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# Biochemical characterization of teak clones for resistant traits to teak defoliator (*Hyblaea puera* Cramer)*

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**Abstract:** The leaf samples of teak clones showing resistance or susceptibility to teak defoliator, *Hyblaea puera* Cramer under laboratory condition were analyzed for biochemical constituents *viz.*, total phenols, nitrogen and potassium. The phenol content among the selected leaves of resistant clones varied from 0.56 to 1.43 per cent. Teak clones recorded gradual increase in phenol content in relation to increase in degree of resistance. The increased per cent nitrogen content recorded increase in the degree of susceptibility. There was no appreciable difference observed among the resistant categories of the teak clones with respect to per cent nitrogen content. The most resistant clone STG-3 and MYHAK-2, of state graft and Haliyal, respectively exhibited highest amount of potassium content (1.73%) as compared to others.

**Key words:** *Hyblaea puera,* Nitrogen, Phenol, Potassium, Teak

## Introduction

About 187 insect species have been observed to feed on the living teak tree in India (Hutacharern and Tubtim, 1995). The majority of these insects are leaf feeders, with a smaller number are sap feeders, stem borers, inflorescence and fruit feeders and root feeders. Among these, the teak defoliator, *Hyblaea puera* Cramer is the most widespread and considered as serious national pest. Its outbreak occurs almost every year in India over extensive areas. The outbreak coincides with the early flushing period of teak, trees usually suffer a total defoliation, and sometimes there is partial defoliation later in the growth season (Nair, 1988). Studies in young teak plantations at Nilambur in Southern India recorded that defoliation by *H. puera* caused loss of 44.1 per cent of the potential wood volume increment (Nair and Mohandas, 1996).

Resistance or susceptibility of plants is the result of a series of interactions between plants and insects, which influence the ultimate degree of establishment of insect population on plants. The morphological or biophysical resistance factors in plants interfere with insect's vision, orientation, locomotion, feeding, mating and oviposition mechanism. Biochemical factors are far more important for imparting resistance to insects. They may be nutrition based or may include non nutritional chemicals, called allelochemics (allelochemicals, semiochemicals and infochemicals) that affect insect behaviour, development, growth or physiology in a number of ways. Foliar poly phenols play a major role for insect resistance in trees. There is a direct relationship between leaf phenols and degree of resistance against insects (Rupa *et al.,* 1993). Phenols, the aromatic compounds with hydroxyl groups are wide spread in plant kingdom. They occur in all parts of the plant. Phenols are said to offer resistance to diseases and pests in plants and grains. Phenols include compounds like tannins, flavonoids *etc.* Nature seems to have endowed many plants with an in built defense mechanism by providing their system with certain biochemicals, which can ward-off insect attacks (Jain *et al.,* 2002). In most phytophagous insects, plant substances acting as feeding inhibitors (biochemicals) determine the host plant specificity. The plants protect themselves with defensive chemicals, thus developing resistance against insects while insects develop specialized chemosensory receptors to identify such defensive chemicals which are unpalatable or detrimental to them.

## Material and methods

The teak clones showing resistance or susceptibility under laboratory condition based on the leaf feeding bioassay were used for estimating the bio-chemical constituents *viz.*, total phenols, nitrogen and potassium. The total phenol was estimated by following Folin and Ciocalteau method and expressed in mg per gram of sample (Folin and Ciocalteau, 1927). The oven dried leaf samples were finely powdered using a grinding machine. About 100 mg of powdered sample was mixed with 10 times volume of 80 per cent ethanol. The extract was centrifuged to homogenate at 10,000 rpm for 20 min. The residues were re-extracted with five times the volume of 80 per cent of ethanol, centrifuged and the supernatant pooled. The supernatant was evaporated to dryness. The residue was dissolved in 5 ml of distilled water. Different aliquots were pipetted out into labeled test tubes and made up the volume to 3 ml. A blank with 3 ml distilled water was maintained. Folin Ciocalteau reagent (0.5 ml) was added and mixed well. After 3 minutes, 2 ml of 20 per cent Sodium carbonate ($Na_2CO_3$) solution was added to each tube. Then the test tubes was placed in a boiling water bath for one minute to get blue colour and cooled. Finally the absorbance at 650 nm against a reagent blank was measured. The concentration of phenols present in the sample was estimated from the standard curve and expressed as mg per g with catechol as standard.

The total nitrogen present in the samples was estimated by following Micro-Kjeldahl method. About 100 mg of the sample was transferred to 30 ml digestion flask. Then 1.9 ± 0.1 g potassium sulphate and 80 ± 10 mg mercuric oxide and 2 ml

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concentrated $H_2SO_4$ was added to the digestion flask. Samples were boiled till the solution became colourless. After cooling the digested sample, it was diluted with a small quantity of distilled ammonia-free water and transferred to the distillation apparatus. The kjeldahl flask was rinsed with successive small quantity of water. Later, 100 ml conical flask containing 5 ml of boric acid solution with a few drops of mixed indicator with the tip of the condenser dipping below the surface of the solution. 10 ml of sodium hydroxide-sodium thiosulphate solution was added to the test solution in the apparatus. Nitrogen in the form of ammonia was collected on boric acid. Titrate the ammonia collected against N / 50 sulphuric acid. From the titre value, the nitrogen content of the sample was calculated using the following formulae.

$$\% \text{ N} = \frac{\text{T.V. x N } H_2SO_4 \text{ x } 50 \text{ x } 0.014 \text{ x } 100}{0.5 \text{ x } 5}$$

Estimation of potassium was done by flame photometric method, also known as flame emission or flame atomic emission. The sample in solution was sprayed into a flame to vaporize, atomize and excited atoms of the element of interest was separated from remainder of emitted radiations using appropriate filters and its intensity was measured. The intensity measurement was related directly to the concentration of the element of intensity usually by comparing with the measured intensities, of a standard or series of standards. The potassium content in the sample was expressed in percentage by following formula.

$$\% \text{ K} = \frac{\text{ppm on graph x } 50 \text{ x } 100}{10^6 \text{ x } 0.5}$$

**Results and discussion**

The phenol content among the selected leaves of resistant clones varied from 0.56 to 1.43 per cent and results revealed significant differences in their mean values (Table 1). Teak clones showed gradual increase in phenol content in relation to increase in degree of resistance. The clones, STG-3 and MYHAK-2 recorded significantly higher concentration of phenol and were on par with each other (1.43 mg/g) with respect to phenol content as compared to other clones. These clones were found significantly different from other clones. STG-6 and MYHD-2 recorded no significant differences in phenol content. Clones under resistant categories *viz.*, $R_1$, $R_2$ and $R_3$ exhibited significant difference among themselves with respect to phenol content.

The phenol content among the seventeen selected leaves of susceptible clones varied from 0.02 to 0.63 mg/g and revealed significant differences in their mean values (Table 2). Susceptible clones showed gradual decrease in phenol content in relation to increase in degree of susceptibility. The phenol content among most susceptible clones ($S_1$) varied from 0.02 to 0.26 mg/g and was on par with each other. Among the susceptible category ($S_2$) the clone ID MYBL-1 and MYHUT-3 were found on par with the other. The phenol content in moderately susceptible clones ($S_3$) varied from 0.52 to 0.63 mg/g.

Table 1. Estimation of bio chemical constituents in resistant teak clones of Karnataka

| Clones | Source/ Origin | Resistance category | Total Phenol content (mg/g) | Nitrogen content (%) | Potassium content (%) |
|---|---|---|---|---|---|
| STG-3 | State graft | $R_1$ | 1.43 | 1.91 | 1.73 |
| MYHAK-2 | Haliyal | $R_1$ | 1.43 | 1.91 | 1.73 |
| MYMK-3 | Mysore | $R_1$ | 1.41 | 1.91 | 1.54 |
| MYSS-2 | Shimoga | $R_2$ | 1.41 | 1.91 | 1.44 |
| MYHV-6 | Haliyal | $R_2$ | 1.40 | 1.91 | 1.51 |
| STG-06 | State graft | $R_2$ | 1.37 | 1.92 | 1.41 |
| MYHD-2 | Haliyal | $R_2$ | 1.37 | 1.93 | 1.41 |
| MYCM-2 | Chikmagalur | $R_2$ | 1.36 | 2.01 | 1.36 |
| MYHV-5 | Haliyal | $R_2$ | 1.34 | 2.01 | 1.36 |
| MYMK-4 | Mysore | $R_2$ | 1.24 | 2.02 | 1.33 |
| MYHUT-8 | Hunsur | $R_3$ | 1.23 | 2.12 | 1.33 |
| MYHAK-1 | Haliyal | $R_3$ | 1.23 | 2.12 | 1.33 |
| MYHUN-5 | Hunsur | $R_3$ | 1.22 | 2.12 | 1.32 |
| MYHD-4 | Haliyal | $R_3$ | 1.15 | 2.12 | 1.32 |
| MYMK-2 | Mysore | $R_3$ | 1.05 | 2.12 | 1.31 |
| MYMK-1 | Mysore | $R_3$ | 1.05 | 2.13 | 1.31 |
| MYCM-1 | Chikmagalur | $R_3$ | 0.98 | 2.14 | 1.27 |
| MYHV-3 | Haliyal | $R_3$ | 0.98 | 2.14 | 1.26 |
| MYHUN-4 | Hunsur | $R_3$ | 0.90 | 2.15 | 1.24 |
| MYSS-1 | Shimoga | $R_3$ | 0.88 | 2.21 | 1.24 |
| MYHV-1 | Haliyal | $R_3$ | 0.82 | 2.22 | 1.22 |
| MYHUN-2 | Hunsur | $R_3$ | 0.88 | 2.31 | 1.17 |
| MYKB-4 | Koppa | $R_3$ | 0.70 | 2.50 | 1.17 |
| MYHV-4 | Haliyal | $R_3$ | 0.56 | 2.50 | 1.14 |
| S.Em.± | - | - | 0.03 | 0.02 | 0.02 |
| C.D. (0.01) | - | - | 0.07 | 0.05 | 0.07 |
| C.V. (%) | - | - | 3.46 | 0.83 | 3.48 |

$R_1$= Most resistant, $R_2$= Resistant, $R_3$= Moderately resistant

Table 2. Estimation of bio chemical constituents in susceptible teak clones of Karnataka

| Clones | Source/ Origin | Susceptibility category | Total Phenol content (mg/g) | Nitrogen content (%) | Potassium content (%) |
|---|---|---|---|---|---|
| STG-12 | State graft | $S_1$ | 0.02 | 2.81 | 0.81 |
| MYCM-47 | Chikmagalur | $S_1$ | 0.02 | 2.81 | 0.81 |
| MYHUT-4 | Hunsur | $S_1$ | 0.20 | 2.75 | 0.83 |
| MYHD-3 | Haliyal | $S_1$ | 0.25 | 2.71 | 0.98 |
| MYCM-3 | Chikmagalur | $S_1$ | 0.26 | 2.61 | 0.97 |
| MYKB-5 | Koppa | $S_1$ | 0.26 | 2.61 | 0.97 |
| MYBL-1 | Bhadravati | $S_2$ | 0.27 | 2.61 | 0.91 |
| MYHUT-3 | Hunsur | $S_2$ | 0.27 | 2.53 | 0.91 |
| MYHV-7 | Haliyal | $S_2$ | 0.29 | 2.53 | 1.00 |
| MYHUN-6 | Hunsur | $S_2$ | 0.45 | 2.52 | 1.01 |
| MYSA-1 | State graft | $S_2$ | 0.48 | 2.52 | 1.01 |
| MYHAK-3 | Shimoga | $S_2$ | 0.48 | 2.51 | 1.02 |
| MYHD-1 | Haliyal | $S_3$ | 0.52 | 2.51 | 1.05 |
| MYKB-1 | Koppa | $S_3$ | 0.54 | 2.51 | 1.07 |
| MYMK-5 | Mysore | $S_3$ | 0.20 | 2.51 | 1.08 |
| MYCM-3 | Chikmagalur | $S_3$ | 0.60 | 2.51 | 1.16 |
| MYKB-2 | Koppa | $S_3$ | 0.63 | 2.51 | 1.17 |
| MYHUT-5 | Hunsur | $S_3$ | 0.63 | 2.20 | 1.17 |
| S.Em.± | - | - | 0.02 | 0.02 | 0.02 |
| C.D. (0.01) | - | - | 0.09 | 0.10 | 0.09 |
| C.V. (%) | - | - | 2.47 | 2.19 | 1.44 |

$S_1$= Most susceptible, $S_2$= Susceptible, $S_3$= Moderately susceptible

The clone MYMK-5 of Mysore division contained 0.20 mg/g phenol and found significantly different from other clones. Clones under susceptible categories *viz.*, $S_1$, $S_2$ and $S_3$ exhibited significant differences among themselves with respect to phenol content (Table 2). Insect resistance in plants is also due to the chemical constituents, which may be qualitative or quantitative. These chemicals occur within certain parts of the plant or specific stages of plant growth. Phenolic chemicals are reported to be toxic to the insects and which influence on the biology of the insects. Study can be comparable with findings of Jain *et al.* (2002) who reported gradual increase in phenol content in relation to increase in degree of resistance. Our results also indicated increase in total phenol content in resistance and decrease in susceptible clones of Karnataka. Roychoudhury *et al.* (2003) opined that phenols have an antibiotic effect on growth of *H. puera.* Jacob and Balu (2007) detected more content of phenol such as Orcinol and Pyrogallol in resistant teak clones as compared to susceptible.

The nitrogen content in leaves of teak clones, among the resistant category varied from 1.91 to 2.50 per cent (Table 1). The nitrogen content increased with the decrease in resistance. There was no appreciable difference observed among the resistant categories of the teak clones with respect to nitrogen content. However, the higher concentration of nitrogen was recorded in the clones MYKB-4 and MYHV-4 of Koppa and Haliyal and exhibited significantly different among all the other clones.

The nitrogen content of susceptible leaves of teak clones varied from 2.20 to 2.81 per cent (Table 2). The nitrogen content increased with increase in the degree of susceptibility. However, no much variation was recorded among the susceptible categories ($S_1$, $S_2$ and $S_3$) of the teak clones with respect to nitrogen content. The lowest concentration of nitrogen was recorded in the clones MYHUT-5 of Hunsur division (2.20 %) and found statistically different among susceptible categories. Study revealed inverse relationship between percentage of nitrogen and susceptibility. High nitrogen content of plant generally favoured insect feeding (Jain *et al.,* 2002). The present findings are comparable with this study where they reported lowest percentage of nitrogen in most of the resistant categories of teak clones.

The most resistant clones STG-3 and MYHAK-2, of state graft and Haliyal, respectively exhibited highest amount of potassium content (1.73%) as compared to others. The result suggests that the potassium content is directly proportional to their degree of resistance (Table 1). The resistant clones of MYMK-4, MYHUT-8 and MYHAK-1 of Mysore, Hunsur and Haliyal respectively, recorded no significant variation among themselves (Table 1). The most susceptible clones STG-12 and MYCM-47 of state graft and Chikmagalur, respectively and MYHUT-4 of Hunsur recorded lower per cent of potassium content as compared to others. All the clones under susceptible category differed from each other with respect to potassium content. High potassium level in plant had negative effects against insect growth (Jain *et al.,* 2002). The results of present study are comparable with the results of Jain *et al.* (2002) who reported high percentage of potassium in most of resistant and low per cent in susceptible categories of the teak clones.

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