Biochemical Parameters to Identify Shot-Hole Borer *(Euwallacea fornicatus)* Resistance in Tea Cultivars: Emphasis on Polyphenols, Catechins, and Caffeine

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Abstract. Shot-hole borer (SHB) (*Euwallacea fornicatus*) is a major pest threatening tea (*Camellia sinensis*) plantations in Sri Lanka. Traditional methods for screening SHB resistance rely on variable levels of natural infestation, which can lead to inconsistent results. This study aimed to develop a screening protocol based on biochemical parameters to identify tea cultivars and accessions resistant to SHB. Resistance levels were evaluated through beetle-settling bioassays and biochemical analyses. The bioassay results demonstrated varying levels of resistance to SHB: Resistant (Accession 43, TRI 2023), Moderately Resistant (TRI 5006, TRI 5002, Accession 208), Moderately Susceptible (TRI 5005, TRI 2025), and Susceptible (Accession 88). Biochemical analysis of bark material revealed that the resistant cultivars, Accession 43 and TRI 2023, had significantly lower caffeine concentrations ($p<0.05$) of 2.46 mg/g and 2.85 mg/g, respectively, before infestation. However, these levels increased to 6.48 mg/g and 5.24 mg/g after infestation. In contrast, the susceptible cultivars (Accession 88, TRI 5005, and TRI 2025) exhibited higher caffeine levels before infestation (6.02 mg/g, 5.31 mg/g, and 5.08 mg/g, respectively), with further increases to 17.5 mg/g, 16.17 mg/g, and 15.75 mg/g, respectively, after infestation. There was a significant reduction ($p<0.05$) in total catechin concentration across all cultivars after infestation, while polyphenol levels varied among the cultivars. Cluster analysis classified the tested cultivars into two distinct groups. The findings indicate significant variation in the responses of secondary metabolites among tea cultivars and suggest that lower caffeine and specific catechin levels could serve as biochemical markers for identifying tea cultivars resistant to SHB infestation.

Keywords: *Camellia sinensis*, Shot-hole Borer, Secondary Metabolites, Pest Resistance, Screening.

1 Introduction

The tea plant (*Camellia sinensis*) belongs to the family Theaceae, and is affected by a range of arthropod pests, and the tea shot-hole borer (SHB) beetle (*Euwallacea fornicatus*) is the most serious pest of tea in Sri Lanka (Walgama, 2012). This pest, which belongs to the family Curculionidae, sub-family Scolytinae, was first encountered in 1892 at Craighead Estate, Nawalapitiya (Austin, 1956; Walgama and Pallemulla, 2005). Tea SHB is well protected because it spends its life cycle within the galleries of living branches. Pruning practices induce SHB damage because newly formed primary branches with a diameter of 1 cm reach the susceptible stage of infestation at 10–12

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months after pruning and continue to remain vulnerable for 24 months after pruning (Senaratne and Mohotti, 2008).

Plant metabolomics is the end product of gene expression and has become a valuable tool for understanding plant responses (Rodriguez et al., 2021). Plants have evolved various defence mechanisms to combat microbial pathogens and herbivores, including programmed cell death, the production of antimicrobial compounds, and cell wall fortification (Kim and Sano, 2008). The three primary categories of secondary metabolites in tea plants are nitrogenous compounds, terpenoids, and phenolic compounds (Jain et al., 2019), which are usually classified by their biosynthetic pathways, each of which plays a role in plant defence. Phenolic compounds, including catechins, flavonoids, lignins, and tannins, are essential for tea plant-herbivore interactions. Caffeine was shown to be an effective repellent and pesticide for slugs and snails (Hollingsworth et al., 2003) and cutworms (*Spodoptera litura*) (Uefuji et al., 2005). Furthermore, caffeine has been shown to reduce oviposition and delay SHB development (Karunaratne et al., 2009), thereby decreasing reproductive potential and lowering egg protein content (Uefuji et al., 2005). However, secondary metabolites can vary in quantity and quality depending on factors such as plant type, growth environment (Fauziah et al., 2022), and growth phase (Jain et al., 2019).

The application of insecticides against the SHB has shown moderate success; however, it has been largely ineffective due to the SHB's hidden lifestyle within the plant (Walgama, 2012). As a result, the most effective strategy to prevent SHB infestation is to plant varieties of tea that are highly resistant or tolerant. Tea cultivars can be categorised into four groups: resistant, moderately resistant, moderately susceptible, and susceptible based on their responses to SHB. Therefore, it is essential to identify SHB-resistant genotypes and to focus breeding programs on these varieties to ensure economic sustainability. Conventional screening of tea cultivars for SHB resistance typically relies on natural infestation levels, which can vary considerably. There is a need to develop an early screening protocol using biochemical parameters for tea breeding programs, as levels of certain compounds differ between resistant and susceptible cultivars. Fernando et al. (2020) demonstrated that caffeine, catechin, and polyphenol contents could effectively distinguish between tea cultivars resistant to *Glyptotermes dilatatus*. Similarly, Lizardo et al. (2022) showed that antioxidant and phenolic levels serve as effective markers for screening tomato varieties against root-knot nematode infestations. These findings support the hypothesis that biochemical profiles vary between resistant and susceptible cultivars, highlighting the potential for using biochemical markers in early resistance screening.

To date, there is no published data on protocols for screening tea cultivars using biochemical parameters. Therefore, it is crucial to understand the intrinsic factors contributing to SHB resistance. Identifying and breeding resistant cultivars will be an essential tool for tea plantations in their efforts to combat SHB and other pest-related challenges. Hence, this study was designed with the specific objectives of evaluating SHB resistance levels in selected Sri Lankan tea cultivars using a laboratory bioassay and comparing total polyphenol, caffeine, and catechin levels in the cultivars before and after SHB infestation.

2 Materials and methods

2.1 Sampling procedure and plant material

Different tea cultivars were selected from the trial plot (UVP 9), Passara, Sri Lanka, where no synthetic pesticides were applied. All cultivars are grown in rows within a plot consisting of 24 plants. The tea cultivars/accessions were chosen based on prior screening results, as shown in Table 1. A Complete Randomised Design (CRD) was used to collect samples for biochemical analysis. Sampling was done in triplicate, with each replicate consisting of branches around 1 cm in diameter (Pencil thickness) and 30 cm in length. Samples were collected before and after natural SHB infestation, with a one-month interval between sampling events (Fernando et al., 2020).

Table 1. Selected tea cultivars/accessions for this study according to cultivar screening results

	Cultivar/Accession	SHB resistance level
T1	43	Resistant
T2	TRI 2023	Moderately resistant
T3	TRI 5006	Moderately resistant
T4	TRI 5002	Moderately resistant
T5	208	Moderately susceptible
T6	88	Moderately susceptible
T7	TRI 5005	Susceptible
T8	TRI 2025	Susceptible

2.2 Laboratory culture of Shot-hole borer beetles

SHB beetles were cultured under laboratory conditions as described by Hewavitharanage et al. (1999), with modifications. Tea barks were collected using the TRI 2025 cultivar at St. Coombes Estate for diet tube preparation. Healthy adult female beetles were collected from Nayapana Estate.

2.3 Beetle-settling bioassay

Fresh plant, disease and SHB non infested (SHB-free) primary stems (30 cm length and pencil thickness in 1cm diameter per cultivar) were collected from field-grown tea plants from UVP9, Passara, Sri Lanka. Each 30 cm stem was divided into three units (10 cm each), placed vertically, and twelve healthy female beetles were introduced, four per stem unit, into the glass jar, which was then covered with a breathable piece of cloth. Those were maintained in an incubator (25±1ºC, 70±2% R.H.). Total nibble points and total gallery count in each stem unit (10 cm length) of each cultivar were recorded after 3 days, and total nibble points and total gallery count per 30 cm length of primary stem were recorded. Experiments were replicated 10 times. Cultivars were categorised into the following groups based on AGC/stem: resistant (AGC 0.0–2.0), moderately resistant (AGC 2.1–4.0), moderately susceptible (AGC 4.1–6.0), and susceptible (AGC >6.0) (Amarasinghe et al., 1999).

2.4 Preparation of crude extract for the total polyphenol (TPP), caffeine, and catechin analysis

The crude extract was prepared according to the method described by Kottawa-Arachchi et al. (2022).

Determination of total polyphenol content. Total polyphenol content was determined using the method explained by Piyasena et al. (2024). The standard curve of gallic acid ranged from 0.5 to 40 µg/ml (Pearson's correlation coefficient: r^2= 0.9997). The final TPP values were expressed as a percentage on a dry matter basis and as mg gallic acid equivalent (GAE) g^{-1} (mg/g).

Quantification of caffeine and catechin. Caffeine and catechin quantification were done by using the ISO 14502-2 (2005) method.

2.5 Statistical analysis

The data was collected and analysed using the SAS Studio (SAS 9.4). The results of the beetle-settling bioassay were tested for normality using the Shapiro-Wilk test, and the one-way Analysis of Variance (ANOVA) followed by mean separation using the Least Significant Difference (LSD) test. First sampling round and second sampling round of SHB-non infested and SHB-infested samples were pooled separately by analysing two-way repeated measures ANOVA. The collected data were subjected to one-way ANOVA for caffeine, total catechin, and polyphenol across cultivars. Mean separation was obtained by LSD. Complete linkage cluster analysis was performed using the data on the tested traits to classify differences among cultivars. The Euclidean distance method was used to measure relationships between the samples (columns), and the results were visualised as a 2D dendrogram. For all statistical analyses, significance was evaluated at 95% confidence interval (α=0.05).

3 Results and discussion

3.1 Beetle-settling bioassay

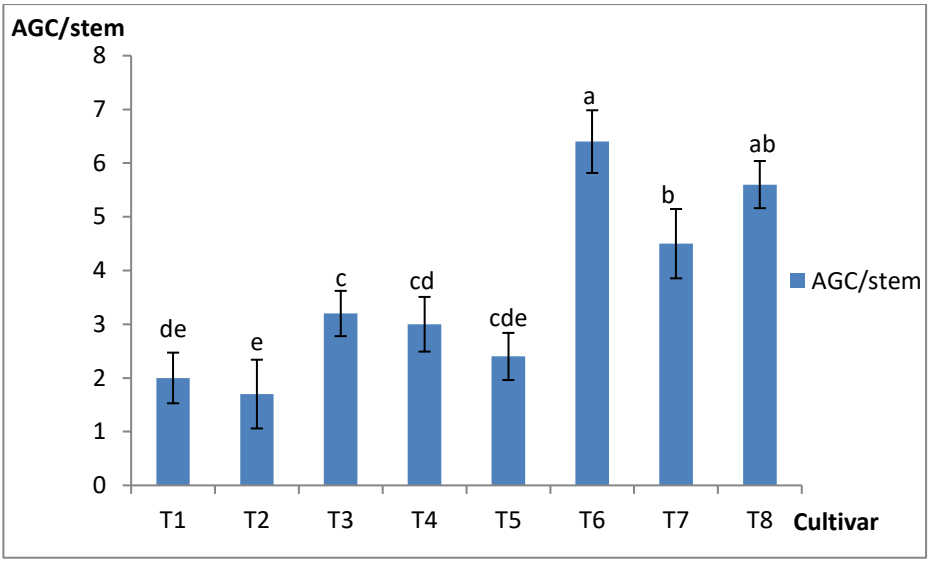

Fig. 1. Mean number of SHB beetles settled in different tea cultivars

Means with the same letters are not significant ($p>0.05$)

The mean number of SHB beetles settled on eight different tea cultivars of T1-T8 is shown in Fig 1. There is a significant difference among cultivars ($p<0.05$). Cultivars T1, T2, and T5 exhibited the lowest means of galleries, indicating potential resistance to SHB. Among them, T2 had the lowest mean gallery count. Moreover, T5 was categorised as moderately susceptible based on field screening results, despite a low gallery count in the bioassay. This may suggest the involvement of factors other than initial beetle attraction under the field conditions, such as post-settlement of biochemical resistance.

In contrast, T6, T8, and T7 recorded the highest mean gallery count, suggesting higher susceptibility to SHB infestation. The statistical analysis confirmed that beetle settlement was significantly lower in T2 than in other cultivars, highlighting its higher resistance. On the other hand, T6 exhibited the highest mean number of galleries, marking it the most susceptible cultivar in this study.

3.2 Caffeine, total catechins, and polyphenol content in the bark of tea cultivars at one-month intervals in non-infested tea plants

There was no significant difference ($p>0.05$) in the caffeine, catechin, and polyphenol content between the monthly interval analyses of SHB non-infested tea bark samples, except for the caffeine content in T3 and T6 cultivars (Table 2).

Table 2. Caffeine, total catechin, and polyphenol concentration (mg/g) in the 1st and 2nd sampling times (at one-month intervals) of eight non-infested tea cultivars

Cultivar/ Accession	Caffeine (mg/g)		Total catechin (mg/g)		Polyphenol (mg/g)	
	1st	2nd	1st	2nd	1st	2nd
T1	2.43±0.0[a]	2.48±0.5[a]	15.64±0.1[a]	13.64±1.8[a]	113.44±5.2[a]	122.14±12.0[a]
T2	3.01±0.0[a]	2.69±0.4[a]	13.41±0.7[a]	12.69±0.4[a]	103.76±3.9[a]	105.87±3.2[a]
T3	7.01±0.1[a]	5.64±0.4[b]	16.64±0.5[a]	19.65±1.5[a]	122.08±2.7[a]	114.9±3.4[a]
T4	5.32±0.2[a]	4.33±0.1[a]	16.79±1.0[a]	17.86±1.2[a]	98.61±2.6[a]	87.93±2.9[a]
T5	2.77±0.0[a]	2.56±0.2[a]	9.09±0.2[a]	13.53±0.8[a]	99.88±3.4[a]	100.1±2.9[a]
T6	6.91±0.1[a]	5.14±0.6[b]	28.28±1.1[a]	25.52±4.7[a]	118.31±1.1[a]	115.61±3.1[a]
T7	5.88±0.1[a]	4.75±0.7[a]	19.24±0.7[a]	17.85±2.9[a]	125.82±3.6[a]	114.38±4.1[a]
T8	5.39±0.2[a]	4.76±0.5[a]	18.93±0.9[a]	14.86±0.5[a]	114.26±2.2[a]	117.12±5.1[a]

Each data point represents the mean of three replicates

Mean + SD of the 1st and 2nd sampling in columns of each phytochemical, followed by different letters, are significantly different at p=0.05. SD: standard deviation of population mean, 1st =1st sampling day of non-infested sample, 2nd = 2nd sampling day of non-infested sample

3.3 Caffeine, total catechins, and polyphenol content in the bark of tea cultivars at one-month intervals in SHB-infested tea plants

There was no significant difference (p>0.05) between caffeine, catechin, and polyphenol content between monthly interval analysis of SHB-infested tea bark samples (Table 3).

Table 3. Caffeine, total catechin, and polyphenol concentration (mg/g) in the 1st and 2nd sampling times (at one-month intervals) of eight SHB-infested tea cultivars

Cultivar/ Accession	Caffeine (mg/g)		Total catechin (mg/g)		Polyphenol (mg/g)	
	1st	2nd	1st	2nd	1st	2nd
T1	5.54±0.7[a]	7.42±3.4[a]	7.76±0.7[a]	6.75±6.1[a]	105.93±8.4[a]	81.97±40.5[a]
T2	4.74±0.3[a]	5.75±3.3[a]	7.98±0.4[a]	8.61±5.8[a]	116.11±1.9[a]	104.41±11.2[a]
T3	10.65±0.9[a]	10.26±2.7[a]	11.08±7.1[a]	14.49±1.3[a]	94.09±37.5[a]	122.58±8.2[a]
T4	11.08±2.4[a]	14.54±3.1[a]	10.87±4.2[a]	19.80±4.0[a]	92.96±6.2[a]	112.46±10.0[a]
T5	10.58±3.0[a]	11.23±0.8[a]	7.14±0.8[a]	8.50±5.9[a]	96.65±2.6[a]	113.17±22.8[a]
T6	16.21±2.1[a]	18.78±2.5[a]	20.61±3.9[a]	24.26±5.3[a]	126.2±7.2[a]	130.28±4.6[a]
T7	13.94±4.9[a]	18.40±4.0[a]	12.85±8.7[a]	20.60±4.6[a]	109.41±40.4[a]	131.35±15.4[a]
T8	14.17±0.6[a]	17.35±2.1[a]	11.84±5.0[a]	17.09±4.9[a]	105.99±31.2[a]	127.85±10.7[a]

Each data point represents the mean of three replicates

Mean + SD of the 1st and 2nd sampling in columns of each phytochemical, followed by different letters, are significantly different at p=0.05. SD: standard deviation of population mean, 1st =1st sampling day of SHB-infested sample, 2nd = 2nd sampling day of SHB-infested sample

3.4 Variation of caffeine, total catechin, and total polyphenol content in SHB non-infested and infested pooled tea bark samples

To evaluate differences in phytochemical composition between SHB non-infested and infested tea bark samples, data obtained from the first and second sampling rounds were pooled separately for each condition (Tables 2 and 3) prior to analysis. This strategy was adopted to better represent the biological stages of infestation rather than short-term temporal variability. Pooling the data reduced within-treatment variability and provided an integrated measure of the average biochemical status of each cultivar under infested and non-infested conditions. A similar approach has been reported by Fernando et al. (2020), who observed no significant monthly variation in caffeine content during their study period; instead, changes in caffeine levels were primarily associated with infestation by low-country live wood termite (LCLWT) rather than temporal fluctuations.

Effect of SHB infestation on caffeine of tea cultivars. The caffeine content (mg g^{-1}) in SHB non infested and infested bark samples from the primary stems of eight tea cultivars (T1–T8) is shown in Fig. 2. Across all cultivars, SHB infestation resulted in a significant ($p<0.05$) increase in caffeine concentration compared to non-infested samples, indicating that caffeine functions as an inducible secondary metabolite in response to herbivore attack (Zhang et al., 2024). Variation in caffeine content between non-infested and infested samples differed significantly among cultivars. Cultivar T6 exhibited the highest caffeine concentration in infested stems (17.5 mg g^{-1}), followed by T7 (16.2 mg g^{-1}) and T8 (15.8 mg g^{-1}). In contrast, the lowest caffeine level in infested samples was recorded in T2 (5.2 mg g^{-1}). Non-infested samples consistently showed lower caffeine contents, particularly in T1 (2.5 mg g^{-1}), T2 (2.9 mg g^{-1}), and T5 (2.7 mg g^{-1}). Notably, SHB-susceptible cultivars such as T6, T7, and T8 exhibited the greatest increases in caffeine content following infestation, whereas SHB-resistant cultivars, including T1 and T2, showed comparatively smaller induction levels. This differential response suggests a cultivar-dependent role of caffeine in defence mechanisms against SHB infestation.

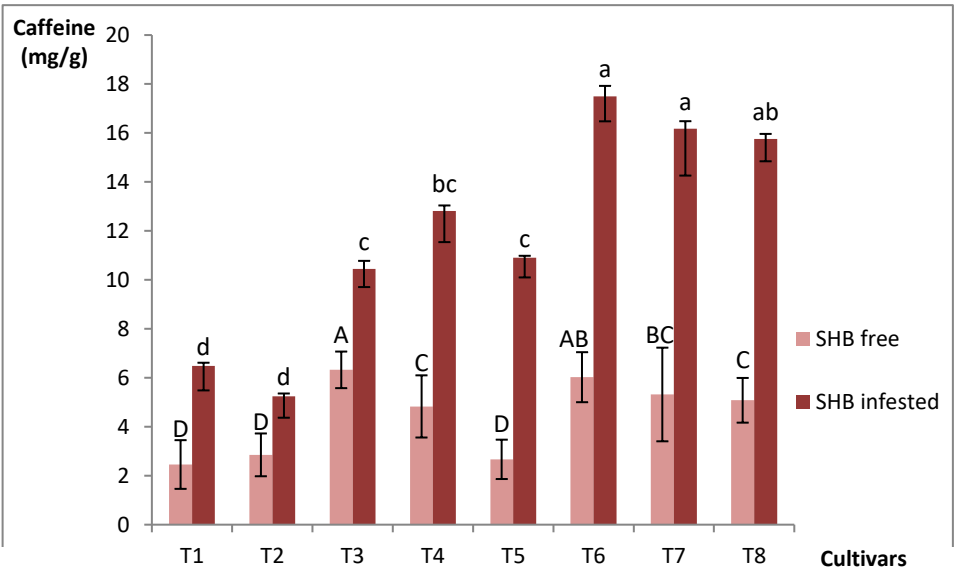

Fig. 2. Caffeine (mg/g) of healthy and infested bark of primary stems of SHB-resistant and susceptible cultivars

Error bars indicate standard errors, reflecting variation within replicates for each cultivar. Different uppercase letters indicate significant differences among cultivars before SHB infestation, while different lowercase letters indicate significant differences after SHB infestation ($p<0.05$).

Effect of SHB infestation on total catechin of tea cultivars. The total catechin content (mg g^{-1}) in the primary stem bark of SHB non-infested and infested tea cultivars (T1–T8) is presented in Fig. 3. Across all cultivars, SHB infestation resulted in a significant reduction ($p<0.05$) in total catechin concentration. The magnitude of catechin decline varied significantly among cultivars, indicating a cultivar-dependent response to SHB attack. Among the non-infested samples, cultivar T6 exhibited the highest catechin content (26.9 mg g^{-1}), followed by T7 (18.6 mg g^{-1}) and T3 (18.2 mg g^{-1}). In SHB-infested samples, catechin concentrations were consistently lower; however, T6 maintained the highest level (22.4 mg g^{-1}), albeit with a marked reduction relative to healthy tissues. The most pronounced decreases were observed in cultivars T1 and T3, where catechin content declined from 14.7 to 7.3 mg g^{-1} and from 18.2 to 12.8 mg g^{-1}, respectively. Cultivar T5 recorded the lowest catechin concentrations under both conditions, with values of 11.3 mg g^{-1} in non-infested samples and 7.8 mg g^{-1} following infestation. The consistent reduction in catechin content across all cultivars after SHB infestation suggests enhanced utilisation or degradation of catechins during plant defence responses, or possible suppression of catechin biosynthesis by the pest.

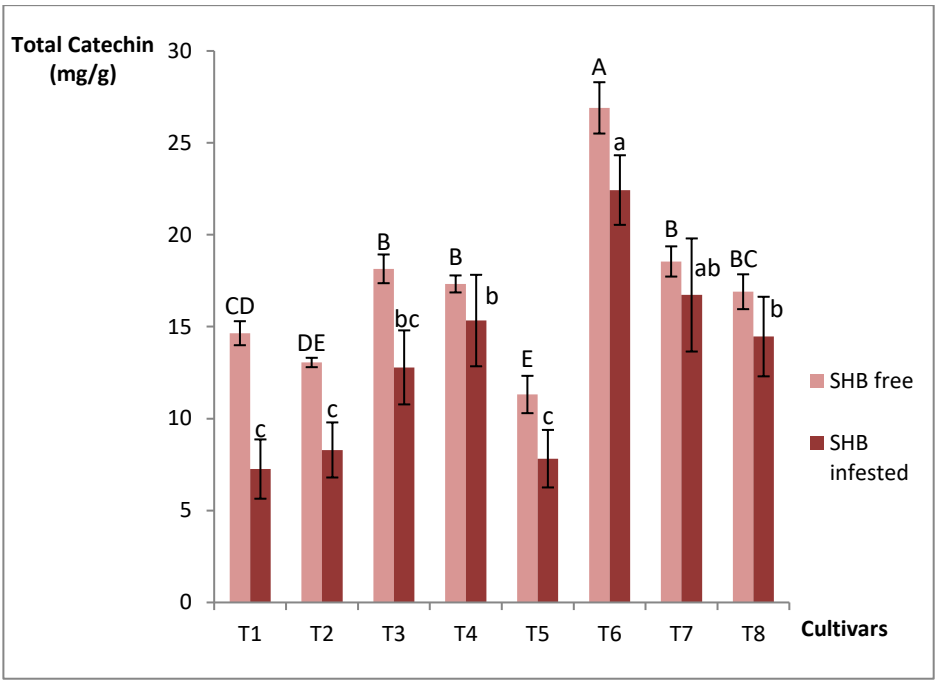

Fig. 3. Total Catechin (mg/g) of healthy and infested bark of primary stems of SHB-resistant and susceptible cultivars

Error bars represent standard errors, indicating variation among replicates for each cultivar. Different uppercase letters indicate significant differences among cultivars before SHB infestation, while different lowercase letters indicate significant differences after SHB infestation ($p<0.05$).

Effect of SHB infestation on the total polyphenol of tea cultivars. Fig. 4 illustrates the total polyphenol content (mg g^{-1}) in the bark of primary branches from SHB non-infested and infested plants across eight tea cultivars (T1–T8). Consistent with the observations for caffeine and total catechin, total polyphenol content did not exhibit a clear or consistent trend in response to SHB infestation. Variation in total polyphenol content among the non-infested samples differed significantly across cultivars. However, in SHB-infested samples, the differences in total polyphenol levels were not statistically significant ($p=0.1927$), indicating a convergence of polyphenol content among cultivars following infestation.

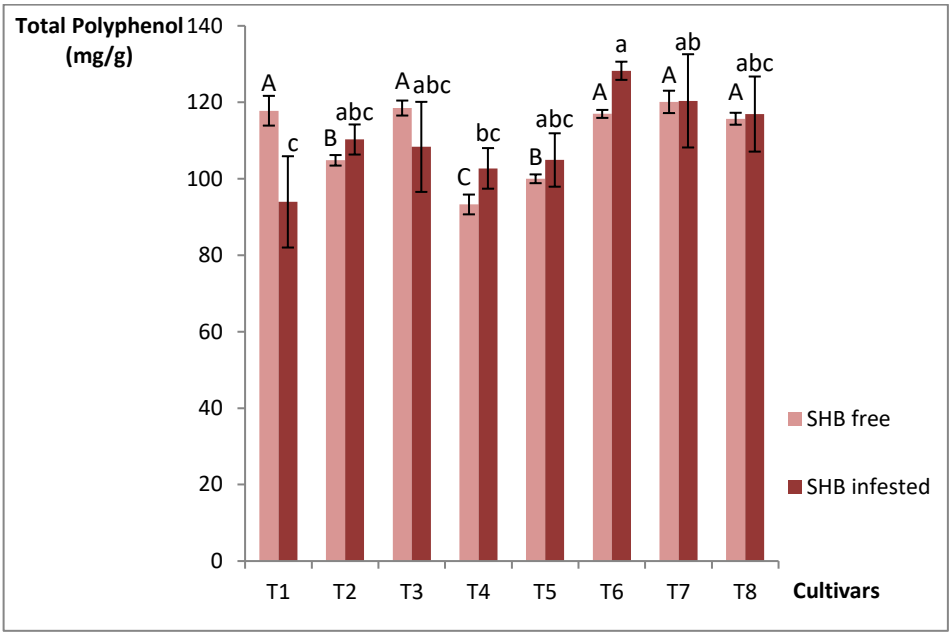

Fig. 4. Total polyphenol (mg/g) of healthy and infested bark of primary stems of SHB-resistant and susceptible cultivars

Error bars represent standard errors, indicating variation among replicates for each cultivar. Different uppercase letters indicate significant differences among cultivars before SHB infestation, while different lowercase letters indicate significant differences after SHB infestation ($p<0.05$).

According to the results, SHB infestation primarily affected the caffeine and total catechin contents of tea cultivars, suggesting a potential role for these compounds in plant defence responses. Caffeine concentration in the bark of infested stems was significantly higher ($p<0.05$) than that observed in healthy stems, indicating inducible accumulation following SHB attack. Similar responses have been reported previously, where invasion by *Ectropis obliqua* triggered enhanced caffeine biosynthesis and accumulation in tea plants (Zhang et al., 2024). Overall, SHB infestation altered the production of key secondary metabolites, including caffeine and total catechins, in tea cultivars. In contrast to caffeine, total catechin content, representing the major fraction of tea polyphenols showed a consistent reduction following infestation. This decrease may reflect the utilisation of catechins in defence-related processes, such as their conversion into esterified forms or other oxidative derivatives involved in plant defence mechanisms (Punyasiri et al., 2005).

Although Zeng et al. (2021) and Hewavitharanage et al. (1999) reported that elevated caffeine levels play an important biological role in defence against *Ectropis obliqua* and SHB, respectively, based on *in vitro* evaluations, the present study revealed a contrasting pattern under field conditions. The SHB-resistant cultivar T2 exhibited comparatively low caffeine content (2.9 mg g^{-1}) relative to the susceptible cultivar T8 (5.0 mg g^{-1}). This observation suggests that high caffeine concentration alone may not be a reliable indicator of resistance. Supporting this view, Punyasiri et al. (2017) reported that tea accessions with elevated caffeine levels were more susceptible to blister blight disease.

Furthermore, Hewavitharanage et al. (1999) demonstrated that caffeine inhibits the growth of the SHB-associated fungus *Fusarium ambrosium*, with delayed growth observed in caffeine-amended media and complete inhibition at 5000 ppm. Interestingly, the addition of tea bark extract to the growth medium partially alleviated the antifungal effect of caffeine, indicating complex biochemical interactions between host plant metabolites and pest-associated microorganisms. These findings suggest that resistance to SHB infestation is likely governed by multifactorial biochemical mechanisms rather than by caffeine content alone.

According to the results, we can conclude that caffeine has an inhibitory effect; however, under plant conditions, there may be conditions favourable to fungal growth and pest survival. Moreover, specific secondary metabolites can act as feeding stimulants or deterrents depending on their concentration (Zhao et al., 2020). For example, rutin, a flavonoid compound, has been shown to stimulate feeding in several *Spodoptera* and *Helicoverpa* species when applied at concentrations between 10^{-4} and 10^{-5} M, whereas, at higher concentrations, it acts as a feeding deterrent (Zhao et al., 2020). This concentration-dependent effect highlights the complexity of plant-insect interactions and suggests that not only the presence but also the amount of specific metabolites can significantly influence herbivore behaviour. In this study, susceptible tea cultivars were observed to contain higher levels of caffeine and catechins than resistant cultivars. This finding may indicate that these compounds, although generally associated with plant defence, could potentially serve as feeding cues or may not exceed the threshold required to deter the Shot-hole borer. Therefore, the role of secondary metabolites in resistance appears to be not only compound-specific but also concentration-dependent. Alternatively, the pest may tolerate or detoxify these compounds to a certain extent, enabling it to feed and develop on cultivars with higher concentrations. Ceja-Navarro et al. (2015) found that the coffee borer beetle (*Hypothenemus hampei*) utilises its gut microbiota to detoxify caffeine, enabling it to feed on caffeine-rich coffee beans. Similarly, Zhang et al. (2020) demonstrated that the gut microbial community of the Camellia weevil (*Curculio chinensis*) can degrade saponins, common defence compounds in tea seeds, thereby allowing the insect to bypass plant defences.

3.5 Complete linkage cluster analysis of non-infested and infested pooled bark samples

A hierarchical cluster analysis (Fig. 5a and 5b) was performed to evaluate the similarity among the eight tea cultivars (T1–T8) based on the concentrations of secondary metabolites (caffeine, total catechins, and polyphenols) quantified before and after SHB infestation. The dendrogram revealed two clusters. The clustering of resistant cultivars T1, T2, and T5 in both dendrograms indicates that they share similar resistance and levels of the tested secondary metabolites. T6 records as an outlier representing the most susceptible one. The variation in the placement of T3 and T4 revealed that their metabolite composition may differ depending on their response to SHB. This indicates that, within the same bioassay series, susceptible and resistant cultivars can be screened for caffeine and total catechin content.

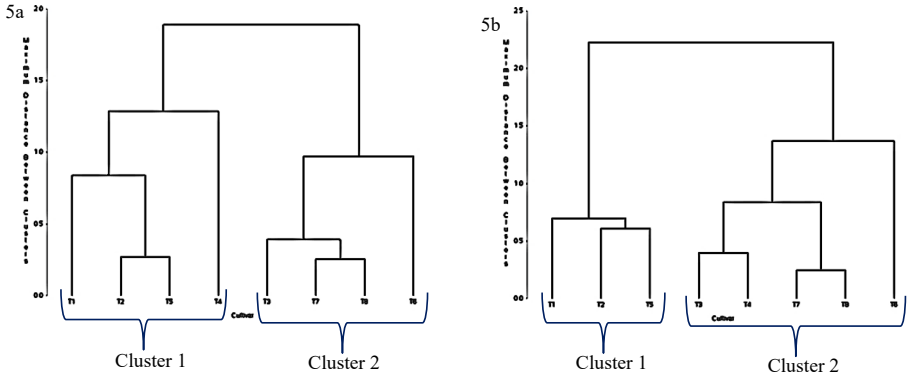

Fig. 5. Hierarchical cluster with average distance between clusters (a): SHB non-infested bark sample, (b) SHB infested bark sample

4 Conclusion

This study demonstrates that SHB infestation significantly alters key secondary metabolites in tea bark, particularly caffeine and total catechins. Caffeine levels increased substantially in infested tissues, whereas total catechin content decreased, reflecting distinct defence-related metabolic responses. Based on these biochemical patterns, tea cultivars could be classified into SHB-resistant and SHB-susceptible groups, supporting the feasibility of early biochemical screening to reduce reliance on prolonged field evaluations.

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Disclosure of Interests. Authors have declared no competing of interest.

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