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ORIGINAL RESEARCH ARTICLE

Study on varietal preference and nutritional indices of tea looper *Hyposidra talaca* (*Lepidoptera*: *Geometridae*) on ten selected tea varieties

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Abstract
To find out the varietal preference of *H. talaca* larvae, leaves of ten selected tea cultivars were provided separately to II, III, IV, and V instars. The food consumption by different larval instars was the maximum on TV20 followed by TV1 > K1/1 > TV22 > TV26 > TV16 > SNT8 > TS520 > HP12 > B157. The nutritional indices showed the highest ECD and ECI values on TV20 followed by other cultivars. However, AD values were inversely proportional to the ECI and ECD. Biochemical analysis of the host leaves indicated higher carbohydrate and lipid concentrations in TV20 which was positively correlated (r = 0.6351) with the mean food consumption, whereas, the lowest carbohydrate and lipid concentration was observed in TS520. The levels of secondary metabolites such as alkaloids (r = -0.6137) and flavonoids (r = -0.7403) showed a negative correlation with larval food consumption. Results of HPLC analysis showed the presence of different types of catechins like, EGC, Catechin, EC, EGCG, ECG, and caffeine in variable quantity in all cultivars that were positively correlated with larval feeding except the Gallic acid which showed a negative correlation (r = -0.5610). Based on the larval food consumption, the susceptible index of cultivars were categorized as 'highly susceptible' (TV20, TV1, K1/1, TV22, TV26, TV16, and SNT8), 'susceptible' (TS520 and HP12), and 'moderately susceptible' (B157). Based on cluster analysis A1 grouped (TS520 and HP12), A2 indicated B157 whereas sub-branches B1 grouped (TV22, TV16, TV26, and SNT8) and B2 grouped (TV1, TV20, and K1/1).

Keywords Tea variety · *Hyposidra talaca* · Food consumption · Secondary metabolites · Nutritional indices · Susceptible index

Introduction

The perennial plant tea *Camellia sinensis* (L.) Kuntze is cultivated on large and small scale in the Assam and Sub-Himalayan region of West Bengal in Northern India. Tea plantation along with shade trees provides shelter and food for diverse arthropod species. The black inch looper, *Hyposidra talaca* Walker, causes significant crop loss in tea growing regions of India especially West Bengal (Dooars and Terai) and Assam and has gained the status of major destructive pest of tea (Sinu et al. 2011; Roy et al. 2017). This widely distributed lepidopteran insect has been reported to attack several host plants viz., cinchona (*Cinchona officinalis*), coffee (*Coffea arabica*) litchi (*Litchi chinensis*), longan (*Dimocarpus longan*), mango (*Mangifera indica*), mangosteen (*Garcinia mangostana*) Hill glory bower (*Clerodendron infortunatum*), Indian mahogany (*Chukrasia tabularis*), Sea derris (*Derris robusta)* (Sinu et al. 2011; Basu Majumdar et al. 2010; Intachat et al. 2001). The developing stages of *H. talaca* consume tender and mature leaves with an average of 12,690.33 cm^2 leaf area leading to significant crop loss besides causing stress to the tea plant (Prasad and Mukhopadhyay 2013). Continuous application of synthetic pesticides for controlling this pest has its limitations leading to

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high tolerance to pesticides, possibility of resurgence, and outbreak of secondary pests. Besides, synthetic chemical pesticides harm the natural enemy populations and pollute environment. To overcome these demerits, research has been focused on the development of alternative methods of sustainable pest management (Sarkar et al. 2019, 2021).

Depending on the leaf structure, texture, and biochemical composition, insects show varietal preference towards their host plant (Maffei et al. 2007). Polyphagous insects voraciously consume nutritious food for their growth, development, and reproduction (Panizzi and Parra 2009). Insects prefer to consume protein and carbohydrate-rich plants, to fulfill their nutrient requirements (Simpson and Raubenheimer 1993). To evade damage by insects, plants apply their defensive mechanism through the production of toxic secondary metabolites or morphological defensive mechanisms like trichomes and latex deposition (War et al. 2013). Larvae of black inch looper exhibit variation in food consumption on different host plants by showing variation in the activity of digestive enzymes (Das and Mukhopadhyay 2014) and show the adaptive fitness in the tea ecosystem. The foraging or feeding behavior along with digestion, absorption, and allocation by polyphagous insects are responsible for meeting their nutrition and development (Karban 2011). The nutritional requirements of an insect vary in quantity and quality depending on the developmental stages.

In non-chemical strategy of pest management, host plant resistance is an important tool, which is globally acceptable. In insect-plant co-evolution, plants always alter and make new approaches to avoid insect pest attacks and insects also apply their counter-adaptations (War and Sharma 2014). The development of induced resistance by herbivorous pest attack makes the host plant unpalatable to arthropod pests (Karban 2011). Majumder et al. (2010) reported the growth development and survival variation of *H. talaca and H. infixaria* on four different tea varieties.

In the present study, the nutritional indices of different life stages of tea looper *H. talaca* when fed on ten selected tea cultivars based on the assessment of variation in food consumption and body weight gain has been studied. Biochemical analysis of all the cultivars was also carried out to correlate their feeding preference and to determine the level of susceptibility among the different tea cultivars. Besides, biophysical characteristics like leaf thickness and trichome density were also assessed. Based on these assessments, the susceptible index and cluster analysis were worked out.

Materials and methods

Selection of tea cultivars

Ten tea cultivars (variety) were selected from the Tocklai Vegetative series (TV1, TV26, TV16, TV22, and TV20), garden series (K1/1, B157, HP12, and SNT8), and seed stock (TS520) for the experiment, collected from experimental plot of Tea Research Association, North Bengal Regional Research & Development Centre.TV1, TV16, TV20, SNT8 (Sanyasithan 8), and HP12 (Hantapara 12) are standard cultivars (having average yield potential and quality); B157 (Bannockburn 157) and K1/1 (Kopati 1/1) are quality/flavored cultivar (having high quality and average yield potential) and TV22 and TV26 yield cultivar (having average yield potential and quality) (Barman 2011).

Laboratory rearing of *H. talaca*

Healthy moths of *H. talaca* were collected from tea fields from the same experimental plot. Egg masses were also collected and kept in separate containers (30 cm × 5 cm) for hatching. The larvae were nurtured on tea leaves in a growth chamber under laboratory conditions (27 ± 2 °C, 70–80% RH, and 16:10 LD photoperiod).

Food consumption and development of larvae of *H. talaca* on different tea cultivars

The leaf samples were collected from 25–30 years old conventionally managed tea fields. For each experiment, the leaf samples were collected from the same plant. Leaf discs (3.2 cm in diameter) were cut from the second or third leaf from the top of the shoot of each cultivar and put in a Tarson sample collecting container (Diameter 4.3, height 5.4 cm). A wet cotton swab was put in the same container for maintaining the moisture. Newly moulted II, III, IV, and V instar larvae of *H. talaca* (larval instars were differentiated by measuring its head capsule, Majumder 2010) were taken from the stock culture and released in each container individually. The food consumption, body weight gain, and weight of fecal matter of each larval instar at 24 h intervals were recorded throughout the larval duration of each instar. Similarly, all the selected cultivars were subjected to parallel bioassay and each experiment was replicated five times for each larval instar. The food utilization parameters like approximate digestibility (AD), efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD), relative consumption rate (RCR), and relative growth rate (RGR) were calculated according to Waldbauer (1968), subsequently modified by Gurusubramanian et al. (1991). The susceptible index was constructed, using TV1 as relative susceptible check (RSC) adopted the methods of Roy et al. 2009; Majumder et al. 2010; Henrich et al.1985.

All these parameters are worked out using the following formulae:

Food consumption (C) = (Initial leaf weight—final leaf weight) – (Average transpiration loss by leaf).

Production (P) = Final body weight—initial body weight.

Assimilation (AS) = Food consumed (C)—Faecal matter (F).

$$AD = \frac{AS}{C} X100$$

$$ECI = \frac{P}{C} X100$$

$$ECD = \frac{P}{AS} X100$$

$$RCR = \frac{C}{mean\ of\ the\ body\ wt.\ of\ a\ stage\ X\ feeding\ period(days)}$$

$$RGR = \frac{P}{mean\ of\ the\ body\ wt.\ of\ a\ stage\ X\ feeding\ period(days)}$$

$$Susceptible\ index(SI) = \frac{Food\ consumption\ on\ test\ cultivar}{food\ consumption\ on\ RSC}$$

Preparation of tea leaf for biochemical analysis

Same aged tea leaves of selected cultivars were collected from TRA experimental plot. Leaves were steamed for 3 min and dried in a hot air oven at 65 °C for 10 h. The dried leaves were blended using a mixture grinder machine for making powder. The biochemical tests were conducted using the dry powder.

Estimation of total carbohydrate

Total carbohydrate was estimated by anthrone reagent and the absorbance was noted at660 nm by UV Spectrophotometer Hedge and Hofreiter (1962).

Estimation of total protein

Total protein was assessed with Bovine Serum Albumin (BSA) as standard, using UV Spectrophotometer at 660 nm by following the standard methodology Lowry et al. (1951).

Estimation of lipid

For the estimation of lipid, Chloroform–methanol mixture was used by following the methodology adopted from Bligh and Dyer (1959); Dutta et al. (2013).

Estimation of total polyphenol

The total polyphenol content was estimated using Folin-Ciocalteu reagent by following the method of ISO/CD 14502–1.2: (2001). Absorbance was taken at 760 nm in an ultraviolet–visible spectrophotometer.

Estimation of total alkaloids

Total alkaloid was determined and measured using acetic acid and ethanol by taken the initial and final weight of the Whatman filter paper by following the method of Harborne, (1973).

Estimation of total flavonoid

Quercetin was used as the standard for the estimation of total flavonoids. The absorbance of the prepared solution was taken at 415 nm against the suitable blank (water) in the ultraviolet–visible spectrophotometer. Method was followed from Akbay et al. (2003); Park et al. (2008).

Estimation of total chlorophyll and carotenoids

Total chlorophyll and carotenoid were assessed at 646.8 nm, 663 nm and 470 nm in the spectrophotometer, where acetone was used as blank. The method followed was developed by Lichtenthaler (1987).

Estimation of individual catechins, gallic acid and caffeine in HPLC

Individual catechins, gallic acid and caffeine was analyzed by using Agilent 1260 infinity series of HPLC fitted with ZORBAX Eclipsed plus Phenyl-Hexyl column (4.6 × 250 mm, 5 µm) and Agilent ZORBAX Eclipsed Plus Phenyl-Hexyl guard column (4.6 × 12.5 mm, 5 µm) following ISO 14502 – 2:2005 (E) method.

Tea Leaf biophysical characters

The tea leaf biophysical characters viz.,trichome density was assessed by taking 1cm^2 area from different portion of a leaf under a dissecting microscope (at 40 × magnification) and leaf thickness were measured by following the methodology of Bindu and Pramanik (2017).

Statistical analyses

Data on larval food consumption and body weight gain were analyzed using one-way ANOVA, means were separated by Tukey's multiple comparison test. A dendogram (Euclidean distance mapping) of selected tea cultivars was constructed based on the feeding and nutritional indices of larva of *H. talaca* by using InStat software.

Results

Food consumption and weight gain by *H. talaca* on selected cultivars

The food consumption and weight gain by the larvae of *H. talaca* were significantly different on the selected tea cultivars. The II instar larvae showed the highest quantity of food consumption (9.2 ± 0.6 mg) (CD (0.01) = 1.950, $F = 12.174$, $df = 9$, $p < 0.0001$) and body weight gain (3.0 ± 0.3 mg) (CD (0.01) = 0.907, $F = 3.729$, $df = 9$, $p < 0.0001$) when TV20 leaf was provided compared with the other cultivars. Food consumption (33.6 ± 4.0 mg) (CD (0.01) = 9.407, $F = 3.779$, $df = 9$, $p < 0.0001$) (Fig. 1) and body weight gain (9.8 ± 0.7 mg) (CD (0.01) = 2.647, $F = 5.900$, $df = 9$, $p < 0.0001$) by III instar larvae were also found to be highest when fed on TV20 followed by TV1, K1/1, TV22, TV26, TV16, SNT8, TS520, HP12 and B157. Similar trends of food consumption (112.0 ± 14.2 mg) and body weight gain (18.2 ± 2.2 mg) by IV instar larvae were recorded when fed on TV20 cultivars whereas, significantly lower food consumption (86.7 ± 7.0 mg) (CD (0.05) = 16.385, $F = 2.242$, $df = 9$, $p < 0.05$) and weight gain (9.6 ± 0.6 mg) (CD (0.01) = 4.217, $F = 11.796$, $df = 9$, $p < 0.0001$) were observed when fed on the cultivar B157 compared to all other selected cultivars. In case of V instar, larval food consumption and body weight gain no significant differences were found among the selected cultivars.

The relative consumption rate (RCR) and relative growth rate (RGR) of *H. talaca* showed a decreasing trend with the advanced larval instars with a gradual increase in AD value while the ECI and ECD values gradually decreased with the advancement of larval instars (Table 1). The nutritional indices of II, III, IV and IV larvae reared on the cultivar TV20 showed the maximum AD values (55.43, 59.50, 62.60 and 66.18% respectively) with corresponding ECD values of (58.82, 49.00, 25.92 and 20.9 respectively) and ECI values (32.61, 29.17, 16.25 and 13.86 respectively) followed by TV1 > K1/1 > TV22 > TV26 > TV16 > SNT8 > TS520 > HP12. Whereas, comparatively lower AD values (51.16, 53.93, 58.82, and 62.96% respectively), ECD values (54.54, 45.15, 18.82, and 16.72 respectively), and ECI values (respectively 39.5, 25.4, 11.1 and10.4) were observed when reared on the cultivars B157.

Susceptible index (Status of host preference on different tea cultivars by *H. talaca*)

Based on the food consumption, the nutritional indices of looper larvae on all the selected tea cultivars, TV20, TV1, K1/1, TV22, TV26, TV16, and SNT8 were graded as 'highly susceptible', with susceptible index (SI) score ranging from 0.90 to1.07. On the other hand, TS520 and HP12 were graded as 'susceptible' (SI 0.80 to 0.89). Among all the selected cultivars, only B157 was found to be 'moderately susceptible' with an SI score of 0.772 (Table 2).

Biochemical profiles of tea cultivars and correlation with food consumption of larvae of *H. talaca*

Primary metabolites

Among all the tested cultivars, TV1 was found to contain a higher amount of carbohydrate (0.737 ± 0.003 mg/g) followed by other tested cultivars K1/1, TV26, B157, HP12, TV16, TV22, SNT8, TV20, and TS520 (Table 3). A higher value of protein was found in the tea cultivar TV20 (10.40 ± 1.2 mg/g) and the lowest value was in TS520 (6.31 ± 0.6 mg/g) compared to the other cultivars studied. In case of lipids, high quantity was observed in the cultivar TV20 (14.6 ± 1.4 mg/g) whereas TV22 contained a comparatively low amount of total lipid (10.8 ± 1.8 g/mg). Chlorophyll, the predominant photosynthetic pigment was present comparatively higher in the cultivar K1/1(0.946 ± 0.9 mg/g) and TV20 (0.912 ± 0.8 mg/g) whereas, lower amount was found in TV16 (0.678 ± 0.07 mg/g).

Primary metabolites play a major role in the food consumption and development of looper. The correlation, as well as the regression equations, revealed a significantly positive correlation ($p < 0.05$) with protein ($r = 0.6351$), lipid ($r = 0.6223$), carbohydrate ($r = 0.5767$) with the food consumption of looper (Table 4). The correlation between chlorophyll and the larval food consumption of looper was found to be positively insignificant.

Secondary metabolites

The cultivar TS520 was found to have the highest polyphenol content (270.2 ± 10.2 mg/g), and the lowest quantity was observed in B157 (190.9 ± 15.5 mg/g) compared to all other tested cultivars (Table 3). Total alkaloid present in B157

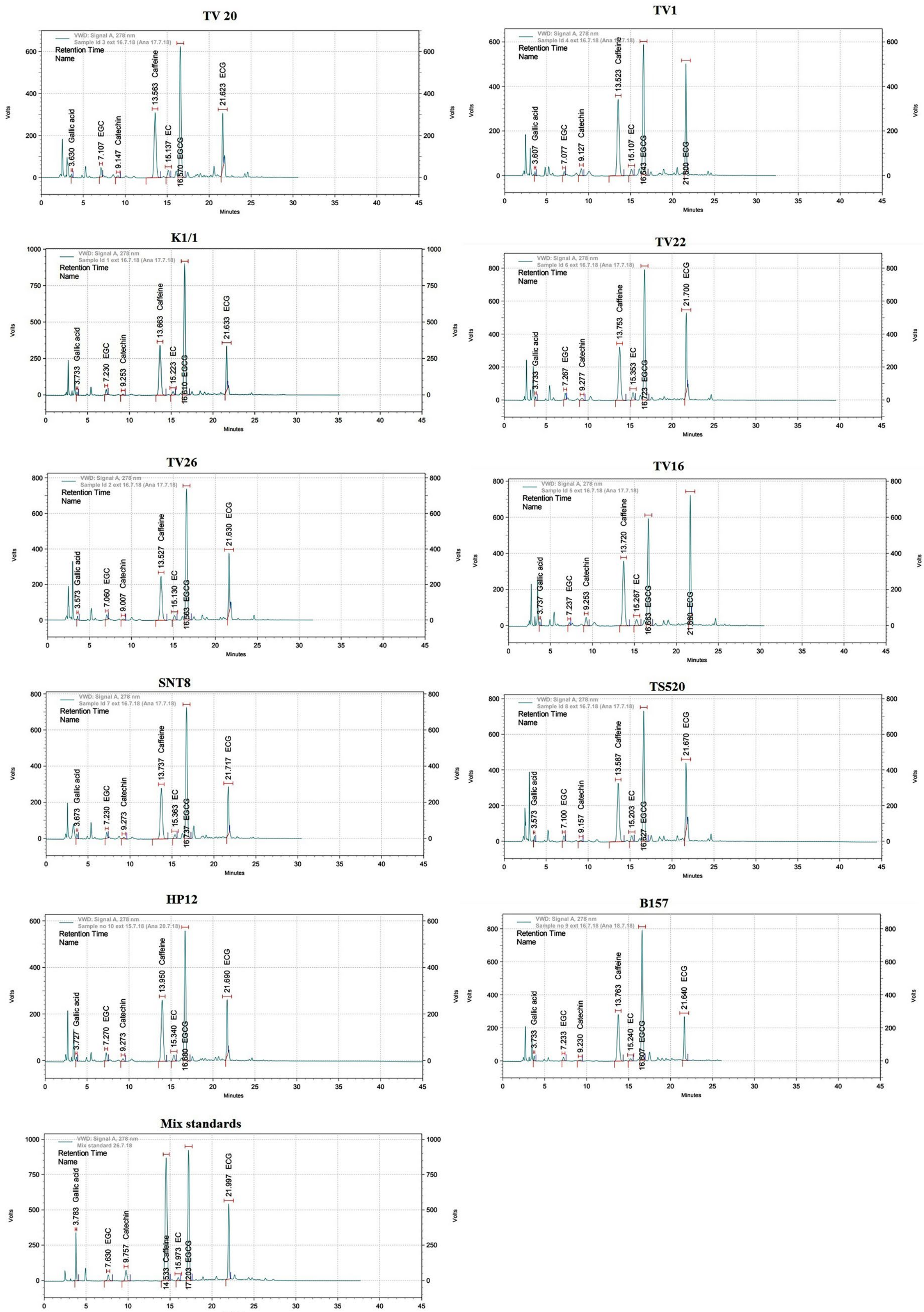

Fig. 1 Chromatograms showing gallic acid, caffeine, catechin mixed standard and catechin profiles in green leaf of different tea cultivars

Table 1 Food consumption of *H. talaca* on tea leaves

Cultivars	Larval stages of *H. talaca*	Food consumption (mg)	Body weight gain (mg)	Nutritional indices				
				RCR	RGR	AD (%)	ECD (%)	ECI (%)
TV20	II	9.2 ± 0.6^{a}	3.0 ± 0.3^{a}	0.300	0.098	55.43	58.82	32.61
	III	33.6 ± 4.0^{a}	9.8 ± 0.7^{a}	0.300	0.088	59.50	49.00	29.17
	IV	112.0 ± 14.2^{a}	18.2 ± 2.2^{a}	0.252	0.040	62.60	25.92	16.25
	V	223.6 ± 24.7^{NS}	31.0 ± 2.0^{NS}	0.084	0.011	66.18	20.91	13.86
TV1	II	9.2 ± 0.7^{a}	3.0 ± 0.3^{a}	0.300	0.098	55.10	59.17	32.60
	III	33.2 ± 4.2^{a}	9.6 ± 1.4^{ab}	0.291	0.086	59.12	48.92	28.91
	IV	111.4 ± 12.9^{ab}	17.8 ± 1.0^{a}	0.254	0.040	62.13	25.57	16.11
	V	222.4 ± 21.1^{NS}	30.6 ± 1.4^{NS}	0.084	0.011	66.09	20.82	13.84
$K_{1/1}$	II	9.0 ± 0.3^{a}	2.9 ± 0.2^{ab}	0.292	0.095	55.00	58.58	32.22
	III	32.8 ± 1.9^{a}	9.2 ± 0.7^{abc}	0.290	0.083	58.53	47.92	28.00
	IV	110.2 ± 3.0^{ab}	17.4 ± 1.1^{a}	0.251	0.039	62.00	25.51	15.93
	V	220.0 ± 18.4^{NS}	29.5 ± 1.8^{NS}	0.080	0.011	65.90	20.34	13.41
TV22	II	8.0 ± 0.5^{ab}	2.4 ± 0.2^{abc}	0.282	0.087	54.87	54.66	30.00
	III	32.0 ± 1.6^{ab}	8.8 ± 0.4^{abc}	0.290	0.080	58.12	47.31	27.52
	IV	108.6 ± 4.5^{abc}	15.2 ± 1.4^{ab}	0.241	0.348	61.69	22.68	14.254
	V	217.0 ± 21.0^{NS}	29.0 ± 1.5^{NS}	0.083	0.011	65.85	20.29	13.42
TV26	II	7.1 ± 0.7^{bc}	2.1 ± 0.2^{bcd}	0.282	0.086	53.52	55.26	30.91
	III	30.8 ± 1.7^{ab}	8.1 ± 0.5^{sbcd}	0.290	0.076	57.79	45.50	26.34
	IV	103.1 ± 2.9^{abcd}	14.3 ± 1.0^{bc}	0.241	0.031	61.45	21.93	12.90
	V	214.8 ± 15.0^{NS}	28.8 ± 0.6^{NS}	0.080	0.011	64.89	20.65	13.41
TV16	II	6.9 ± 0.5^{bc}	2.0 ± 0.2^{cd}	0.282	0.088	52.17	55.55	31.93
	III	30.5 ± 1.2^{ab}	8.0 ± 0.4^{bcd}	0.290	0.076	55.40	47.33	26.22
	IV	102.0 ± 3.9^{abcd}	13.1 ± 0.8^{bc}	0.240	0.031	59.31	21.65	12.81
	V	212.5 ± 14.4^{NS}	28.5 ± 0.5^{NS}	0.080	0.011	64.47	20.87	13.43
SNT8	II	6.0 ± 0.4^{cd}	1.71 ± 0.2^{cd}	0.261	0.091	52.00	54.98	35.02
	III	28.8 ± 1.8^{abc}	7.4 ± 0.5^{cde}	0.302	0.078	54.86	46.83	25.71
	IV	98.0 ± 3.1^{abcd}	12.0 ± 0.9^{c}	0.240	0.029	60.20	20.33	12.20
	V	208.0 ± 19.8^{NS}	27.0 ± 1.8^{NS}	0.110	0.014	63.94	20.30	12.91
TS520	II	5.8 ± 0.6^{cd}	1.65 ± 0.2^{cd}	0.260	0.095	51.89	54.81	36.22
	III	25.7 ± 2.4^{bcd}	6.5 ± 0.7^{def}	0.290	0.074	54.47	46.42	25.34
	IV	95.0 ± 5.6^{bcd}	11.5 ± 0.4^{cd}	0.232	0.004	60.15	20.12	12.12
	V	200.0 ± 11.8^{NS}	25.3 ± 1.7^{NS}	0.080	0.010	63.50	19.92	12.90
HP12	II	5.0 ± 0.4^{de}	1.41 ± 0.2^{cd}	0.251	0.096	51.60	54.65	38.01
	III	23.4 ± 1.7^{cd}	5.8 ± 0.6^{ef}	0.312	0.078	54.27	45.66	24.82
	IV	91.0 ± 8.0^{cd}	10.4 ± 0.6^{de}	0.232	0.021	59.34	19.25	11.42
	V	194.3 ± 15.9^{NS}	24.6 ± 3.6^{NS}	0.080	0.009	63.04	18.44	11.64
B157	II	4.3 ± 0.5^{e}	1.4 ± 0.2^{d}	0.241	0.093	51.16	54.54	39.52
	III	19.3 ± 1.2^{d}	4.9 ± 0.4^{f}	0.282	0.071	53.93	45.15	25.43
	IV	86.7 ± 7.0^{d}	9.6 ± 0.6^{e}	0.250	0.021	58.82	18.82	11.12
	V	189.2 ± 4.8^{NS}	24.1 ± 2.6^{NS}	0.080	0.008	62.96	16.72	10.41

Values (Food consumption and Body weight gain) represent means ± SD of 5 replications. The same alphabets of same instar looper are not significantly different

was (35.7 ± 2.8 mg/g) followed by SNT8, TV16, TV26, TV22, TS520, K1/1, HP12, TV1, and with the lowest value in TV20 (25.6 ± 5.2 mg/g). Among all of the selected tea cultivars, the amount of flavonoid recorded was as high (0.15 ± 0.003 mg/g) in B157 followed by TV26, SNT8, TS520, HP12, TV1, TV16, and TV20 whereas low value recorded in K1/1 (0.10 ± 0.005 mg/g). However, the carotenoids were recorded as highest in K1/1 (0.277 ± 0.03 mg/g)

Table 2 Susceptible index of the selected tea cultivars

Cultivars	Susceptible index*	Status**
TV20	1.009	HS
TV1	1.000	HS
K1/1	0.989	HS
TV22	0.969	HS
TV26	0.948	HS
TV16	0.938	HS
SNT8	0.908	HS
TS520	0.870	S
HP12	0.837	S
B157	0.772	MS

Highly susceptible (HS) 0.90–1.0, Susceptible (S) 0.80–0.89, Moderately Susceptible (MS) 0.70–0.79

compared to TV20, TV22, B157, TV26, HP12, SNT8, TV16, and TS520 whereas, the lowest was present in TV1 (0.178 ± 0.03 mg/g).

Individual catechins, gallic acid and caffeine using HPLC

Quantitative analysis of EGC, Catechin, EC, EGCG, ECG, Caffeine and Gallic acid using HPLC revealed that the EGC ranged between 2.480% to 4.382% among the tested cultivars (Table 5). Catechins content found in TV16 (1.794%) was the highest whereas; the lowest quantity was recorded in K1/1 (0.714%) among all the selected cultivars. The amount of EC was present maximum in TV22 (1.855%) and minimum in B157 (0.942%), whereas, highest EGCG value was recorded in K1/1 (11.31%) and the lowest value in HP12 (6.633%). In the cultivar TV16, ECG was recorded as (4.929%) followed by other cultivars, whereas HP12 contained the lowest quantity (1.737%). (Fig. 2). The gallic acid was found to be on the higher side (0.199%) in the case of B157, followed by other cultivars, with a minimum quantity found in HP12 (0.162%).

The correlation coefficient

The statistical analysis indicated that, the secondary metabolites namely alkaloids (r = -0.6137) and flavonoids (r = -0.7403) showing a negatively significant correlation with food consumption of looper of *H. talaca* (Table 4) whereas, polyphenol and carotenoid showed a positive correlation with the food consumption of looper. All types of catechins (EGC, Catechin, EC, EGCG, ECG) and caffeine were found to be positively correlated with the feeding of looper on the selected cultivars except Gallic acid (r = -0.5610) (Table 6).

Tea leaf biophysical characters

In the present investigation, no significant variation in leaf thickness and trichome density was observed among the cultivars (Table 7) which ranged between (0.212 to 0.248 µm and 103.3 to 134.3 number/cm^2). The regression equation showed a negative correlation (r = -0.7624) between the leaf thickness and food consumption of looper (Table 8). It was also evident from the results that, trichome density of B157 was found to be maximum (134.3 ± 9.3/cm^2) and minimum in TV22 (103.3 ± 6.0/cm^2) (Table 7). Trichome density showed a negatively significant correlation (r = -0.8024) with larval food consumption.

Table 3 Quantitative estimation of tea leaf primary and secondary metabolites

Cultivars	Total polyphenols (mg/g)	Total alkaloids (mg/g)	Total flavonoids (mg/g)	Carbohydrate (mg/g)	Protein (mg/g)	Lipids (mg/g)	Chlorophyll (mg/g)	Carotenoids (mg/g)
TV20	228.7 ± 21.1	25.6 ± 5.2	0.12 ± 0.007	0.687 ± 0.05	10.40 ± 1.2	14.6 ± 1.4	0.912 ± 0.08	0.267 ± 0.04
TV1	266.6 ± 15.2	25.8 ± 3.8	0.13 ± 0.004	0.786 ± 0.003	10.10 ± 1.1	14.2 ± 1.5	0.604 ± 0.06	0.178 ± 0.03
K1/1	205.4 ± 15.0	27.4 ± 3.7	0.10 ± 0.005	0.774 ± 0.005	7.71 ± 0.9	12.4 ± 1.2	0.946 ± 0.09	0.277 ± 0.03
TV22	213.9 ± 11.3	29.8 ± 2.9	0.11 ± 0.005	0.756 ± 0.008	9.26 ± 0.9	11.3 ± 1.8	0.889 ± 0.08	0.256 ± 0.04
TV26	217.2 ± 17.3	30.4 ± 3.5	0.14 ± 0.001	0.698 ± 0.006	10.83 ± 1.8	12.8 ± 1.2	0.821 ± 0.07	0.233 ± 0.02
TV16	259.2 ± 20.1	31.8 ± 3.5	0.13 ± 0.003	0.678 ± 0.001	9.42 ± 1.9	12.3 ± 1.1	0.678 ± 0.07	0.201 ± 0.03
SNT8	264.2 ± 21.3	33.8 ± 4.2	0.14 ± 0.001	0.643 ± 0.002	7.21 ± 0.7	12.4 ± 1.2	0.788 ± 0.08	0.218 ± 0.02
TS520	270.0 ± 10.2	29.4 ± 2.9	0.14 ± 0.004	0.664 ± 0.003	6.31 ± 0.6	9.4 ± 0.8	0.711 ± 0.09	0.198 ± 0.01
HP12	241. ± 14.5	26.8 ± 2.6	0.14 ± 0.003	0.583 ± 0.007	8.35 ± 0.8	12.2 ± 1.2	0.746 ± 0.06	0.220 ± 0.02
B157	190.9 ± 15.5	35.7 ± 2.8	0.15 ± 0.003	0.703 ± 0.007	7.20 ± 0.8	11.2 ± 1.2	0.838 ± 0.08	0.241 ± 0.02

Values represent mean ± SD of five replications

Table 4 Correlation between food consumption of *H. talaca* and biochemical compositions tea leaves

Biochemical compositions	Components	Correlation coefficient (r)	Regression equation
Primary metabolites	Protein	0.6351*	$y = 0.1352x - 3.036$, $R^2 = 0.403$
	Lipid	0.6223*	$y = 0.127x + 1.265$, $R^2 = 0.387$
	Carbohydrate	0.5767*	$y = 0.005x + 0.269$, $R^2 = 0.332$
	Chlorophyll	0.1414	$y = 0.002x + 0.608$, $R^2 = 0.02$
Secondary metabolites	Polyphenol	0.1356	$y = 0.1093x + 227.21$, $R^2 = 0.001$
	Alkaloid	-0.6137*	$y = -0.2866x + 54.489$, $R^2 = 0.376$
	Flavonoid	-0.7403*	$y = -0.0016x + 0.2681$, $R^2 = 0.548$
	Carotenoids	0.1874	$y = 0.0008x + 0.1574$, $R^2 = 0.035$

*Valuesare significant at $p < .05$

Cluster analysis

For a better understanding of the varietal preference, a dendogram was constructed based on the mean values of all larval food consumption, body weight gain, and nutritional indices of *H. talaca* (Fig. 3). The dendogram revealed two different clusters A and B which are further sub-branched as A1, A2, and B1, B2. In each four sub-branch different varieties were included based on the variation of larval food consumption, body weight gain, and nutritional indices. A1 grouped TS520 and HP12 as 'susceptible' category whereas A2 indicated B157 as 'moderately susceptible' category. Sub-branched B1 (TV22, TV16, TV26, and SNT8) and B2 (TV1, TV20, and K1/1) are categorized as 'highly susceptible' cultivars.

Discussion

The development of pest-resistant variety of any crop imparts a crucial role in the IPM program (Shah et al. 2008; Wilson and Huffaker 1976) and insect-resistant cultivars of tea would offer a significant element of IPM (Roy et al. 2009; Majumder et al. 2010). In the current study, the feeding preference of *H. talaca* on the selected tea cultivars was evaluated based on larval food consumption and utilization. The lowest food consumption and utilization was noticed in young larval instars of *H talaca*, which gradually increased with the advancement of larval instars. The nutritional requirements of insects vary based on their growth and development which are characteristically reflected in feeding behavior (Browne and Raubenheimer 2003). The food consumption and development of II, III, IV, and V instar larvae of tea looper significantly varied among the tested cultivars. The order of preference of cultivar was TV20 followed by TV1 > K1/1 > TV22 > TV26 > TV16 > SNT 8 > TS520 > HP12 whereas, the lowest consumption and body weight gain were observed on B157. A similar trend was observed by Majumder et al. (2010) who had worked on the variation in growth and survival of larvae of a related looper species *H. infixaria* maintained in four different tea clones, where, they observed the shorter larval duration of 15. 78 days when reared on the clone TV25 than 18.14 days on TV1, 18.00 days on TV9 and 17.00 days on Teen Ali 17.

Table 5 Percentage of weight of EGC, Catechin, EC, EGCG, ECG, Caffeine and Gallic acid

Cultivars	Catechins						
	EGC (%)	Catechin (%)	EC (%)	EGCG (%)	ECG (%)	Caffeine (%)	Gallic acid (%)
TV20	4.382	0.803	1.484	7.912	1.877	3.125	0.162
TV1	2.639	1.380	1.373	7.392	3.498	3.431	0.166
K1/1	3.837	0.714	1.210	11.31	2.174	3.394	0.176
TV22	3.927	0.980	1.855	9.793	3.397	3.331	0.175
TV26	3.255	0.753	1.278	9.504	2.353	2.519	0.182
TV16	2.480	1.794	1.645	7.575	4.929	3.625	0.172
SNT8	3.568	0.792	1.112	8.953	1.990	2.947	0.194
TS520	3.474	0.771	1.477	9.345	2.681	3.366	0.195
HP12	3.852	0.829	1.276	6.633	1.737	2.663	0.162
B157	2.987	0.716	0.942	9.499	2.109	2.818	0.199

Values represent mean of five replications

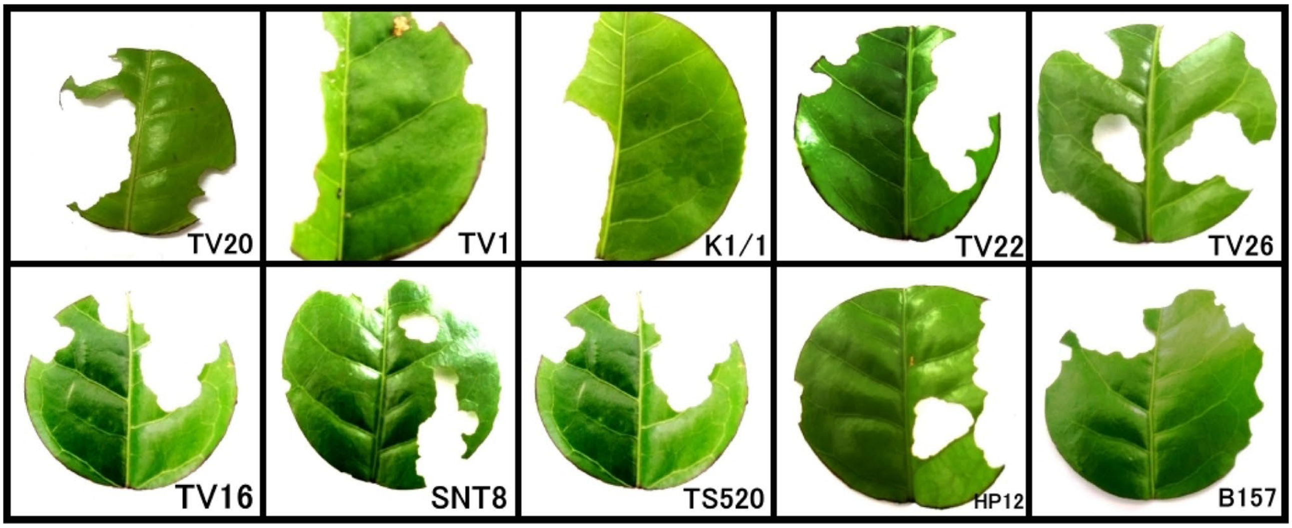

Fig. 2 Leaf discs selected cultivars showing feeding by III instar larvae of *H. talaca*

Noticeable variations were observed within the nutritional indices mainly in ECI and ECD values when *H. talaca* fed on the ten alternative tea cultivars. The values of ECD depend on the extent of food ingestion and approximate digestibility (Naseri et al. 2010). In the present study, the ECD and ECI were recorded as higher in II instar larva compared to the later instars. Our findings are in agreement with the findings of Kumar and Ahmad (2006) who had observed relatively lower values of ECI during the advancement of larval instars. The value of ECD and ECI were also fluctuating when *H. talaca* was reared on TV20 followed by TV1, K1/1, TV22, TV26, TV16, SNT8, TS520, and HP12 whereas it was low on B157. The ECI was also found to vary due to the digestibility of food and the relative amount of the digestible portion of food which is transformed to body mass and energy required for physiological activities of the developing larvae (Abdel- Rahman and Al-Mozini 2007). The RGR and RCR values were also found to be high in young larval instar and gradually decreased with the advancement of stage of the larvae with an order II > III > IV > Vwhen larvae of *H. talaca* fed on these tested cultivars. The AD values increased proportionally with the larval development with the highest in TV20 followed by B157 cultivar. Our results are in line with the results of Gurusubramanian et al. (1991) and Raman et al. (1994) who had reported that AD and ECI was inversely correlated with the larval age of *Heliothis armigera*.

The results of the susceptible index study revealed that the cultivars TV20, TV1, K1/1, TV22, TV26, TV16, and SNT8 were highly susceptible whereas TS520 and HP12 as 'susceptible' and B157 as categorized as 'moderately susceptible'. This may be attributed to the variation in the primary and secondary metabolites of the selected cultivars. The correlation of protein (r = 0.6351), lipid

Table 6 Regression equation and correlation between food consumption and percentage of weight of EGC, Catechin, EC, EGCG, ECG, Caffeine and Gallic acid

Biochemical compositions	Correlation coefficient (r)	Regression equation
EGC	0.1852	y = 0.015x + 2.107, R^2 = 0.034
Catechin	0.2884	y = 0.014x-0.272, R^2 = 0.083
EC	0.5149	y = 0.018x-0.256, R^2 = 0.265
EGCG	0.0789	y = 0.015x + 7.475, R^2 = 0.006
ECG	0.2840	y = 0.039x-0.703, R^2 = 0.080
Caffeine	0.4635	y = 0.023x + 1.082, R^2 = 0.214
Gallic acid	-0.5610	y = -0.001x + 0.271, R^2 = 0.314

Table 7 Biophysical characters of tea leaf

Cultivars	Leaf thickness (µm)	Trichome density (No./cm^2)
TV20	0.224 ± 0.002	104.33 ± 7.3
TV1	0.215 ± 0.004	111.6 ± 7.2
K1/1	0.235 ± 0.002	118.0 ± 9.9
TV22	0.227 ± 0.001	103.3 ± 6.0
TV26	0.212 ± 0.002	125.3 ± 8.6
TV16	0.223 ± 0.005	123.0 ± 9.6
SNT8	0.239 ± 0.001	128.3 ± 9.9
TS520	0.240 ± 0.002	131.3 ± 9.3
HP12	0.241 ± 0.002	125.7 ± 7.8
B157	0.248 ± 0.002	134.3 ± 9.3

Values represent mean (± SE) of five replications

Table 8 Regression equation and correlation between food consumption of *H. talaca* and leaf morphology

Correlation between food consumption of *H. talaca* and leaf characteristics		
Parameters	Correlation coefficient (r)	Regression equation
Thickness of leaf	-0.7624*	y = -0.001x + 0.339, R^2 = 0.581
Trichome density	-0.8024*	y = -1.203x + 224.79, R^2 = 0.643

*Values are significant at p < .05

(r = 0.5767), carbohydrate (r = 0.6223) and chlorophyll (r = 0.1414) with all the larval mean food consumption showed a positive relation. However, no significant difference was noticed in the fifth instar larval feeding on the tested cultivars. Proteins and carbohydrates present at a variable quantity in an individual plant depending on the type of tissue and age of leaves (Mattson 1980; Yeoh et al. 1992; Sattelmacher et al. 1994). Thus, it is a challenge for herbivorous insects to find, select and consume food possessing presence of a high concentration of proteins and carbohydrates (Gall and Behmer 2014). Abundance of polyphenols, catechins, total carbohydrates and proteins in tea shoots seem to encourage the degree of attack by insect pest (Roy et al. 2009). In the present study among all the secondary metabolites, alkaloid (r = -0.6137), flavonoid (r = -0.7403), and gallic acid (r = -0.5610) showed a negative correlation with the feeding of *H. talaca* on the tested cultivars. Plants produce secondary metabolites like alkaloid, flavonoid, etc., when they encounter insect herbivores or pathogen attacks (Grayer, 2005; Ahmed et al. 2017). Gallic acid produced by plants act as insect repellent (Sarvamangala et al. 2014). Ananthakrishnan (1997) reported that gallic and salicylic acids show an important role in insect-plant interactions which also have significant application in biotechnology in crop protection. Polyphenol, carotenoids, EGC, catechin, EC, EGCG, ECG, caffeine are positively correlated with the mean food consumption of larval instars of *H. talaca*. This could be due to their abundance in all the selected tea cultivars. *Hyposidra* spp. could exploit detoxifying enzymes to overcome the secondary metabolites and the concentration of allelochemicals to complete their life cycle on both the host plants (*Schima wallichii* and *Camellia sinensis*) (Das and Mukhopadhyay 2014). The trichome density (r = -0.8024) and leaf thickness (r = -0.7624) also negatively co-related with the food consumption of larvae of *H. talaca* on the tested cultivars. Results of the present investigation showed maximum food consumption on TV20 in which trichome density and leaf thickness were low whereas reverse spectacle was observed in B157. Apart from secondary metabolites plants also use morphological defensive structures like waxes, trichomes, and latices to make the herbivores feeding more problematic (Hägg et al. 2013). The results of the cluster analysis revealed that, the cultivars grouped within cluster B and sub-branch A1 could be attributed to the presence of higher number of trichomes and biochemical profile similarities whereas B157 was grouped to the sub-branch A2 due to the variation in the biochemical profiles and morphological characteristics of the tested cultivars.

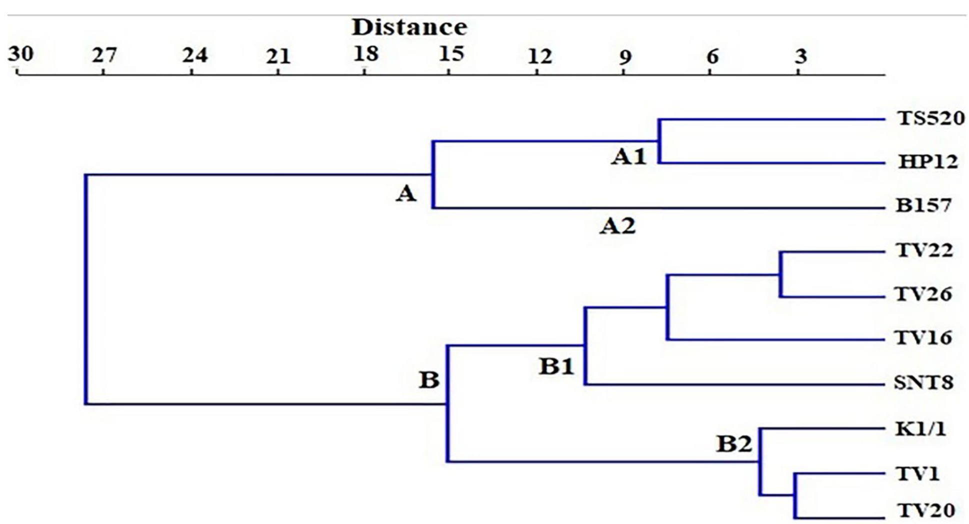

Fig. 3 Dendogram based on the mean of all larval food consumption, body weight gain and nutritional indices of *H. talaca* fed on different tea cultivars

Conclusion

The current study provides basic information about the degree of infestation by the insect and awareness to take the necessary protection strategy for the the tested cultivars to keep the *H. talaca* population below the economic injury level (ETL). For a better understanding of the *H. talaca*-tea cultivar interaction, further studies are needed, for minimizing the attack of *H. talaca* in the tea plant in an effective manner.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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