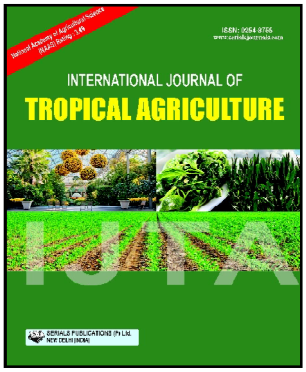

INTERNATIONAL JOURNAL OF TROPICAL AGRICULTURE

ISSN : 0254-8755

available at http: www.serialsjournals.com

Volume 36 • Number 1 • 2018

Distribution of Flavonoids, Phenols and Related Enzymes in *Musa* Cultivars and their Role in Resisting the Infestation and Causing Mortality in *Odoiporus longicollis* [Olivier]

Ajitha T., Kavitha K. J., Shabith Raj K. and Evans D.A.*

* *Department of Zoology, University College, Thiruvananthapuram 695034, Corresponding author E-mail: drevansda@gmail.com*

Abstract: Distribution of total phenols (TP), total flavonoids (TF) and activity of enzymes such as Phenylalanine ammonia lyase (PAL), Polyphenol oxidase (PPO), and Peroxidase (PO) in different cultivars of *Musa* showed high variation and have great influence on the pest status of cultivars in relation to infestation by *Odoiporus longicollis* [Olivier]. Under field condition the pest showed extreme specificity in selection of host plants and they selectively avoided those cultivars which possessed very high activity of PAL, PPO, PO and high contents of TP and TF. Pest susceptible cultivars possessed very low activity of the three enzymes and very low amount of TP and TF. Pest status and genome constitution did not show any correlation in the present study using 26 cultivars. As the cultivar *Amrithsagar* (AAA) was susceptible to infestation by *O. longcollis*, another cultivar *Yangambi* (AAA) was highly resistant to pest. While *Bonda bathesa* and *Cuba* (both ABB) showed susceptibility to this pest, *Karpooravally*, *Peyan* and *Saba* (all ABB) showed non preference by this pest. Rearing of larvae of *O. longicollis* in cultivars possessing very high activity of PAL, PPO and PO and very high contents of TP and TF has resulted mortality of larvae in seven days. Mother weevils are highly judicious in selecting host plants for oviposition and their selective avoidance of resistant cultivars (R) may be by sensing the cultivar specific volatile molecules, which needs detailed study.

Key words: *Odoiporus longicollis*, *Musa* cultivars, pest resistance, total phenol, total flavonoids, Phenylalanine ammonia lyase, Polyphenol oxidase, Peroxidase.

INTRODUCTION

Banana and plantain are clumped perennial herbs. India is one of the major biodiversity centres of plantain and contributes 15% of the banana production in the world (Alagesan *et al.*, 2016). In India 70% of banana production is contributed by small scale farmers (Frison *et al.,* 1998). It can be cultivated throughout the year and it can be used both as vegetable and fruit. Some rare cultivars have cultural importance in Kerala, such as *Kadali* (AA), in temple worship, *Kappa* (AAA), in marriage ceremony (Kavitha *et al*., 2015 b) and certain cultivar of certain places are so unique and thereby they got GI tag and one such banana is a unique *Nendran* (AAB) cultivar and is called as *Kazhchakula* from Guruvayoor, Kerala, which has special place of worship in Guruvayur temple.

One of the reasons for extreme diversity of *Musa* cultivars in India is postulated as natural hybridization of *Musa acuminata* triploid (hybrid) with *Musa balbisiana*, which has resulted preponderance of diversity particularly in cultivars with AAB and ABB genome constitution (Price, 1995). India is endowed with considerable diversity for edible banana cultivars in the North Eastern and Western Ghats region. Diversity among *Musa* cultivars in India is vast owing to diverse agro-climatic conditions from dry arid zones to humid tropical and cold temperate zones. The local cultivar diversity is more conspicuous in South India, especially in the states of Kerala, Tamil Nadu and Karnataka and cultivar variability decreases from extreme South towards the North (Amalraj, 1992). The South Indian states especially Kerala and Tamil Nadu are endowed with delicate edible diploid cultivars (AA) which can be nurtured only in sheltered humid environments (Uma & Sathiamoorthy, 2002). The wealth of banana cultivars in the coast of Kerala is unequalled by any other banana growing regions in the world (Nayar, 1962).

One of the major constraints in banana production is infestation by pests. It is attacked by different insect pests among which banana pseudostem borer *O. longicollis* is a key pest limiting the production and productivity of banana and plantains (Ostmark, 1974; Visalakshi *et al.,* 1989). It is a monophagous pest, and both the adults and larvae cause severe damage to banana plants (Shukla and Kumar, 1970). Long life span of adults and larval endophytic behaviour are the major factors limiting the efficiency of conventional methods of control, especially chemical control using insecticides (Shanmugam *et al.,* 2013). Almost all common edible cultivars are affected by the weevil which showed extreme specificity to some cultivars and extreme non preference to some others (Prasad, 1987; Gawel, 1991; Kavitha *et al*., 2015a). The severity of loss is greater when infestation occurs at early vegetative stage (Visalakshi *et al.,* 1989) and cultivars such as *Nendran, Palayankodan* and *Red Kappa* are highly susceptible (HS) to infestation by *O. longicollis* (Kavitha *et al., 2015 a&b*).

Organic compounds generated from primary metabolites that do not have any direct function in growth and development are categorized as secondary metabolites (SM). These SM are specific in one species or a taxonomically related group of species. Biosynthesis and degradation of secondary metabolites play important roles in ecology and physiology of organism in which they occur (Waller and Nowack, 1978; Waller and Dermer, 1981). Chief ecological function is to defend host plants against herbivory or infestation (Harborne, 1982). So the quantity and pattern of distribution of important SM obviously indicate the status of host plant resistance.

Field study conducted to identify the pest resistant *Musa* cultivars in different sites of Kerala has resulted in the identification of 24 cultivars in a remote village and among them nine were highly resistant to attack by insect pests (Kavitha *et al.,*2015 a & b) and one of the reason for pest resistance was identified as presence of extremely high content of Phenols, Flavonoids and high activity of related enzymes such as Phenylalanine ammonia lyase (PAL), Peroxidase (PO) and Polyphenol oxidase (PPO) (Kavitha *et al.,* 2017a). Further study in various agro ecosystems of Kerala has resulted in the identification of another 26 cultivars and their genome constitution and pest status in relation to *O. longicollis* under field condition was studied (Ajitha *et al.*, 2017). Studies done by the investigators showed that farmers are not interested in cultivating *Musa* cultivars with long duration to set flower, small fruit bunch, less palatable fruits and those with low market value due to non familiarity among the public (Kavitha *et al.*, 2015b). The above 26 cultivars are all with low commercial viability and hence they were rare in the agro ecosystem but at the same time many of them did not show attack by *O. longicollis* in field condition. The defense mechanism behind the non preference shown by *O. longicollis* on these 26 cultivars was reported in this communication.

MATERIALS AND METHOD

Collection of *Musa* cultivars

Kerala state has banana procuring centers in all Grama panchayaths, which ensure reasonable market value to the farmers. Regularly visited these quasi governmental organizations, which are under the Department of Agriculture, Government of Kerala and interacted with farmers, about the different types of *Musa* cultivars in their local place and procured suckers of cultivars, which are not common or rare in their locality. Suckers were planted in the campus of University College, Thiruvananthapuram and maintained with organic manure. No synthetic fertilizers were provided. The cultivars such as *Cuba*, *Bondabathesa, Lady's finger* and *Yangambi,* were collected from an enthusiastic farmer of Kerala (Mr.Vinod Sahadevan, Parassala, Thiruvananthapuram District) who maintains more than 150 cultivars of *Musa* collected from various Agricultural Universities of India. All other cultivars are indigenous to Kerala.

Leaf sample collection

Tender cigar leaf, possessing 20 to 30 cm length was cut from the tip and kept in ice cold condition till weighing and processing.

Assay of enzymes, Phenols and Flavonoids

All estimations were done as described in the standard techniques. Total phenols (Mayr *et al.,* 1995), Total flavonoids (Chang *et al.,* 2002) and assay of enzymes such as Phenylalanine Ammonia lyase (Whetten and Sederoff, 1992), Polyphenol oxidase (Mayer *et al.,* 1965), Peroxidase (Hammer Schimist *et al.*, 1982). Activity of enzymes was expressed as units/mg protein.

Rearing of *O. longicollis* larvae in *Musa* cultivars

Cultivars which never showed infestation by *O. longicollis* in field conditions and those possessed very high activity of PAL, PO, PPO and bearing very high contents of TP and TF were used for this study. Those cultivars possessed very low contents of TP and TF, and very low activity of PAL, PO and PPO were used as control. Four month old cultivars with pseudostem of 25 to 30 cm circumference, whose crown was chopped down at a height of 100cm above the ground. A small depression was made on the free cut end of live pseudostem and seven *O. longicollis* larvae (third instar) were put in to it (third instar larvae are voracious feeders than younger instars, moderately large in size and easy to handle). The larvae were allowed to bore into the pseudostem and cut end was covered with a piece of mosquito net. In order to prevent the entry of rain water, the cut end was closed by a piece of plastic, if there is rain. On the seventh day the live pseudostem (live stump) was cut 15 cm below the first cut and the larvae were carefully dissected out. Those cultivars which caused complete mortality of larvae within seven days were called Resistant (R) and those cultivars on which no mortality was observed were designated as Susceptible (S).

Statistical analysis

Five leaf samples from each cultivar were evaluated. The data was analyzed by one way analysis of variance (ANOVA). The values with level of significance $p<0.05$.

RESULTS

Musa cultivars numbered as 1-5 showed very low content of TP when compared to cultivars 15-26 (Fig.1). The content of TP in cultivars 6-14 was slightly but significantly higher than (1-5) but lower than 15-26 groups. The quantitative distribution of TF in the 26 cultivars also showed a same pattern as that of TP., viz. cultivars 1-5 showed lowest amount of TF and cultivars 15-26 showed highest amount (Fig. 2).

Activity of PAL showed very high variation among the 26 *Musa* cultivars (Fig. 3). Activity of PAL was very low in cultivars 1-5, but cultivars 15-26 have maintained highly elevated activity of this enzyme. *Musa* cultivars 6-14 showed only a moderate activity of PAL, which was higher than that of the first category of cultivars included in 1-5 and lower than the cultivars 15-26 (Fig. 3).

Activity of PPO was very low in *Musa* cultivars which are susceptible (1-5) to infestation by *O. longicollis*. Slightly elevated activity of PPO was observed in cultivars sporadic attack (6-14) by this pest and the activity of PPO was very high in cultivars (15-26) which did not show any infestation by this pest. The pattern of activity was almost identical in case of another related enzyme PO (Fig. 5).

Rearing of *O. longicollis* larvae in *Musa* cultivars which showed very high activity of PAL, PPO and PO and very high content of TP and TF has resulted mortality of larvae after 7th day and were termed as Resistant (R). Under the field condition cultivars (15-26) showed no infestation by *O. longicollis*. Cultivars 1-5 showed infestation by this pest and were termed as Susceptible (S) and cultivars 6-14 showed sporadic attack by this pest and are designated as Non preferred (NP). The NP cultivars which are existing in the agro ecosystem as lone clones and are surrounded by abundance of commercially viable cultivars (CVC) which are all highly susceptible (HS), the pest showed no interest on attacking them. Rearing of larvae in NP cultivars did not result mortality after seven days.

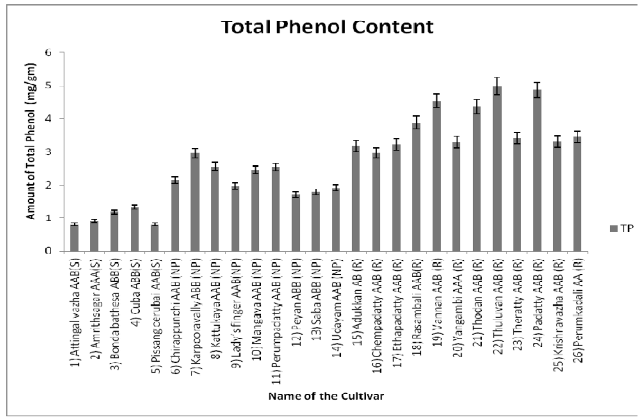

Figure 1: Amount of total phenols in different cultivars of *Musa* and their significance in relation to pest status

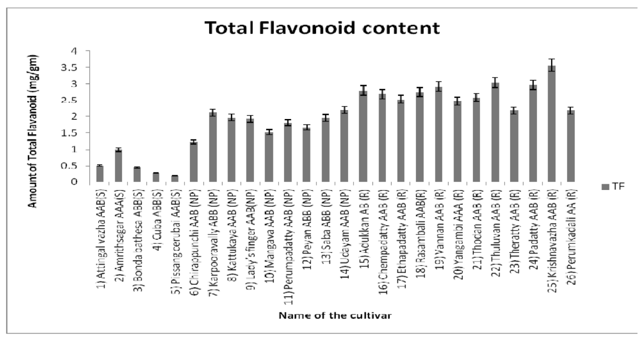

Figure 2: Amount of total flavonoids in different cultivars of *Musa* and their role in categorizing the cultivars as Susceptible, Non-prefered and Resistant to infestation by *O. longicollis*.

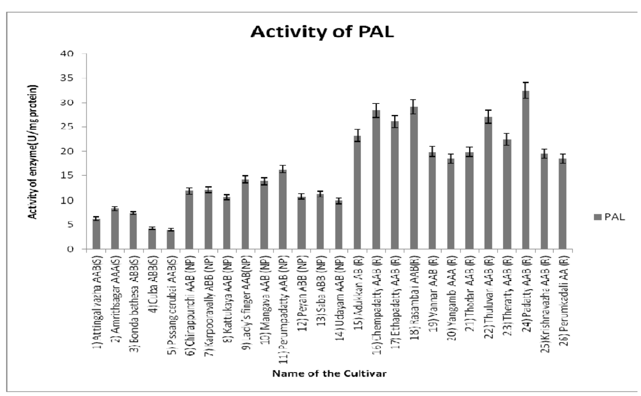

Figure 3: Activity of Phenyl alanine ammonia lyase in different cultivars of *Musa* and their pest status in relation to *O. longicollis*.

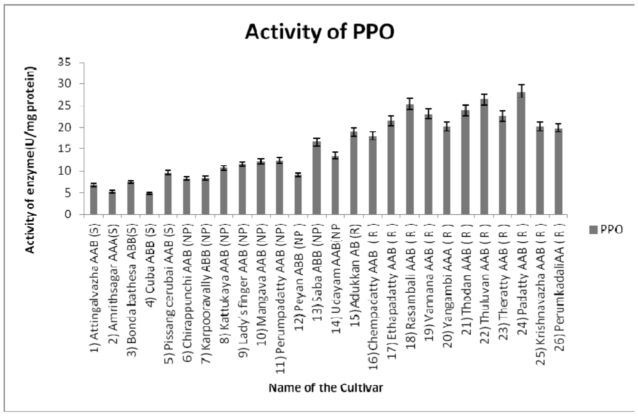

Figure 4: Activity of Polyphenol oxidase in *Musa* cultivars and the pest status in relation to infestation by *O. longicollis*.

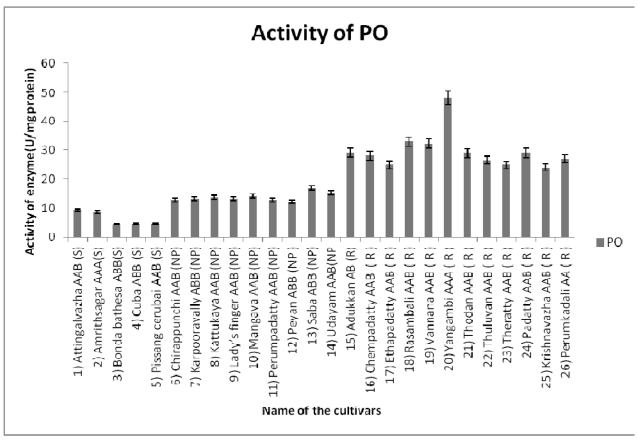

Figure 5: Activity of Peroxidase in *Musa* cultivars and the pest status of them in relation to *O. longicollis* infestation

DISCUSSION

Genome constitution of all the 26 *Musa* cultivars discussed in this paper has been studied and their pest status in relation to *O. longicollis* under the field condition has been documented (Ajitha *et al.,* 2017). No commercial cultivation was done on any of the 26 cultivars and were located as lone clones in a neglected state. Most of the farmers in Kerala who are fully engaged in *Musa* cultivation are small scale farmers and they do not have their own land for cultivation. So they cultivate *Musa* cultivars in a land on lease for one year contract with owner of the land, and hence they are very particular to plant *Musa* cultivars which can give harvest within one year (Kavitha *et al.*, 2017c).

Agro ecosystem of Kerala are with abundance of *Musa* cultivars and farmers are very particular in selecting commercially viable cultivars (CVC) only for their sustenance and they are very much interested in CVC which are able to give harvest within one year. Other traits preferred by farmers are large fruit bunch, high market value, palatable taste and high demand among the public. So agro ecosystems of Kerala are with abundance of CVC such as *Nendran* (AAB), *Kappa* or *Red Banana* (AAA), *Palayankodan* (AAB) and *Njalippovan* (AB) and the pest status of *Njalippovan* is NP and other three are S. (Kavitha, *et al.,* 2017 a,b&c, 2015a and b). *O.longicollis* exhibited extreme specificity in selecting the host plant for oviposition (Padmanabhan and Sundararaju, 1999), which was confirmed through field study by the investigator's team and *Nendran*, *Kappa* and *Palayankodan* were found to be the most preferred cultivar by this pest (Kavitha *et al.*,2015a). In the agro ecosystem the three cultivars are abundant and *O. longicollis* showed extreme preference on them and can be referred them as highly susceptible (HS). In presence of HS cultivars the pest showed no interest on other cultivars. Quantitative estimation of TP, TF, and activity of PAL, PO and PPO in the above three cultivars proved that the above constituent were least minimum in these CVC (Kavitha *et al.*, 2017b). In the present investigation, cultivars numbered as 1-5 showed very low content of TP, TF, PAL, PPO and PO, but their distribution in agro ecosystem was either scanty or rare. So no aggressive infestation by *O. longicollis* was noticed on these plants. Field study in Kerala proved that selective cultivation of HS cultivars which are also CVC have resulted many of the agro ecosystems as true breeding sites of *O. longicollis* (Kavitha *et al.*, 2017 c). Rearing of *O. longicollis* larvae in the pseudostem of these cultivars did not result in any problem and they complete life cycle as usual.

Musa cultivars described as numbers 6-14 possessed preferably higher content of TP and TF and in the agro ecosystem they did not show any infestation by this pest (Ajitha.*et al.,* 2017). The activity of enzymes and the secondary metabolites which are designated as NP are equal to the *Musa* cultivars which were categorized as NP cultivars by *O. longicollis* as described in the previous paper (Kavitha *et al.,* 2017b). Peculiarity of NP cultivars is that even though they were cultivated for commercial purpose in large numbers. *O. longicollis* preferentially avoid them in presence of *Nendran, Palayankodan* and *Kappa* (all three are HS) which are abundant in the agro ecosystem (Kavitha.*et al.,* 2015a) and on which the pest showed extreme preference (Padmanabhan and Sundararaju 1999; Kavitha.*et al.,* 2015b).

The cultivars described as 15-26 possessed very high content of TP, TF together with very high activity of PAL, PO and PPO. Rearing *O. longicollis* in these cultivars has resulted mortality of larvae within 7 days. Previous study by the investigation in another set of *Musa* cultivars showed that rearing of *O. longicollis* in some pest resistant cultivar has resulted wide spread cytopathological changes in the hemocytes of larvae (Kavitha *et al.,* 2016) leading to mortality of them and such cultivars designated as resistant cultivars. In the present study, cultivars numbered as between 15-26 caused immobility on fourth day and death on sixth or seventh day of keeping them in these cultivars.

Yangambi, the 20th cultivar in the present study was reported as possessing very high content of phenols and is resistant to infestation by Nematodes (Fogain 1996; Valette *et al.,* 1997). Increased activity of PO and PAL were reported in brinjal having resistance against nematodes (Rajasekhar *et al.,* 1997; Sirohi and Dasgupta 1993). It has been established that PAL is a very important enzymes involved in the plant defense mechanism which is evolved in phenyl propaniod pathway which imparts resistance against various types of pests (Ramesh Kumar *et al.,* 2012). Many investigators have reported the importance of PAL, PO, PPO in many crop plants and these enzymes showed elevated activity under the influence of pest infestation (Felip Otalvaria *et al.,* 2002, Valette *et al.*, 1998).

Agricultural practices chiefly aimed only at financial gain has resulted sharp depletion of diversity of many crop plants. In case of *Musa* cultivars also farmers are extremely selective in cultivating commercially viable cultivars and eliminating those cultivars which are not commercially viable. Commercially less viable cultivars at the same time, possessed many desirable traits such as strong defense mechanism against various pests, folk lore use against various ailment and in many cultural activities. So conservation of such commercially less viable crop plants has prime importance, especially for deciphering the mechanism of pest resistance.

ACKNOWLEDGEMENT

Authors are grateful to Kerala State Council for Science Technology and Environment for financial assistance and infrastructure development of laboratory to carry out this work.

REFERENCE

Ajitha,T., Kavitha,K.J, Shabith Raj, K., Murugan,K and Evans, D.A. (2017). Pest status of certain *Musa* cultivars of Kerala with special reference to infestation by *Odoiporus longicollis* [Olivier] and genome constitution of the cultivars. *Journal of Entomological Research* 41(4), 399-404.

Alagesan, A., Tharani, G., Padmanbhan, B., Sivavijayakumar, T., Manvannan, S. (2016). Screening and characterization of developing resistant cultivar against *O.longicollis* (Olivier) (coleopteran: *Curculionidae*) using reference genotypes in India. *Inter J. pharmacy and pharmaceutical science.* **8**(7): 223-226.

Amalraj,V.A. (1992). Collecting banana germplasm in South India. *Plant Genetic Resources Newsletter.* **88-89**: 64-66.

Chang, C., Yang, M., Wen, H., and Chern, J. (2002). Estimation of total flavanoid content in propolis by two complementary colorimetric methods. *J. Food Drug Analysis*. **10**: 178-182.

Felipe Otalwaro., Fernando Echeverri., Winston, Q., Fernando, T. and Bernd, S. (2002). Correlation between phenylphenalenone phytoalexins and phytopathological properties in *Musa* and the role of a dihydrophenylphenalene triol. *Molecules*. **7**: 331-340.

Fogain, R. (1996). Evaluation of *Musa* spp. for susceptibility to nematodes and study of resistance mechanisms. *Acta Hort*. **540**: 215-224.

Frison, E.A., Orjeda, G., and Sharrock, S.L. (1997). PROMUSA: A global programme for *Mus* improvement. *Proceedings of a meeting held in Gosier, Guadeloupe*. International Network for Improvement of Banana and Plantain, Montpellier, France. The World Bank, Washington, USA.

Gawel, N. and Jarret, R.L. (1991). Cytoplasmic and genetic diversity in Bananas and plantains. *Euphytica*, **52**:19-23.

Harborne, J. B. (1982). Introduction to Ecological Biochemistry, 2nd Edition. Academic Press, New York.

Hammer schmidt, R., Nuckles, E. M. and Kuc, J. (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Collectotrichum lagenarium*. *Physiology and Plant Pathology*, **20**: 79-82.

Kavitha, K.J., Evans, D.A. and Murugan, K. (2015a). Screening of Wild and Cultivars of Banana of Kerala, India using Score Card Method and its Resistance against *O.longicollis* (Olivier). *Phytomorphology* ., **65** (3&4): 121-126.

Kavitha, K.J., Murugan, K. and Evans, D.A. (2015b). Allelopathic interactions of certain Musa cultivars against *O.longicollis*., *Entomon.,* **40** : 209-220.

Kavitha, K.J., Murugan, K. and Evans, D.A. (2016). Cytopathological and haematological changes in *O.longicollis* (Olivier) grub by *Attinkombu* and *Thenkaali*, the two pest resistant *Musa* cultivars identified in Kerala., *Journal of Entomological Research.,* **40** : 27-33.

Kavitha, K.J., Murugan, K. and Evans, D.A. (2017a). Diversity, Genome classification, Pest status and Genetic Polymorphism of *Musa* Cultivars identified in Chittar Panchayath of Pathanamthitta District, India, *Proceedings of Kerala Science Congress*, 28-30, January, 2017.

Kavitha, K.J., Shabith Raj, K., Murugan, K. and Evans, D.A. (2018). Distribution of Secondary Metabolites and Activity of related enzymes in *Musa* Cultivars and their role in defense against *O. longicollis* (Olivier) Journal of Allelopathy (in press).

Kavitha, K.J., Ajitha, T., Shabith Raj, K. and Evans, D.A. (2017b). Diversity, Genome constitution, Commercial viability and Pest status of 60 Musa cultivars identified in Kerala., *Proceedings of Third National Biodiversity Congress, KSBB*, Thiruvananthapuram.

Mayer, A.M., Harel, E., and Shaul, R.B. (1965). Assay of catechol oxidase a critical comparison of methods. Phytochemistry, **5**: 783–789.

Mayr, V., Treeter, D., Santo, S., Buelga, C., Bauer, H., and Feucht, W. (1995). Developmental changes in the phenol concentration of golden delicious apple fruits and leaves. *Phytochemistry*, **38**: 1151-1155.

Nayar, T. G. (1962). Banana in India. The Fact Technical Society, Udyogamandal, Kerala, India.

Otsmark,H.E. (1974). Economic Insect Pests of Banana. *Ann.Rev.Entomol.* **19: 161**-176.

Price, N. S. (1995). The origin and development of banana and plantain cultivation. In: Bananas and Plantains. Gowen,S.(Ed). Chapman and Hall, London. Pp1-12.

Padmanabhan, B., and Sathiamoorthy, S., (2001). Musa pest fact, **5**: 8-11.

Padmanaban, B and Sundararaju, B, P. (1999). Occurrence of banana weevil borers (Coleoptera: *Curculionidae*) in Tamil Nadu. Insect Environment**, 5**(3):135.

Prasad,B. and Singh, OlL. (1987). Insecct pest of Banana and their incidence in Manipur, *Indian J. Hill Farm.*, **1:71**-73.

Rajasekar, S. P., Gangul, A. K. and Swain, S. C. (1997). Quantitative changes in superoxide dismutase, catalase and peroxidase with reference to resistance in tomato to *Meloidogyne incognita*. *Indian J. Nematol.* **27**: 79-85.

Rameshkumar, A., Kumar, N., Poornima, K. and Sooryanathasundaram, K.(2012). Screening of invitro derived mutants of banana against nematodes. *African J. Biotech.* **11**: 15451-15455.

Shanmugam, P. S., Indhuumathi, K., and Tamilselvan, N. (2013). Suitability of semiochemical and chemical methods for the management of Banana pseudostem weevil, O. longicollis Oliver (Coleoptera: *Curculionidae*). *J. Ent. Res.* **37**(1): 1-3.

Sirohi, A. and Dasgupta, D. R. (1993). Mechanism of resistance in cowpea to root-knot nematode, *Meloidogyne incognita* race-early induction of phenylalanine ammonia lyase and chlorogenic acid. *Indian J. Nematol.* **23**: 37-41.

Shukla, G. S., and Kumar, K. (1969). *Sci. Cult.*, **35**: 481-482.

Uma, S., and S. Sathiamoorthy. (2002). Names and Synonyms of Bananas and Plantains of India. National Research Centre for Banana, Indian Council of Agricultural Research (ICAR), Tiruchirapalli, India.

Valette, C., Nicole, M., Sarah, J. L., Boisseau, M., Boher, B., Fargette, M. and Geiger, J. P. (1997) Ultrastructure and cytochemisrty of interactions between banana and the nematode *Radopholus similis*. *Fundam. Appl. Nematol.* **20**: 65-77.

Visalakshi, A., *Nair*, G. M., Beevi, S. N. and Amma, A.M.K. (1989). Occurrence of *Odoiporus longicollis* Olivier. (Coleoptera:*Curculionidae*) as a pest in Kerala. *Entomon.,* **14**(3): 367-368.

Waller, G. R. and Dermer, O. C. (1981). Biochemistry of Plants. **7**:317-402. Academic Press, New York.

Waller, G. R. and Nowacki, E. R. (1978). Alkaloid Biology and metabolism in Plants. Plenum Press, New York.

Whetten, R.W., Sederoff, R.R. (1992). Phylalanine ammonia lyase from lobololly pine. Purification of the enzyme and isolation of complementary DNA clones. *Plant Physiol.* **98**, 380-386.