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Allelopathic interactions of certain *Musa* cultivars against *Odoiporus longicollis* (Olivier)

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ABSTRACT: *Odoiporus longicollis* is a major pest of the banana (*Musa)* cultivars that enormously feeds on the pseudostem and causes serious damage to banana cultivation. It is a monophagous pest of banana, which showed extreme preference to some commercially viable cultivars such as *Nendran*, (AAB) *Palayankodan,(AAB) Red banana (AAA)* and extreme non preference to some commercially non viable cultivars such as *Kadali, Kannan, Aattinkombu (*all *AA* types*)* and *Thenkaali (AAB).* Field study, diversity analysis and pest status of *Musa* cultivars in Chittar panchayt of Pathanamthitta district (Kerala) by Arc GIS software showed that maximum diversity of cultivars with minimum pest attack was seen in those wards which are ecotones with respect to forest and agro ecosystem. Rearing of *O.longicollis* larvae in *Thenkaali* and *Aattinkombu* has resulted mortality of them evidenced by hyperprotenemia and hyperuricemia of haemolymph. The HPTLC study has revealed that pseudostem of *Aattinkombu* and *Thenkaali* possessed three additional compounds which were not present in pest sensitive cultivars. The differential distribution of secondary metabolites in the pseudostem of the above two cultivars can also be felt by difference in the smell of freshly cut pseudostem.

KEYWORDS: *Odoiporus longicollis*, resistant *Musa* cultivars, ecotones, hyperproteineaemia, allelopathic ineractions

INTRODUCTION

Bananas (*Musa* spp) are the major food crop globally cultivated and consumed in more than 100 countries throughout the tropics and subtropics. They provide a staple food for millions of people (Tiwari *et al*., 2006). They are also providing a well balanced diet and also contribute to the livelihood through crop production, processing and marketing. In developing countries

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they are the most important food crop after rice, wheat and maize (INIBAP, 2000). They are the cheapest, plentiful and the most nourishing of all the fruits and are consumed by the rich and poor alike. The plants are considered as a symbol of prosperity and fertility due to its place as a token of goodwill in various religious practices and ceremonial functions (Agrawal *et al*., 2007).

Banana is attacked by a number of pests, that includes rhizome weevil *Cosmopolites sordidius* (Germer), banana aphid *Pentalonia nigronervosa* (Coq.), fruit and leaf scarring beetle *Nodostomata subcostatum* (Coq.) burrowing nematode *Radopholus similis*, among which major key pest is the banana pseudo stem borer, *Odoiporus longicollis* (Olivier), a monophagous pest of banana and plantains limiting the production and productivity, posing serious threats to the cultivation of bananas (Visalakshi *et al*.,1989). It was estimated that banana pseudo stem borer caused 10-90 percent yield loss depending on the growth stage of the crop and management efficiency (Padmanabhan and Sathiamoorthy, 2001).

Field study conducted in Chittar Panchayat of Pathanamthitta District has resulted in the identification of 21 cultivars of *Musa* .Genome classification and their pest status in relation to *O.longicollis* was carried out. Majority of the cultivars identified were triploid (either AAA or AAB), four are diploid with AA constitution and only one *Njalipoovan* with AB genetic constitution (Kavitha *et al*., 2015a) This pest exhibited extreme preference to commercially viable cultivars and extreme non preference to some commercially non viable and less common cultivars. Among the four cultivars which are resistant to pest, three (*Aattinkombu, Kannan* and *Kadali*) are diploid with AA type and only one *Thenkaali* is triploid with AAB type (Kavitha *et al*., 2015a) Farmers are reluctant to cultivate the commercially non viable and pest resistant cultivars because of some practical problems. All these aspects described in this paper.

MATERIALS AND METHODS

The study was carried out in Chittar Grama Panchayat, Pathanamthitta District. It is geographically located between 09Ú 18' - 09Ú20' of north longitude and 076Ú 53'- 076Ú 57' of east latitude. It is a high range area and elevation ranging from 59m to 334m above the sea level. The area enjoys a tropical climate with 2922 mm annual rainfall. Annual temperature range between 18 °C (64 °F) and 35 °C (95 °F) (The Statistics department, Pathanamthitta, 2004).The soil type was laterite. The South West region of the Panchayat falls into forest region and North East region consists of rubber plantation. The banana plots selected for the study falls in these two regions .The Panchayat consists of 13 wards, and five sites from each wards were selected based on accessibility and the agriculture records, available in the Panchayat office. A total of 65 sites were selected and latitude and longitude of sites were taken by using GPS (Geographical Positioning System).

Construction of map using ArcGIS Software

A total of 65 study sites from 13 wards were selected for the present study (5/ward). Local

farmers were consulted to identify the important banana growing areas within the Panchayat. Using Global Positioning System (GPS) during the field survey, the locations of each of these sites were collected. This information was used to construct maps using Arc GIS.

ArcGIS 9 (established in May 2004) is geographic information system (GIS) for working with maps and geographic information. It is used for creating and using maps; compiling geographic data; analyzing mapped information; sharing and discovering geographic information; using maps and geographic information in a range of applications; and managing geographic information in a database. The system provides an infrastructure for making maps and geographic information available throughout an organization, across a community, and openly on the Web. Today, GIS has evolved into a crucial tool for science based problem solving (Arc View GIS, 1996). The digitalized map of Chittar Panchayat was taken from the toposheet of Pathanamthitta prepared by Land Use Board, Vikas Bhavan (Toposheet No. 58 C /15/SE, Scale 1:25000, First Edition) .Fig 1Map showing land use Fig 2- Map showing study area were prepared from CED (Centre For Environment and Development, Thozhuvankodu, Trivandrum).

Study on the Diversity of *Musa* Cultivars

Various cultivars of *Musa* grown by the farmers in various fields were observed thoroughly for distinguishing features such as length and width of leaf, shape and colour of the pedicel, colour of the pseudostem, general appearance of the fruit bunch, peduncle of the fruit bunch, shape of the male flower buds, bract shape, and local name of the cultivar was recorded by interaction with the farmers.

Diversity Analysis

Diversity analysis was carried out by three standard methods such as Shannon Wiener diversity index, Simpson's diversity index, Species richness and Abundance (Southwood, 1978; Fager, 1972).

Experimental maintenance of larvae

The developing stages of banana stem weevil *O. longicollis* were collected from infected clones of common cultivars of *Musa* from the fields. Three month old *Musa* cultivar such as *Palayankodan*, a variety to which the pest showed strong preference to infestation, *Aattinkombu* and *Thenkaali*, two cultivars to which the pest showed no preference for infestation were taken for the study.

The crown of the plants were chopped down and a small depression was made at the cut end and third instar larvae (ten numbers) of *O. longicollis* were put into the depression made at the top of the live stump and observed for 4, 8 and 10 days. Number of live larvae after appropriate duration was observed for pupation, mortality and difference in their sizes from that of the control. The live larvae were collected and used for further studies.

The larvae were cleaned and placed on ice for immobilization. The hemolymph for biochemical studies was isolated by making a small cut on the neck of the larvae. Standard procedures were used for biochemical estimations- protein (Lowry *et al*., 1951), total free amino acid (TFAA) (Spies *et al*., 1957) uric acid (Standard Assay Kit) and SDS-PAGE (Laemmli, 1970). 10% acrylamide concentration was used for the separating gel and 4% for the stacking gel.

HPTLC analysis of the banana pseudostem: Banana pseudostem powder (2gm) was extracted in 95% ethanol for 8 hours. The extract was dried in vacuum rotary evaporator and the dried sample was dissolved in 2 ml of methanol.5 µl of solution was loaded on a pre coated (Silica gel, $60F_{254}$, 2mm thick) plate (Merk).The solvent mixture of chloroform: methanol (8.8:1.2). Anisaldihyde-Sulphuric acid mixture as location reagent .The plate with sample was heated as 105^0C for 5 minutes, Visualized and photographed. Data of quantitative estimation were analyzed statistically by ANOVA (Daniel, 2006).

RESULTS AND DISCUSSION

The land use map of Chittar pnchayat of Pathanamthitta District (Fig. 1) and the 65 study sites in that area is shown in Fig.2.All the 13 wards of this panchyat possessed commercial cultivation of *Musa* sp. The distribution and diversity of *Musa* cultivars in different wards of Chittar pnchayat is shown in Table 1.Three wards which shares boundery with Konni forest division of Kerala cited as 7,8 and 9 in this table showed rich diversity of *Musa* cultivars. The pest attack on cultivars were minimum in the above three wards. The pest status of these 21 cultivars were studied in field condition (Kavitha *et al* 2015a)and also by rearing the larvae in live stumps of different cultivars in experimental fields. The cultivars which caused 100% mortality of *O.longicollis* larvae on 10^{th} day of experimental maintenance was considered as pest resistant and they never showed any symptoms of pest attack under field condition (Kavitha *et al*.,2015b).Through field study we could prove that cultivars which are bearing large fruit bunches short duration to set flower, short duration to harvest with high market value were preferred by the farmers and they were *Nendran, Palayankodan (both AAB), Robusta ,Red banana(both AAA)* and *Njaalipoovan (AB)* and these cultivars were widely cultivated in the fields. The less common cultivars such as *Kadali, Aattinkombu, Kannan (all AA) and Thenkaali (AAB)* , were seen only within the premises of houses as lone clones and no commercial cultivation was seen in any of the study places. Interactions with farmers have proved that long duration for harvest, small size of the fruit bunch and low market value were the reasons which make the above cultivars as economically non viable. No symptoms of pest attack were seen on these pest resistant cultivars, such as *Kadali, Thenkaali, Aattinkombu* and *Kannan* (Fig.3).The above four cultivars took long duration to sprout once the suckers were separated from the mother and planted in a new site. Among the four resistant cultivars the *Kadali* cultivar is common because of its importance in temple worship (Shing, 2002).The plant and fruit bunch (*Kadali*) were very small and hence commercial cultivation was not common (Sunderbabu, 1983).

One of the very interesting features observed in the field study was that the cultivars such as

Fig 1.Land use map of Chittar Panchayat

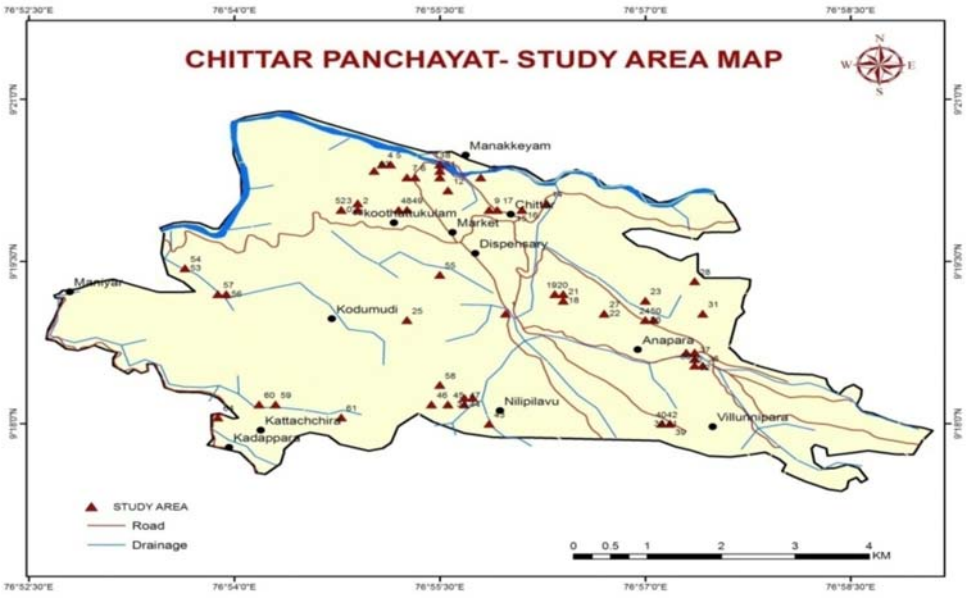

Fig 2.Study area map of Chittar Panchayat

Kannan

Aattinkombu

Thenkaali

Kadali

Fig3.Pest Resistant Cultivars

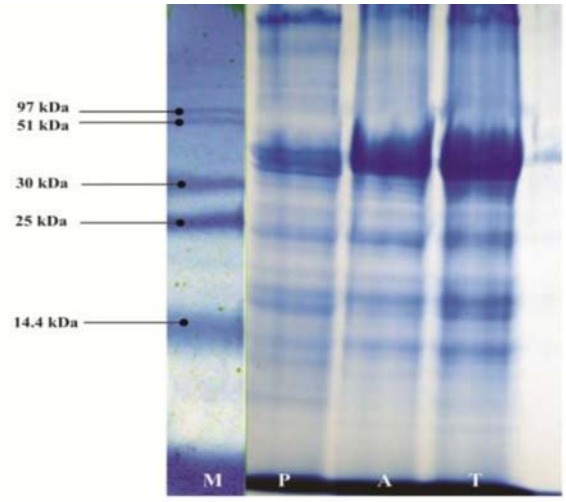

Fig 4. Electropherogram of hemolymph of *O. longicollis* larvae reared in resistant and susceptible Musa cultivars

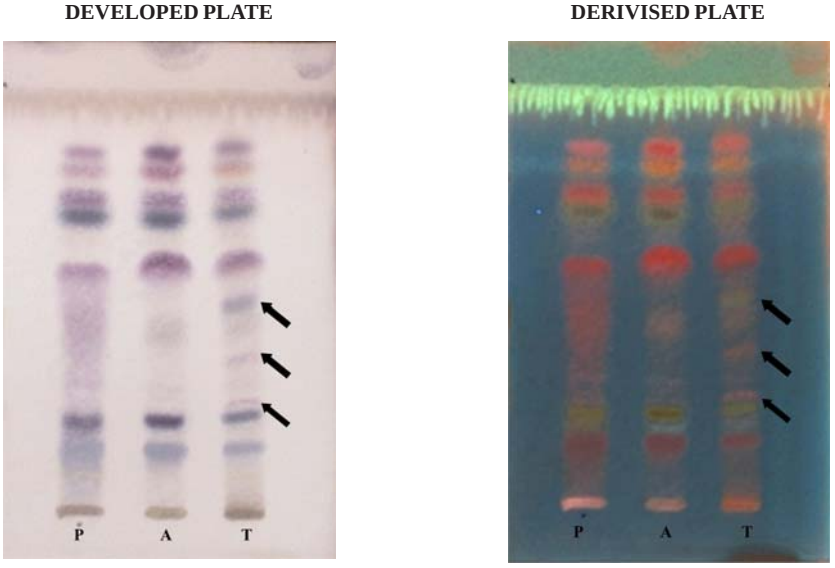

Fig 5.HPTLC Analysis Of Pseudostem of Three Musa Cultiars

P – *Palayankodan* A – *Aattinkombu* T – *Thenkaali*

Table 1.Comparison of *Musa* diversity in different wards of Chittar Panchayat

DIVERSITY STATISTICS				
WARD No.	**ABUNDANCE**	**SPECIES RICHNESS*1**	**SIMPSON INDEX*2**	**SHANNON WEINER INDEX*3**
1	22	12	0.18	2.22
2	20	12	0.14	2.95
3	42	10	0.25	1.61
4	37	11	0.26	1.92
5	52	9	0.32	1.44
6	34	10	0.21	2.16
7	18	19	0.09	4.27
8	20	15	0.10	4.00
9	15	17	0.08	4.62
10	43	13	0.24	1.70
11	50	6	0.32	1.40
12	22	15	0.14	4.23
13	21	12	0.10	2.21

*1 Number of different cultivars

*2 $D = \Sigma n(n-1) / N(N-1)$
(Values ranges from 0–1. **'0'** infinite diversity,**'1'** no diversity)

*3 $H' = \Sigma pi(ln\ pi)$
(Larger H = High diversity, values ranges from 0–5)

Red banana Palayankodan, and Nendran with very large pseudostem, leaves and fruit bunch are possessing high incidence of pest attack. On the other hand *Aattinkombu, Kadali* ,*Kannan* were very small with respect to their pseudostem, fruit bunch and leaves. The literature survey very well agreed with our observation and showed that *Nendran Red banana* and *Palayankodan* are triploid (Wang, 2010).It was well known that plants which are triploid or polyploids possessed high vegetative growth and at the same time the secondary metabolites present in them are very low. This may be the reasons for high incidence of pest attack in such cultivars *(*Wyiniger, 1962) and there are previous reports that. *O.longicollis* possessed extreme host specificity (Karr, 1983).

TABLE 2.Total Protein, Total Free Amino Acid (TFAA) And Uric Acid Levels in the Hemolymph of *Odoiporus longicollis* Maintained In Three *Musa* cultivars

Cultivars in which larvae were maintained*	LARVAL HEMOLYMPH		
	Total Protein (µg/ml)	Total Free Amino Acid (µg/ml)	Uric Acid (µg/100 ml)
Palayankodan	320.11 ± 6.52	498.07 ± 7.82	7.89 ± 0.68
Attinkombu	460.06 ± 7.39	587 ± 8.61	9.801 ± 0.91
Thenkali	500.1 ± 8.26	383 ± 6.76	10.99 ± 0.99

Larvae were maintained in four days in the pseudostem
Values are Mean ± S.D. All values are significant at 0.05 levels on compiring with control (*Palayankodan*) n=6

O.longicollis larvae maintained in *Palayankodan* has successfully pupated, but the larvae maintained in *Thenkaali* and *Aattinkombu* showed significant changes from control such as weakness and absence of wriggling movements. When they were carefully dissected out from the pseudostem on the fourth day and kept on a fresh pseudostem they were unable to bore into it indicating extreme weakness. Hyperproteinaemia and hyperururicemia together with increase in Total free amino acids (TFAA) were observed in larvae maintained *Aattinkombu* for four days (Table.2). Hyperproteinaemia and hyperururicemia together with decrease in TFAA were observed in larvae maintained *Thenkaali* for four days (Table.2). Increased amount of uric acid in intoxicated larvae of both cultivars may due to increased catabolism of amino acids or nucleotides. More than 85% of larvae were dead on the eighth day of maintenance and 100% mortality of larvae was seen on the 10 th day of maintenance in *Thenkaali* and *Aattinkombu*.

Hyperproteinaemia of hemolymph can be considered as generalized stress response of insect larvae, which were subjected to various types of stress. Hyperproteinaemia was observed in other Coleopteran larvae such as *Oryctes rhinoceros* in the presence of various stress conditions such as infection of *Bacillus thuringiensis*, exposure to cold shock, and infection by ectoparasitic mite *poecilochirus* sp., and antigen challenge (Adhira *et al*., 2010, Adhira, 2015). It is known that uric acid is produced principally in the cells of the fat body and is released into the hemolymph, which is then transported to the malphigian tubules to be excreted out. Our findings are agreeing with the observations of investigators on *Spodoptera litura* under various stress condition (Tripathy and Singh, 2002).

SDS-PAGE of hemolymph of *O.longicollis* larvae maintained in *Thenkaali* and *Aattinkombu* were proved that there was elevation of protein content in quantitative estimations and also evidenced by increase in the thickness of many protein bands. Many small polypeptides are

vanished or lost during the course of toxicity, complete disappearance of a series of low molecular weight protein were observed in larvae maintained in non-preferred varieties which was attested through GEL-DOC analysis of the electrophorogram (Fig.4). In GEL-DOC analysis it is clear that larvae in *Thenkaali* cultivar showed protein band with molecular weight 16.97, 29.78,41.64 and 226.03 are not seen in *Palayankodan*.Similarly larvae maintained in *Aattinkombu* showde protein band with molecular weight 21.96, 27.58, 49.06 and 124.56 are not found in *Palayankodan*.

The High Performance Thin Layer Chromatography (HPLC) study has revealed that pseudostem of *Palayankodan*, *Aattinkombu* and *Thenkaali* has 9-10 compounds which are common in three varieties which gave positive reaction with colouring reagent, Anisaldehyde-Sulphuric acid mixture. *Thenkaali*, a pest resistant cultivars possessed three additional compounds which gave positive reaction to the above colouring reagent, but *Aattinkombu* exhibited compounds almost identical to *Palayankodan* and at the same time, some compounds in excess quantities than *Palayankodan* (Fig.5). It was reported by other investigators that the pest had least affinity towards certain *Musa* cultivars (Tiwari, 1971). The differential distribution of secondary metabolites in the pseudostem of the above two cultivars can also be felt by difference in the host plant odour. It may be the reason for selective preference of the pest. The non-preference of *O. longicollis* towards *Thenkaali, Aattinkombu* may be the presence of certain volatile secondary metabolites. The chromatogram of the pseudo stem of the three varieties such as *Palayankodan, Thenkaaali and Aattinkombu* has revealed that observed non-preference *by O. longicollis* may be due to the presence of additional compounds in their pseudo stem. The odour differences of the sap of these three cultivars were noticed. The mother weevil *(O.longicollis*) with sensitive antennae could differentiate the most favorable host plant for their next generation and this may be the reason for no symptoms of pest attack on *Thenkaali and Aattinkombu*.

In India (Isahaque, 1978) showed that the banana cultivars such as 'Bhimkal',Kaskal and Jhajee were completely free of infestation by *O. longicollis*. Resistance in these three varieties appeared to be connected with their broad, thick and compact leaf sheaths and pseudostems, although chemical antibiosis may also have been a contributory factor. Vevai (1971) has repoted that some cultivars with excess phenolic compounds showed no pest attack in field condition.

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