Plant Extracts as Alternative Botanical Insecticides f r Control the Grasshopper *Heteracris littoralis* Romb. (Orthoptera : Acrididae) with reference to histological changes on the reproductive system

By

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Abstract

In the present study , alchoholic extract 85 % of five different medicinal plants that possess insecticidal activity against other insec pests, namely , *Euphorbia pulchrrima* and *Euphorbia cotimfabia* (Euphorbiacae) *Dodonea viscosa* (Sapindaceae), *Schinus trbinlitolia* (Anacardiaceae) and *Eucalyptus rostata* (Myrtaceae) , were tested for toxic effects against 1St nymphal instars of the grasshopper *Heteracris littoralis*. The toxic activity according the LC_{50} values could be arranged in the order that *E. pulchrrima = E.cotinfabia >D.viscosa > Eucalyptus rostata > Chinus trbinlitolia* , The effect of LC_{50} values of the most effective extracts (*E. pulchrrima , E.cotinfabia* and *D.viscosa*) were tested on some biological aspects of *H. littoralis* treated 1St nymphal instars . Results cleared that there was statistical variable numbers of reduction in preoviposition ,Oviposition and postoviposiyion periods f the adult offspring resulting from the treated nymphs . Highly reduction in the deposited eggs,

adults longevity and egg hatchability. Also normal dev lopment was exhibited . However water ethanolic extract of the tested plants h d a toxic effect and retarding the development of *H.littoralis* . Histological changes on the reproductive system of both male and female adults resulting from the treated nymphs that detected by light microscopy has been discussed .

Key words : Grasshopper , *H. littoralis* , Plant extracts , Histopathological changes , insecticidal properties , Medicinal plants , Orthoptera .

Introduction

In recent years ,the increasing information on hazardous effect of synthetic insecticides on plant and animal health has alarmed scientists to seek some alternative ways , which are ecofriendly . About 450 species of insect pests and mites have been developed resistance to one or more major synthetic pesticides (Georghiou,1986) [1] . Botanical insecticides are one of the best alternatives for these hazardous chemicals . They are plant – drive insecticides ,either naturally occurring plant materials or the pro cts simply derived from such plants (Gupta *et al* , 2005) [2] . A number of medicinal plant species like *Euphorbia* sp. , *Dodonea vescosa* , *Eucalyptus sp*. and *Chinus trbinlitolia* etc , are known to possess insecticidal properties (Sharma and Gupta 2009 [3] ;Mazen *et al*, 2009 [4] ; Oparaeke,2004 [5] ; Uwaezuoke, 2002 [6] and Cruze *et al* 2000 [7]) . The grasshopper *H. littoralis* considered one of the most harmful pests to different cultivated crops in Egypt . Its economic importance comes from attacking many vegetable cultivated areas even tr es , feeding on it and causing great losses in quantity and quality of the attacked crops . In some cases thousands of cultivated hectares may be attacked by the swarms of grasshopper leaving it as a divested desert .The economic injury of *H.littoralis*

in Egypt had been documented by Mistikawy (1929) [8] and Nakhla (1957) [9] . The main aim of the present study was to evaluate the potential use of the organic extracts of Euphorbia sp . , *Dodonea* , *Eucalyptus* and *Chinus* in the *H.littoralis* control , so that they can be used in the integrated handling of pests . Exposure of sublethal doses of the insecticides greatly affect the development of gonads in insects (Ghazawi et al ., 2007 [10]; Habluetzel et al ., 2007 [11] and Senthil et al ., 2008 [12]) . To attain this purpose histopathological studies of females and males gonads of normal and treated *H . littoralis* grasshopper were conducted . We could concluded that artificial diet when mixed with the promising toxic extracts may be use as a toxic bait for controlling the insect .

Materials & methods

Adults and nymphs of *H . littoralis* were collected from Giza governorate , Egypt . The colony was raised in laboratory stock and reared in electrical heated wooden cages at constant temperature af 30 ± 1 C° with fluctuating relative humidity (50 – 70 %) . Insects were fed on synthetic diet mentioned by Sharaby *et al.*, (2010) [13] . For Oviposition , cages were supplied with suitable ovipositional pots . These pots were examined every da and , when laid in , were removed to glass jars (ca 100 ,c.c) , hatched hoppers were transferred to large jars (ca.7000 ages . c.c) . After the fourth or fifth moult , hoppers were released in the larges cages .

Biological notes were recorded including the developmental duration of each nymphal instars , number of instars , pre -Oviposition period , number of eggs per egg – pod , number of egg – pods per female , Oviposition period and the

duration of the post – Oviposition period as well as effect of the treatment on development and reproduction .

T o study reproduction and longevity , ten pairs of newly emerged nymphs were used in pairs , each pair was placed in large glass jars . Each jar was provided with an ovipositional pot and supplied with p e of synthetic diet for feeding replacing it every four days or when consumed . The experiment was conducted at 30 C^{o} and L.D 12:12 ., and the relative humidity fluctuated between 50 - 60% .

Plant extracts preparation :

Five medicinal plants were collected from the ga of National Research Centre , Egypt . The plant parts were dried in shad place then minced into powder in an elecreric mill . Each plant was extracted separately. Soxhelet extraction was used. Known weight (500g) of plant powder of each plant species was filled into the Soxhlet apparatus . A cotton plug was used at the place of thimble to stop the entry of the crude material into the siphoning tube . The required solvent (ethanolic alcohol 85%) was filled up five times more than total amount of the sample material into the flas of the apparatus . The apparatus was then connected with the water supply to the condenser . The temperature of the heating mantle was maintained at 65-70 C^{o} . The process was carried out for 6-8 hours for each sample . The extracts were filtered to remove particular matter. Each extract was dried under reduced pressure using Rota vapor. The evaporated material was weighed and stored in the refrigerator for further use . The desired stock solution of each extract was made by adding more solvent until the plant material w s dissolved completely

Bioassay tests :

For determine the LC_{50} concentration of the different plant extracts on the ^{1St} nymphal instars of *H. littoralis* , five descending concentrations that permit the computation of LC_{50} was diluted on the basis of weigh / volume (25 ,12.5 , 6.25 , 3.13 , 1.56 %) from each plant extract were prepared by mixing known weight from the extract with 100 ml. diet during the diet preparation , one drop of Triton x100 was added for obtained the desired concentration . The treated diet poured into blasic box and kept in refrigerator till use. A piece of the treated diet was introduced into jars with containing 1^{St} nymphal instars for feeding on it for five days then remained treated diet replaced by untreated one , number of dead insects were counted each day after treatment till ten day (the end point) for calculating LC_{50} values . For each concentration , 25 individuals were tested in five replicates , 5 nymphs each . Controls were fed on untreated diet . LC_{50} were determined according to Finney (1971) [14] and mortality percent was corrected according to Abbott' s formula (Abbott, 1925) [15] . After determined the LC_{50} concentration values of each extract , different biological aspects of the resulted Insects have been recorded . The newly emerged 1^{St} nymphal instars were fed on diet mixed with the prepared concentration of the extracts for five days then the diet replaced by untreated one till reached to the adult stage . The remaining adults were noticed for egg Oviposition and egg hatching . The different biological aspects were recorded for each plant extract, fore each test , 200 insects were used. All data were subjected to Statistical analysis of Variance (ANOVA) SPSS Computer Program . To differentiate between means , Duncan' s (1965) [16] multiple range test (P = 0.05) was used .

Histological examination :

The newly emerged adults resulted from the treated 1St nymphal instars , were used . Ovarioles and sperm tubes of treated insects and contr l groups were histologically examined . Specimens were dissected in 0.9 % Nacl solution and fixed in Bouin' s solution for 24 hrs (Humason , 1962) [17] then dehydrated in ascending alcoholic series and cleared in xylen for few seconds then specimens were infiltrated in three changes of paraffin wax each lasted 20 minutes. Paraffin blocks were prepared and 6 u longitudinal or cross section were cut and stained with Ehrlich' s acid haematoxylin and alcoholic eosin . The stained sections were dehydrated , cleared and mounted using D.P.X . for examination .

Results & discussion

The toxicity of different plant extracts on the 1St nymphal instars of *H. littoralis* was shown in Table(1) , according to the LC_{50} values it could be arranged as follows, *E . pulchrrima* 3.61 nearly equal to 3.75 for *E.cotimfabia* > *D.vescosa* 13.32 > *Eucalyptus rostata* 14.07 > *schinus terbinulitilia* 23.18 mg/100 ml . diet . The variable in toxicity may be due to that the insect consumed great amount from the treated diet with an extract than the other ,this depending on the concentration of the oxic constituents of each extract. Finally we could be concluded that the most toxic extracts were the two *Euphorbia* species followed by *Dodonea* and *Eucalyptus* , while *Schinus* has the lowest toxicity .

The effect of LC_{50} values of the most three effective extracts (*E. pulchrrima* , *E. cotimfabia* and *D. vescosa*) were evaluated on some biological aspects of the treated 1St nymphal instars of *H.littoralis* after feeding on the treated diet for

five days then on untreated diet till reached the adult e . Data shown in Table(2) cleared that there were no statistical significance fferences for the 1^{St} , 2^{nd} , 3^{rd} , 4^{th} and 5^{th} nymphal periods of all extracts and the control , the total nymphal period lasted 45 – 48 days for the treated diet while it was 41 day for the control . Preoviposition , Oviposition and po toviposition periods have not significantly different for *E. pulchrrima* and *D. vescosa* , while adults that resulted from nymphs fed on treated diet with LC_{50} of *E . cotimfabia* extract could not laid egg and completely inhibit reproduction . This may be related to the hormonal effect of the extract on the reproductive system of the insect. Passerini and Hill (1993) [18] working on the locust , *Kraussaria angulifera* , reported that effect of neem can be displayedas growth regulators activity ,causing difficulty and inhibition in molting or incomplete sclerotization. Mohammed and EL-Gammal (2002) [19] reported that azadirachtin caused prolongation in duration of the last instars nymphs of *S.gregaria* .

The number of female fecundity greatly significantly decreased for *E. pulchrrima* 58.6 and *D.vescosa* 54 than the control 151 egg/ female, respectively. Adult longevity significantly affected by the treatments of all extracts than the control , they lasted 90 days for the control decreased to 63 – 64 day for treated diet . Mortality percent through the life span for all extracts treated diet reached to 46 -56 %at different stages (from 1^{St} nymphal instars till adult stage) , malformation reached to 42-54% of the treated diet . Percentages of egg hatchability significantly reduced for *E.pulchrrima* and *D. vescosa* comparing with the control ,they were 46.6 %, 52.2% and 93.8% respectively . Life span also affected by feeding on diet mixed with LC_{50} of the tested extracts, for the control diet it lasted 148.6 days redused to 111.4, 128 , 129.8 days for *E. cotimfabia , D.vescoca* and *E . pulchrrima* , respectively .

From the foregoing results it could be concluded that 85 % ethanolic extract of E. *pulchrrima* , *E. cotimfabia* and *D. vescosa* had promising effect in disrupting the development and metamorphosis of *H. littoralis* . Our results agreed With that mentioned by Schwartz (1965) [20] on *Hippelates pusio* after tepa treatment , on *Poecilocerus pictus* after chemosterilant treatment , Valbonesi *et al* (2007) [21] on the biting louse *Damalinia limbata* after treatment with the botanical insecticide neem Azal , on *Chrotogonus terachypterus* after treatment with cypermethrin (Shakeet and ; 2009) [22] . Ghazawi (2005) [23] recorded that lower doses of azadirachtin treatment the4th, 5th and 6th nymphal instars of *H . littoralis* . caused prolongation of the insect duration , leading finally to death . In few cases malformed adults were able to emerge.

Morphological changes in the reproductive system:

All five tested extracts caused some histological cha s on the reproductive system of *H. littoralis* nearly by the same way .

D. vescoca **extract was selected for studying the effect of its sub lethal concentration (LC_{50}) on the histological changes in the reproductive system of the adults that resulted from the treated 1St nymphal instars after fed on the treated diet . A considerable reduction in the size of female ovaries (Fig 2B) and male testes (Fig 3B) was observed comparing with the control ones (F2A& 3A) , morphological changes of the ovary was noticed more than that of the testis . The ovary and testis were irregular in shape their white color turned to yellow as a result of shrinkage or rupture o the external sheath , all gonads become fused , and appears as an undifferentiat mass . The impact on the gonads may lead to a completely destroyed and thin , in turn , could lead to incomplete mating , non – production of eggs or sperms and consequently complete the sterility .Size of gonads of male and female were affected in fruit**

flies , melon and oriental fruit flies , when steriliz with tepa (Irving *et al* , 1985) [24] . Medina *et al* . (2004) [25] reported that azadirachtin affect the ovarioles of *Chrysoperla carnea* (Neuroptera : Chrysopidae) , thus growing follicle in treated female were significantly smaller an that those of the control .

Histological changes in female ovary .

The female has a pair of white ovaries each ovary is composed of panoistic type of egg tubes called ovariole . An ovariole differentiated into four zones , the terminal filament is composed of stand of cells containing prominent nuclei Germarium occupied by the oogonia which are different ated into young oocyte and mass of prefollicular tissue scattered along the periphery . various stages of oocytes development occurred in vitellarium Pedicel is the basal portion of the ovariole and thin walled fine duct leading to the uct . Histological changes were observed in the treated ovariole , follicular epithelial cells degenerated and became thick and multiplied to fill the ovariole cavity , appearance of vacuoles (Fig 8) , damaged of yolk droplets, contracting ooplasm forming vacuoles and spaces, contracting nucleus cells (Fig 9) comparing with the control ovariole (Fig 5 , 6 , 7) .

The same observations were recorded by Nath *et al* . (1975) [26] in *Locosta migratoria* after treatment with tepa , Saxena and Aditya , (1974) [27] in *Poecilocerus pictus* after treatment with some chemosterilants , and in *H . littoralis* after treatment with azadirachtin (Ghazawi et al, 2007) [10] .

Fig 9 cleared partially abolished Oocyte growth and clumping as a few eggs were developed , damage to the oocytes probably as a result of inter rence with the vitellogenesis process . This may eventually explain blocking of developmental process of ovarioles and consequently the shrinkage of ovary .

Contracting and disappear nucleus , damage of the ooc e and space around them , vacuoles appeared inside it were also observed . The oocyte changed in shape (Fig 10, 11) , some of them were clumped together (Fig 9) .The oocyte cytoplasm was not homogeneous , rupture muscular layer lined the ovariole (Fig 12) .

Histological changes in male testes .

The male reproductive system of *H. littoralis* *is* composed of pair of testes each consisted of a large number of slender tubule called follicles . A testicular tubule may be differentiated into four zone the Germarium occupied by spermatogonial cells (Fig 13) , zone of growth filled by spermatocytes (Fig 13 & 14) , zone of maturation contained spermatids and zone of transformation in which spermatids developed into spermatozoa (Fig 15) .

In the treated testes there were loosing in germ cells and formation of spaces occurred in the spermatogonial zone (Fig 16) . In growth and maturation zone Spermatocytes showed ubnormal shape and disruption , disintegration of cytoplasm forming vacuoles , reduction division and disruption in spermatocytes , some elongate cells were observed (Fig 17) . In transformation zone (Fig 18 , 19 , 20 , 21) spermatids observed clumping in irregular shape formation vacuoles and spaces increased , hyper plasia and some pycnotic cells noticed in (Fig 20) , clumping of ubnormal spermatids and spermatozoa in (Fig 21) disruption in all zones of the testicular tubule occurred (Fig 22) comparing with the control (Fig 15) . The hypertrophied spermatozoa are called elongate cells , these elongated cells suggest physiological inactivation of spermatozoa . These histological changes in the *H. littoralis* gonads when the 1^{St} nymphal instars fed on diet mixed with the tested extracts mainly affe the earlier stages of development which severely damaged and reflect directly on

the adult gonads and its fertility . We could concluded that the tested plant extracts could be utilized in the control of *H .littoralis* grasshopper effectively .

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