Role of *Pityrogramma calomelanos* (L.) Link in dietary and nutritional indices of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae)

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ABSTRACT

The polyphagous, ubiquitous, destructive pest, *Spodoptera litura* (F.) affects 150 host plant species and notably their larval forms are notorious. Extensive feeding and rapid reproduction cycles led the farmers and industrialists to seek the effective chemical and synthetic pesticides. Random and inappropriate usages of pesticides imposed a negative impact on non-target organisms, environment and abiotic factors and also pest resurgence to the particular pesticides. To overcome this incidence, biopesticides are practiced as an alternative source. Plant botanicals play a huge role as insect growth regulators (IGRs) by inhibiting regular moulting cycle, development and survival of insect pests. Also, the silverback fern, *Pityrogramma calomelanos* are reported with several secondary metabolites which are known for their pesticidal properties. Hence, the present work was framed to study the dietary responses as well as nutritional indices of *S. litura* treated with crude extract of *P. calomelanos*. The treated larvae showed dietary and post ingestion responses prior to moulting and death. On the 4th day of treatment, reduction in food intake (522.89 mg), larval weight gain (41.02 mg), ECI (56.25 %), ECD (46.30 %), AD (47.21 %) and RGR (3.06 mg/mg/day) was observed as dose dependent manner except the feacal production (378.18 mg) as compared to control. Therefore, the ethanolic extract of a fern, *P. calomelanos* can be recommended as a biopesticides for its post ingestion and anti-nutritional effects on *S. litura* larvae.

Keywords: *Pityrogramma calomelanos, Spodoptera litura, Dietary parameters, Nutritional indices*

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INTRODUCTION

Insects from the order Lepidoptera comprised of beneficial and harmful insects. Most of them are pestiferous insects in their larval forms. Among the destructive pests, the tobacco cutworm, *Spodoptera litura* (Fab.) is an ubiquitous, polyphagous and multivoltine insect infests more than 150 host species *viz*., cotton, tobacco and groundnut etc., in the Asian tropics (Edwin *et al*., 2021). Severe incidence of pest attacks causes yield loss in many countries including India, China, Japan and Southeast Asia (Wan *et al*., 2014). The larvae extensively feed the crops and resulted in complete stripping of the plants. Synthetic chemical pesticides widely used to control insect pests. Inappropriate usage of insecticides has caused the insects to develop resistance and several adverse effects on food, soil,

ground water and air as well as carcinogenic, teratogenic and great threats to both human and environmental health (Garriga and Caballero, 2011; Tong *et al*., 2013). To reconcile these effects, biopesticides were introduced as an alternative. Plant extracts and plant based natural products in insect pest management programs are received much attention due to less environmental pollution, pest resistance and resurgence, and undesirable effects to the non-target organisms caused by unsystematic use of synthetic pesticides. Plant botanicals have the ability to protect from herbivores including lepidopteran pests. Pteridophytes (ferns) are rich source of secondary metabolites which are well known for their pesticidal activity (Xavier *et al*., 2016). *Pityrogramma calomela*nos (L.) is a silverback fern, reported with alkaloids, steroids, tannins, flavonoids, terpenoids and phenolic compounds (Princy *et al*., 2022a). These secondary metabolites synergistically acts over the insects, seize its metabolism and the steroidal compounds alters the insects endocrine system *viz*., ecdysteroid and juvenile hormone titers (Ghoneim and Hamadah, 2017). Feeding and reproduction in insects are closely related to nutritional factors which have impact on the rate of growth, survival and development. The amount and quality of food consumed by a larva influences its performance, growth rate, developmental time, body weight and survival. Therefore, an understanding of the nutritional indices in relation to the rate of ingestion, digestion and conversion by the growing larvae would be useful (Slansky and Scriber, 1985). Also, reduction in feeding activity of an insect may reduce normal development, weight gain and increase mortality. In insects, the physiological events that are linked to food consumption and utilization appear to be controlled by neural, endocrine and secretogogue mechanisms. With regard to the botanical influences on food metabolism of insects, many authors (Senthil-Nathan *et al*., 2006a) reported that the reduction of food consumption caused by botanicals has been reliant upon the insect species, type of botanical, and the concentration. Therefore, the current work was conducted to assess and investigate its dietary parameters and nutritional indices on the food consumption and utilization of *S. litura* larvae.

MATERIALS AND METHODS

Collection and Extraction of *P. calomelanos*

P. calomelanos collected from Parassala, (8.3394^{o} N, 77.1517^{o} E) Trivandrum, Kerala, India and was identified by Dr. V. Irudayaraj, the eminent pteridologist (Rtd), Department of Botany, St. Xavier's College (Autonomous), Palayamkottai. Voucher specimen (SXC/CPRC/FN/32) was prepared and deposited in Crop Protection and Research Centre (CPRC), Department of Zoology, St. Xavier's College (Autonomous), Palayamkottai. The fern was washed thrice in tap water to remove dust and debris and were shade dried for two weeks. The dried material was partially ground in a domestic grinder and stored in refrigerator for further use. Soxhlet extraction was used to collect the crude extract from the dried material (Princy *et al*., 2022a).

Insect rearing

Egg masses of *S. litura* were collected from Bhendi field at Veeravanallur, Tirunelveli, Tamil Nadu. Freshly hatched neonate larvae were fed with tender castor leaves (*Ricinus communis*) up to pre-pupal stage. Care was taken to avoid overcrowding and strict sanitation was maintained to prevent any infection. After pupation, the pupae were placed inside the oviposition chamber for adult emergence and oviposition. Cotton soaked with 10 % (w/v) sugar solution fortified with multivitamins was provided as feed for the newly emerged adults. Castor leaves were kept inside the chamber for laying eggs. Adult female laid creamy white eggs covered with scales under the leaves. Eggs were carefully removed from the chamber and kept in petridish with tender castor leaves and cotton soaked with water (27 ± 2^{o}C); 14 D:10 L hrs (Photophase : Scotophase); RH = 75 %). Eggs were hatched into neonates and the laboratory reared larvae were used for the study (Princy *et al*., 2022b).

Bioassay and Nutritional indices

Food utilization experiment was conducted in the freshly moulted, 9-day old, 4 hrs starved, third instar larvae of *S. litura*. Pre-weighed larvae (10 larvae/treatment; 5 replications) were introduced into the separate plastic containers (1.4 L) and allowed to feed on 5 g of castor leaves treated with various concentrations (250, 500, 1000, 1500, 2000 and 2500 ppm) of crude extract of *P. calomelanos*. The treated and control category leaves were air dried for 5 min. A gravimetric technique was used to determine weight gain, food consumption and excreta production. Containers were cleaned on daily basis to avoid any infection. The left-over leaves and excreta were weighed for further studies and after the stipulated period of the experimental exposure (4 days) the animals were fed with normal diet (untreated castor leaves). Observations were made on larval weight, residual diet and faecal matter was compared with the last recorded value. Nutritional indices (ECI, ECD, AD and RGR) were calculated by Datta *et al*. (2019) using wet weights.

Efficiency of conversion of ingested food (ECI) = P/E,

Efficiency of conversion of digested food (ECD) = P/ (E - F),

Approximate digestibility (AD) = (E - F)/E,

Relative growth rate (RGR) = P/ (A×T).

Where A= mean wet weight of larvae over unit time,

E= wet weight of food consumed,

F = wet weight of feaces produced,

P = weight gain of larvae, and

T= duration of feeding period.

Statistical analysis

Data were subjected to multivariate analysis of variance (MANOVA) and means were separated by Tukey's HSD (Honestly Significant Difference) at $p \leq 0.05$. Values were represented as mean ± SD. Statistical analysis of experimental data was carried out using SPSS software for Windows version 16.0 (Kaur *et al*., 2019).

RESULTS

Dietary parameters

The food consumption by *S. litura* treated with the crude extract of *P. calomelanos* is presented in Table 1. It shows that, next to control, maximum food intake was observed in 250 ppm (635.89 mg) followed by 609.25 mg in 500 ppm at 96 hrs. Decreased food intake was found in 2500 ppm (522.89 mg). On the other hand, food intake was gradually increased during the experimental period of four days (F 21, 128 = 9.804; p = 0.000).

The defecation rate of *S. litura* treated with crude extract of *P. calomelanos* is shown in Table 1. Irrespective of the treatment, all the experimental animals showed gradually increase in feacal weight from 24 hrs to 96 hrs. At the fourth day of treatment, feacal weight was between 405.4 mg (250 ppm) to 378.18 mg (2500 ppm; F21, 128 = 1.984; p = 0.011).

Daily weight gain by the experimental *S. litura* larvae treated with crude extract of *P. calomelanos* was represented in Table 1 and it showed reduced weight gain of 70.31 mg in 250 ppm as compared to control (71.8 mg) and in higher concentration (2500 ppm), it was 41.02 mg at 96 hrs (F 21, 128 = 0.429; p = 0.778). Maximum of 107.34 mg (250 ppm) weight was gained at 72 hrs of treatment.

Nutritional indices

Impact of crude extract of *P. calomelanos* on the efficiency of converting ingested food, converting ingested food into digested food, approximate digestibility and relative growth rate by *S. litura* larvae was calculated and presented in Table 2. The larvae showed reduced ECI as compared to control and it was gradually increased up to 72 hrs and also a downfall was recorded at 96 hrs. At the end of 4th day, maximum (75.44 %) and minimum (56.25 %) ECI was obtained in 250 and 2500 ppm respectively (F (21, 128) = 19.145; p = 0.000). The treated larvae showed gradual increase in ECD from 24 hrs to 72 hrs and a declined ECD at 96 hrs. In the 4th day of treatment, reduced ECD (60.79 %) was observed in 250 ppm and it was 46.30 % in 2500 ppm. In contrast, maximum ECD of 62.52 % was observed in control at 96 hrs (F 21, 128 = 3.393; p = 0.000). The maximum AD was observed in 24 hrs followed by 48 hrs and it was decreased at 72 hrs and slightly increased AD was recorded at 96 hrs. As compared to control

(65.52 %), reduced AD (47.21 %) was obtained in 2500 ppm and in least concentration it was 61.72% at 96 hrs of treatment (F 21, 128 = 0.560; p = 0.024). Crude extract of *P. calomelanos* treated larvae showed maximum RGR at 72 hrs followed by 96 hrs. When compared to the control (3.69 mg/mg/day), experimented larvae recorded with low RGR (3.06 mg/mg/day) in 2500 ppm whereas 250 ppm showed 3.53 mg/mg/day (F (21, 128) = 1.081; p = 0.050). With the use of Wilk's criterion, statistically significant interaction was found between the dietary and nutritional indices of *S. litura* treated with crude extract of *P. calomelanos* (F (147, 825.194) = 4.036; p = 0.000; Wilk's Λ = 0.027).

DISCUSSION

Dietary responses

Reduced food ingestion, feacal excretion and weight gain due to the action of various insect growth regulators (IGRs) and plant extracts had been estimated in different insect species (Linton *et al*., 1997; Richter *et al*., 1997; Bream *et al*., 1999). Irrespective to the experimental concentrations, a detrimental effect on the food intake of 3rd instar *S. litura* larvae were found in

Table 1. Dietary parameters of *S. litura* larvae treated with crude extract of *P. calomelanos*

Concentration (ppm)	24 hrs	48 hrs	72 hrs	96 hrs
Food Intake (mg)				
Water	342.49 ± 3.14^{bcdefg}	341.21 ± 3.08^{bcdefg}	572.43 ± 2.42^{cdefg}	654.04 ± 1.56^{bg}
250	321.80 ± 2.27^{ag}	326.42 ± 1.06^{adefg}	485.26 ± 1.22^{cdefg}	635.89 ± 1.98^{acdefg}
500	302.84 ± 1.07^{ag}	306.60 ± 2.32^{adefg}	461.20 ± 1.34^{abfg}	609.25 ± 3.39^{b}
1000	296.20 ± 2.63^{ag}	294.59 ± 1.48^{abcfg}	448.88 ± 1.93^{abg}	592.18 ± 1.05^{b}
1500	289.61 ± 2.49^{ag}	286.20 ± 1.39^{abcg}	417.42 ± 1.45^{ab}	576.72 ± 2.62^{b}
2000	285.03 ± 1.36^{ag}	277.00 ± 2.00^{abcdg}	405.58 ± 1.27^{abc}	559.60 ± 1.29^{b}
2500	277.24 ± 1.09^{abcdef}	269.09 ± 1.45^{abcdef}	390.27 ± 1.41^{abcd}	522.89 ± 1.75^{ab}
Feacal Weight (mg)				
Water	186.21 ± 1.24b^{cdefg}	152.36 ± 1.10^{bcdefg}	221.95 ± 1.97^{bcdefg}	311.61 ± 1.89^{bcdefg}
250	179.10 ± 1.28^{a}	242.22 ± 1.74^{a}	312.96 ± 1.39^{a}	405.40 ± 1.06^{acdefg}
500	161.00 ± 0.60^{a}	227.12 ± 1.25^{a}	308.43 ± 1.75^{a}	395.12 ± 1.29^{abg}
1000	153.48 ± 1.42^{a}	217.80 ± 1.92^{a}	294.81 ± 1.33^{a}	391.70 ± 2.88^{ab}
1500	149.20 ± 1.30^{a}	214.22 ± 1.49^{a}	292.27 ± 0.87^{a}	386.00 ± 1.01^{ab}
2000	134.80 ± 1.64^{a}	208.90 ± 1.14^{a}	288.93 ± 1.96^{a}	384.93 ± 1.25^{ab}
2500	121.82 ± 1.42^{a}	194.92 ± 0.76^{a}	281.51 ± 1.88^{a}	378.18 ± 2.61^{ab}
Weight Gain (mg)				
Water	42.56 ± 2.78	59.96 ± 3.85	113.40 ± 3.89	71.80 ± 1.66
250	35.83 ± 2.27	55.38 ± 1.07	107.34 ± 1.55	70.31 ± 1.86
500	34.66 ± 1.05	51.11 ± 1.13	100.80 ± 3.77	63.20 ± 1.36
1000	33.84 ± 1.73	48.94 ± 2.02	99.51 ± 1.42	61.11 ± 2.06
1500	31.91 ± 0.58	47.52 ± 1.56	96.03 ± 1.19	53.40 ± 0.59
2000	27.84 ± 0.49	40.91 ± 1.03	89.63 ± 1.11	49.62 ± 0.44
2500	25.52 ± 0.26	38.67 ± 1.14	85.19 ± 1.34	41.02 ± 1.31

Within each concentration, values followed by the alphabet(s) show significant difference (MANOVA, Tukey's HSD, $\alpha \leq 0.05$).

Table 2. Nutritional indices of *S. litura* larvae treated with crude extract of *P. calomelanos*

Concentration (ppm)	24 hrs	48 hrs	72 hrs	96 hrs
Efficient Conversion of Ingested food (ECI in %)				
Water	43.56 ± 2.49^{g}	51.48 ± 1.95^{bg}	91.86 ± 1.79^{defg}	78.18 ± 1.25^{defg}
250	40.23 ± 1.24^{defg}	47.00 ± 3.19^{acdefg}	88.14 ± 1.33^{defg}	75.44 ± 3.36^{defg}
500	37.70 ± 1.04^{g}	45.86 ± 2.26^{bfg}	85.24 ± 1.43^{efg}	72.28 ± 5.66^{efg}
1000	34.21 ± 1.49^{b}	41.79 ± 1.18^{bg}	81.03 ± 1.42^{abfg}	69.82 ± 2.32^{abfg}
1500	31.69 ± 1.10^{b}	37.83 ± 0.90^{bg}	78.31 ± 3.44^{abcg}	65.75 ± 2.82^{abcg}
2000	29.51 ± 2.92^{b}	33.12 ± 0.87^{bc}	74.79 ± 1.11^{abcd}	60.51 ± 0.54^{abcdg}
2500	26.16 ± 1.79^{abc}	30.59 ± 0.84^{abcde}	70.34 ± 2.96^{abcde}	56.25 ± 2.03^{abcdef}
Efficient Conversion of Digested food (ECD in %)				
Water	35.94 ± 4.57^{bcde}	42.62 ± 2.77^{bcdef}	75.00 ± 3.55^{bcdefg}	62.52 ± 3.07^{bcdeg}
250	33.27 ± 1.04^{acdefg}	40.82 ± 1.95^{acdefg}	73.44 ± 1.38^{acdefg}	60.79 ± 2.12^{acdefg}
500	31.03 ± 0.85^{abfg}	38.27 ± 3.12^{abfg}	71.14 ± 2.17^{abcefg}	58.32 ± 3.03^{abefg}
1000	29.83 ± 1.45^{abfg}	35.10 ± 1.26^{abfg}	68.89 ± 1.85^{abcefg}	55.12 ± 1.84^{abfg}
1500	27.90 ± 0.79^{abg}	32.35 ± 1.35^{abg}	65.57 ± 3.46^{abcdg}	52.63 ± 1.88^{abcg}
2000	23.04 ± 0.23^{bcd}	29.43 ± 1.49^{abcd}	63.20 ± 1.65^{abcdeg}	49.77 ± 1.72^{bcdg}
2500	20.03 ± 0.31^{bcde}	26.42 ± 1.42^{bcde}	61.21 ±1.74^{abcdef}	46.30 ± 1.73^{abcdef}
Approximate Digestibility (AD in %)				
Water	87.81 ± 2.15	67.61 ± 1.72	55.84 ± 1.99	65.52 ± 1.81
250	82.43 ± 1.48	65.18 ± 1.56	51.89 ± 1.07	61.72 ± 1.32^{g}
500	79.92 ± 1.30	64.10 ± 1.56	49.89 ± 1.51	58.45 ± 1.21
1000	76.23 ± 1.57	61.62 ± 1.70	47.32 ± 1.28	56.09 ± 1.21
1500	73.32 ± 1.17	58.31 ± 1.21	45.86 ± 1.21	54.90 ± 1.78
2000	70.06 ± 1.48	56.62 ± 1.63	43.63 ± 1.43	50.97 ± 1.73
2500	68.31 ± 1.41	53.23 ± 1.57	41.13 ± 0.93	47.21 ± 1.43
Relative Growth Rate (RGR in mg/mg/day)				
Water	2.39 ± 0.75	3.13 ± 1.44^{b}	5.77 ± 1.92	3.69 ± 1.78
250	2.31 ± 0.38^{g}	3.09 ± 0.61^{ag}	5.67 ± 1.47	3.53 ± 1.32
500	2.28 ± 0.22^{g}	3.04 ± 0.08	5.61 ± 1.48	3.46 ± 0.07
1000	2.20 ± 0.05^{g}	2.99 ± 0.24	5.55 ± 0.28	3.39 ± 0.02
1500	2.15 ± 0.09	2.95 ± 0.01	5.49 ± 0.06	3.25 ± 0.07
2000	2.09 ± 0.02	2.91 ± 0.24	5.44 ± 0.03	3.13 ± 0.18
2500	2.02 ± 0.04^{bcd}	2.86 ± 0.08^{a}	5.37 ± 0.25	3.06 ± 0.13

Within each concentration, values followed by the alphabet(s) show significant difference (MANOVA, Tukey's HSD, $\alpha \leq 0.05$).

the present study. The amount of food consumption and excretion heavily plays a role in weight gain of the larvae. The poor diet intake and watery defecation are the major factors for the poor weight gain, also it affect the insects with demoultability and ecdysial failure due to the insufficient/abnormal hormone titers. In the present study, food intake, feaces production and weight gained by the 4th and 5th instar larvae exhibited strong and synergistic action of crude extract of *P. calomelanos*. These results agreed with the findings of Huang *et al*. (2000). At 96 hrs, significant reduction in food intake (522.89 mg) and relatively increased feacal excretion (378.18 mg) was observed in 2500 ppm. The highest feacal excreta might be due to the improper digestion and absorption of food materials by the insect. Owing to the poor absorption, the larvae recorded significant weight gain (41.02 mg) as compared to control (71.80 mg). Wei *et al*., (2000) recorded similar results in 5th instar nymphs of *Schistocerca gregaria* (Desert locust).

Nutritional Indices

Food utilization indices of insects characteristically calculated for ECI, ECD and AD (expressed as percentages) and RGR (mg/mg/day). Nutritional analyses revealed that the extract also acts as a chronic toxic substance, when ingested by larvae (Slansky, 1985). The crude extract of *P. calomelanos* treated *S. litura* larvae shows, reduced ECI, ECD, AD and RGR in a dose dependent manner. ECI is a complete measure of an insect's capacity to utilize the food that it ingests for growth. The ECI and ECD decreased in the larvae of *S. litura* when fed with various concentrations of crude extract of *P. calomelanos*. Decreased ECI is an indication of more food being metabolized for energy to perform defensive functions or to detoxify the toxic effect of the diets and less energy being converted into body mass (Koul *et al*., 2003). At 96 hrs, the low ECI (56.25 %) values as compared to control (78.18 %) in the *S. litura* larvae and it could be due to the energetic cost for detoxification or due to impaired metabolism. The latter can have an adverse effect on insects' food conversion efficiency (Aljabr *et al*., 2017). Decrease in ECD (46.30 %) values usually results from either the presence of toxins in the diet or due to the lack or unsuitability of food constituents required by the insects for proper growth (Koul *et al*., 2004). Low ECI and ECD probably by the switching of biomass production towards detoxification process (Wheeler and Isman, 2001). AD was drastically decreased in the *S. litura* larvae fed with treated leaves. This reduced AD in turn shows lacking of essential nutrients and poor food intake (Koul *et al*., 2003). Low ECI and ECD account for low RGR (3.06 mg/mg/day) of *S. litura* larvae. Reduced RGR was also observed after treatment with *Melia azedarach* (Chinaberry tree) on fall armyworm, *Spodoptera frugiperda* and rice leafroller, *Cnaphalocrocis medinalis* (Senthil-Nathan *et al*., 2006b). Our results are in accordance with the findings of Datta *et al*. (2019) who also reported a significant decline in nutritional parameters *viz*. ECI, ECD, AD and RGR in *S. litura* larvae in response to crude plant extracts. Punia *et al*. (2020) also reported similar anti-nutritional effect of plant secondary metabolites against *S. litura*. Likewise, Princy *et al*. (2022b), found the similar results in *S. litura* larvae treated with phytoecdysteroid fraction of *P. calomelanos*. The present study revealed that, *P. calomelanos* heavily impact on the food intake, feacal excretion, weight gain by the larvae, efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD), approximate digestibility (AD), and relative growth rate (RGR). Hence *P. calomelanos* ethanolic extract (crude) can be explored in *S. litura* management. Although, the bioactive components and their mode of action remain to be elucidated.

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