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Extract Bioefficiency of Five *Euphorbia* spp. (Euphorbaceae) on Crimson-Speckled Moth, *Utetheisa pulchella* L. (Lepidoptera, Acritidae) Growth and Development

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ABSTRACT

In the course of screening for novel naturally occurring insecticides from plants, the ethanol extract of the Arial parts of 5 *Euphorbia* weeds was found to show insecticidal activity against *Utetheisa Pulchella* L. Extracts from *E. granulata* L., *E. petiolata* L., *E. peplis* L., *E. nutans* Lag. and *E. heliscopia* L. possessed bioinsecticidal activity against immature stages with LC_{50} values between 84.0 and 480 ppm. Although all could be viable insecticides, extracts of *E. granulata*, *E. petiolata* and *E. peplis* are by far the most likely to succeed.

Significant observations about wings malformations were reported, they varied between fair and heavy curly wings and eclusion failure. Histological effect of the applied *Euphorbia* extracts was tested by *E. granulata* extract, the extract induces separation of peritrophic matrix and necrosis of epidermal cells. The extract affects the metabolic activities of prepupa midgut which is represented by notable variation in chromatin colour of epidermal nuclei.

Keywords: *Euphorbia* spp., *Utetheisa Pulchella*, mortality, malformations, sensitivity.

تأثير مستخلص خمسة أنواع من الجنس *Euphorbia* (Euphorbaceae) في تطور ونمو العثة *Utetheisa pulchella* L. (Lepidoptera, Acritidae)

الملخص

من خلال التوجه إلى مسح النباتات للحصول على مبيدات حشرية طبيعية مستخرجة منها، فقد كان المستخلص الأيثانولي للأجزاء الهوائية لخمسة أدغال من جنس أم الحليب (العائلة السوسبية) ذات فعاليـة أباديـة لعثـة الحنـاء *Utetheisa pulchella*. كانـت تراكيـز مستخلـصات كـل مـن *Euphorbia granulata* و *E. petiolata* و *E. peplis* و *E. nutans* و *E. heliscopia* التي تسببت في قتل 50% من الأطوار غير البالغة للآفة قد تفاوتـت قيمتها بين 84.0 إلى 480.0 جزء في المليون. وعلى الرغم من فعالية هذه المستخلصات جميعاً، فان مستخلصات *E. granulata* و *E. petiolata* و *E. peplis* كانت ذات فعالية أكثر لاستخدامها بنجاح.

سجلات ملاحظات مميزة بالمقارنة مع المجموعة الضابطة عن تشوه الأجنحة بدرجات من التجعـد وفـشل البزوغ. أما التأثير النسيجي لمستخلصات نباتات أم الحليب، فقد اختير منها مستخلص *E. granulata*، وقـد أحـدث المستخلص انفصال غشاء حول الغذاء وتآكل خلايا البشرة في القناة الهضمية الوسطى في اليرقات. وكان من تـأثير المستخلص في طور قبل العذراء تغير لون المادة الكروماتينية، إذ يكون اللون عميقاً في المجموعة الـضابطة وازرق فاتح في مجموعة المعاملة.

الكلمات الدالة: *Utetheisa Pulchella*, *Euphorbia* spp.، نسبة القتل، التشوهات، الحساسية.

INTRODUCTION

The injudicious use of chemical pesticides has resulted in multiple problems such as increase in the insect resistance to insecticides, emergence of new pests, minor pestes becoming major pest and pesticide pollution to the environment (Kannaiyan,1999). Many plants provide an alternative to currently used pesticides for the control of plant pests, and they have bioactive chemicals (Kim *et al.*, 2005). Certain plants by nature act as antifeedants, oviposition deterrents, ovicidal, inhibition of egg hatchability, larvicidal and insect growth regulators (David, 2008). Jacobson (1982) stated that Dioscorides (A.D.4 0–90) listed the juices of *Euphorbia* spp. as plant poisons. Many *Euphorbia* spp. are used in folk medicine and over the past twenty years, they have received considerable phytochemical and biological attention (Shi *et al*., 1999; Wu *et al.*, 2009).

The family Euphorbaceae (Spurge family) consists of 300 genera and 5000 species (Webester,1994). The genus *Euphorbia* is the largest genus in the family Euphorbaceae with over 2000 species (Jassbi, 2006). The Euphorbaceae is well known in Iraq and represent 5 genera and 46 species, *Euphorbia* genus is dispersal with more 40 wild species, the famous species are *E. heliscopia, E. peplis, E. prostarta* and *E. granulate*, all species of *Euphorbia* are the very broad Arabic names; the lubbaina, om alhalib, Khanag aldajaj… because of their poisonous milky latex which protects these plants from herbivorous animals(AL-Musawi, 1987; Al-Sultan and Hussein, 2006; Haba *et al*., 2009).

Euphorbia extracts possess some insecticidal activities, Vanderplank (1945) study their effect on adult Diptera, *E. peplus* extract was proved to be toxic to a mosquito larvae (Gayar *et al*., 1971). *E. fischeriana* has antifeeding activity against stored-product insects (Geng *et al*., 2011). *E. heterophylla* extract affects the Bonacroftian filariasis vector; *Culex quinquefasciatus* (Kuppusamy and Murugan, 2008).

Numerous ingredients had been isolated from *Euphorbia* species extracts, Jain *et al*., (2008) and Al- Younis and Abdullah (2009) identified flavinoids and phenolic acids from several species of *Euphorbia* genus including *E.granulata* and *E.heliscopia*. Different triterpenoids and diterpenoids were isolated from various *Euphorbia* species (Kurpadanam and Srim, 1999; Shizuri *et al*., 1983 ; Abdel-Monem *et al*., 2008).

Crimson-speckled moth, *Utetheisa pulchella* (Acritidae) is a common migrant pest between plains and middle heights in Iraq, it has been known to attack cultivated plants and may defoliate their host plants with multiple generations (Wiltshire, 1957). *U. pulchella* is

polyphagous leaf feeder pest and its host plants range are: *Heliotrobium ramosissimum* (Boraginaceac), *Launaea cassiniana* (Asteraceae), *Gossypium* sp. (Malvaceae), *Ricinus communs* (Euphorbaceae), *Lawsonia incamis* (Lgthraceae), *Medicago sativa* (Fabaceae) and the host plants of solonaceae are *lycopersicum esculentum, Solanum melongenia* and *Wathania somnifera* (AL-Ahmadi and Salem, 1995).

In the present study an attempt has been made to develop a new botanical insecticide throughout evaluating the insecticidal activity of five medicinal plants from euphorbian family namely *E. granulata, E. petiolata, E. peplis, E. nutans and E. heliscopia*.

MATERIALS AND METHODS

Insects

In the late August, the larvae and egg patches of the migrant crimson-speckled moth, *Utetheisa pulchella* was seen in the fields near Mosul city/ north Iraq. They were observed on helitropic weed, *Heliotropium* spp. and clover, *Medicago sativa.* Parts of the host plant with pest larvae and egg-patches were collected and introduced to the laboratory and incubated eggs and the growing larvae kept in the incubator under 27±1ºC, relative humidity 65±5 and photoperiod 8:16. For refresh the host plant foliages, they were replaced every two days.

Extraction Method

All the *Euphorbia* spp. in this study are herbal weeds, the whole plants were collected through May till July and dried in a shadow place, then, crushed and grounded with electric mill. 50 gm of each powdered plant species were macerated with 150ml of 96% ethanolic alcohol for 48 hrs., then, the extract was filtrated with goose cloth, as well as filter papers No.1 for two times. By rounding; the solvent was released. For chlorophyll separation as possible, the ethanolic extract was dissolved again with v/v petroleum ether and ethanol in the separation funnel and repeated for two times. The ethanol extract for each *Euphorbia* sp. was rounded and dried until experimental treatment, the stock solution was prepared in 1000 ppm with ethanol solvent. Extraction was modified after Kaushik and Siani (2008).

Design of the experiments

New foliages of the wild host plant, *Heliotropium ramosissimum* were dipped in the applied concentration for 20 sec. and dried in open place, then, the treated foliages implanted in 100 cm^3 bottles filled with distilled water. To prevent water from evaporation, the bottles were sealed with cotton plugs, the bottles were kept in 500 ml flasks to prevent larvae escaping, the flask's opening was shut with a goose cover.

According to the primary experiments, the following concentrations: 40,80,200 and 300 ppm were chosen, the experiments were designed with three replications for each concentration and five of the pest larvae transformed for each replicate. Fourth instar larvae were applied to the plant extracts.

After seven days of exposure time, dead and alive larvae are counted and the alive ones kept to complete their development for separating normal and malformed adults. In addition to dead larvae, dead prepupae, pupae and permanent larvae are represented dead immature stages and total mortality.

Histological preparation

To investigate the extract effect on the midgut of the immature stages, the 7 day 5th instar treated larvae and prepupae were fixed in Boin solution for six days. Slides preparation and staining had been done after Mekhlif (2004).

Sensitivity ratio

Sensitivity ratio is equal 1.0 for the lowermost Lc_{50} plant extract or positive control. Then, relative sensitivity for all the last plant extract by consider any one of them as denominator and Lc_{50} for the lowermost numerator (Mekhlif, 2004).

Statistical analysis

Mortality of the treated pest was corrected using Abbott´s formula (Abbott, 1925), Lc_{50} (Lethal concentrations causing 50% mortality) was calculated using probit analysis (Finney, 1971). Data from dead immature stages were subjected to analysis of variance, two-way ANOVA. Also Duncan test for multiple comparisons is carried out.

RESULTS AND DISCUSSION

Bioactive effect of extracts

Mortality

In table 1, death of the immature stages (larvae, prepupae and pupae) of the pest *Utetheisa pulchella* were significantly increased as the concentration of the applied euphorbian leaf extract and positive control increased from 40-200 ppm, but immature stages death were not significantly different as the concentration increased from 200 to 300 ppm. However, *Euphorbia* spp. extracts and the positive control (neem oil) were behaved as phytoecdysteroid hormones with sigmoid curves, whereas, calibration curves were resulted through application of the synthetic insecticides (Baker *et al.,* 2000; Mekhlif, 2007).

Moreover, the death of the immature stages and their mortality percentage were depended on *Euphorbia* species (Table 1 and 2). Thus, extracts of the weeds *E. granulata and E. petiolata* can be canditated for preparing effective bioinsecticidies

Sensitivity ratio

It was found that the mortality of the pest varied according to the extract source. Table 2 and fig. 1 were illustrated this result, neem oil induces growth disturbances as insect growth regulator (Zebitz, 1984). Fundamentally to comparison between Schmutterer and Sing (1995) and the present plates, it can be reported that the active metabolites in *Euphorbia* spp. extracts are insect growth regulators.

Table 2 shows the differences in phytoinsecticidal activity between the extracts of *Euphorbia* spp. However, *E. nutans* and *E. heliscopia* are nearly 10 times less toxic than neem oil. Also, neem oil more toxic than *E. granulate, E. petiolata* and *E. peplis* by 2 , 3 and 5 folds receptivity.

Table 1: Number of *Utetheisa pulchella* immature stages death, their host plant is treated with *Euphorbia* species.

Plant extract	Concentration (ppm)				
	40	**80**	**200**	**300**	**Mean**
E. heliscopia	1.0 h-j	1.33 g-i	2.0 e-h	2.0 e-h	1.58 D
E. nutans	0.67 i-k	1.67 f-h	2.33 d-g	2.33 d-g	1.75 CD
E. peplis	1.33 g-i	2.0 e-h	2.33 d-g	3.0 b-d	2.17 C
E. petiolata	1.67 f-h	2.67 c-f	3.33 a-d	3.33 a-d	2.75 B
E. granulata	2.0 e-h	2.67 c-f	3.33 a-d	3.67 a-c	2.92 B
Neem oil	2.67 c-f	3.33 a-d	4.0 ab	4.33 a	3.56 A
Control	0.33 j	Zero k	Zero k	Zero k	0.08 E
Mean	1.38 b	1.95 ab	2.48 a	2.67 a	

* Each replicate 5 larvae.

** Numbers with the same letters are not significantly different ($p>0.05$, Duncan's multiple range test).

Table 2: Immature stages mortality percentage and sensitivity ratio, host plant of the pest, *Utetheisa pulchella* applied with *Euphorbia* spp. extracts.

Plant extract	Mortality at the concentrations (ppm)				Lc_{50}(ppm)	Sensitivity ratio to Neem oil
	40	**80**	**200**	**300**		
Neem oil*	49.9	64.3	78.6	85.7	42.0	1.0
E. granulata	35.7	49.9	64.3	71.4	84.0	0.5
E. petiolata	28.5	42.9	57.1	57.1	135	0.31
E. peplis	21.4	35.7	42.9	57.1	240	0.18
E. nutans	7.1	28.5	42.9	42.9	420	0.1
E. heliscopia	14.3	21.4	35.7	35.7	480	0.09

* neem oil is positive control.

Histological effect

To evaluate the effect of the *Euphorbia* spp. extracts on the internal organs and morphology of *U. pulchella*, the extract of *E. granulata* as more effective was chosen. For this purpose, sections of the midgut were prepared:

Larva midgut

One of the first systematic effects of the bioactive metabolites of the extract is the gradual histolysis of epidermis cells and peritrophic matrix of the midgut. Priorly, peritrophic matrix separates from the epidermis and it lyses later by increasing the concentration to 200 ppm (Fig. 2). Peritrophic matrix is permeable to the nutrients forward the epidermal cells, and protects them by avoiding the fraction with food particles

(Wigglesworth, 1974). Also, the extract gradually degrades the epidermal cells. Therefore, food absorbance is significantly decreased and that will affect the pest metamorphosis and growth parameters.

Prepupa midgut

The present study is the first attempt to emphasize the internal organs of prepupal stage, due to the importance of this stage in Noctuid family. However, full grown larva prevents feeding and seeks for substratum shelter for contrast pupal cell, through that time and later prepupa passes with dramatic changes before pupation. Effect of sublethal concentration of the extract transforms into prepupa. Therefore, it was found bioactive molecules of the extract interferes with cellular function of midgut epidermis. Fig. 3a represents section in control prepupa midgut which indicates an active protein synthesis through dense chromatin staining of the nuclei of the epidermal cells, in comparison pale colour staining in Fig. 3b.

CONCLUSIONS

In view of the results mentioned above, it was concluded that plants produce diverse phytochemicals which mostly secondary metabolites synthesized by the plant for defensive purposes. These phytochemicals were applied to IPM programmes through direct death and interfere with growth and development (Rembold, 1995).

The investigated *Euphorbia* spp. are weeds and can be easily cultivated with high production with low agricultural operation. the spurge plants; *E. granulate, E. peplis* and *E. nutans* are endemic weeds in farms, whereas *E. heliscopia* and *E. petiolata* are grown in rain fed aired area.

Foliages treatment with *Euphorbia* spp. extract is reducing *Utetheisa pulchella* population by significant mortality of immature stages and induction of adults malformation (Fig. 4). The present finding gives promise for development of new generations of unharmful and degradable bioinsecticides.

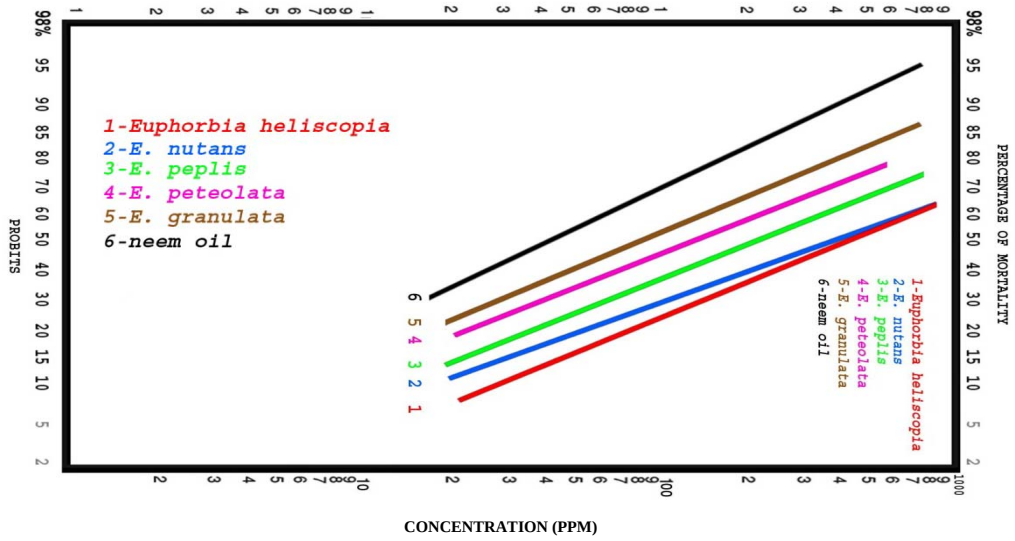

Fig. 1: Toxicity of the extracts of five *Euphorbia* spp. against the pest *Utetheisa pulchella* L. in compared with neem oil.

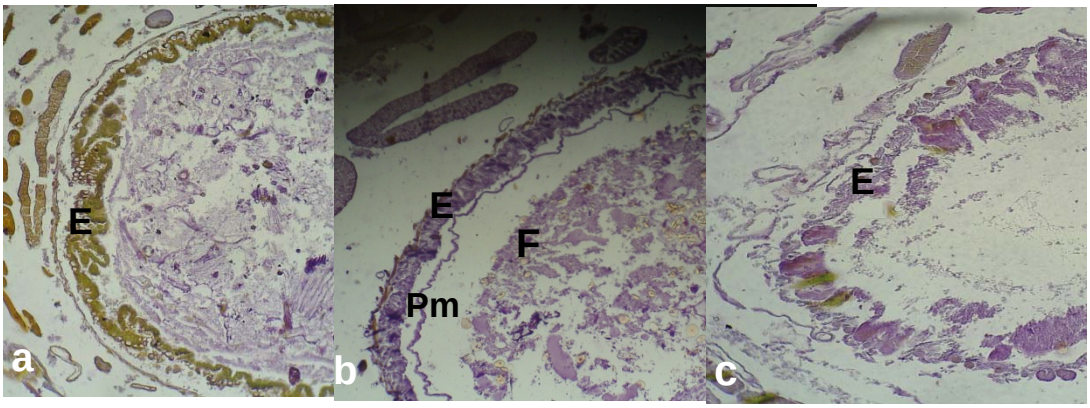

Fig. 2: Light microscope section through the midgut of five- day 5th instar *Utetheisa pulchella* larva: a – untreated control, b- treated with 40 ppm *Euphorbia granulate* extract, C-treated with 200 ppm, E – epidermis, F- food, Pm – peritrophic matrix. 100x.

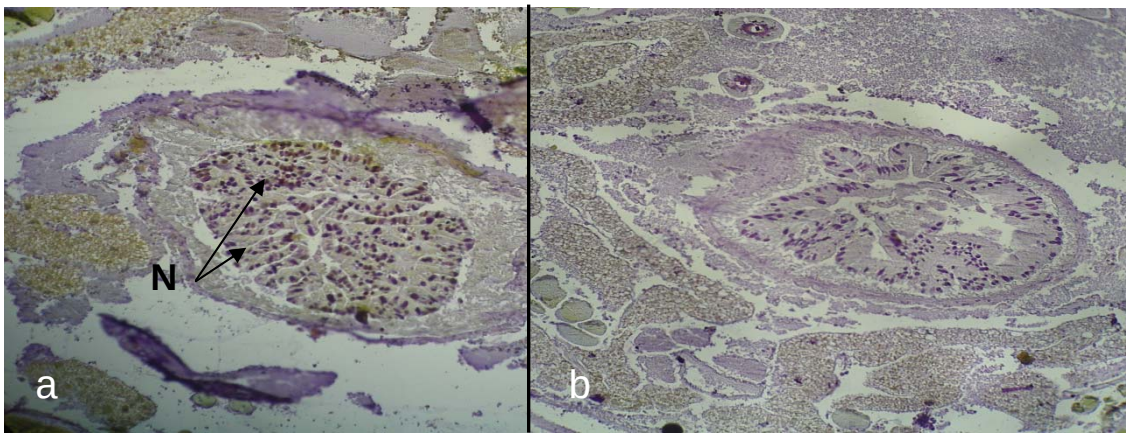

Fig. 3: section of *Utetheisa pulchella* prepupa midgut, diet in the larva stage treated with 40 ppm *Euphorbia granulate* extract, a- control, b- treated, N- nuclei of epidermal cells. 100x.

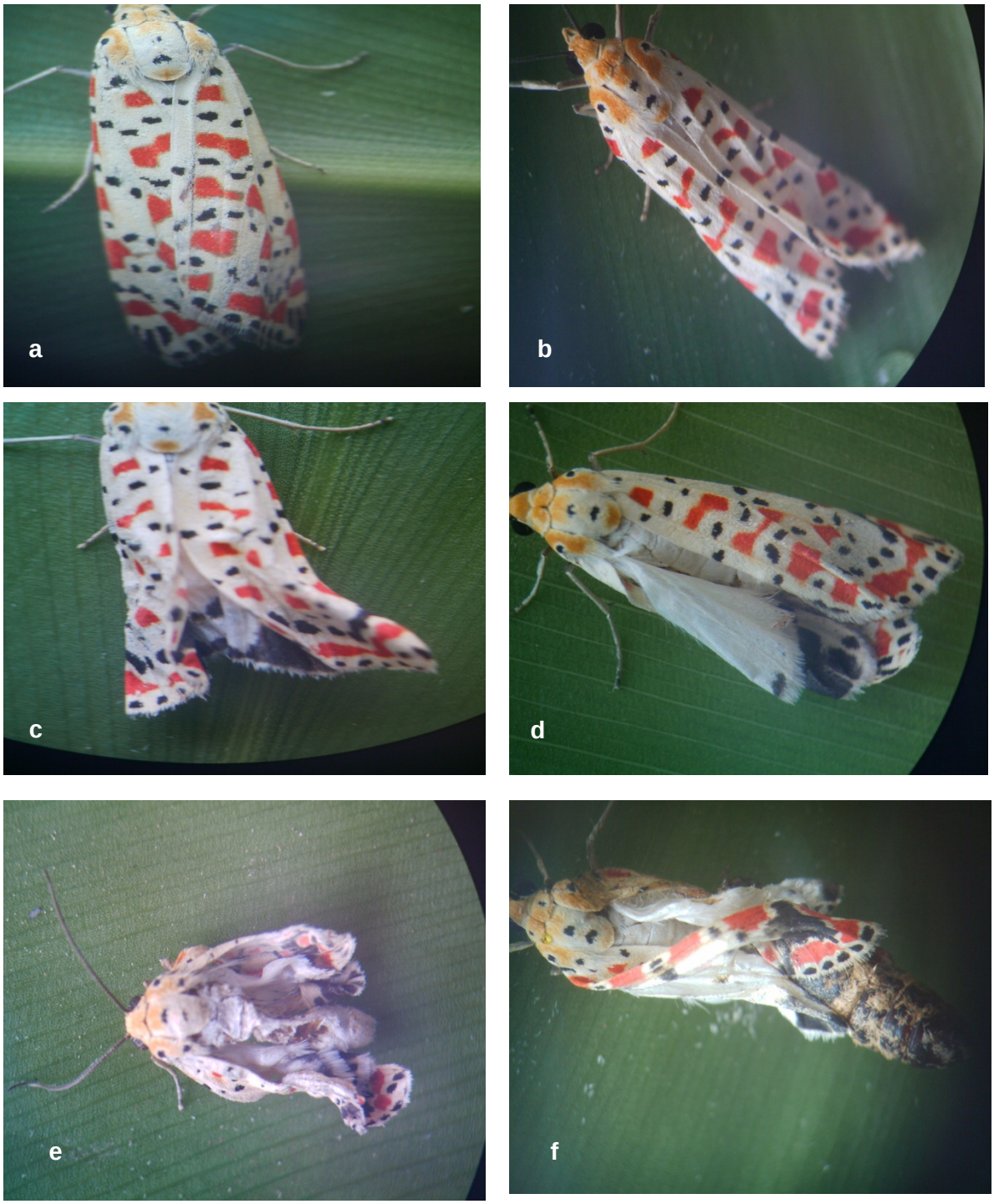

Fig. 4: Adults of *Utetheisa pulchella* cannot close their wings completely, they developed from larvae treated with 40 ppm *Euphorbia granulate* extract a- normal closed wings, b , c- mild corrugated, d- diverged wings, e- very curly wings , f- eclusion failure. 4x.

REFERENCES

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol*., **18**, 265-267.

Abdel-Monem, A.R.; Abdel-Sattar, E.; Harraz, F.M.; petereit, F. (2008). Chemical investigation of *Euphorbia Schimperi* C- Presl. *Res. Nat. Prod*., **2** (2),39-45.

AL-Ahmadi, A.Z.; Salem, M.M.(1995). "Entomofauna of Saudia Arabia". Part II Checklist of Phytophagous Insects. Academic Pub. and Press, king Saud University Press. pp. 181-310.

AL-Musawi, A.(1987). "Plant Taxonomy". Mosul University. Press, Iraq, pp.230-232.

Al-Sultan, S.I.; Hussein, Y.A. (2006). A cute toxicity of *Euphorbia heliscopia* in rats. *Pak. J. Nutr.,* **5** (2), 135-140.

AL-Younis, N. K.; Abdullah, A. F. (2009). Isolation and antibacterial evaluation of plant extracts from some medicinal plants in Kurdistan region, *J. Duhok University*, **12**(1), 250-255.

Baker. K. D.; Warren, J.T.; thummel, C.S.; Gilbert, L.I.; Mangelsdorf, D.J.(2000). Transicriptional activation of the *Drosophila* ecdyson receptor by insect and plant ecdysteroids. *Insect Biochem. and Molecular Biol.* **30**,1037-1043.

David, V. (2008). Biotechnological approaches in IPM and their impact on environment. *J. Bioinsecticides,* **1** (1),1-5.

Finney, D.J. (1971). "Probit Analysis :A Statistical Treatment of Sigmoid Response Curve " 3rd edn., Cambridge Univ. Press, Cambridge, UK, pp. 25-353.

Gayar, F.H., Shazli, A.Y. and Abbassy, M.A. (1971). Toxicity of *Euphorbia peplus* L. (Euphorbaceae) to insects. *J. Appl. Entomal.*, **68**, issue 1-4, pp. 63-65.

Geng, Z. F.; Liu, Z. L.; Wang, C. F.; Liu, Q. Z.; Shem, S.M.; Lin, Z.M.; Du, S.S.; Deng, Z.W.(2011). Feeding deterrents against two grain storage insects from *Euphorbia fischeriana*. *Molecules,* **16**, 466-478.

Haba, H.; Lavau, C.; harkat, H.; Magid, A.A.; marcourt, L.; Benkhalid, M. (2009). Diterpenoids and triterpenoids from *Euphorbia guyoniana*. *Phytochem.*, **68**, 255-260

Jacobson, M. (1982). Plants, insects and man-their interrelationships. *Econom. Bot.*, **36**, 346-354.

Jain, R.; Jindal, C.; Sing, S.; Datta, A. (2008). Phormaceutical compositions comprising an extract of *E. prostrata* US patent/ patent no. US 7, 731,412 B2 May 13.

Jassbi, A.R. (2006). Chemistry and biological activity of secondary metabolites in *Euphorbia* from Iran. *Phytochem.*, **67**, 1977-1984.

Kannaiyan, S.(1999). Botanical in pest control. In augural address in the training programme on botanicals in pest management, Dec. 1-10, 1999, TN Agricultural University, Coimbatore India.

Kaushik, R.; Saini, P. (2008). Larvicidal activity of leaf extract of *Millingtonia hortensis* (Family: Bignoniaceae) against *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti. J. Vector Brone Dis*. **45**, 66-69.

Kim, H.G.; Jeon, J.H.; Kim, M.K.; lee, H.S. (2005). Pharmacological ectsofaseron aldehyde isolated from *Acorusgram eusrhizome*. *Food Science Biotech.,* **14**,685-688.

Kuppusamy, C.; Murugan, K. (2008). Mosquitocidal effect of *E.heterophylla* L. against the banacroftin filariasis vector; *Culex quinquefasciatus* Say (Diptera: Culicidae). *Int. J. Integrative Biol*., **4** (1), 33-39.

Kurpadanam, V.G.; Srim, G. (1999). Cyclonivulialol a new cycloartane epoxy triterpenoids from *Euphorbia buch-ham*. *Indian J. of Chem.,* **30**,989-993.

Mekhlif, A. F. (2004). Effect of chinaberry tree, *Melia azedarach* L. extraxt on the reproduction and development of the sugar beet. Armyworm, *Spodoptera exigua* (Hub.) (Lepidoptera: Noctuidae). Ph.D. thesis, College of Science, Mosul University (in Arabic).

Mekhlif, A.F. (2007). Efficiacy of enriched *Melia azedarch* L. extract on immature stages of the pest *Spodoptera cilium latebrosa* (Guerine) (Lepidoptera: Noctuidae). *J. Pharaceutical Sci.,* **3**, 63-68.

Rembold, H. (1995). Growth and development. In: Schmutterer, H.(ed). (1995). "The neem tree-source of unique natural products for investigated pest management, medicine, industry and other purposes". VCH verlagsgesells chaft mbH, D-69451 Weiheim. pp. 177-194.

Schmutterer, H.; Singh, R. (1995). List of insects susceptible to neem products. In: schmutterer, H.(ed). (1995). "The Neem Tree-Source of Unique Natural Products for Integrated Pest Management, Medicine, Industry and Other Purposes" VCH , Weinheim. pp. 330-351.

Shi, Y.P.; Jai, Z.J.; Ma, B.; Saleh, S.D.; Laham, J. (1999). Ingenane diterpenes from *Euphorbia petiolata*. *Plant Med*., **62**, 260-262.

Shizuri, Y.; Kosemura,S.; Yamanura, S.; Ohba, S. I. M.; Saito, Y.(1983). Isolation and structures of heliscopinolides, new diterpens from *Euphorbia heliscopia*. *Chem. Lett*., **12**, 65-68.

Vanderplank, F.L. (1945). Insecticidal properties of *Euphorbia* extracts. *Nature*, **156**,182.

Webester, G. (1994). Systematic of the Euphorbaceae, *Introdut. Annal. Miss. Bot. Gard*., **81**, 33-144.

Wigglesworth, V.B. (1974). "Insect Physiology". 7th edn., Chapman and Hall, London, pp. 50-52.

Wiltshire, E.P. (1957). "The Lepidoptera of Iraq". Addard and Son, limited Bartholomew Press, Dorking, 50p.

Wu, Q.C.; Tang. Y.P.; Ding, A.W.; You, F.Q.; Zhang, L.; Duan, J.A. (2009). C- NMR data of three important diterpenes isolated from *Euphorbia* species. *Molecules,* **14**,4445-4475.

Zebitz, C. P. W. (1984). Effect of some crude and azadirachtin- enriched neem) *Azadirachta indica*) seed kernel extracts on larvae of *Aedes aegypti*. *Entomologia experimentalis et applicata*, **35**, 11-16.