

CONTROL OF *EPILACHNA VIGINTIOCTOPUNTATA* FAB. (COLEOPTERA: COCCINELLIDAE) USING SOME INDIGENOUS PLANT EXTRACTS

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Abstract: *Epilachna vigintioctopunctata* is an important pest that causes considerable economic losses to many crops including egg-plants. The crude aqueous extracts of leaves from three indigenous plants namely *Ricinus communis*, *Calotropis procera* and *Datura metel* were used against this beetle aiming at its control under laboratory conditions. Larvicidal bioassays of the extracts showed the following order of toxicity: *R. communis* (LC₅₀=18.40%) > *C. procera* (LC₅₀=23.70%) > *D. metel* (LC₅₀=29.61%). Subsequent data on some vital life-history traits were promising because the extracts significantly reduced both oviposition and egg-hatch, prolonged larval duration (P<0.001), and inhibited pupae formation and adult emergence (P<0.05). However, female ratio was not significantly affected by the treatments. Relevance of these findings on the control of this phytophagous species has been discussed.

Key words: *Epilachna*, life-history traits, indigenous plants, aqueous extract, phytochemicals.

mi vsk: *Epilachna vigintioctopunctata* n^o te_b M^omn A_b ZK f^ote_b i^o Z^oY^oneif b^oed m^o j i G^oK^oU A^oib^oo^oK^ov^oi^ox c^oZ^o½ M^otel Y^oM^oti GB c^oZ^o½ i^ob^oq^os^of^oY^oi D^of^oi^o t^ok^o w^oZ^ob^oi^oU^o t^o k^oq^o D^ow^o h^oY^o: *Ricinus communis* (t^oi^oo), *Calotropis procera* (A^ov^oK^o) I *Datura metel* (a^oy^oZ^oi^ov)-G^oi c^oi^oZ^ov^oi i^ob^oh^of^om^o g^oj^o v^oq^ob^o K^oi^ov n^ot^oq^ot^o, t^oh^o t^oj^o v^oi LC₅₀ μg 18.40% > 23.70% > 29.61% | c^oo^oB d^oj^o v^od^oj n^ot^oZ t^o L^ov h^oq^o t^oh, e^oe^ou^oZ i^ob^oh^of^om^o t^oj^o v^o R^ox^oe^ob-P^ot^oi^o i^o n^oe^of^ob^oe^oc^oh^oe^ot^oq^o t^oh^og^o w^og^o c^ov^oo^o v^o i w^og^o c^ou^oi^o U^ot^ob^oi n^ov^oi Z^oi^or^oc^oh^oe^oY^of^ote^o K^og^ov^oq | G^og^ob^ou^oK^o j^o v^of^op^o k^ov^o n^o N^oh^oq^o Z^oK^oi, j^o v^of^op^o n^ot^oZ w^oc^oD^oc^ov %Z^oi^ox G^oe^os c^oY^o½ c^oZ^o½ c^ou^oi Y^oZ n^ot^oZ e^ov^oa^oi m^oo^o K^ot^oi | Z^ot^oe^o g^o A^ob^oc^ov^ot^oZ^oi t^oq^ot^o A^ov^ot^oj v^op^o i^ob^oh^of^om^o t^oj^o v^oi t^oZ^og^o t^oK^oi^ob^o M^oi^oZ^oY^oc^of^ote^o t^o L^ov h^oq^ou^ob | e^oZ^og^oi^ob M^otel Y^oM^oti d^oj^o v^od^oj GB q^ou^oZ^oK^oi c^oZ^o½ u^oi i^ob^oq^os^of^oY^o i^ok^of^ote^o m^oe^ou^o,^o Z^ov A^ov^ot^oj v^oP^ob^ov K^oi^ov n^ot^oq^ot^o |

Introduction

Indiscriminate use of pesticides for insect pest management results in various environmental and ecological problems such as pest resistance and outbreak of secondary pests (Hagen and Franz 1973), long persistence, bio-accumulation and health hazards (Bhaduri *et al.* 1989) and environmental pollution (Devi *et al.* 1986; Fishwick 1988). Substitutes are being strongly conceived whereby researchers are now paying much emphasis on the biologically active indigenous plant products because they are environmentally safe, biodegradable and cost effective (Saxena *et al.* 1983; Ghani 1998). Plant extracts contain botanical insecticides or phytochemicals that could be used to repel, deter feeding, or limit reproduction and survival of various insect pest species including coccinellid ladybird beetles (Rajagopal and Trivedi 1989; Mondal and Ghatak, 2007; Ghatak and Mondal 2008; Swaminathan *et al.* 2010).

The 28-spotted potato ladybird or Hadda beetle, *Epilachna vigintioctopunctata* Fab. (syn. *Henosepilachna vigintioctopunctata* Fab.) (Coleoptera: Coccinellidae), is one of the devastating pests of

vegetable crops in Bangladesh (Alam 1969). It is fairly common throughout the country and causes considerable damage to a number of solanaceous, cucurbitaceous and leguminous crops (Anam *et al.* 2006; Rahaman *et al.* 2008). Using various plant extracts, attempts have been made to save such crops as potato (Rajagopal and Trivedi 1989), brinjal (Sreedevi *et al.* 1993; Ghatak and Mondal 2008), oilseed (Ahmed 2007; Ahmed *et al.* 2010), cucumber (Mondal and Ghatak 2007; 2009) and bitter gourd (Rahaman *et al.* 2008) against the attacks of the beetle.

Various plant parts possess defense chemicals that play roles against insect herbivores (Konno *et al.* 2006; Ali *et al.* 2011). Ahmed (2007) reported that aqueous extract sprays of the castor oil plant *Ricinus communis* L. (F. Euphorbiaceae) reduced *Epilachna* attack on foliage and capitulum of sunflower *Helianthus annulus* and consequently increased the oilseed crop. Subsequently, methanol extracts of *R. communis* were found to have larvicidal properties against the mosquitoes *Aedes aegypti* (Zahir *et al.* 2009) and *Anopheles arabiensis* and *Culex quinquefasciatus* (Elimam *et al.* 2009a).

Aqueous extracts of the milkweed *Calotropis procera* (Aiton) (F. Asclepiadaceae) were shown to have oviposition deterrant, larvicidal and ovicidal activities against mosquito (Singh *et al.* 2005; Kabir *et al.* 2011), reduced population build-up in *Henosepilachna elaterii* (Ahmed *et al.* 2006) and controlled *Anopheles* and *Culex* populations (Elimam *et al.* 2009b). While leaf extracts of the downy thorn apple *Datura metel* L. (F. Solanaceae) were found effective against the stored product beetles *Callosobruchus chinensis* (Rajapakse and Senanayake (1997) and *Trogoderma granarium* (Dwivedi and Kumar 1999). Keeping the aforesaid strategies in view, effects of crude aqueous extracts of leaves of the above three indigenous plants were assessed on the larvicidal bioassays and some vital life-history traits in *E. vigintioctopunctata*.

Materials and Methods

Test insects: Adults of *E. vigintioctopunctata* were collected from the leaves of three available egg-plants viz. *Solanum melongena* (Begun), *S. nigrum* (Futi Begun) and *S. indicum* (Tit Begun). The experimental beetles were identified by the presence of characteristic black spots on their elytra (Dieke, 1947). For stock culturing, techniques described by Ahmed (1983) were adopted. Newly emerged adults were mated singly in separate plastic containers (5cm × 7.5cm) that had fresh middle-aged leaves of the host plants as food. The containers were covered with fine muslin cloth to prevent escaping of the beetles. Third-instar larvae were transferred from the stock cultures into rearing cages (80cm × 45cm × 40cm) for emergence of the adults.

Test plants and their extracts: Considering the availability, handling convenience and efficacy, three species of indigenous plants viz. *C. procera*, *R. communis* and *D. metel* were selected for the present experiments. The plants usually grow abundantly in arid and semi-arid regions without irrigation, fertilizer, pesticide, or other agronomic practices. Identities of the plants were confirmed at the Department of Botany, University of Rajshahi, and voucher specimens have been preserved as herbarium sheets for future reference. Fresh and middle-aged leaves were collected from Rajshahi University Campus and around Rajshahi Metropolitan areas, brought to the laboratory, rinsed in tap water, cut into small pieces, and dried in the shade at ambient temperature (35±1°C) and uncontrolled relative humidity (75±5%) for about three weeks. The dried specimens were then ground with the help of an electric blender to form fine powder, sieved, sealed in reagent bottles and refrigerated at 4°C until extraction was made.

Crude extracts were made in distilled water as follows. For each plant, a stock solution was prepared by taking 10g of the leaf dust in a 250ml conical flask to which 100ml distilled water was added, soaked, and kept in an electric shaker for 24h. The foliage aqueous extracts thus obtained were filtered, sealed and refrigerated at 4°C until used. Initially a pilot experiment was conducted with the plant extracts to select the doses. Finally, the extracts were diluted volumetrically to obtain 10%, 20% and 30% solutions by pipetting 25ml, 50ml and 75ml stock solutions, respectively into 250ml distilled water in separate reagent bottles. For control groups, only distilled water was used.

Treatment protocol: For treatments *S. indicum* (F. Solanaceae) leaves were drenched into each dose of the extracts for a few minutes, dried at room temperature and placed on a piece of moistened sponge kept at the bottom of the plastic containers. For each dose and plant species, 20 third-instar larvae of *E. vigintioctopunctata* were placed on the treated leaves and the larvicidal effects of each extract were monitored by counting the number of dead larvae at 24h intervals up to three days (72h). Finally, the larvicidal efficacy was determined by the use of the median lethal concentration (LC₅₀) for each extract. To study the life-history traits, 10-day oviposition, egg-hatch (%), larval duration (days), numbers of pupae formed and adults emerged, and female ratio (*i.e.* number of females ÷ total number of adults) were recorded. Single pair-matings of newly emerged beetles were made in plastic containers. Each dose was replicated five times and controls were maintained for comparisons.

Statistical procedures: Cumulative larval mortality data recorded up to 72h post treatment were loaded on to the *GWBASIC* Probit Analysis software to determine LC₅₀ values, 95% fiducial limits (lower and upper) and regression equations. In addition, one-way ANOVA, followed by the least significant difference (LSD) tests using SPSS for Windows (version 11.5) were performed to analyze the significance of the data on life-history traits.

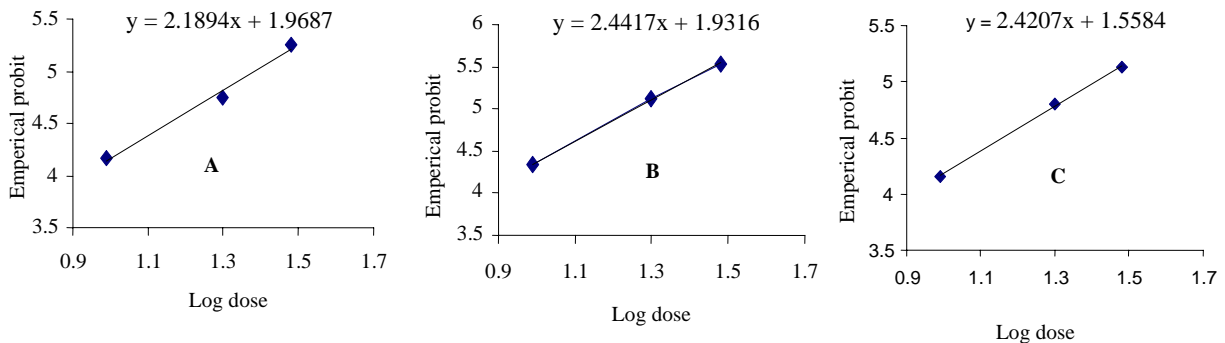
Results and Discussion

Larvicidal efficacy of the plant extracts

The LC₅₀ values from 72hrs dose-mortality data on the third-instar larvae of *E. vigintioctopunctata* were estimated to be 18.40%, 23.70% and 29.61% for *R. communis*, *C. procera* and *D. metel*, respectively (Table 1). Results indicate that *R. communis* was the most toxic whereas *D. metel* was the least toxic against the beetles. Dose-mortality responses of the three extracts are graphically represented in Fig. 1.

Table 1. Estimated LC₅₀ values, 95% confidence limits, regression equations and χ^2 values for the aqueous extracts of leaves from three plant species against third-instar larvae of *E. vigintioctopunctata*.

Plant species	LC ₅₀ (%)	95% confidence limits		Regression equations	χ^2 values (df)
		Lower (%)	Upper (%)		
<i>R. communis</i>	18.40	13.51	25.06	$Y = 2.1894x + 1.9687$	2.57 (1)
<i>C. procera</i>	23.70	16.36	34.34	$Y = 2.4417x + 1.9316$	9.66 (1)
<i>D. metel</i>	29.61	17.15	51.15	$Y = 2.4207x + 1.5584$	0.69 (1)

**Fig. 1.** Probit regression lines for the aqueous extracts of leaves of *R. communis* (A), *C. procera* (B) and *D. metel* (C) on the third-instar larvae of *E. vigintioctopunctata*.**Table 2.** Efficacy of the aqueous extracts of leaves derived from three plant species against some life-history traits in *E. vigintioctopunctata*.

Plant species (LC ₅₀) ¹	Oviposition*	Egg-hatch (%)	Larval duration (d)	Female ratio**
Control (0.00%)	37.87±5.46 ^a	67.59±2.47 ^a	13.20±0.17 ^a	0.50±0.05 ^a
<i>R. communis</i> (18.40%)	25.67±5.52 ^b	52.27±11.82 ^b	15.17±0.80 ^b	0.44±0.06 ^a
<i>C. procera</i> (23.70%)	20.93±1.86 ^c	62.10±9.35 ^b	13.53±0.80 ^a	0.50±0.04 ^a
<i>D. metel</i> (29.61%)	36.20±5.21 ^a	65.25±3.03 ^a	13.87±0.47 ^a	0.45±0.02 ^a
F-ratios (df= 2, 57)	11.72***	5.99***	9.57***	0.88ns

¹Estimates are shown in Fig. 1; *10-day egg-layings; All values are mean±SD of 5 replicates; Dissimilar superscripts indicate significant difference by LSD tests at $P < 0.05$; df= degrees of freedom; ***= $P < 0.001$; ns= not significant.

Effects of leaf extracts on life-history traits

(a) Oviposition: The oviposition of *E. vigintioctopunctata* females reared on the host leaves treated with the plant extracts reduced significantly ($F_{2, 57} = 11.72$; $P < 0.001$) compared to the control females (Table 2). The highest reduction in egg-laying was recorded for *C. procera* in a dose-dependent manner.

(b) Egg-hatch: The hatchability of eggs laid on treated host leaves reduced significantly from about 68% in the control to 52%, 62% and 65% in the treated lines ($F_{2, 57}$

=5.99; $P < 0.001$), indicating a dose-dependent inhibition in egg-hatching in *E. vigintioctopunctata*. Similar to oviposition deterrent, the most potent extract inhibiting egg-hatch was of *R. communis*. Relatively poor inhibition was recorded for *D. metel* and *C. procera*.

(c) Larval duration: The overall larval duration was significantly prolonged in the treated lines ($F_{2, 57} = 9.57$; $P < 0.001$), where *R. communis* also had the maximum effect. However, differences among the control and other two treatments were not statistically significant.

(d) Pupae formation: The formation of pupae in *E. vigintioctopunctata* was significantly inhibited by the leaf extracts ($F_{2, 57} = 4.42$; $P < 0.05$; Fig. 2), where *R. communis* and *C. procera* showed much more drastic effects than that of *D. metel*.

(e) Adult emergence: Significantly fewer adults emerged from the treated lines in comparison with the control ($F_{2, 57} = 4.87$; $P < 0.05$; Fig. 2). As in pupae formation, *R. communis* and *C. procera* had more pronounced inhibition effects on adult emergence in *E. vigintioctopunctata*.

(f) Female ratio: Unlike the all above life-history traits, female ratio in the treated lines did not differ significantly from the control (Table 2). From the present investigation it was obvious that there was no significant effect of leaf extracts on the female ratio of the beetles. All treated and control lines showed a more or less similar trend.

The present results clearly demonstrate that the foliage extracts of the three indigenous plant species possess remarkable larvicidal properties against *E. vigintioctopunctata* at around 18-30% concentrations. In addition, the extracts were found to have oviposition deterrent as well as egg-hatch inhibition potentials, which culminated in lengthened larval duration followed by significantly reduced pupae formation and adult emergence. Apparently, these achievements could be attributed to the chemical components like terpenoids and tocopherol present in *R. communis* (Okonkwo and Okoye 1992), propane alkaloids in *D. metel* (Rajapakse and Senanayake 1997) and steroid cardiac aglycones in *C. procera* (Amin *et al.* 2000).

A number of studies have been conducted in the past to establish the effectiveness of these plant extracts against various coleopteran pest insects. *R. communis* extracts, for example, were found to suppress the pulse beetles *C. maculatus* (Okonkwo and Okoye 1992) and *C. chinensis* (Bhargava and Meena 2000; Upasani *et al.* 2003). Likewise, Amin *et al.* (2000) and Mollah (2004) experimented with *C. procera* extracts against the lesser grain borer *Rhyzopertha dominica* and *C. maculatus*; while extracts of *D. metel* were reported to have reduced the population growth and development of the flour beetle *T. castaneum* (Khalequzzaman and Islam 1992), *C. chinensis* (Rajapakse and Senanayake 1997), the Khapra beetle *Trogoderma granarium* (Dwivedi and Kumar 1999) and *R. dominica* (Mahal 2002).

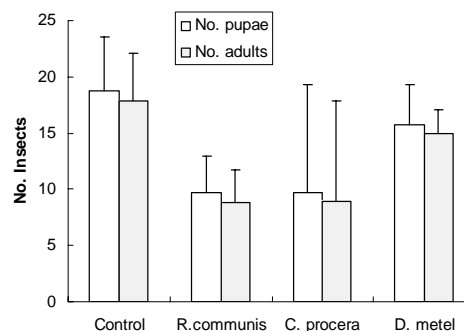


Fig. 2. Effects of aqueous extracts of leaves from three indigenous plants on the number of pupae formed and adults emerged in *E. vigintioctopunctata*.

Over the last couple of decades, efforts directed at controlling *Epilachna* beetles infesting various cash crops showed promising results. Rajagopal and Trivedi (1989) observed the repellent and antifeedant properties of neem, mahua and groundnut cakes against *E. vigintioctopunctata* attacking potato whereas Sreedevi *et al.* (1993) and Mehta *et al.* (1995) reported success of some plant extracts against *H. vigintioctopunctata* infesting brinjal. The aqueous extracts of the leaf, flower and root of *C. procera* against *H. elaterii* on cucurbit leaves proved much effective because 5% extract resulted in 100% protection where no larvae of the pest survived and 1% and 2.5% extracts highly reduced fecundity and longevity (Ahmed *et al.* 2006). Anam *et al.* (2006) reported that neem oil treatments prolonged larval and pupal periods in *E. dodecastigma*, in which some treated larvae never pupated; pupal recovery and adult emergence were greatly reduced and the botanical acted as a food deterrent. These findings corroborate the present results in that the extracts were found to have larvicidal, oviposition deterrent and pupal and adulticidal effects against *E. vigintioctopunctata*.

Ten percent aqueous extract spray of *R. communis* leaves was reported to reduce *Epilachna* attack on foliage and capitulum of sunflower, consequently increasing the yield of oilseed crop (Ahmed 2007) and aqueous seed extracts of *Annona squamosa* (5ml/L), *Azadirachtin indica* (6 ml/L) and petroleum ether extract of *Acorus calamus* (2 ml/L) reduced population build-up of *H. vigintioctopunctata* infesting cucumber up to 53.24%, 41.67% and 33.16%, respectively (Mondal and Ghatak 2007), while 10% turmeric and neem seed kernel dusts were found most effective against *Epilachna* on brinjal (Sankari and Narayanasamy 2007). Moreover, using extracts of indigenous plants Ghatak and Mondal (2008) and

Mondal and Ghatak (2009) demonstrated excellent suppression of *H. vigintioctopunctata* attacking brinjal and cucumber, respectively. *Tephrosia* leaf extracts (20g/100 ml water) were shown to kill adults and inhibit pupae formation by *Epilachna* on bitter melon (Rahaman *et al.* 2008), whereas yield of sunflower crop was increased significantly by spraying 5% *R. communis* extracts against *E. varivestis* (Ahmed *et al.* 2010). Recently, Swaminathan *et al.* (2010) have demonstrated the antifeedant and lethal effects of *A. indica*, *Pongamia glabra* and *Madhuca latifolia* on *H. vigintioctopunctata*. The above results are very similar to the present ones owing to the fact that the present extracts were capable of inducing higher larval mortality, inhibited both egg-laying and egg-hatching, increased immature developmental period, and reduced the number of pupae and adults in the experimental *E. vigintioctopunctata* under laboratory conditions.

The present data suggest that the shaker aqueous extracts of the three indigenous plants could be potent agents against *Epilachna* beetles. The prime merit of such botanical insecticides is that these could easily and cheaply be prepared by the farmers and/or manufactured by small-scale industries as crude or partially purified forms. However, future comprehensive investigations at field levels are needed to ascertain the eco-friendly nature of the extracts under study.

Conclusion

Larvicidal bioassays with crude aqueous leaf extracts of three plants *viz.* *R. communis*, *C. procera* and *D. metel* from Rajshahi University Campus showed significant toxicity against the experimental *Epilachna* beetles. Vital life-history traits like egg-laying, hatchability, larval duration, pupae formation and adult emergence were also adversely affected by the extract treatments.

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