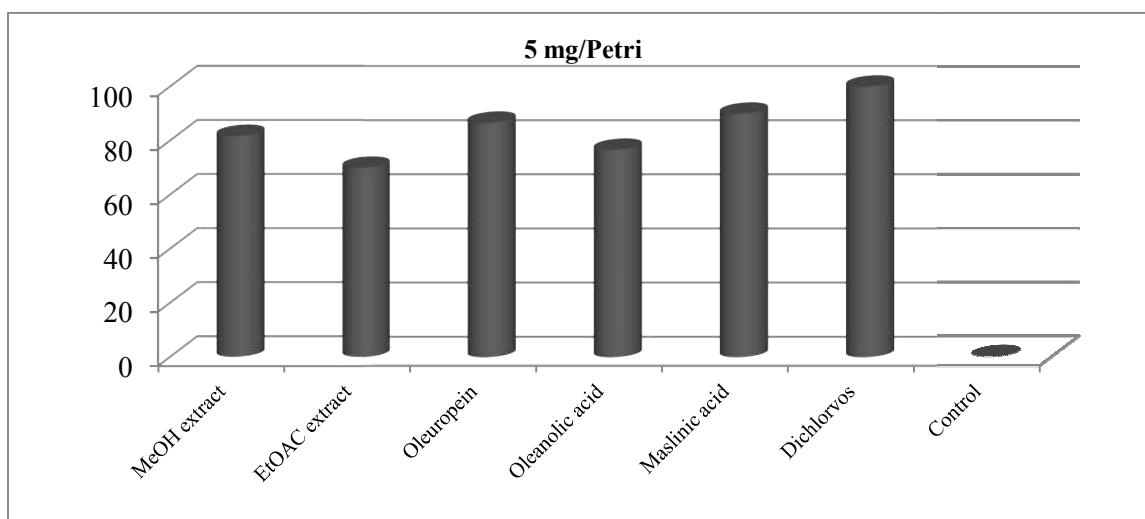
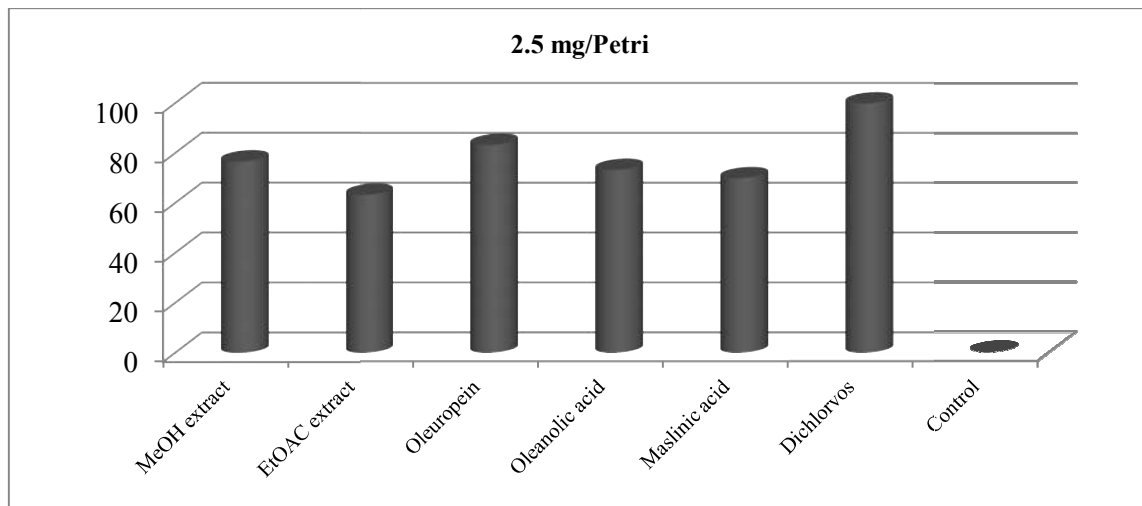


Figure S4: Toxic effects of the extracts and the pure metabolites of the olive leaf against *A. obtectus* adults 96h of treatments