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Short communication

Effects of *Melia azedarach* L. extract on the teak defoliator *Hyblaea puera* Cramer (Lepidoptera: Hyblaeidae)

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Abstract

Methanolic extracts from leaves and seeds of chinaberry tree, *Melia azedarach* L. (Meliaceae) were tested against the larvae of *Hyblaea puera* (*H. puera*) Cramer (Lepidoptera: Hyblaeidae) under laboratory conditions. This insect defoliates teak, and is considered as a major pest that strongly influences the development of the teak tree. Chinaberry extracts were found to affect the growth, feeding and oviposition of *H. puera*. In general, the seed extracts showed high bioactivity at all doses, while the leaf extract, proved to be active, only at the higher doses. Our laboratory experiment showed that the seed extract suppressed the larval activity of *H. puera* even at low doses. Gross dietary utilization (efficiency of conversion of ingested and digested food) of *H. puera* decreased after treatment in the diet. The growth of surviving larvae decreased, and no late fourth and early fifth instars completed development on higher dose treatment of both leafs and seed extracts. Food consumption, digestion, relative consumption rate (RCR), efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD), and relative growth rate (RGR) values declined significantly, but concurrently a significant increase in approximate digestibility (AD) was observed. Clear dose–response relationships were established, with the highest dose of 4% seed extract evoking 94% feeding deterrence. Larvae that were chronically exposed *M. azedarach* extract showed a reduction in weight (65–84%). The less expensive and naturally occurring biopesticide may be an alternative for synthetic pesticides in order to protect forest trees.

Keywords: Teak; Defoliator; *Hyblaea*; Biology; Food utilization; Toxicity; Antifeedant

1. Introduction

Teak (*Tectona grandis* (*T. grandis*) Linn. f.) has been recognized for centuries as the finest hardwood in the world because of its strength, durability, pest and rot resistance, attractiveness, and workability. With the expansion of teak plantations, pest problems also arose. The most serious is the teak defoliator *Hyblaea puera* (*H. puera*) Cramer (Lepidoptera: Hyblaeidae). *H. puera* is a common defoliator of teak that can be found between the West Indies and Fiji (Nair, 1988). Apart from teak, there are a large number of alternative host plants for these polyphagous caterpillars. Though this damage is very severe, expensive and limited control measure are available.

The Meliaceae plant family is known to contain a variety of compounds that show insecticidal, antifeedant, growth regulating, and development modifying properties (Champagne et al., 1989; Schmutterer, 1990; Mordue (Luntz) and Blackwell, 1993; Senthil Nathan and Kalaivani, 2005; Senthil Nathan et al., 2004, 2005a, b, c). One member of the Meliaceae, known as

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Chinaberry or Persian lilac tree, is a deciduous tree that is native to northwestern India, and has long been recognized for its insecticidal properties but is yet to be wholly analysed. Fruit extracts of *M. azedarach* elicit a variety of effects in insects, such as growth retardation, reduced fecundity, moulting disorders, morphogenetic defects, and changes of behaviour (Ascher et al., 1995). The antifeedant effects of *M. azedarach* extracts are known for many insects (Saxena et al., 1984; Schmidt et al., 1998; Juan et al., 2000; Carpinella et al., 2003). Recently, the promotion of botanicals as environmentally friendly pesticides, microbial sprays, and insect growth regulators has been of major concern in the presence of other control measures such as beneficial insects, all of which necessitates an integration of supervised control (Ascher et al., 1995). In the present study, the bioactivity of leaf and seed extracts of *M. azedarach* has been tested against *H. puera.*

2. Materials and methods

2.1. Laboratory mass culture of H. puera

H. puera larvae were collected from natural forest trees of *T. grandis*, Siruvani hills, Western Ghats, Coimbatore district, Tamil Nadu, India. Insect colonies were maintained in the laboratory at $27\pm2\,^{\circ}C$; 10:14LD; 85% RH. The culture was initiated with partly grown larvae from the field. *H. puera* larvae were reared in insect cages and fed ad libitum on the leaves of *T. grandis*. The moths were fed with 10% sucrose solution fortified with a few drops of vitamin mixture (Multidec®drops, Ashok Pharmaceuticals, Chennai 600024, India) to enhance egg production. After two days the leaves of teak plants were removed from the oviposition cage. The leaf portions containing the eggs were clipped and placed on moist filter paper in a petri dish. These eggs were used to maintain the culture.

2.2. Methanolic extract of leaves and seeds of M. azedarach

Leaves and seeds of *M. azedarach* were collected from five trees in the natural forests of Kolli hills, Namakkal District, Tamil Nadu, India. Methanol extracts of the seeds and leaves were obtained according to the following methodology. First, the plant seeds and leaves were crushed to fine particle size and shade dried at room temperature. Extraction was carried out according to the procedure described in Warthen et al. (1984). In a 1000 ml flask, 100 g of crushed and dried plant materials in 1000 ml of methanol were stirred for 3 h. After leaving the methanolic solution to rest overnight, it was filtered through Whatman No. 40 filter paper. The solid filtration residues were extracted again as above, and the two filtrates were combined. The solvent was removed by vacuum evaporation in a rotary evaporator. An oily dark red residue from seeds and a dark green colour residue from leaves were obtained. These crude extracts were used to prepare the stock solutions.

2.3. Preparation of stock solution

A known amount (100 mg/ml) of filtered crude extract obtained from the above process was serially diluted to obtained the desired concentration. The stock solution of concentration 100 mg/ml was serially diluted to prepare test solutions of 0.25%, 0.50%, 1.0%, 2.0%, and 4.0%. One drop of emulsifier (0.005%) (Tween 20, Sigma Chemical Company) was added with the seed and leaf extracts to ensure complete solubility of the material in water.

2.4. Bioassays and treatments

Bioassays were performed with first to fifth instar *H. puera* using concentrations from 0.25% to 4% of *M. azedarach* extract (30 larvae/concentration, five replicates). The effective concentration (EC_{50}) was calculated using Probit analysis (Finney, 1971). Fresh teak leaves were sprayed with different concentrations of seed and leaf extracts of *M. azedarach* and air dried. The formulations were applied to leaves with a regulator-controlled spray applicator. Control leaves were treated with 1% methanol alone. The 4 h starved first to fifth larval instar were individually fed leaves with different concentrations of seed and leaf extracts. Every 24 h, the uneaten leaves were removed and placed with fresh treated leaves (30 larvae/concentration, five replicates). The insects were maintained at $27\pm2\,^{\circ}C$ throughout the test and checked daily until pupation. Then each pupa was removed from the test container, placed in a clean container, and observed for emergence. The days from moulting of the larvae to pupation and to adulthood were noted. Fecundity was assessed by counting the number of eggs laid during the life span in control and experimental insects. The larval and pupal duration of treated and control individuals were compared and the developmental rate was determined (Murugan et al., 1999).

2.5. Quantitative food utilization efficiency measures

A gravimetric technique was used to determine weight gain, food consumption, and faeces produced. All weights were measured using a monopan balance, accurate to 0.1 mg. The newly moulted fifth instar larvae were starved for 4 h. After measuring the initial weight of the larvae, they were individually introduced into separate containers. The larvae (30 larvae/concentration, five replicates, totally $n = 150$) were allowed to

feed on weighed quantities of extract- treated and untreated *T. grandis* leaves for a period of 24 h. Larvae were again weighed. The difference in weight of the larvae gives the fresh weight gained during the period of study. Sample larvae were weighed, oven dried (48 h at 60 °C), and reweighed to establish a percentage dry weight of the experimental larvae. The leaves remaining at the end of each day were oven dried and reweighed to establish a percentage dry weight conversion value to allow estimation of the dry weight of the diet given to the larvae. The quantity of food ingested was estimated by subtracting the diet (dry weight) remaining at the end of each experiment from the total dry weight of the diet provided. Faeces were collected daily and weighed, and then oven dried and reweighed to estimate the dry weight of excreta. The experiment was continued for four days and observations were recorded every 24 h.

Consumption, growth rates, and post-ingestive food utilization efficiencies (all based on dry weight) were calculated in the traditional manner (Waldbauer, 1964, 1968; Slansky and Scriber, 1985; Senthil Nathan et al., 2005b, c; Senthil Nathan and Kalaivani, 2005). Consumption index (CI) = E/TA, Relative growth rate (RGR) = P/TA, Relative consumption rate (RCR) = E/(T–A), Approximate digestibility, (AD) = 100(E–F)/E, Efficiency of conversion of ingested food (ECI) = 100 P/E, Efficiency of conversion of digested food (ECD) = 100 P/(E–F), where A = mean dry weight of animal during T, E = dry weight of food eaten, F = dry weight of faeces produced, P = dry weight gain of insect and, T = duration of experimental period.

2.6. Antifeedant deterrence bioassays

Antifeedant activity was assayed using a leaf cut choice test (Senthil Nathan et al., 2005b, c) in a 15 cm diameter petri-dish lined with a moist filter paper disc. Three cm^2 long leaf discs from teak plants were treated on each side, with various concentrations (0.25% to 4%). Control leaf cuts were treated with 1% methanol. The leaf discs were dried at room temperature, and then 4 h starved fourth instars of *H. puera* were introduced into each arena containing one treated and untreated leaf discs in alternate position line with moist filter paper disc. Experiments were carried out with two larvae petri-dishes in five replicates. Consumption was recorded using a digitizing leaf area meter (Model LI-3000, Li-cor, USA) after 24 h. The index of feeding deterrence (IFD) was calculated as

$$\mathrm{IFD} = \frac{C - T}{C + T} \quad (1)$$

where '*C*' is the consumption of control leaf cut and '*T*' is the treated leaf cut.

2.7. Statistical analysis

The effective concentration was calculated by Probit analysis (Finney, 1971). Data from nutritional, feeding deterrence, and biology experiments were subjected to analysis of variance (ANOVA of arcsine square root transformed percentages). Differences between the treatments were determined by Tukey's multiple range test ($P \leqslant 0.05$) (Snedecor and Cochran, 1989; SAS Institute, 2001).

3. Results and discussion

Teak is one of the most important tropical hardwood forest species in the international market because of its high-quality timber. As the sustainable supply of teak from natural forests declines and the demand continues to increase, the general trend in the future of teak growing will be towards increasing production by way of protecting it from pest and diseases. Botanicals tested in the present study were reported to be ecofriendly and are non-toxic to vertebrates (Al-Sharook et al., 1991). An EC_{50} value of leaf and seed extract of *M. azedarach* against *H. puera* was shown in Fig. 1. Seed extracts were most potent in all experiments with least EC_{50} (0.4,0.9,1.3,1.6, and 1.9% first to fifth instars respectively).

Larval duration was extended at higher doses of leaf and seed extracts. The same trend was noticed in pupal stages. Pupal duration was similarly extended in the seed extracts treatment compared to the leaf extract treatment, but not significantly different (Table 1). Adult longevity was greatly reduced according to the control by both leaf and seed extract. Fecundity was also reduced by the plant extract according to the control (Table 1). Affected larvae had a characteristic dark brownish black colouration. Growth regulatory effects such as the formation of larval–pupal intermediates,

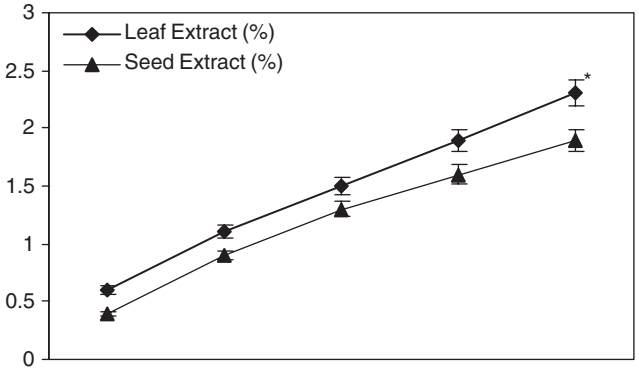

Fig. 1. Effective concentrations (EC_{50}) of *M azedarach* against first to fifth instar larvae of *H puera*. *Values are mean of five replicates and ± standard error.

Table 1
Life cycle of *H. puera* after treatment with leaf and seed extracts of *M. azedarach*

Treatment	Total larval duration* (days)	Total pupal duration* (days)	Adult longevity (days)*		Fecundity* (no. of eggs laid by the female)	Mean (±SE) larval weight (mg)* fifth instar	Mean (±SE) pupal weight* (mg)
			Male	Female			
Control	14.5±1.3^{bc}	10.4±0.7^{d}	8.2±0.7^{a}	9.8±0.7^{a}	544±41^{a}	42.3±2.4^{a}	44.9±3.1^{a}
Leaf extract (%)							
0.5	15.4±1.2^{b}	11.9±0.8^{d}	7.8±0.5^{a}	9.1±0.7^{a}	441±31^{b}	35.2±2.2^{b}	36.3±3.1^{ab}
1	16.7±1.3^{b}	13.4±0.9^{c}	6.8±0.4^{b}	8.1±0.7^{ab}	312±25^{c}	30.7±2.1^{b}	27.9±1.9^{b}
2	18.9±1.5^{a}	15.1±1.0^{bc}	6.2±0.5^{b}	7.4±0.5^{b}	218±21^{d}	23.9±1.9^{c}	21.8±2.2^{c}
4	20.8±1.6^{a}	16.3±1.1^{a}	5.6±0.4^{b}	6.8±0.5^{b}	151±18^{e}	17.7±1.6^{c}	17.9±1.1^{c}
Seed extract (%)							
0.5	15.9±1.2^{b}	12.6±0.7^{c}	7.3±0.4^{ab}	8.6±0.7^{a}	398±34^{b}	33.0±3.5^{b}	32.1±2.7^{b}
1	17.6±1.3^{b}	14.2±1.2^{c}	6.5±0.3^{b}	7.7±0.5^{b}	278±27^{cd}	26.5±1.7^{bc}	24.4±1.8^{bc}
2	19.7±1.7^{a}	15.7±1.2^{b}	5.4±0.2^{bc}	6.4±0.4^{b}	179±22de	20.1±1.7^{c}	20.0±1.9^{c}
4	21.6±1.8^{a}	17.1±1.3^{a}	4.2±0.2^{c}	5.3±0.4^{c}	108±12^{f}	14.6±1.6^{d}	14.9±1.1^{d}

*Within columns, means followed by the same letter do not differ significantly (Tukey's test, $P \leqslant 0.05$).

Table 2
Nutritional indices of fifth instar larvae of *H. puera* after treatment with leaf and seed extracts of *M. azedarach*

Treatment	RGR* (mg/mg/day)	CI* (mg/mg/day)	AD* (%)	ECI* (%)	ECD* (%)
Control	1.63±0.18^{a}	9.51±0.72^{a}	50.16±3.7^{bc}	17.15±1.46^{a}	34.25±2.3^{a}
Leaf extract (%)					
0.5	1.12±0.11^{b}	7.54±0.56^{ab}	52.25±3.7^{b}	14.96±1.29^{a}	28.64±2.1^{ab}
1	0.82±0.06^{c}	6.76±0.41^{b}	53.87±3.2^{ab}	12.18±1.12^{b}	22.61±1.5^{b}
2	0.49±0.03^{d}	5.11±0.32^{b}	54.95±3.8^{a}	9.67±0.76^{b}	17.61±1.2^{c}
4	0.34±0.02^{e}	4.21±0.27^{c}	56.92±3.9^{a}	8.16±0.69^{bc}	14.35±1.1^{c}
Seed extract (%)					
0.5	0.94±0.08^{b}	7.04±0.46^{b}	53.10±3.4^{b}	13.37±1.29^{ab}	25.19±2.2^{b}
1	0.62±0.04^{cd}	5.98±0.29^{b}	54.21±3.8^{a}	10.44±0.91^{b}	19.27±1.6^{bc}
2	0.40±0.03^{e}	4.73±0.22^{bc}	55.71±3.4^{a}	8.65±0.74^{b}	15.54±1.3^{c}
4	0.29±0.01^{ef}	3.92±0.19^{c}	57.69±4.1^{a}	7.64±0.63^{c}	13.26±1.1^{cd}

Within columns, means followed by the same letter do not differ significantly (Tukey's test, $P \leqslant 0.05$).
CI, consumption index; RGR, relative growth rate; AD, approximate digestibility; ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food.

deformed wings, and abdomen occurred in all treatments. These effects were more predominant at higher concentrations.

Dietary utilization by *H. puera* was severely affected when fed on teak leaves treated with. *M. azedarach*. The adverse effect of *M. azedarach* extract on the feeding and growth of *H. puera* was evident from the nutritional experiment. Both the consumption and relative growth rate of fifth instar *H. puera* were reduced by Chinaberry extract (Table 2). The CI and RGR of the treated fifth instar larvae remained significantly at a lower level than their control counterparts. Food consumption, RCR, digestion, ECI, and ECD values declined significantly while AD increased significantly following treatment with *M. azedarach* (Table 2). The present finding showing reduced growth rate during fifth instar, with extended span of development in treated larvae, is in confirmation with earlier findings (Senthil Nathan et al., 2005b, c) It may be inferred from the study that an extended larval period is coupled with lower RCR, which is more likely due to longer retention of food in the gut for maximization of AD to meet the increased demand for nutrients (Reynolds et al., 1985; Senthil Nathan et al., 2005b, c). Seed extract caused the highest feeding deterrence index. Seed and leaf extract both at 2% and 4% concentration significantly prevented females from laying egg (Fig. 2). The adverse effect on ovarian development, fecundity, and viability from Chinaberry extract is due to its interference with either the synthesis of vitellogenic protein or its uptake by oocytes (Murugan et al., 1999). The conclusions of this study indicate that plant extracts such as *M. azedarach* leaf and seed extracts affect the pest insect, and it may be an effective alternative to conventional synthetic

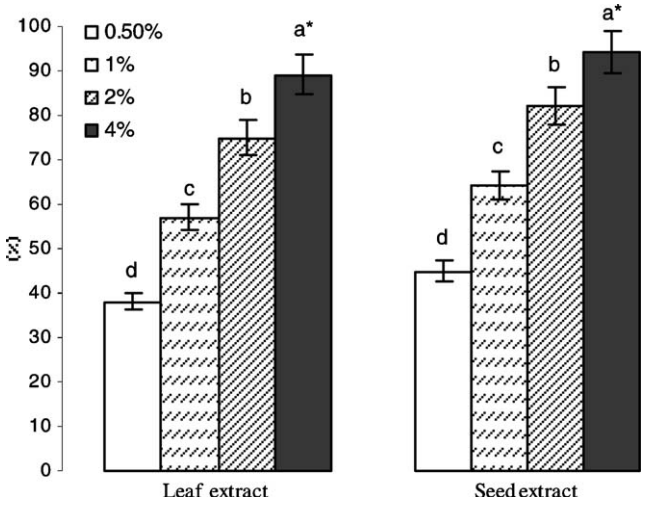

Fig. 2. Feeding deterrence index of fourth instar larvae of *H puera* after treatment with *M. azedarach*. Means (± (SE) standard error) followed by the same letters above bars indicate no significant difference ($P \leqslant 0.05$) in a Tukey test.

insecticides for the control of teak defoliator. The use of plant extracts or botanical pesticides may play a more prominent role in integrated pest control programmes in the future.

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