Teak defoliator (Hyblaea puera Cramer) outbreak happens in teak (Tectona grandis) plantations with ten
der foliage. Present study identified biochemical and volatile profile of teak tender leaves as opposed to mature
leaf in different seasons, so as to identify the cues detected by the female moth to identify tender teak leaves. In biochemistry, there
was no significant difference in oil, ash, total sugar, reducing sugars and non reducing sugars. Six volatiles compounds were
present in the tender foliage; while only two were found in the mature teak leaves. Tender teak leaves analyzed during pre-outbreak,
outbreak and post outbreak showed the presence of six, six and one volatile respectively. Through this study it is understood that host
plant recognition in Teak defoliator is mediated by the cocktail of volatile compounds. Development of the right combination of the
volatiles and behavioral testing using it would open new avenues in controlling incipient outbreak.

Introduction
Insects are single major link between the plants and animals at high-
er trophic level (Bernays and Chapman, 1994). They have been evolving together for one hundred million years ago, with different
kinds of interactions that are studied today in every branch of biology, from biochemistry and genetics to behavior and ecol-
y. Majority of the insect species depend on plant parts and consume ten per centage of plant annual production. Insects
exactlying choose their host plants on which they deposit eggs (Schoonhoven, 1968; Schoonhoven, 2006). Usually plants are
protected from insect herbivore or pest attack but most insects are highly specialized feeders as to overcome the plant defenses.

Host plant searching is essential for phytophagous insects be-
cause eclosion of adults from pupae may occur away from the
host plants. To locate a host plant, the insect needs to move towards
it and contact it to examine the characters (Beck, 1965;
Kennedy, 1977). The physical and chemical properties of plants
play major role in the selection, rejection and acceptance of
plants by phytophagous insects (Hsiao, 1969). The first chemi-
 cal that detected by insect from a plant is volatiles. All plants re-
lease volatiles through open stomata especially in active growth
phase and have little control over this (Bernays, and Chapman,
1994; Gibbs, 1974).

The components of the volatile organic compounds (VOCs) that
insects use to locate their host plants are often complex and spe-
cific (Schoonhoven, 1968; Schoonhoven, 2006). Detailed study
on VOCs features will facilitate to understand the underlying
mechanism of insect attraction towards specific plants and in
turn can use as an insect trap for pest management. The current
study was designed to understand the chemical cues originating
from teak (Tectona grandis) which would attract phytophagous
insects associated with it. The relationship between teak leaf
VOCs and its major pest Hyblaea puera (Lepidoptera: Hyblaei-
daе) commonly known as teak defoliator was analyzed, com-
pared and studied.

Materials and methods
Teak leaves of different maturity collected through different
seasons were studied for its biochemical and volatile com-
 pounds. The method used to detect teak leaf volatiles was GCMS
(Head Space) analysis. Fresh leaves of tender and mature teak
leaves were collected between 9.30 am and 10 am from lower
branch of a single teak tree. Maturity of the leaves was decided
according to their position in the branch. The 1st and 5th posi-
tioned leaves were treated as tender and mature respectively.
The leaves were plucked from the petioles by minimum damage
and placed in glass bottles with rubber caps, supplied with the
GCMS (head space), and were sealed and transported immedi-
ately to the laboratory.

The collected leaf materials were subjected to volatile analysis
using Head Space GCMS Varian MS #1. The column used was
VF5MS. Following was the program configuration selected for
the volatile analysis. Injection mode – GC Head space, syringe
– 1ml headspace, syringe temperature - 25°C, sample agitator
- agitate and heat, agitator temperature - 50°C, incubation time
0.25 min, incubation rpm – 250, agitator on – 10 sec agitator
off – 2sec, plunger fill speed – 100.000ml/sec, Fill stokes – 3,
viscosity delay – 1.00sec, injector- front, preinjection delay –
0.500sec, plunger injection speed – 250.000µl/sec, post injec-
tion delay – 0.500 sec, syringe flush time – 30 sec, and GC cycle
time – 0.30 min.

For the analysis of biochemical contents of teak leaves, mature
and tender teak leaves were collected from teak plantation. The
collected leaves were immediately transferred in to plastic bags,
sealed and brought to the laboratory for biochemical analysis.
Carbohydrate (Anthrone method) (James, 1999; Sadasivam
and Manickam, 1996), total phenols (Folin Ciocalteau reagent)
(William et al, 2005; Sadasivam and Manickam, 1996), total
sugar (Lane and Eynon method), reducing sugar (Lane and Eyn-
on method) (Eynon, and Lane,1923), amino acid (diff-
ference between total sugar and reducing sugar),total oil (ex-
traction with petroleum ether) (Lehninger et al.,1993), crude
fiber (acid alkali treatment) (Williet al., 2005; Sadasivam
and Manickam, 1996), nitrogen and protein Micro-Kjeldahl
method (James, 1999; Sadasivam and Manickam, 1996), total
sodium (Toluene distillation method) (William et al., 2005;
Sadasivam and Manickam, 1996), Sodium and potassium (Atomic Absorption Spectro photometer), moisture
content (Toluene distillation method) (William et al., 2005;
Sadasivam and Manickam, 1996) and pH (using pH meter) were
tested.

Results
Volatile analysis
It was observed that the tender leaf holds more volatiles than the
mature ones. Figure 1,2, and 3 presents the retention time of
volatile compounds in tender and mature teak leaves during three
different seasons- pre monsoon, monsoon and post mon-
soon.

The leaves collected during February and July months had more
number of volatiles than in those collected in November. In Febru-
ary the leaves had six compounds - ethanol, 2-methoxy, 1R-α
pinene, β-phellandrene, sabinene, 1-4 methanol-1-x cycloex and α-
thujene. In July, ethanol, 2-methoxy, 1R- α- pinene, β- phel-
landrene, sabine and Caryophyllene were found in the leaves. Only one volatile compound (Caryophyllene) was present in November. The amount of ethanol, 2-methoxy increased to 4.48 per cent, 1R-α-pinene to 93.14 per cent, and sabine to 0.637 in July. Caryophyllene and α - Caryophyllene was newly recorded in the month of July. 1-4 methanol-1-x cyclo and α - thujene were absent in July. Only one compound, Caryophyllene was present in the month of November.

In the month of February the mature leaf contained three volatiles ethanol, 2-methoxy-; β- phellandrene and 1-4 methanol-1-x cyclo. In July, the amount of ethanol, 2-methoxy- showed a very high increase to 85.84 per cent and 1R-α-pinene was newly found.

Biochemical contents of tender and mature teak leaves

The biochemical contents were different in mature and tender teak leaves (Table 1). It can be seen that except for oil, sugars, sodium, potassium, phenol and tannin, there was marked difference in the content of all other compounds. Carbohydrate, protein, ash, nitrogen, lignin and moisture were high in the tender leaves as compared to that in the mature leaves. Fiber content and pH value were more in mature leaves. But there was no significant difference in the biochemical composition of teak leaves, which were collected during two different periods.

Discussion

In this study we exploited insect host finding cues from teak (Tectona grandis). Leaf biochemical parameters and volatile organic compounds (VOCs) in different seasons were analyzed and compared. The seasonal variations observed in the combination of VOCs from teak are imperative for pest incidence study in teak plantation. The outbreak of defoliator (Hyblaea puera) a major pest of teak, is seasonal and the infestation starts after the pre monsoon shower when teak tree are in new flushing (Nair, 2007; Nair and Mohandas, 1996). It is evident that some of the VOCs detected through this study are capable of attracting the defoliator moths towards the teak plantation as they had been reported to be insect attractants by earlier workers. This information supports H.puera immigration to teak plantation from a distant location (Chandrasekhar et al., 2004; Nair, 1986).

The characteristic odor of a plant is produced by a group of chemically related volatiles, such as terpenoides in conifers, sulfiides from onion and garlic. The first chemicals that insects detect from the host plant are volatiles (Bernays and Chapman, 1994). These slightly volatile compounds make aerial bouquets that enable them to find the host plant from a distance. Among, olfactory signals are making insect to take off and move towards the odor from host plant (Bernays and Chapman, 1994).

A large number of insect pests utilize plant volatiles in host location (Metcalf and Metcalf, 1992; Metcalf, 1987). The identified teak leaf volatiles and their relation on teak defoliator behavior could eventually provide tools for managing this pest in future. Present study identified six teak leaf volatiles, amongst four of them are generally act as cue in insect outbreak causation as kairomones and oviposition / feeding stimulants. Further studies on this would open up new avenues in mass trapping of immigrant pest moths towards teak plantation so as to control incipient outbreaks.

Acknowledgement

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Figures and Pictures

Figure 1: a. Volatiles from tender teak leaf in preout-break period of defoliator

Figure 1: b. Volatiles from mature teak leaf in preout-break period of defoliator

Figure 1: retention time of teak leaf volatiles in pre-outbreak period of teak defoliator
Figure 2: a. Volatiles from tender teak leaf in outbreak period of defoliator

Figure 2: b. Volatiles from mature teak leaf in outbreak period of defoliator

Figure 2: Retention time of teak leaf volatiles
a. Mature teak leaf (February)
b. Mature teak leaf (July)

Figure 3: retention time of teak leaf volatiles in post outbreak period of teak defoliator

Table: 1. Biochemical contents of tender and mature teak leaves in different seasons

<table>
<thead>
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<th>Sl. No</th>
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<th>Post out break</th>
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<td>Tender</td>
<td>Mature</td>
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<tr>
<td>1</td>
<td>Carbohydrate (per cent /wt)</td>
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<td>Ash (per cent /wt)</td>
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<td>Tot sugar (per cent /wt)</td>
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