



Impact of green leafy vegetable extracts on the mortality of pulse beetle, *Callosobruchus maculatus* F. in cowpea

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Abstract

An investigation was carried out in the laboratory to evaluate the efficacy of petroleum ether extracts of selected green leafy vegetables against the notorious pulse beetle, *C. maculatus* F. in cowpea, *Vigna unguiculata* L. The powdered leaves of selected plants (*Centella asiatica*, *Alternanthera sessilis*, *Eclipta alba*, *Phyllanthus amarus* and *Solanum trilobatum*) were Soxhlet extracted using petroleum ether as solvent. 25 g of cowpea were weighed, kept in individual polythene bags and treated with 1 %, 3 % and 5 % concentration of leaf extracts. An untreated and acetone treated control were maintained simultaneously. 5 pairs of newly emerged insects were introduced into each bag. Regular observations were made at every 24 hours interval for twelve days on adult mortality and only 2nd, 5th and 7th day observations were taken for analysis. The data were subjected to two-way analysis of variance and the mean were separated using Duncan's Multiple Range Test (DMRT). The values of percentage mortality were transformed to ARC sine before ANOVA. The overall result indicated that the significant variation was observed for the treatments and control. Cent per cent mortality of *C. maculatus* was registered in all the three doses (1%, 3% and 5%) of *Alternanthera* on 5 day of observations. Among the treatments, the petroleum ether extract of *Solanum* recorded highest percentage of mortality, the mean value being 67.77 per cent on 2 DAT and 87.77 per cent on 5 DAT. The findings of the present investigation indicates that botanical derivatives might be useful as insect control agents for commercial use. Petroleum ether extracts of *Alternanthera* and *Solanum* are an inexpensive and effective technique and its easy adaptability will give an additional advantage of acceptance of this technology by the farmers.

Keywords: *Callosobruchus maculatus*, *Vigna unguiculata*, mortality, *Centella asiatica*, *Alternanthera sessilis*, *Eclipta alba*, *Phyllanthus amarus*, *Solanum trilobatum*

Introduction

The world cannot revolve around without an atom. Likewise it cannot live without food. Agriculture is the backbone of Indian economy. The survival of man depends mainly on the food production and not on the feats achieved in the fields of service sector, industrial sector and so on. The earth is the producer of everything required for a man's selfish needs. Now agriculture has become a sick industry because of scarcity of labourers, water, electricity, fertilizers, and land, over population, conversion of agricultural lands into habitats, educational institutions, industrial estates and economic zones. Global warming too has its impact on the sharp fall in food production as the climate change and agriculture are interrelated processes. Global warming have significant impacts on agriculture and other abiotic factors such as temperature, carbon-di-oxide, glacial run-off etc. (Lakshminarayanan, 2011) [8]. Ensuring food security has an issue of key importance to countries with different degrees of economic development, while the agricultural sector plays an important role in improving food availability (Pawlak and Kołodziejczak, 2020) [20].

The food grains, Viz., cereals and pulses are the staple agricultural products alleviating the hunger. The food grains are difficult to store as it is infested and contaminated by insects, micro-organisms and rodents. The search for the safe method of protecting the grains is the need of the hour. Green leafy vegetables are reported to possess antioxidant activity (Shahjahan *et al.*, 2005) [15], hepato protective

activity (Shahjahan *et al.*, 2004) [14], anti ulcerogenic activity (Amir and Kumar, 2004) [2], ovicidal activity against mosquitoes (Rajkumar and Jebanesan, 2004) [11], skin repellent activity against *Anopheles stephensi* (Rajkumar and Jebanesan, 2005) [12], antifungal activity (Venkatesan and Ravi, 2004) [21], antibacterial activity (Karthikumar *et al.*, 2007) [6] and antiviral activity (Khin *et al.*, 1978) [7].

However the report on the insecticidal activity of green leafy vegetables were meager. Therefore, the present study was undertaken to evaluate the efficacy of selected green leafy vegetables as protectants against pulse beetles. The petroleum ether was selected as solvent to extract the residues from locally available plants such as *Centella asiatica*, *Alternanthera sessilis*, *Eclipta alba*, *Phyllanthus amarus*, *Solanum trilobatum* against *Callosobruchus maculatus* in cowpea. By considering the above facts, the present study was conducted to evaluate the efficacy of selected green leafy vegetable extracts to manage the pulse beetle *C. maculatus*

Materials and Methods

An investigation was carried out in the laboratory to evaluate the efficacy of leaf extracts as an insecticide against the pests of pulses. The materials and methods used for the present study are described under the following headings

Rearing of test insects

Adults of *Callosobruchus* species were collected from

households, and they were identified under Stereo Binocular Microscope (Karl Zeiss Stemi DV4) using the keys of Southgate and Howe (1958) [16] and Halstead (1963) [5]. Ten pairs of test insects, *Callosobruchus maculatus* were collected from the stock and released into separate pre-sterilized jars containing cowpea seeds.

Adults were removed after seven days, and seeds bearing eggs were placed in a Biological Oxygen Demand (BOD) incubator at $30^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and $70\% \pm 5\%$ RH until adult emergence. First-generation progeny (F_1) was used for bio-assay studies.

Collection of plants

Fresh leaves of selected plants shown in Table 1 were collected from Nilgiris to screen its insecticidal efficacy.

Table 1: Plants evaluated for its insecticidal efficacy

S.No.	Botanical Name	Tamil Name	Family Name
1.	<i>Centella asiatica</i>	Vallarai	Apiaceae
2.	<i>Alternanthera sessilis</i>	Ponnanganni	Amaranthaceae
3.	<i>Eclipta alba</i>	Karisalanganni	Asteraceae
4.	<i>Phyllanthus amarus</i>	Keelanalli	Euphorbiaceae
5.	<i>Solanum trilobatum</i>	Thuduvalai	Solanaceae

Table 2: Impact of selected green leafy vegetable extracts on the mortality of *C. maculatus* on 2 DAT

Treatment	1%	3%	5%	T – Mean
Control	0.00 ^c	0.00 ^d	0.00 ^c	0.00
Acetone control	0.00 ^c	0.00 ^d	0.00 ^c	0.00
<i>Centella asiatica</i>	33.33 (35.21) ^{ab}	30.00 (33.21) ^b	50.00 (44.99) ^b	37.77 (37.80)
<i>Alternanthera sessilis</i>	20.00 (26.07) ^b	23.33 (28.78) ^{bc}	30.00 (33.21) ^b	24.44 (29.35)
<i>Eclipta alba</i>	3.33 (6.14) ^c	13.33 (17.71) ^c	6.61 (12.28) ^c	7.74 (12.04)
<i>Phyllanthus amarus</i>	0.00 ^c	16.66 (19.92) ^{bc}	36.66 (37.41) ^b	17.77 (19.02)
<i>Solanum trilobatum</i>	50.00 (49.99) ^a	60.00 (51.14) ^a	93.33 (77.70) ^a	67.77 (57.95)
C – Mean	15.23 (16.06)	20.47 (21.53)	30.95 (29.33)	22.22 (22.31)

In a column, means followed by a common letter are not significantly different at the five per cent level by DMRT. Figures in parenthesis are ARC sine transformed, values before ANOVA.

Table 3

SV	DF	SS	MS	F
REP (R)	2	53.57	26.70	< 1
Treatment	20	28449.55	1422.48	22.39**
Treat (T)	6	24045.80	4007.63	63.09**
Conc (C)	2	1868.94	934.48	14.71**
TXC	12	2534.81	211.23	3.33**
Error	40	2541.07	63.52	
Total	62	31044.20		

** Significant at one per cent level

Table 4: Impact of selected green leafy vegetable extracts on the mortality of *C. maculatus* on 5 DAT

Treatment	1%	3%	5%	T – Mean
Control	23.33 (28.78) ^c	23.33 (28.78) ^d	23.33 (28.78) ^c	23.33 (28.78) ^d
Acetone control	20.00 (26.07) ^c	20.00 (26.07) ^d	20.00 (26.07) ^c	20.00 (26.07) ^d
<i>Centella asiatica</i>	53.33 (46.92) ^{bc}	50.00 (44.99) ^{cd}	76.66 (61.71) ^b	60.00 (51.21) ^c
<i>Alternanthera sessilis</i>	100.00 (90.00) ^a	100.00 (90.00) ^a	100.00 (90.00) ^a	100.00 (90.00) ^a
<i>Eclipta alba</i>	76.66 (65.85) ^b	90.00 (78.92) ^{ab}	86.66 (68.85) ^{ab}	84.44 (71.21) ^b
<i>Phyllanthus amarus</i>	53.33 (46.92) ^{bc}	73.33 (59.00) ^{bc}	100.00 (90.00) ^a	75.55 (65.30) ^b
<i>Solanum trilobatum</i>	70.00 (56.78) ^b	93.33 (77.70) ^{ab}	100.00 (90.00) ^a	87.77(74.83)
C – Mean	56.66 (51.61)	64.28 (57.92)	84.44 (75.55)	64.76 (66.77)

In a column, means followed by a common letter are not significantly different at the five per cent level by DMRT. Figures in parenthesis are ARC sine transformed values before ANOVA.

Table 5

SV	DF	SS	MS	F
REP (R)	2	203.77	101.88	< 1
Treatment	20	58771.42	2938.57	0.61 ^{NS}
Treat (T)	6	52415.87	8735.97	131.03**
Conc (C)	2	2752.39	1376.19	20.64**
TXC	12	3603.17	300.26	4.50**
Error	40	2800.00	66.66	
Total	62	61571.43		

** Significant at one per cent level; NS – Not Significant

Table 6: Impact of selected green leafy vegetable extracts on the mortality of *C. maculatus* on 7 DAT

Treatment	1%	3%	5%
Control	63.33	63.33	63.33
Acetone control	70.00	70.00	70.00
<i>Centella asiatica</i>	100.00	100.00	100.00
<i>Alternanthera sessilis</i>	100.00	100.00	100.00
<i>Eclipta alba</i>	100.00	100.00	100.00
<i>Phyllanthus amarus</i>	100.00	100.00	100.00
<i>Solanum trilobatum</i>	100.00	100.00	100.00

Extraction procedure

The fresh leaves of selected plants were collected, washed, and shade dried. The dried leaves were pulverized in an electric pulverizer, passed through a sieve, and retained in packets.

Ten grams of each leaf powder was subjected to soxhlet extraction using petroleum ether as solvent. The extracts thus obtained were distilled, and residues were separated by evaporation in the water bath at 60°C and refrigerated.

Bioassay Studies

Experimental design

A laboratory experiment was laid in a Completely Randomized Design (CRD). Three replications from each concentration, untreated control and an acetone-treated control, were maintained simultaneously. The desired concentration from the residue, i.e., 1%, 3%, and 5% concentrations of leaf extracts, were prepared using acetone as a solvent.

25 g of disinfected cowpea seeds were taken in polythene bags in which different doses of extracts were added using micropipettes. The packets were shaken for 5 minutes and exposed to air for 5 hours. The acetone evaporates, leaving a thin film of residue on the seed coat. All the treatments and the control were replicated three times. Five pairs of newly emerged adults were released into each packet. The polythene bags were tightly closed with rubber bands. The mortality of *C. maculatus* was observed from both the treatments and control and recorded.

Percentage mortality of *C. maculatus*

Observations on the mortality of insects were recorded for each treatment and control at every 24 hours interval for twelve days. The dead and moribund insects were removed from grain at the time of counting. Percentage mortality on each day was calculated using the following formula.

$$\text{Percentage mortality of insects} = \frac{\text{Dead insects}}{\text{Insects introduced}} \times 100$$

Statistical Analysis

The data on percentage adult mortality was transformed into ARC sine values before subjecting to ANOVA. The mean difference of each variable was further analyzed using Duncan's Multiple Range Test (DMRT). To determine the significance of the comparative efficacy of different leaf extracts on adult mortality, Two-way analysis of variance (ANOVA) was used. The mean values thus obtained were grouped using DMRT.

Results and Discussion

The impact of selected green leafy vegetables on cowpea against *C. maculatus* was discussed under the following headings. Statistical analysis of the data on mortality of *C. maculatus* revealed that significant variation exists among the concentration, treatments, and control.

Impact of selected green leafy vegetable extracts on the mortality of *C. maculatus* on 2 DAT (Day After Treatment)

All the three dosages of *Solanum* recorded maximum mortality in comparison with other treatments. Dose-dependent variation was also observed in this treatment. The highest mortality was in a 5 percent concentration of

Solanum (93.33%), followed by 3 percent (60%) and 1 percent (50%). Next to *Solanum*, the mortality was high in *Centella* at a 5 percent dose level (50%) which was on par with that of *Phyllanthus* (36.66%) and *Alternanthera* (30%) at 5 percent concentration. The moderate insecticidal activity was noticed in *Alternanthera* (Table – 2). However, the control and *Phyllanthus* (1% dose) recorded no death of *C. maculatus* up to 2 DAT (2 Day After Treatment).

Impact of selected green leafy vegetable extracts on the mortality of *C. maculatus* on 5 DAT

Cent percent mortality was observed in *Alternanthera* at all concentrations on the 5th Day After Treatment, (Table – 3) although moderate activity was recorded on 2 DAT. *Phyllanthus* and *Solanum* at 5 percent concentration also recorded 100 percent mortality. The other treatments recorded a significant increase in the percentage mortality ranging from 50 to 93.33 percent. In comparison, the control acetone treated control produced 23.33 and 20.00 percent mortality, respectively.

Impact of selected green leafy vegetable extracts on the mortality of *C. Maculatus* on 7 dat

The percentage mortality on 7 DAT revealed the complete death of insects in all the treatments. However, in control and acetone treated control, few insects were alive and the percentage registered were 63.33 and 70.00, respectively (Table 4).

Comparative analysis of the data on the mortality of *C. maculatus* by the petroleum ether extracts of *Centella asiatica*, *Alternanthera sessilis*, *Eclipta alba*, *Phyllanthus amarus* and *Solanum trilobatum* revealed that the significant increase in the percentage mortality in all the treatment in comparison with control. The reason for selecting petroleum ether as solvent for present investigation is its increased insecticidal effect on insects, as stated by many investigators. The significant role played by solvents in extraction was reported by Srivastava and Mann (1999) [17]. The increased effect of petroleum ether extract over acetone was reported by Lakwah *et al.* (1996) [9]. Teotia and Pandey (1979) [19] also reported that petroleum ether extract was the most toxic among various extracts, followed by ether and alcohol extracts. Bhaduri *et al.* (1985) [14] reported the superiority of petroleum ether extract over benzene and alcohol extracts.

In the present study, the petroleum ether extracts of *Solanum* and *Alternanthera* were more effective in bringing about the highest percentage of mortality. The impact of leaf extracts on adult mortality was attributed to several factors reported by earlier workers. Adult mortality due to abrasive effect on pest cuticle as described by Mathur *et al.* (1985) [10], or contact toxicity as suggested by Talukdar and House (1994) [18] or to the abrasive effect on pest cuticle interfering with respiratory mechanism causing suffocation and death of insects as reported by Agarwal *et al.* (1988) [11] or to the antifeedent effect as suggested by Saxena *et al.* (1992) [13] or to the presence of volatile substances which possess insecticidal property (Anuradha *et al.*, 2002) [3].

Conclusion

Post-harvest losses to the grains due to the insect pests are the major threat to food availability. Identification of simple measures to combat insect pest problems helps the farmers to safeguards their harvested goods. The present study

recommends the usage of *Alternanthera and Solanum* as insecticide to manage the pulse beetle, *Callosobruchus maculatus* infestation as they are edible, easily available, harmless to both the grain and human being

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