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Research article

Screening of different clones of *Gmelina arborea* Roxb. with reference to *Calopepla leayana* Latr. to identify the resistant clones through morphological and chemical study

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Abstract: The field and lab study was targeted to identify the resistant clones of *Gmelina arborea* aganist the defoliator *Calopepla leayana* and evaluate the existence of most suseptible and less susceptible clones. Study analyzes through morphological individuality of different genotypes with reference to C. *leayana* along with the confirmatory study of different clones and identified most susceptible and less susceptible clones through leaf micromorphology and chemical study. Resistant clones were identified through morphological study as most susceptible (08) and less susceptible (07) numbers of clones. Among them following six clones were identified as most susceptible GA006, GA 002 and GA 081 and GA 023, GA 034, GA 095 less susceptible. Where leaf micromorphology and chemical study was also resulted same for the selection of above clones.

Keywords: Clone, Selection, Insect resistant, Morphological, Chemical.

INTRODUCTION

Though the species has a number of promising attributes, it also exhibits major drawbacks of poor stem form, low productivity and susceptibility to insect pests etc. *Calopepla leayana* Latr. (leaf defoliator) the mostly attacked insect pest of the species has constantly been a hurdle for a successful plantation. In field and laboratory observation these insects are responsible for the complete demolishing of plantations in very large areas. From December to March the adult is being feeds on leaves during the high growth period gregariously and completely defoliates the leaves in a plantation within a very short period. So, the study of screening of resistant genotypes would be an extremely useful and ecologically sound technique to control *C. leayana* and it is very necessary to manage the insect pest. Several scientists during the last century attention are being paid to the sustained use of these characters at a systematic level (Banerji & Das, 1972; Kumar & Jain, 1986; Rao *et al*., 1987; Thakur, 1988; Sastry & Kannabiran, 1994). In addition, attacks by various pests and diseases have prevented its planting on a large scale, especially within its natural distribution area (Greaves, 1981). A panel of experts of FAO (1969) on Forest Genetic Resources assigned top priority for improved utilization and conservation to *Gmelina arborea* Roxb. This reflected the fact that many tree planters considered the species to be very promising due to ease and cheapness of establishment, rapid early growth, expectations of early returns and promising wood characteristics, including high durability and good yield and quality of pulp. There are good numbers of examples of the existence of genetic variation within species, some of which can be used as the base for insect resistance. Painter (1958) suggests that the search for sources of insect resistance and the use of varieties and cultivars having such resistance in reducing the populations of insects and the damage done by them is an important part of any insect control project. In the northern Shan State of Myanmar, about 8000 ha plantations of were written off way back in 1936 due to severe attack of *Craspedonta leayana* (Hutacharern, 1990; Baksha, 1990).

It is a well-known fact that to protect from herbivore attack, plants produce specialized morphological structures. It is reported by Baruah *et al*. (2018) as a confirmatory study of the selected clones under the same project that due to this main fidelity, micromorphological characters are epidermal cell feature, trichome character, stomotal behaviour, foliar venation pattern can give extra evidence for genetic and specific delimitation of plants. Moreover, plants also confront the herbivores both directly by affecting host plant preference or survival and reproductive success (direct defense) and indirectly through other species such as natural enemies of the insect pests (indirect defense) (Dudareva *et al*., 2006; Howe & Jander, 2008; Arimura *et al*., 2009). Production of toxic chemicals such as terpenoids, alkaloids, anthocyanins, phenols and quinones kills or retarded the development of the herbivores. Direct defenses include any plant traits that themselves affect the susceptibility of host plants to insect attacks and affect the herbivores biology such as mechanical protection on the surface of the plants or production of toxic chemicals such as terpenoids, alkaloids, anthocyanins,

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phenols, and quinones that either kill or retard the development of the herbivores (Kessler & Baldwin, 2002). Indirect defenses against insects are mediated by the release of a blend of volatiles that specifically attracts natural enemies of the herbivores or by providing food and housing to enhance the effectiveness of the natural enemies. Natural enemies restrain the population of the insect and able to decrease the plant injury caused by the insect. In addition to the above study, leaf micromorphology study (Baruah *et al.*, 2018) and chemical evaluation was done for confirmation of morphologically selected genotypes.

Hence, it is designed to evaluate the nutritive content of different clones of the plant to determine their susceptibility against insect. Secondary metabolites could lead to the identification of new signaling molecules involved in plant resistance against herbivores and other stresses. Plant secondary metabolites (PSM) are organic compounds that are not essential for normal plant growth and development and are often produced as by-products during the synthesis of primary metabolic products (Herbert, 1989). Some PSMs perform physiological functions within the plant such as serving as a tool for the transport and storage of nitrogen, as UV-protectants and as attractants for pollinating and seed dispersing animals (Wink, 1999). However, the main function of PSMs is thought to serve as chemical defense against pathogens and herbivores (Bennett & Wallsgrove, 1994; Theis & Lerdau, 2003; Mao *et al*., 2007). Some flavonoids are inhibitors of regulatory enzymes such as calcium dependent ATPase (Salunke *et al*., 2005). Lignin and other phenolics can strengthen cell walls and therefore can be anti-nutritional (Brodeur-Campbell *et al*., 2006; Schroeder *et al*., 2006). Some phenolics and sesquiterpenes along with other volatiles can repel herbivores from oviposition on host plants (Henzell & Hall, 1974; De Moraes *et al*., 2001; Huber *et al*., 2006). In the present study, the various secondary metabolites content of different clones of the plant were estimated to understand their ability of plants to compete and survive against being eaten by herbivores and against being infected by microbial pathogens.

MATERIALS AND METHODS

For assessment of different clones of *Gmelina arborea* by the attack of key pests *Calopepla leayana* observations were made on the incidence of pest on selected clones through population dynamics and damage studies**.** Incidence of the targeted pest on individual ramet of 70 clones was assessed for first period of three years continuously at the starting of the project. The intensity of the attack of the pest on each clone was estimated using the modified Wellendrof (1989). During the active insect period from March to October, fortnightly observations was made on the incidence of *Calopepla leayana* on different colnes in the Naharani, Golaghat. Defoliation and borer attack intensity was rated through index method of insect infestation in all the ramets of each clone. Health score for trees compared with Rohrmoser severity scale (Mac Dicken *et al.*, 1991).

For controlled condition studies, insects was maintained in the laboratory for conducting various experiments during the investigation. Clones grouped under three categories based on the severity scale were selected for life cycle assessment under controlled conditions to correlate and confirm with field observations, that the impact of the clones on life cycle duration and productiveness of the pest. The culture of *Calopepla leayana* adults and eggs were collected from field stations and reared in the laboratory. Freshly cut leaves collected from the selective clones were provided every day to the starved III instar larvae of the targeted pests in the laboratory for feeding for a fixed period of time 24 hours still pupation. As all the clones were found susceptible to *Calopepla leayana*, in that only 7 clones found less susceptible to the targeted pest, representative clones selected out of the most susceptible and moderately susceptible and less susceptible (3 clones in each category) subjected to the feeding test exhibited the variations in quantum of food intake by the targeted pest. The experiment was conducted in a randomized block design with 3-5 replications. The area fed (cm^2) by the larvae on each clone was measured and compared among the clones. Number of days took to complete the life cycle was assessed in the laboratory condition. The actual eaten and leftover leaf area of each clone was calculated with leaf area meter and analyzed statistically.

After completion of morphological study, chemical study started with young and mature 4-5 leaves were collected of six selected clones from the experimental field Naharoni. Collections were made randomly in replicated manner from selected clones and dried leaves were grinded to coarse powder. Powdered plant materials were soaked in methanol for 24 hrs. The extract was filtered and after concentration through evaporation under reduced pressure is completed by rotary vacuum evaporator, dried and kept in air tight container. These freshly prepared dried leaf extract of different clones were subjected to estimate for various nutrient compositions like alkaloids, flavonoids, tannins, phenol, carbohydrate and protein etc.

Crude alkaloids determination

Crude alkaloid was determined gravimetrically for phytochemical analysis (Harborne, 1973). 2.5 g of the sample was weighed and 100 ml of 10% acetic acid and ethanol was added. It was incubated for 4h at room temperature. Then this solution was filtered and concentrated up to 1/4 of the original volume using a water bath. Concentrated ammonium

hydroxide was added drop by drop in to the extract until it is precipitated. The settled down precipitates were collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried in an oven at 60°C and the percentage of alkaloid is expressed mathematically as,

$$\% \text{ of alkaloid} = \frac{\text{Weight of alkaloid}}{\text{Weight of sample}} \times 100$$

Total protein estimation

The total protein content of the samples was estimated by following the method developed by (Lowry *et al*., 1951). Extraction was carried out with Phosphate Buffer (0.1 M, p^H -7.6). 500 mg of sample was ground in pastel mortar with 20 ml of Phosphate buffer. The samples were kept overnight to fulfill the complete extraction of protein. These were centrifuged at 10000 rpm for 20 minutes. The supernatant was used for protein analysis and reading was recorded in a UV-Vis Spectrophotometer at 660 nm.

Total carbohydrate estimation

The total carbohydrate content of the samples is estimated by the Anthrone method (Hedge, 1962). 100 mg dried samples were hydrolyzed with 2.5 N HCl for about 3 hours in a boiling water bath. To neutralize the extracts sodium carbonate was added. Then the extracts were centrifuged and supernatant were collected. The residue was washed for three times with distilled water and supernatant wee pooled and final volume make up to 100 ml. From this 0.5 ml of the extracts was taken and volume make up to 1ml with distilled water. The above solution was added by anthrone reagent of 4 ml. UV-Vis spectrophotometer at 630 nm was used to taken the absorbance.

Total phenol content estimation

Total Phenolic content was determined spectrophotometrically according to the Folin –Ciocalteu's method (Lachman *et al.*, 1998) with slight modification. Briefly, 0.5 ml of the sample was pipette into a 10 ml volumetric flask containing 0.5 ml of Folin–Ciocalteu's reagent, 5 ml of distilled water and 1.5 ml of Na_2CO_3 solution (w = 20 %), and the volume was made up with distilled water. At the time of oxidation of phenolic compounds, phosophomolybdic and phosphotungstic acid, contained in Folin- Ciocalteu's reagent, were reduced to blue coloured molybdenum and tungsten oxides. After two hours the absorbance of blue coloration was measured at λ = 765 nm against a blank sample. The measurements were compared to a standard curve of prepared gallic acid solutions (20, 40, 60, 80, 100 mg l^{-1}) and expressed as milligrams of gallic acid equivalents per 100 g from the equation.

Determination of total flavonoids

For determination of flavonoid aluminium chloride method was used (Olajire & Azeez, 2011). The methodology has been followed and quercetin was used as standard and flavonoid contents were measured as quercetin equivalent and calibration curve of quercetin was drawn. For this purpose, 1 ml of standard or extract solution (0.1, 0.5, 1.0, 2.5, 5.0 mg ml^{-1}) was taken in 10 ml volumetric flask, 5 ml of distilled water and 0.3 ml of 5% $NaNo_2$. After 5min, 0.3 ml 10% $AlCl_3$ added to the mixture. At the 6^{th} min 2 ml of 1M NaOH was added sequentially and the volume made up to 10ml with distilled water. The test solution was vigorously shaken. The absorbance was noted at 510 nm using UV-Visible spectrophotometer after 10 mints of incubation.

Determination of total tannin

Tannin is estimated by following the methodology of Burns (1971). 0.5 g of the powdered bark was taken in a 100 ml beaker. 50-ml Methanol was added in it. Mix occasionally by swirling after 20-28 hrs. The solution was centrifuged at 2000 rpm for 20 minutes and filtered. The supernatant liquid was collected in 100 ml beaker and the volume was made up to 50 ml. 0.2 ml of the sample extract was transferred to a test tube and made up the volume to 1 ml by the addition of methanol. Quickly add 5 ml vanillin hydrochloride reagent mix equal volume of 8% hydrochloride in methanol and 4% vanillin in methanol). The solution must be mixed just before use and avoid using oven if it is slightly colored. The solution was shaken well and the absorbance was read at 500 nm after 20min. Blank was prepared with vanillin hydrochloride reagent alone. Standard graph was prepared by using 20-100 µg Catechin using the dilute stock solution. The tannin content of the samples as g E. Catechin. 100 g^{-1} DM from the standard graph was calculated. All the analytical determinations were performed in triplicates, with an estimation of the averages and standard deviations. To determine the relationship between the total amount of phenolic and the antioxidant activity, the correlation coefficient was calculated and a regression analysis was performed.

RESULTS

Results obtained for screening of clone through morphological characteristics of *Calopepla leayana* shows that, the intensity of the attack of the pestson each clone in Naharoni experimental trial.The clones were classified in to three categories *viz*. Severely attacked, Moderately attacked and less attacked or negligible attack (Table 1).

Table 1. Defoliation score and severity scale of *Calopepla leayana.*

Level	Description	
	% of Defoliation	Severity scale
Severe	60-100	Most susceptible (attacked leaves dominate)
Medium	10-60	Moderately susceptible (equal occurrence of attacked & un attacked leaves)
Negligible	1-10	Un attacked (attack symptoms negligible/ Nil)

Among the total seventy (70) clones, eight clones were severely attacked, 55 clones were moderately attacked and 7 clones were less attacked (Table 2).

Table 2. Classification of *Gmelina arborea* Rox. clones based on intensity of attack by *Calopepla leayana*.

Most susceptible (8)	Moderately susceptible (55)	Less susceptible (7)
GA 015, GA 004, GA 111, GA 093, GA 002,GA 006, GA 020, GA 081	GA 022, GA 021, GA 071, GA 045, GA 044, GA 007, GA 003, GA 037, GA 114, GA 072, GA 026, GA 027, GA 031, GA 028, GA 009, GA 029, GA 038, GA 033, GA 016, GA 025, GA 085, GA 005, GA 030, GA 088, GA 011, GA 077, GA 097, GA 014, GA 107, GA 102, GA 103, GA 036, GA 099, GA 073, GA 075, GA 043, GA 113, GA 042, GA 105, GA 101, GA 094, GA 017, GA 106, GA 110, GA 108, GA 040, GA 096, GA 115, GA 079, GA 104, GA 116, GA 039, GA 098, GA 112, GA 008.	GA 019, GA 100, GA 109, GA 095 GA 034, GA 023, GA 086

As all the clones were found susceptible to *Calopepla leayana*, in that only seven (07) clones found less susceptible to the targeted pest. Representative clones selected out of the most susceptible and moderately suscetible and less susceptible (3 clones in each category) subjected to the feeding test exhibited the variations in quantum of food intake by the targeted pest. The variation of leaf area damage between the clones tested in most susceptible category, *viz*. GA 006,GA 081, and GA 002 showed 7.16 to 7.40 cm^2. The variation of leaf area damage between the clones tested in moderately susceptible category, *viz*. GA 110, GA 031 and GA 016 showed 5.73 to 6.04 cm^2. The variation of leaf area damage between the clones tested in most susceptible category, *viz*. GA 002, GA 006 and GA 081 showed 7.16 to 7.40 cm^2, in moderately susceptible category, *viz*. GA 016, GA 031 and GA 110, showed 5.73 to 6.04 cm^2 and less susceptible category, *viz*. GA 023, GA 095 and GA 034 showed 3.01 to 3.45 cm^2 (Table 3).

Table 3. Leaf area damage by *Calopepla leayana* on *Gmelina arborea* Rox. clones.

CLONE	CATEGORY	AREA FED (sq.cm/larva)
GA006	Most susceptible	**7.40 ±0.02**
GA 002	Most susceptible	7.16 ±0.10
GA 081	Most susceptible	7.22 ±0.20
GA 016	Moderately susceptible	6.04 ±0.05
GA 031	Moderately susceptible	5.89 ±0.09
GA 110	Moderately susceptible	5.73 ±0.02
GA 023	Less susceptible	3.45 ±0.05
GA 095	Less susceptible	3.11 ±0.07
GA 034	Less susceptible	**3.01 ±0.01**

Three clones in each category were subjected to the life cylcle period test. The result indicates that the larvae fed with the highly susceptible clone leaves completed the life cycle period of 33-36 days. Whereas the larvae fed with the less susceptible clone leaves completed the life cycle period of 45-47 days. The life cycle of the *Calopepla leayana* larvae fed with the moderately susceptible clone leaves completes the life cycle with in the period of 38-39 days (Table 4).

Table 4. Life cycle period of *Calopepla leayana* on different clones of *Gmelina arborea* Rox.

CLONE	CATEGORY	Life cycle period (indays)
GA 095	Less susceptible	45 ±1.00
GA 023	Less susceptible	44 ±1.00
GA 034	Less susceptible	47 ±1.00
GA 016	Moderately susceptible	39 ±1.00
GA 031	Moderately susceptible	38 ±1.70
GA 110	Moderately susceptible	39 ±1.70
GA 081	Most susceptible	33 ±1.00
GA 006	Most susceptible	36 ±1.00
GA 002	Most susceptible	35 ±1.70

The field and lab studies with the objectives conducted so far to identify the resistant candidates of *Gmelina arborea* for the defoliator *Calopepla leayana* expressed the existence of less susceptible candidates. Still, it may not be conclude

that the less susceptible clones for *Calopepla leayana* are to be considered as fully resistant clones. Hence, as for confirmation, the foliar study reported by Baruah *et al*. (2018) under this investigation and chemical study was done from the leaves of selected clones of the species.

Results of the proximate analysis for nutrient composition of different clones for total carbohydrate, content, total protein and the secondary metabolites study *i.e*. alkaloid, total phenol, total flavanoid and tannin were presented in table 5. The average percentage of carbohydrate content was varying widely. The clone GA081 possesses highest 48.27% and GA095 possess lowest 31.15%. In case of total protein content, GA 081 was possess highest 22.30% and lowest in GA095 (16.73%) made it highest survivalist against insect. Similarly, total phenolics content of various clones ranges from 0.20 to 0.48 mg GAE/100g. Clone GA034 shows the highest amount of phenolic content while the lowest content was observed in clone GA006. The highest amounts of flavonoids are reported in clone GA095 with 0.39 mg Quer/100g. The least values of flavonoids are observed in clone 002 (0.25 mg Quer/100g). Estimation of tannins content. The clone GA 034 showed highest amounts of tannin (0.21 mg/100g) and least concentrations are observed in clone GA006 (0.06 mg/100 g). Determination of alkaloids, the gravimetric analysis for total alkaloid contents in different clones of *Gmelina arborea* exhibited that highest alkaloid contents were present in clone GA95 (0.10%) and the lowest was found for clone 81 (0.06%). As a defensive mechanism phenols act against herbivores, and also for the microorganism present and make challenging the plant. The amount of total phenolic content of the different clones of *Gmelina arborea* can be arranged in descending order *viz*, 34>23>95>081>002>006. High value of phenol in clone GA34 makes it better repellent from insects than the other clones. To determine the amount of protein present is calculated by plotting the value in a standard curve of Bovine Serum Albumin (BSA) (Fig. 1) the amount of carbohydrate present was calculated by plotting the value in a standard curve of Standard Glucose solution (Fig. 2) and standard curve of Gallic acid (Fig. 3). From this study it is seen that the clones GA095, GA034, GA023 contains secondary metabolite in higher amount and nutrient content in less amount compared to the clones GA006, GA002, GA081, therefore the clones GA095, GA034, GA023 are less susceptible by the insects and the clones GA006, GA002, GA081 are highly susceptible by the insects.

Table 5. Nutritional and secondary analysis of secondary metabolites of different clones of *Gmelina arborea* Roxb.

Clone	Susceptibility	Phenol (mg gallic acid equivalents/100g extract)	Protein (%)	Carbohydrate (%)	Flavanoid (mg of Quercetin/100g)	Tannin (mg/100g)	Alkaloid (%)
GA095	Less	0.39	16.73	31.15	0.39	0.14	0.10
GA023	susceptible	0.45	18.32	37.51	0.30	0.19	0.09
GA034		0.48	17.77	42.15	0.35	0.21	0.09
GA006	Highly	0.20	18.73	43.18	0.29	0.06	0.07
GA002	susceptible	0.25	20.20	44.51	0.25	0.09	0.07
GA081		0.32	22.30	48.27	0.31	0.08	0.06

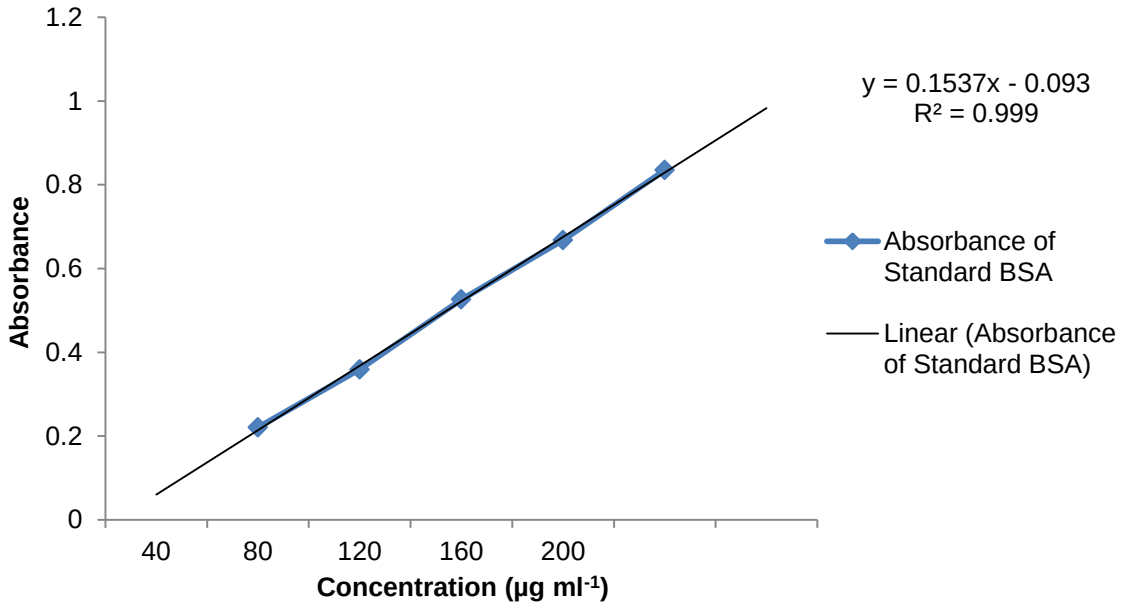

Figure 1. Graph showing absorbance of standard BSA.

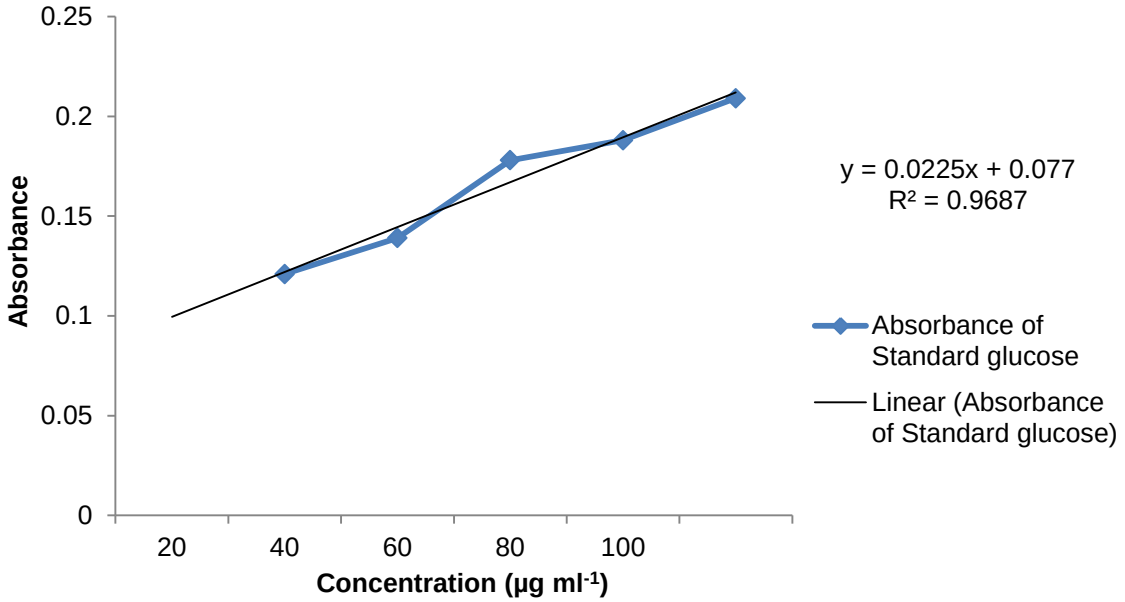

Figure 2. Graph showing absorbance of standard Glucose.

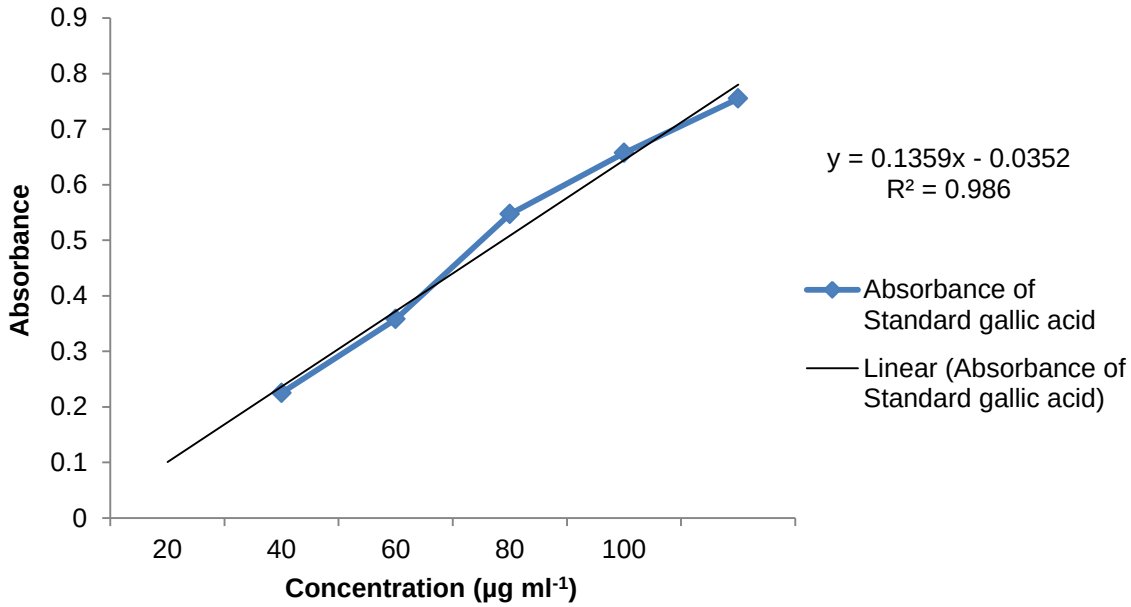

Figure 3. Graph showing absorbance of standard Gallic acid.

The result possess from screening of clone with reference to *Calopepla leayana*, which shows similar with insects infestation, foliar morphology and chemical analysis study. These were recorded and selected for most susceptible and less susceptible clones for insect infestation.

DISCUSSION

It is reported that the number of non-native, invasive pathogen and insect species continues to increase and they can have a significant negative effect on the health and biodiversity of native forest ecosystems, urban forests and forest plantations, which in turn can have large economic impacts (Pimentel *et al.*, 2000; Roy *et al.*, 2014; Campbell & Schlarbaum, 2014; Lovett *et al*., 2016). Screening assays is to enable identification of the full range of resistance phenotypes. Such screening trials can provide clues about the variety of resistance responses present in a population and their potential inheritance (Sniezko *et al.*, 2014). Naturally occurring host resistance tree populations can be maintained through the process of resistance development through selection and breeding (Sniezko & Koch, 2017). Screening of clones through selection and classification of weevil resistance in white pine (*Pinus strobus* L.) progenies was reported by Gerhold (1962) and describe the important measures to the degree of resistance. Our attempt to develop resistant clone through breeding program is also studied by Garrett (1972) to develop long-term breeding program for the development of weevil resistance in white pine. The program includes replicated testing of progenies over several sites and year, the establishment of clonal tests with promising selections. The trees may remain leafless for about four

during in the growing season, and repeated defoliation may even kill the attacked trees (Nair, 2001). Morgan *et al.* (1955) stated that for insect attack certain characteristic of resin are under a high degree of genetic control where, the *Populus* spp. supports a good clonal variation in case of insect resistance. It is found that in the plains of Assam Valley the pest remains active from March/April to October/November with four complete generations and suggested that only those stocks to be grown on large scale which displays resistance to an acceptable degree and in the experimental trial at RFRI, it was found that stocks established with different plus trees showed rich diversity in morphological features and resistance to *Calopepla leayana* that the clones had higher hair per unit area were least preferred by the insect (Singh *et al*., 2003). The ratio of protein and poly-phenol determines the degree of resistance in teak, whereas a higher ratio makes them susceptible. Jain *et al.* (2002) studied protein and poly-phenol contents of clonal leaves, and concluded that protein is inversely and poly-phenol is directly proportional to resistance imparted.

To select resistant plant varieties secondary metabolites should be main criteria to be taken into consideration. Jain *et al.* (2002) studied protein and poly-phenol contents of clonal leaves, and concluded that protein is inversely and poly-phenol is directly proportional to resistance imparted. The clones which are rich source of proteins and carbohydrates are highly eaten by the insects and which are rich source of secondary metabolites are less eaten by the insects. The clones which possess highest number of secondary metabolites are good for survival against the insect. Considering the available data on the chemical ecology of secondary metabolites, it can be assume that these compounds can indeed contribute to the protection of a plant against microorganism and other hervivores in concert with other chemicals and morphological features. The clones which are rich source of proteins and carbohydrates are highly eaten by the insects and which are rich source of secondary metabolites are less eaten by the insects. In case of Teak, the ratio of protein and poly-phenol determines the degree of resistance, whereas the higher ratio makes them susceptible to insects. Widespread group of defensive compound among the secondary metabolites plant phenols represent one of the most common compounds and play a major role in plant defense mechanism against insects (Sharma *et al.*, 2009; Usha & Jyothsna, 2010; War *et al.*, 2011). Phenols also act a defensive constituent against the herbivours, microorganism presents and challenging the plant. They could be an important part of the plants defense system against pests and diseases including root parasitic nematodes (Wuyts *et al*., 2006). The amount of total phenolic content of the different clones of *Gmelina arborea* can be arranged in descending order *viz*, 34>23>95>081>002>006. High value of phenol in clone 34 makes it better repellent from the insects than the other clones. Flavonoids are one of the largest classes of plant phenolic, perform very different functions in plant system including pigmentation and defense (Kondo *et al*., 1992). Treutter (2006) reported that flavonoids defend the plants against seneral biotic and abiotic stresses, UV radiations, pathogens and insect pests. Simmonds (2003) also found that flavonoids and isoflavonoids protect the plant against insect pests by controlling the behavior, growth and development of insect. Clone GA095 possesses the highest flavonoid contents. Therefore, flavonoid contents in relatively higher amount in clone GA095 would justify its comparative advantage over other clones in terms of protection of the plants from the insects. Considering the effect of Tannins, it has a strong deleterious effect on phytophagous effect insects which affect the insect growth and development through bindings of proteins and reduces the nutrient absorption efficiency and cause midgut lesions (Sharma & Agarwal, 1983, Barbehenn & Peter, 2011). In addition to this tannins are astringent, bitter polyphenols and act as feeding deterrents to several insect pests. The high value of tannin in clone GA 34 makes it better repellent from the insects than the other clones. The alkaloids are one group of the compound that seems to play a role in natural resistance mechanism of some plant species. In general, pyrrolizidine alkaloids (PAs) are toxic to at some level and serve in defense against microbial infection and hervivoral attack. Now, most of the alkaloids are believed to function as defensive elements against predators, because of their general toxicity and deterrence capability (Robinson, 1980; Harborne, 1988; Hartmann, 1991). The nutritional requirements of insects are similar to other animals, and proteins and carbohydrates are particularly important dietary nutrients (Behmer, 2009; Simpson & Raubenheimer, 2012) for the insects. The insects utilize plant proteins and carbohydrates for their nutritional requirements. The clones which are rich source of proteins and carbohydrates are highly eaten by the insects and which are rich source of secondary metabolites are less eaten by the insects. The clones which possess the highest number of secondary metabolites are good for survival against insect.

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