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A Biochemical Approach to identify Resistant Tea Cultivars to Low Country Live Wood Termite (*Glyptotermes dilatatus*)

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ABSTRACT

Pest damage to the tea bush is a serious issue for Sri Lankan tea industry. Low Country Live Wood Termite (LCLWT), *Glyptotermes dilatatus* is one of the most economically important pests as they attack the low grown teas which have the highest contribution to the Sri Lankan tea production. Introducing resistant cultivars with high yield is the most promising method among various methods of controlling this pest. Therefore, screening new cultivars is important to introduce resistant cultivars to the grower. This research was conducted to study the feasibility of using caffeine, total catechin and total polyphenol contents as biochemical markers to identify resistant cultivars for LCLWT. Healthy and rotted stems of four cultivars (TRI 4042, TRI 4049, TRI 3025, TRI 3069) which were susceptible and resistant to LCLWT were collected separately. Caffeine content had increased in all cultivars upon infestation. Resistant cultivars had high caffeine in the healthy stems. Total catechin and total polyphenol content decreased in all the cultivars upon infestation and high amounts were observed in resistant cultivars in healthy stems. There were two clusters according to the biochemical parameters considered in hierarchical cluster analysis. Resistant and susceptible cultivars to LCLWT are divided into clusters separately in *k*-means cluster analysis. This confirms that the caffeine, total catechin and total polyphenol contents in healthy tea stems can be used as biochemical markers to identify resistant cultivars for LCLWT.

Keywords: Cultivar screening semio-chemicals, Low country live wood termite

INTRODUCTION

Low Country Live Wood Termite (*Glyptotermes dilalatus*) is a major pest of tea in Sri Lanka. As its name implies, it is restricted to the low elevation of below 610 m amsl. The colony formation of LCLWT occurs by the dispersion of winged reproductive alates from the parent colony. Swarming occurs simultaneously in most colonies as it depends on the weather factors such as temperature and humidity. Therefore, there is an ample opportunity for the mixing of alates of both sexes from different nests. Behavior after 'pairing off' involves wing shedding and searching for a nest site. Field observations confirmed that the alates of *G. dilatatus* are attractive to the rotted stumps of tea and initiate the colony in it (Senanayaka *et al.,* 2015).

Rotted stumps are formed due to invasion of wood rotting fungi through pruned stems, sun-scorch damage, and cankers. Thus, these die-back and wood-rot predispose the tea bushes to live wood termites damage (Sivapalan and Senarathne, 1977). Even a susceptible cultivar won't get infested until the first prune in vegetatively propagated (VP) tea. At the time of first prune, there is a possibility to create a soft spot which helps the nest founders to enter through (Anon., 2003). After entering the tea bush and initiate the colony, they make it way down towards the collar, feeding on the non-conducting heart wood. The galleries would extend into the conducting tissue causing water stress during dry periods with the spread of gallery system and increase of colony population. This may result in a wilting of single branch with mature leaves acquiring a coppery texture. These branches may clearly visible in the field or die depending on the length of the drought (Vitarana and Mohotti, 2008). At the initial stage, symptoms of the infestation are not visible as the damage to non-conductive tissues will not cause any visible debilitation of the plant. The symptoms can be seen only after the galleries traverse across the conducting tissues of vascular ring, which restrict the movements of water and nutrients within the plant. With this damage, water stress wilting of branches can be seen. The pitted appearance is seen as a result of healing up due to the galleries reached to the bark and damaged the bark (Vitarana and Mohotti, 2008). Furthermore, when the trunk is fully colonized the second generation alates start to emerge in dispersal.

The effect of LCLWT damage on the productivity of the plant can be seen mostly in the third pruning cycle and thereafter. As fourth pruning cycle shows a rapid colonization, yield decline from fourth cycle to fifth cycle due to LCLWT is significant (Vitarana *et al.,* 2003). This can occur due to the branches that fall off with a mechanical damage as they are heavily infested and cannot recover from pruning. Additionally, older galleries may fill with fecal matter and these galleries may invade by the scavengers. The galleries extend into the conducting tissues and interfere with bush physiology causing debilitation. Further, these debilitations in bushes result in casualties which affect the productivity of the surviving bushes (Vitarana *et al.,* 2003). The yield loss due to LCLWT can goes up to 3000 kg/ha of made tea when termite infestation reached up to 50% and stand for 10 years due to above reasons (Sands, 1977).

Integrated pest management approach is recommended to control the termite damage. The management methods should be adopted depending on the infestation levels of LCLWT. Fresh infestations in new clearings can be prevented by keeping good standard of pruning from the first pruning. Good clean up of dead wood and snags at every pruning and coating large prune cuts with a fungicide or a wound dressing to prevent wood rotting fungus attack is recommended. As *Gliricidia sepium* is a

diversionary host of LCLWT, planting *Gliricidia* can be done in new clearings (Sivapalan, *et al*, 1977). When a gallery is detected in a branch it should be cut progressively until the healthy wood is exposed (sanitary pruning). If the collar is infested, it should be completely uprooted as there is no chance of salvage. The uprooted fields should be replanted with resistant cultivars after planting mana grass (*Cybopogan confertiflorus*) at least for two years. The best method to prevent LCLWT infestation in prone areas is planting high resistant or tolerant cultivars for LCLWT. Therefore, paying more attention to resistant cultivars in IPM strategies is beneficial.

Under the same environmental conditions, the damage and infestation of LCLWT differ from cultivar to cultivar. The resistant cultivars are the ones that are less damaged. Secondary metabolites of tea plants play a major role in pest resistance. In various plants they have shown different resistant activities such as anti-termitic properties, anti-feedent activity, repellence and insect growth regulation (Dungani *et al.,* 2012). It has been reported that mature tea stems contain polyphenols, caffeine, saponin and volatile compounds as secondary metabolites (Teranishi and Hornstein, 1995). Therefore, this study was conducted to study the Total Polyphenol content, Caffeine content and Total Catechin content of tea stems of known recommended cultivars to determine their effect on the resistance of tea cultivars to LCLWT. As resistant plants react to pest infestations immediately than susceptible plants, the difference in these parameters after LCLWT termite infestation was determined. When the conventional cultivar screening is considered, it is done by examining six randomly selected bushes per replicate per cultivar and degree of damage sustained by tea bush by termites qualified in relation to the rate of infestation progresses in bush frame. According to that, the degree of damage had been classified into three levels (Vitarana *et al*., 2003).

They are;

Heavy damage – bush collar had been colonized,

Medium damage – Infestation had entered the foundation frame present in the previous two cycles but not yet advanced into the collar, and

Light damage – colony is confined to stems on which the cuts of the last cycle prune.

There are drawbacks of the conventional screening compared to biochemical cultivar screening. Some of them are time consuming and requires high number of replicates. Further they involve high cost and the reliability depends on level of natural infestations.

MATERIALS AND METHODS

Sample collection

The experiment was conducted at the St. Joachim Estate – Rathnapura. The cultivars were planted in 1997. The selected cultivars for the study were; TRI 3025, TRI 4049, TRI 3069 (Resistant High Yielder) and TRI 4042 (Susceptible). Randomized complete block design was used to collect samples. Three replicates from each cultivar were randomly collected and both healthy stems and rotted stems were sampled. Monthly sampling was done covering both wet and dry seasons.

Sample preparation

Primary branches of tea bushes with 2-3 cm diameter were taken as the termite infestation is commonly occur in this condition. Both healthy stems and infested stems were collected. Sample preparation was done at the laboratory and the stem samples were de-barked and the middle portions of the stems were taken. Then the stems were cut into small pieces (1 cm x 1 cm) and air dried for over-night and ground using a stem grinder. Then the samples were further ground to get the powder

form. The ground samples were sieved using 0.5 µm sieve and stored in a refrigerator at -20 °C until the analysis were carried out. Methanol extraction was done for the analysis of Caffeine, total Polyphenol and total catechin contents. About 0.50 g of sieved stem samples (exact weight was recorded) was measured into a centrifuge tube and extracted to 10 ml of 70% hot Methanol (70 °C) and this was taken for further analysis.

Determination of total polyphenol content

Determination of total polyphenol content was done using ISO 14502-1 after optimization for stems. Exactly 1.00 ml of extraction was diluted to 10.00 ml. Then 1.00 ml of diluted extract was taken and 5.00 ml of freshly prepared 10% Folin - Ciocalteu reagent was added. After 3-5 min., 4.00 ml of 7.5% Sodium Carbonate was added, mixed well and kept for 1 hour until the blue color is developed. Gallic acid 1ppm stock solution was taken and prepared a standard series for the standard curve. The analysis was done in triplicates. The Absorbance of this solution was taken at 765 nm using UV-Visible spectrophotometer.

Determination of caffeine content

Caffeine concentration was determined using a UV-Visible spectrophotometer method described by Quan *et al*., (2007). The methanol extract (10 ml) was taken in to 25 ml volumetric flask. Then 4 ml of 0.1 M HCl and 1 ml of 50% Lead Acetate was added accordingly. Then top up the volume up to the mark in the volumetric flask with distilled water and kept for 20 minutes. Then the sample was filtered to a beaker using No 4-filter paper. The filtrate (20 ml) was measured and added to 50 ml volumetric flask. Then 0.2 ml of 9% H_2SO_4 was added and top up the volume up to the mark in the volumetric flask with distilled water and kept for 20 minutes. The sample was filtered to a beaker using No. 1 filter paper. Caffeine (1 ppm) stock solution was used to prepare standard series for the curve and as the control. The analysis was done in triplicates. Absorbance of the filtrate was measured at wave length, 274 nm using Varian spectrophotometer.

Determination of total catechin content

Total catechin content was determined using the method described by Wanyika *et al*., (2010). From the methanol extract, 1 ml was measured into a 10 ml volumetric flask and top up with distilled water. From that diluted solution, 1 ml was measured into a graduated test tube. 4 ml of pre prepared vanillin solution was added, and the mixture was put in an ice bath for 15 minutes. Catechin 1 ppm stock solution was taken and prepared a standard series as control and for the standard curve. The analysis was done in replicates. The absorbance was measured at 500 nm wave length using the spectrophotometer.

Cluster analysis was done using R software to determine whether these parameters are suitable for the identification of resistant cultivars. A hierarchical cluster with average distances and a k-means cluster analysis was done to separate the two groups.

RESULTS AND DISCUSSION

Resistance to pests is a special factor that considered when recommending a cultivar in tea breeding programmes in Sri Lanka as planting resistant cultivars is the best control method that is available to control perennial pests like Low Country Live Wood Termite (*Glyptotermes dilatatus*). Therefore, availability of a reliable cultivar screening method is important as at the end it effects on the plant's survival in the field in the presence of pests.

Secondary metabolites of a plant play a role of defense against pest and diseases. These can be divided into three major classes considering

their chemical structures. Those are terpenoids, phenolics and alkaloids.

Caffeine is an alkaloid with antifungal, antifeedant and antimicrobial properties. As the termite infestation related with the wood rotting fungal attacks LCLWT resistant and susceptible cultivars were tested for Caffeine in their primary branches.

As there was no significant difference between the monthly caffeine during considered period, the average values were calculated and compared. Figure 1 shows the resistant cultivars contain high concentration of caffeine comparative to the susceptible cultivar. There was no significant difference (p=0.3653) of caffeine between susceptible and resistant cultivars for LCLWT. In general, the resistant cultivars contain more than 0.004% caffeine.

This provides evidence to prove that caffeine plays a major role in managing the termite's ability to infest the plants. It has been confirmed that semio-chemicals emitted by rotted or decaying wood of tea plant influences the attraction of LCLWT to the tea plant (Senanayake *et. al.,* 2014). Therefore, the rotted stems were collected and analyzed for the caffeine content (Figure 1).

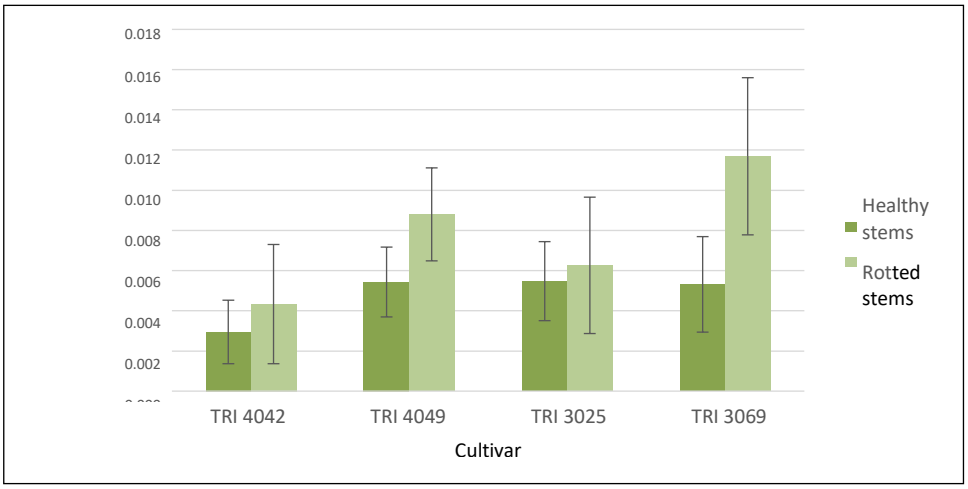

Figure 1. Caffeine % (w/w) of healthy and rotted stems of LCLWT susceptible and resistant cultivars

Caffeine is a defense chemical plant produces to protect the plant from pest attacks and the results of this study proves it. The caffeine in rotted stems is high, compared to that in the healthy stems irrespective to the cultivar's susceptibility or resistance to the pest. Figure 1 shows that the caffeine content in rotted stems are higher than the caffeine content in healthy stems. But there is no significant difference (p=0.056) between cultivars for the caffeine content in rotted stems. The high caffeine content influences the growth of wood rotting fungus, and this eventually leads to a defense against LCLWT as they don't have entry points to the plant stem without wood rots.

Ashihara *et al*., (2008) and Kumar *et al.,* (1995) have proposed that the growth of *Monacrosporium ambrosium,* the symbiont of the *Xyleborus fornicatus,* inhibits by caffeine and accumulation of caffeine after beetle attack is a defense strategy. Here also it plays the similar role in tea plant stem by increasing caffeine concentration with the attack of wood rotting fungus. Phenolic compounds which are synthesize in plants possess biological prosperities like antifeedant, antimicrobial, antifungal. Similarly, in tea plant they act as defense chemicals by avoiding pest attacks.

As there was no significant difference between the monthly total polyphenolic content during considered period, the average values were calculated and compared (Figure 2). The total polyphenolic content is low in susceptible cultivar compared to the resistant cultivars. Similarly, as caffeine, total polyphenol concentration did not show any significant difference ($p = 0.205$) between cultivars but higher than 0.5% in resistant cultivars for LCLWT.

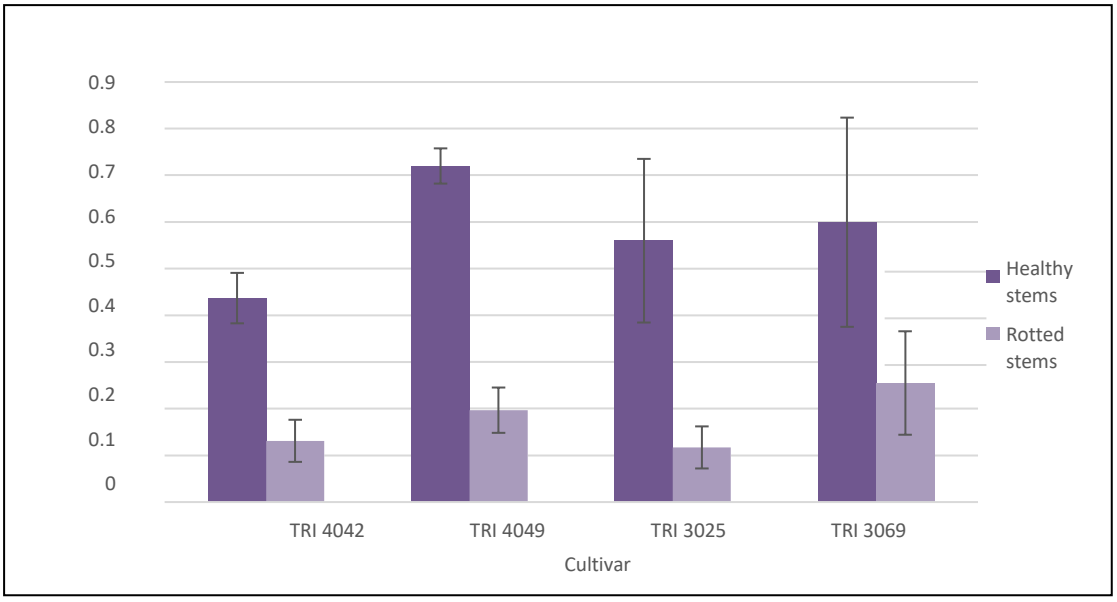

Figure 2. Total Polyphenolic content % (w/w) of healthy and rotted stems of LCLWT susceptible and resistant cultivars

Polyphenols generally stored in leaves and barks of plants as they are toxic to some pests as herbivores. Therefore, the pest attacks can be managed with high concentration of polyphenols or by increasing concentrations as a response (Kulbat, 2016). On the other hand, depending on the situation or the pest, the polyphenol levels can be decreased as well (Constabel & Barbehenn, 2008). In Figure 2 the total polyphenol levels have decreased in rotted stems than in healthy stems. At this point the main defensive role is performed by caffeine and polyphenols interferes with it. Therefore, the levels are decreasing to support the defensive mechanism. On the other hand, some groups of polyphenols convert into other compounds for example to esters, to support the defensive mechanism (Punyasiri *et al.,* 2005). Flavanols is a group of polyphenols that involved in plant defense directly. Especially catechins and proanthocyanidins are very popular in defense mechanisms (Treutter, 2006).

Even though there is no significant difference ($p = 0.188$) the total catechin content in resistant cultivars are higher than that of susceptible cultivars as shown in Figure 3. Catechins are known to available in high concentrations in pest resistant plants. Punyasiri *et al.,* (2005) state that the catechin levels are significantly higher in *Exobasidium vexans* resistant cultivars than that of susceptible cultivars. Similarly, as the LCLWT infestation is a secondary attack that follows the wood rotting fungi infestation, the catechin levels are high in LCLWT resistant cultivars.

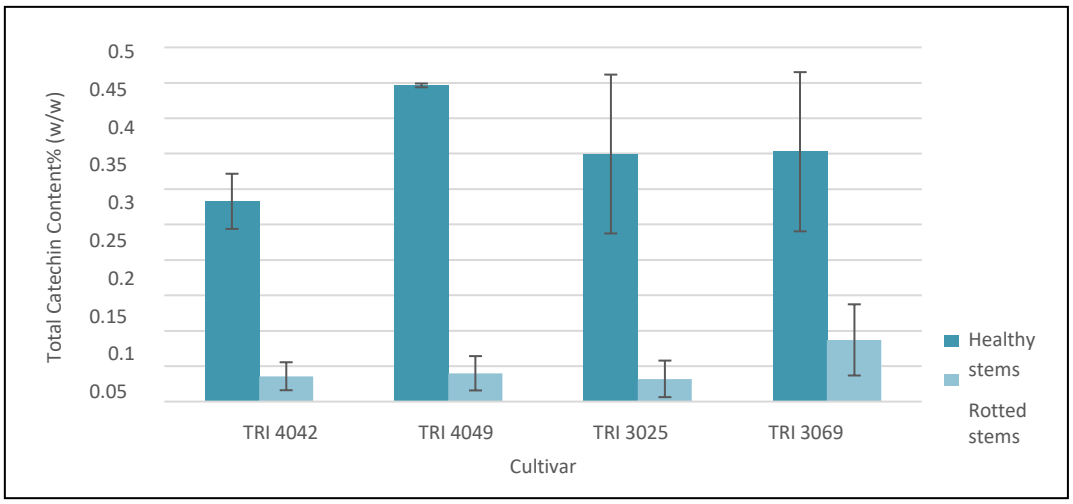

Figure 3. Total Catechin Content % (w/w) of healthy and rotted stems of LCLWT susceptible and resistant cultivars

The catechin levels go down in the infested stems than that of healthy stems (Figure 3) irrespective of the resistance or susceptibility (Punyasiri *et al.,* 2005) reported that the catechins convert into their esters with the maturity of *Exobassidium vexans* infestation and the levels goes down. Likewise, the catechin levels decrease with the wood rotting fungi infestation.

As all these chemicals shows a pattern with the resistance or susceptibility of cultivars to LCLWT, attempts were made to use these chemicals to screen cultivars in tea breeding program. A hierarchical cluster analysis was done, and the results (Figure 4) shows that TRI 4042 the susceptible cultivar as a separate cluster from the other cultivars. The other three cultivars are closely related and TRI 3025 and TRI 3069 comes as a different cluster which confirms the field observations made through years on their resistance. TRI 4049 as the most resistant cultivar out of considered cultivars comes as a different branch confirming its high resistance.

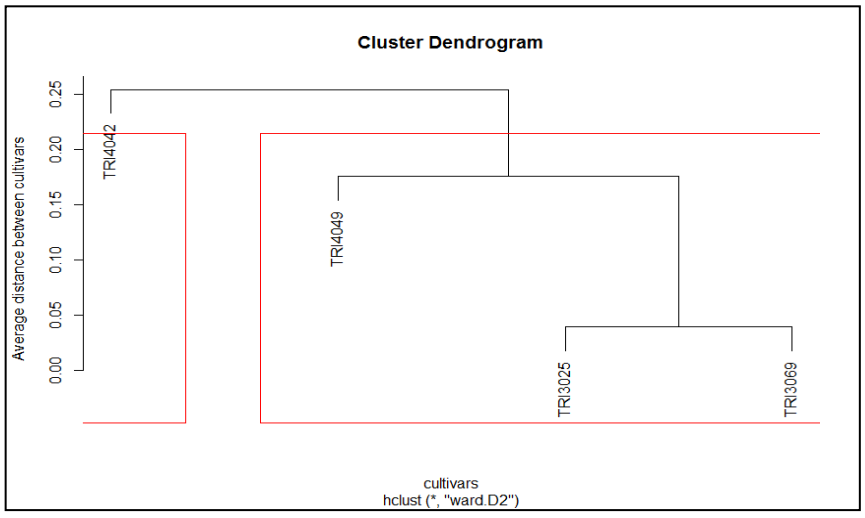

Figure 4. Hierarchical cluster with average distance between clusters *versus* cultivar

A k-means cluster analysis (Figure 5) was done for further confirmation of results and it shows two clear clusters separately for susceptible cultivars and resistant cultivars in TRI 4000 series. TRI 3025 and TRI 3069 show an overlap with the susceptible cultivar in few data points but in most cases, these are in the resistant cluster. This means that within the same series, the susceptible and resistant cultivars can be screened using these three parameters, but to screen in between series more parameters must be included.

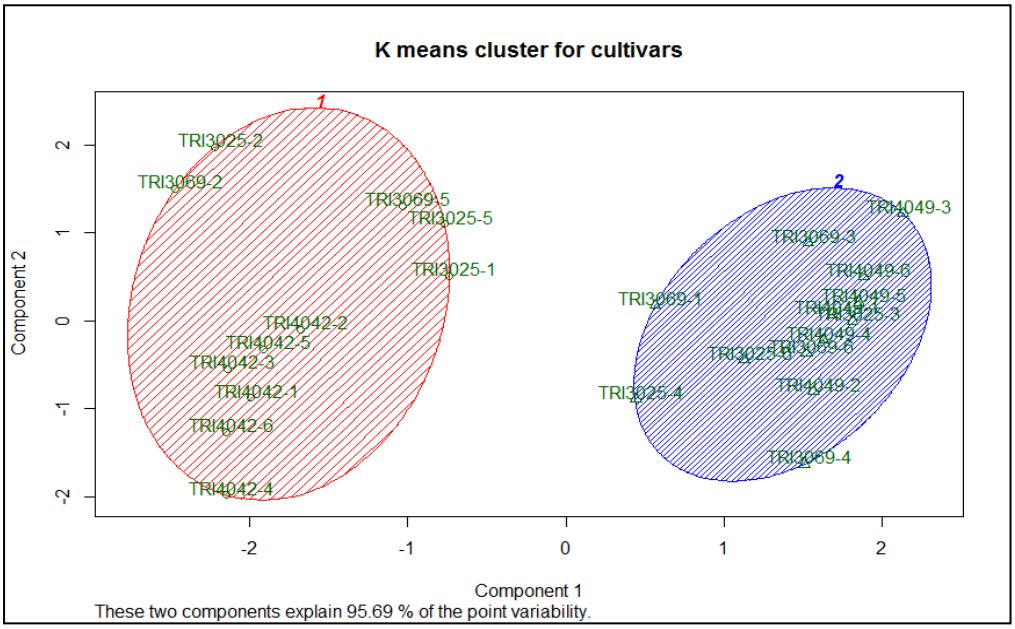

Figure 5. K-means cluster for cultivars; Component 1 *vs* Component 2 in PCA;
Cluster 1: Susceptible cultivars and Cluster 2: Resistant cultivars

CONCLUSION

Caffeine content, total Polyphenol content and Catechin content can be used as biochemical markers to identify resistant cultivars for LCLWT. The screening can be fine-tuned by using physical parameters like light intensity, sanitary conditions of the field as light intensity affects on the concentration of above chemicals and LCLWT infestation is low in properly maintained fields. These three parameters would be enough to identify cultivars with high resistance like TRI 4049. Therefore, it can be concluded that these parameters can be used to identify resistant cultivars to LCLWT.

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REFERENCES

Anon. (2003). T.R.I. Advisory Circular. No. PM 3.

Ashihara, H., Sano, H. and Crozier, A. (2008). Caffeine and related purine alkaloids: Biosynthesis, catabolism, function and genetic engineering, Journal of Phytochemistry 69 (4), 841-856.

Constabel, C.P. and Barbehenn, R. (2008). Induced Plant Resistance to Herbivory, Schaller (eds). Springer Science+Business Media B.V. pp. 253 - 270.

Dungani, R., Bhat, I.H., Abdul Khalil, H.P.S., Naif, A. and Hermawan, D. (2012). Evaluation of Antitermitic Activity of Different Extracts Obtained from Indonesian Teakwood (Tectona grandis L.f), Journal of Bio-Resources 7(2), 1452-1461.

Kulbat, K. (2016). The role of phenolic compounds in plant resistance. Journal of Biotechnology and Food Sciences 80 (2), 97-108.

Kumar, N.S., Hewavitharanage, P. and Adikaram, N.K.B. (1995). Attack on tea by *Xyleborus fornicatus*: inhibition of the symbiote, *Monacrosporium ambrosium* by caffeine, Phytochemistry 40, 1113-1116.

Punyasiri P.A.N., Abeysinghe I.S.B. and Kumar V. (2005). Preformed and Induced Chemical Resistance of Tea Leaf Against *Exobasidium vexans* Infection. J. Chem. Ecol. 31(6), pp.1315-1324.

Quan P.T., Hang T.V., Ha, N.H., Giang B.L. (2007). Total Polyphenols, Total Catechins Content and DPPH Free Radical Scavenger Activity of Several Types of Vietnam Commercial Green Tea. Journal of Science & Technology Development 10, No.10, pp 5-11.

Sands, W.A., (1977). The role of termites in tropical agriculture. Journal of Outlook on Agriculture 9 (3), 136-143.

Senanayake P.D., Vitana B.S., Mohotti K. and Paranagama, P.A. (2014). Evaluation of the response of LCLWT, *Glyptotermes dilatatus* Bugnion & pop off (*Isoptera:Kalotermitidae*) for semio-chemicals present in rotted and healthy stems of tea cultivars. Proceedings of the 5th Symposium on Plantation Crop Research. pp.123-133.

Senanayake, P.D., Mohotti, K., and Paranagama, P.A. (2015). Bioactive constituents of wood rot extract of tea, *Camellia sinensis* L.O. Kuntze against alates of low country live wood termite *Glyptotermes dilatatus* Bugnion and Popoff (*Isoptera: Kalotermitidae*), Springer Plus volume 4, Article number: 711.

Sivapalan, P. and Senaratne, K.A.D.W. (1977). Some Aspects of the Biology of the Tea Termite, *Glyptotermes dilatatus.* Int. J. Pest Manag., 23 (1), 9-12.

Sivapalan, P., Senaratne, K.A.D.W. and Karunaratne, A.A.C. (1977). Observations on the Occurrence and Behaviour of Live-wood Termites (*Glyptotermes dilatatus*) in Low-country Tea Fields. Int.l J. Pest Manag. 23 (1), 5-8.

Teranishi, R.and Hornstein, I. Eds., (1995). Food Review Int, Special Issue on Tea, 480-525.

Treutter, D. (2006), Significance of flavonoids in plant resistance: a review, Environment Chemistry Letter, 4: 147. https://doi.org/ 10.1007/s10311-006-0068-8.

Vitarana, S.I. and Mohotti, K.M. (2008). Management of tea termites (In): *Handbook on Tea* (Eds). Zoysa, A.K.N., Tea Research Institute in Sri Lanka. pp.231-241.

Vitarana S.I., Bandaranayake, J., Hassim, A.R.M., Jayasinghe, H.D. and Samarasinghe, S.M. (2003). The Low Country Live Wood Termite and Yield Decline of Clonal Tea in Sri Lanka Eds. Modder, W.M.D., pp. 341- 360. Twentieth century tea research in Sri Lanka.

Wanyika, H.N., Gatebe, E.G., Gitu, L.M., Ngumba, E.K. and Maritim, C.W. (2010). Determination of caffeine content of tea and instant coffee brands found in the Kenyan market. Afr. J. Food Sci. 4(6), pp.353–358, http://www.academic journals. org/ajfs