ANTIFEEDANT AND TOXICITY EFFECTS OF SOME PLANT EXTRACTS ON *YPONOMEUTA MALINELLUS* ZELL. (*LEP.: YPONOMEUTIDAE*)

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Abstract: The insecticidal and antifeedant activity of extracts derived from different plants of the *Liguidamber orientalis* Mill. (*Hamamelidaceae*), *Tanacetum vulgare* L. (*Compositae*), *Achillea coarctata* Willd. (*Compositae*), *Buxus sempervirens* L. (*Buxaceae*), *Diospyros kaki* L. (*Ebenaceae*), *Arum italicum* Mill. (*Araceae*), *Achilea biebersteinii* Willd. (*Compositae*), *Origanum vulgare* L. (*Labiatae*), *Hypericum androsaemum* L. (*Hypericaceae*) and *Ocimum basilicum* L. (*Labiatae*). are reported. The 70% alcohol extracts of plants were tested for toxicity against the 3–4th instar larvae of the *Yponomeuta malinellus* Zell. (*Lepidoptera.: Yponomeutidae*). Antifeedant activity of the extracts was assessed through tests conducted on the larvae of *Y. malinellus* by the feeding protection bioassay. In tests carried out on the larvae of *Y. malinellus, L. orientalis, O. basilicum* and *A. coarctata* extracts showed high toxicity within 48 hour LC_{50}'s of 75, 75 and 65% respectively. The toxicity effects of the other extracts were determined as 60, 50, 50, 50, 45, 45 and 40% within the same period, respectively. No mortality was noticed in control groups. Alcohol extract from *L. orientalis, T. vulgare* and *B. sempervirens* showed high antifeedant activity (80.90, 46.12) on the larvae of *Y. malinellus*. In addition to both *T. vulgare* and *L. orientalis* extracts caused decrease consumption of food per 1 mg of larvae body weight decrease showed high –8.465, –0.845, mg respectively.
The highest consumption (557.6 mg) was observed with alcohol extract from, *D. kaki* whereas the minimum one was using alcohol extract from *L. orientali*. The other tested extracts showed similar activity.

Key words: Botanical insecticides, *Y. malinellus,* Plant Extracts

INTRODUCTION

In the last fifty years insect pests have mainly been controlled with synthetic insecticides Prior to the discovery of the organochlorine and organophosphate insecticides in the late 1930s and early 1940s, botanical insecticides were important

products for pest management in industrialized countries (Isman 1997). Most insecticidal compounds fall within four main classes, the organochlorines, organophosphates, the carbamates and pyrethroids. Out of these the major classes in use today are organophosphates and carbamates (Ware 1982; Dorow 1993). There are problems of pesticide resistance and negative effects on non-target organisms including man and the environment (Rembold 1984; Franzen 1993; FAO 1992). The organic, synthetic insecticides are more hazardous to handle, leave toxic residues in food products, not easily biodegradable, besides their influence on the environment is deleterious. Unlike synthetic that kill both pests and predators outright the natural insecticides are relatively inactive against the latter.

The botanical insecticides are generally pest-specific and are relatively harmless to non-target organisms including man. They are also biodegradable and harmless to the environment (Jacobson 1975). Furthermore, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise an array of chemical compounds which act concertedly on both behavioral and physiological processes. Thus the chances of pests developing resistance to such substances are less likely (Saxena 1987). One plant species may possess substances with a wide range of activities, for example extracts from the the neem tree *Azadirachta indica* are antifeedant, antioviposition, repellent and growth-regulating (Rembold 1994; Schmutterrer 1995). In contrast, the toxicity of conventional synthetic insecticides is mainly restricted to neuro-muscular function (Ware 1982). Conventional synthetic insecticides require special safety procedures and equipment during production and application despite precautions, exposure to humans, the environment (Franzen 1993) and food (FAO 1992). The synthetic insecticides are expensive and have in many cases only produced moderate results along with major ecological damage (Franzen 1993). In contrast, the low toxicity of botanical insecticides makes processing and application of the product inexpensive. In many cases, the materials are locally available and affordable (Childs et al. 2001).

The supply of natural insecticides could be made continuous at a cheaper rate by regular cultivation The use of organochlorine insecticides has been banned in developed countries and alternative methods of insect pest control are being investigated (Rembold 1984). Botanicals are a promising source of pest control compounds. The pool of plants possessing insecticidal substances is enormous (FAO 1992). These have generated extraordinary interest in recent years as potential sources of natural insect control agents. Today over 2000 species of plants are known that possess some insecticidal activity (Jacobson 1975).

The importation of plant material or derivatives thereof for use as insecticides represented a considerable enterprise: for example, over 6700 (U.S.) tons of *Derris elliptica* roots was imported into the USA from southeast Asia in 1947, but this decreased to 1500 tons in 1963 (Wink 1993).

This reflects the extent to which botanicals have been displaced by synthetic insecticides. The trend continues: in 1990, imports of pyrethrum in the USA totaled just over 350 tons (Gentry 1993). Also, some botanical insecticides that had enjoyed use in North America and Western Europe have lost their regulatory status as approved products. These include nicotine (from *Nicotiana tabacum*), quassin (from

Quassia amara and *Picrasma excelsa*), and ryania (from *Ryania speciosa*). As a consequence, the only botanicals in wide use in North America and Europe are pyrethrum (from *Chrysanthemum cinerariaefolium*) and rotenone (from *Derris* spp. and *Lonchocarpus* spp.), although neem (*Azadirachta indica* A. Juss.) is approved for use in the USA and regulatory approval is pending in Canada and Germany. At best, botanical insecticides presently constitute 1% of the world insecticide market, but annual sales growth in the range of 10–15% is entirely possible. The impact of botanicals will perhaps be most noticeable in the home-and-garden sector, where they might conceivably achieve as much as a 25% market share within 5 years (Isman 1997).

The main purpose of the agricultural studies is to increase the yield of product per hectare. The apple small ermine moth (*Yponomeuta malinellus* Zell.**)** causes more or less serious defoliation at the tips of branches of apple trees whose leaves, superficially gnawed assume a reddish color. The young fruit included within the webs show traces of bites. When defoliation is severe, the summer growth of the fruit is stopped and the latter fall prematurely.

Infestations are frequently spaced out by several years and are witnessed particularly in older orchards in Turkey (The Ministry of Agriculture of Turkey 1995). This pest damages the apple leaves during spring and summer. It causes approximately 50–60% economic damage particularly on apple production per year in Turkey. Up to now, chemical substances such as Carbaryl 85 WP , Etrimfos 520g/l EC , Malathion 250 g/l EC, Fenitrothion 40 WP and Trichlorphon 80 (SP) have been utilized to control this pest (The Ministry of Agriculture of Turkey 1995).

The purpose of this study was to test the extracts of ten wild-growing plants in respect of their antifeedant properties and their toxicity effect on develop of *Yponomeuta malinellus*

MATERIAL AND METHODS

Collection of insects

Larvae of *Y. malinellus* were colleced from April to July 2002 in agricultural fields in the vicinity of Amasya. The larvae were taken to the laboratory, placed individually and in batches in plastic boxes and fed with the leaf of apple trees .

Preparation of plant extracts:

Fresh and twigs of *Ligýidamber orientalis* Mill. (*Hamamelidaceae*), *Tanacetum vulgare* L (*Compositae*), *Achillea coarctata* Willd. (*Compositae*), *Buxus sempervirens* L. (*Buxaceae*), *Diospyros kaki* L. (*Ebenaceae*), *Arum italicum* Mill. (*Araceae*), *Achilea biebersteinii* Willd. (*Compositae*), *Origanum vulgare* L., (*Labiatae*), *Hyperic*ý*m androsaemum* L. (*Hypericaceae*) and *Ocimum basilicum* L. (*Labiatae*) were collected in Black sea region Turkey in April 2002. A few plants sample were obtained from local markets. The identification of this plants were carried out according to flora of Turkey (Valsaraj et al. 1997). Fresh leaves, stems and flowers of the plants were dried at 40°C for 5–6 hour. The extracts of plant were prepared according to the methods described by Holopainen et al. (1988) and Valsaraj et al. (1997). with slight modification. Dried leaves, stems and flowers of each plant were one by one extracted with, ethanol, 1:5

ratio (50 g powder plant material: solvent) at room temperature. The extracts were kept at 4°C for a day, and they were filtered, and then the solutions were dried with an evaporator (BIBBY RE 100 B.) The crude extracts were stored at –20°C until used.

Biological Assays

The caught insects were taken from the gardens to the laboratory with appropriate boxes; larvae were reared in groups of 20 larvae in containers. Containers were punched to permit air flow. Each group was fed for 48 hr with fresh the leaves of apple.

To determine the intensity of caterpillar feeding, the weighed fresh the leaves of apple trees were immersed in the prepared extracts for about 3 second. After air dring, they were placed into glass containers of 100 mm in diameter for each type of different plants extracts agents (Rakowski and Ignatowicz 1997). Two controls were taken, the first with 95% ethanol and the other without any treatment as a check. Twenty about 2–3rd instar larvae were placed on diet in each container for each assay. Each experiment variant including to the formula Kiełczewski et al. (1979): coefficient = (C-T):(C+T) × 100, Where: T – weight of food eaten by caterpillars in experimental variant, C – weight of food eaten in control variant Changes in body weight of tested caterpillars and the amount of eaten food per 1mg of body weight increase were also noted. After 48 hr, the larvae received fresh diet every 24 hr (Lipa and Wiland 1972). First control group treated with ethanol and second control group without any treatment for the first 48 hr and then fresh diet every 24 hr, finally dead larvae were removed (Thiery and Frachon 1997).

All larvae tested were kept at 26 ± 2°C and 60% RH on a 12:12 hr photoperiod (Lipa 1975; Ben-Dov et al. 1995). Dead larvae were removed immediately, and bioassay checked daily till 8th day. Data were evaluated by using Abbott's formula. All tests were made triplicate.

RESULTS

Absolute deterrence coefficient

The analysis of absolute deterrence coefficient factor (ADC), showing relations the amount of eaten food in tested and control variants (Tab. 1), indicates the strongest effect of alcohol extract from *L. orientalis* (coefficient) = 80.9, that practically means a complete lack of feeding, considering the weight loss of leaves because drying). Feeding of *Y. malinellus* caterpillars was also limited by alcohol extract from *T. vulgare* and *B. sempervirens* the coefficient being 46–40. The deterrence coefficient effects of the other tested plants (*O. basilicum*, *A. italicum* and *A. coarctata*) were determined as 32.93, 26.95 and 24.6 within the same period, respectively

Both the alcohol extracts from, *A. biebersteinii*, *H. androsaemum* and *O. vulgare* showed a far weaker deterrent effect. In the case of other extract the values of coefficient was the same Cont I; 95% ethanol (coefficient) = 1.03–1.57). But the alcohol extract from *D. kaki* was showed to negatively values of coefficient which means a stimulating effect on feeding of *Y. malinellus* caterpillars (coefficient) = –8.03)

There were no important differences in the value of absolute deterrence coefficient between alcohol extracts from *O. basilicum, A. italicum, A. biebersteinii, A. coarctata, H. androsaemum, D. kaki* and *O. vulgare*. The alcohol extracts from *L. orientalis, T. vulgare* and *B.sempervirens* were an exception

Change in body weight of caterpillars

The values of the weight changes of tested caterpillars are expressed as percentage relating to the dry control (Tab. 1). The highest caterpillar weight increase obtained within after 48 hours was observed in the case of alcohol extracts from *D. kaki, O. vulgare, A. biebersteinii, A. coarctata* and *H. androsaemum* was approximately one- two times as lowly as (positive way) in the control not significantly different from the control). In the most cases alcohol extracts from *L. orientalis, T. vulgare* and *B.sempervirens,* the decrease in caterpillars body weight ranged from –8.465 to –0.275 mg (–17.42, –8.17). The results were significantly different from the control (Tab. 1).

Table 1. Antifeedant effects of ten differenet plant extracts on feeding of of *Yponomeuta malinellus* caterpillars

Plant	Parts used	Food Placed on dish (in mg)	Total Food consumption (in mg)	Absolute coefficient of deterence	Food consumption per the larva (in mg)	After 48 hours change body weight per one larva (in mg)	After 48 hours % change body weight per one larva (in mg)
Liguidamber orientalis	Flowers, stems and leaves	1000	50	80.90	2.50	–8.465	–17.42
Tanacetum vulgare	Flowers, stems and leaves	1000	175	46.12	8.75	–0.845	–1.90
Achillea coarctata	Flowers, stems and leaves	1000	286.9	24.65	14.34	5.195	13.04
Hypericum androsaemum	Flowers, stems and leaves	1000	439.1	3.89	21.95	5.480	13.38
Buxus sempervirens	Flowers, stems and leaves	1000	208.0	40.11	10.40	–0.275	–8.17
Diospyros kaki	Flowers, stems and leaves	1000	557.6	–8.03	27.88	8.940	21.24
Arum italicum	Flowers, stems and leaves	1000	273.1	26.95	13.65	4.175	10.21
Origanum vulgare	Flowers, stems and leaves	1000	465.0	1.03	23.25	6.150	15.37
Ocimum basilicum	Flowers, stems and leaves	1000	239.5	32.93	11.97	0.865	2.15
Achillea biebersteinii	Flowers, stems and leaves	1000	419.9	6.72	20.99	5.875	14.12
Cont I; 95% ethanol	–	1000	460.0	1.57	23.00	11.125	27.05
Control II; any treatment (the leaves of apple)	–	1000	474.7	–	23.87	12.150	30.29

Food consumption per 1 mg of body weight

The analysis of food used in mg per larvae was as the highest consumption (22.80 mg) was observed with alcohol extract from *D. kaki* and the minimum consumption (2.50 mg) was with alcohol extract from *L. orientalis*. The other tested extracts showed similar consumption (Tab. 1).

Table 2. Toxicity effects of ten differenet plant extracts on *Y. malinellus* caterpillars

Plant	Parts used	Dead larvae	Dead pupae	Total dead
Liguidamber orientalis	Flowers, stems and leaves	14	1	15
Tanacetum vulgare	Flowers, stems and leaves	3	7	10
Achillea coarctata	Flowers, stems and leaves	9	4	13
Hypericum androsaemum	Flowers, stems and leaves	2	8	10
Buxus sempervirens	Flowers, stems and leaves	3	6	9
Diospyros kaki	Flowers, stems and leaves	4	6	10
Arum italicum	Flowers, stems and leaves	5	7	12
Origanum vulgare	Flowers, stems and leaves	2	6	8
Ocimum basilicum	Flowers, stems and leaves	4	11	15
Achillea biebersteinii	Flowers, stems and leaves	3	6	9
Control I; 95% ethanol	–	1	–	1
Control II; any treatment (the leaves of apple)	–	–	–	–
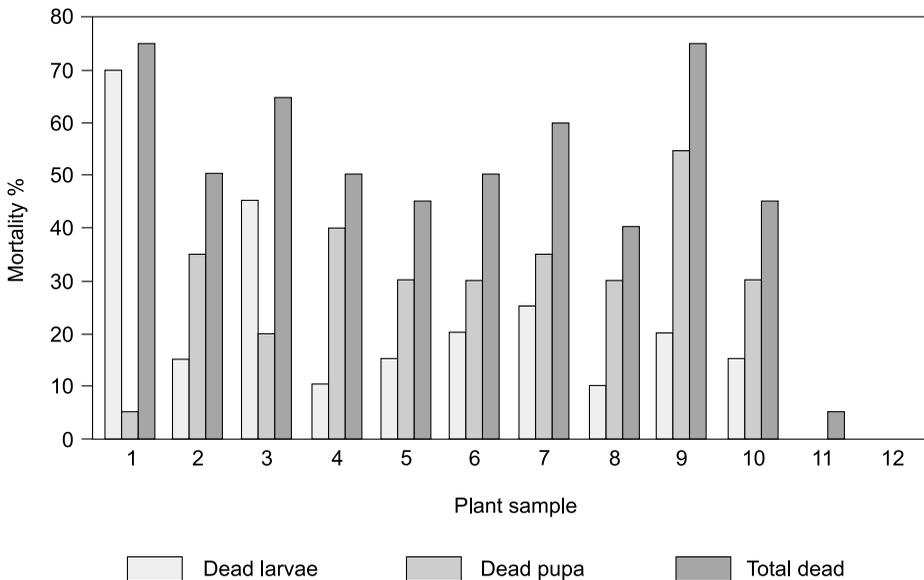
Fig. 1. The toxicity effects of the plant extracts on *Y. malinellus* caterpillars: 1; *L. orientalis,* 2; *T. vulgare,* 3; *A. coarctata,* 4; *H. androsaemum,* 5; *B. Sempervirens,* 6; *D. kaki ,* 7; *A. italicum,* 8; *O. vulgare,* 9; *O. Basilicum,* 10; *A. biebersteini,* 11; Cont I; 95% ethanol, 12; Control II; any treatment (the leaves of apple)

Toxicity to Larvae

The results of the toxicity of *L. orientalis, T.vulgare, A. coarctata, H. androsaemum, B. sempervirens, D. kaki , A. italicum, O. vulgare, O. basilicum* and *A. biebersteini* alcohol extracts to 3–4th instar larvae of *Y. malinellus* is presented in table 2 and figure 1. In this test, the alcohol extracts from *L. orientalis, O. basilicum* and *A. coarctata* showed high toxicity respectively 75, 75 and 65% against *Y. malinellus* larvae. Similarly, these alcohol extracts from *A. coarctata* and *L. orientalis* had high toxic activity on *Y. malinellus* larvae, but was weakly toxic activity against *Y. malinellus* pupae as seen in table 2 and figure 1. In spite of the alcohol extract from *O. basilicum* had high toxic activity on *Y. malinellus* pupae, but was weakly toxic activity against *Y. malinellus* larvae. The toxicity effects of the other extracts from *A.italicum, T.vulgare, H. androsaemum, D. kaki, B. sempervirens, A. biebersteini* and *O. vulgare* as 60, 50, 50, 50, 45, 45 and 40% within the same period, respectively. No mortality was determined on control groups. The equations of the regression lines from probity mortality versus log dosage plots and the lower and upper confidence limits of the LC_{50}'s of each extract are also shown on figure 1.

DISCUSSION

The results of the presented study are interesting. The findings indicate the importance of traditional knowledge in science. The alcohol extract from*, L. orientalis, O. basilicum, B. sempervirens* and *T. vulgare* have been shown to possess insecticidal and insect repellent components and these protect the apple tree from insect damage. Particularly the alcohol extract from *L. orientalis* showed the strong deterrent effect to *Y. malinellus,* manifest in their high weight decrease**.** However this extract showed high toxicity effect against *Y. malinellu*s but did not show against the pupae of *Y. malinellus.* Similarly the extract of *T.vulgare* showed the strong deterrent effect against the larvae of *Y. malinellu*s but this extract did not show toxicity effect against the pupae and larvae of *Y. malinellus*

Both *L orientalis* and *T. vulgare* have antifeedant insectidical effect on larvae of *Y. malinellus*. *T. vulgare* include tansy that reportedly contains about 100 compounds, primarily monoterpenoids such as thujone, camphor and borneol Schearer (1984). Tansy has been tested as a feeding deterrent against several insect species. Schearer (1984) and Panasiuk (1984), reported "avoidance behavior" by Colarado potato beetles exposed to various components of tansy extracts. Hough-Goldstein (1990), Brewer and Ball (1981), found that an aqueous extract of tansy painted on broccoli leaf disk significantly reduced feeding by the imported cabbageworm, *Artogeia rapae* (L.) and diamondback moth, *Plutella xylostella* (L.) but not by the more polyhagous cabbage looper, *Trichoplusia ni* (Hübner). Amonkar et al. (1970) demonstrated toxicity of crude methanolic extract of garlic against mosquito larvae.

The alcohol extract from *B. sempervirens* showed deterrent effect against *Y. malinellus,* manifested in their high weight decrease. However this extract showed low toxicity effect against the larvae of *Y. malinellus.* The alcohol extract from *O. basilicum* had weakly antifeedant effect on the larvae. As similarly this result was found by Hough-Goldstein (1990). But this extract showed high toxicity effect against the pupae of *Y. malinellus* but did not show against the larvae of *Y. malinellus*

(Fig. 1, Tab. 2). The alcohol extract from *A. coarctata* had a very weak antifeedant effect on larvae of *Y. malinellus*. However, this extract showed toxicity effect against larvae of *Y. malinellus*. The other crude ethanol extracts from *H. androsaemum, D. kaki, A. italicum, O. vulgare* and *A. biebersteini* showed similar toxicity and antifeedant effects on larvae of *Y. malinellus*.

Natural antifeedants are mainly plant natural products of various chemical groups. Particularly effective insect antifeedants are triterpenes (van Beek and Grood 1986; Grood and van Beek 1987), sesquiterpene lactones, alkaloids (Nawrot et al. 1986), cucurbitacines, quinines and phenols (Norris 1986). Some plant families include numerous species containing bioactive substances. These may be volatile oils especially terpenes tansy (Wieczorek 1996; Mwangi and Rembold 1988). It is possible that the extracts of plant use in presented study can contain this compounds. If any effective extract is determined, this substance can be isolated, purifical and used against larvae of *Y. malinellus*. The results obtained in this study, suggested that further studies should concentrate on the activity of the extracts of plant. These plants are easily available in nature.

Today, the environmental safety of an insecticide is considered to be of paramount importance. An insecticide does not have to cause high mortality on target organisms in order to be acceptable (Schmutterrer 1995). Antifeedant and growth-inhibiting activity can therefore be incorporated into other insect control techniques in the strategy of integrated pest management (IPM). It would be interesting to investigate whether *L. orientalis, T.vulgare, O. basilicum, B. sempervirens* and *A. coarctata* contain substances similar to the antifeedant, and growth inhibiting compounds present in the fruits of *Azadirachta indica* (Rembold 1984; Schmutterrer 1995) and *Melia volkensii* (Amonkar and Reeves 1970; Nawrot et al. 1986). Testing the toxicity from *Y. malinellus*. could be also of importance. The following conclusions can be made from the results of this study. The alcohol extracts from *L. orientalis, T.vulgare, O. basilicum, B. sempervirens* and *A. coarctata* have toxic effects on the larvae of *Y. malinellus*. In addition, the alcohol extracts from *L. orientalis* and *T.vulgare* have highest toxicity and antifeedant activity.

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POLISH SUMMARY

ZASTOSOWANIE NIEKTÓRYCH EKSTRAKTÓW ROŚLINNYCH PRZECIW ŻEROWANIU *YPONOMEUTA MALINELLUS* ZELL. (*LEP.: YPONOMEUTIDAE*) I OKREŚLENIE ICH TOKSYCZNOŚCI

W niniejszej pracy omówiona została aktywność insektycydowa oraz przeciw żerowaniu owadów ekstraktów otrzymanych z różnych roślin: *Liguidamber orientalis* Mill. *(Hamamelidaceae)*, *Tanacetum vulgare* L. *(Compositae)*, *Achillea coarctata* Willd. *(Compositae)*, *Buxus sempervirens* L. *(Buxaceae)*, *Diosperos kaki* L. *(Ebenaceae)*, *Arum italicum* Mill. *(Araceae)*, *Achilea biebersteinii* Willd. *(Compositae)*, *Origanum vulgare* L. *(Labiatae)*, *Hypericum androsaemum* L. *(Hypericaceae)* and *Ocimum basilicum* L. *(Labiatae)*. Roślinne, 70% ekstrakty alkoholowe były testowane w kierunku toksyczności na larwach *Yponomeuta malinellus* Zell. *(Lepidoptera.: Yponomeutidae)* w 3–4 stadium rozwoju. Aktywność ekstraktów przeciw żerowaniu określano poddając larwy *Y. malinellus* 'feeding protection bioassay'. W testach przeprowadzonych na larwach *Y. malinellus*, ekstrakty z *L. orientalis*, *O. basilicum*, i *A. coarctata* wykazały wysoką toksycznaość w ciągu 48 godzin a wartości LC$_{50}$ wynosiły odpowiednio 75, 75 i 65%. Toksyczność pozostałych ekstraktów w tym samym czasie wynosiła odpowiednio 60, 50, 50, 50, 45, 45 i 40%. W grupie kontrolnej nie zaobserwowano żadnej śmiertelności larw. Alkoholowe ekstrakty z *L. orientalis*, *T. vulgare*, oraz *B. sempervirens* wykazały aktywność przeciw żerowaniu (80,90, 46,12) larw *Y. malinellus*. Dodatkowo, oba ekstrakty z *B. sempervirens* i *L. orientalis* powodowały obniżenie konsumpcji w przeliczeniu na 1 mg masy ciała larwy odpowiednio, –8,465 i –0,845 mg.

Najwyższą konsumpcję (557,6 mg) obserwowano w przypadku ekstraktu alkoholowego z *D. kaki* a najniższą dla ekstraktu z *L. orientali*. Pozostałe ekstrakty wykazały podobną aktywność.