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Secondary metabolites of *Musa* cultivars confer resistance against infestation by stem weevil, *Odoiporus longicollis* (Olivier) (Coleoptera: Dryophthoridae)

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ABSTRACT: Banana, popularly known in Kerala as *Nendran* has diverse cultivars of indigenous and exotic or hybrid types. All *Nendran* cultivars are highly susceptible to infestation by *Odoiporus longicollis* Olivier (Coleoptera: Dryophthoridae), and they possess very low content of secondary metabolites (SM) such as total phenols (TP) and total flavonoids (TF). Activities of enzymes related to the synthesis of SM such as Phenylalanine Ammonia Lyase (PAL), Polyphenol Oxidase (PPO) and Peroxidase (PO) showed very low activity in *Nendran* cultivars and this may be one of the reasons for their susceptibility to infestation by *O. longicollis*. *Yangambi*, a *Musa* cultivar which is resistant to infestation by *O. longicollis* possessed very high content of TP, TF and elevated activity of PAL, PPO and PO. Under field condition, cultivar *Yangambi* did not show any symptoms of attack by this pest and rearing of larvae of *O. longicollis* in *Yangambi* resulted mortality within one week and wide spread cytopathological changes in the hemocytes and enzymatic changes in the hemolymph. Hemocytopaenia together with selective enhancement in the population of granulocytes and selective decrease in the population of plasmatocytes were observed in differential count. Cytopathological changes such as lack of cell membrane integrity, lack of nuclear membrane integrity and degeneration of cytoplasm was observed in hemocytes of larvae maintained in *Yangambi.* Intoxicated larvae showed sharp decrease in the contents of Trehalose through the elevated activity of Trehalase. Significant elevation of fat body glycogen and inhibition of glycogen phosphorylase was also observed in affected larvae. Sharp elevation of lactic acid through elevated activity of lactic acid dehydrogenase and inability to utilize glucose are other adverse effects caused by this pest resistant cultivar on the pest. Even though *Yangambi* is not a commercially viable *Musa* cultivar, the conservation of such cultivars is very much essential for knowing the molecular mechanism of pest resistance, which may help in the management of *O. longicollis* in an eco-friendly way.

KEY WORDS: *Odoiporus longicollis, Musa* cultivar *Yangambi*, resistance, secondary metabolites

INTRODUCTION

Banana is the major agriculture crop of Kerala state, India and globally India is the largest producer of this agricultural commodity. *Nendran* (AAB) cultivar of *Musa* is the most abundant and economically highly viable cultivar of Kerala (Kavitha *et al*., 2017) because of the desirable qualities such as short duration to set flower, large palatable ripe fruits, high commercial viability and comparatively good keeping quality. Field study conducted in various sites of Kerala proved that

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Nendran cultivars are the most pest susceptible *Musa* cultivars (Kavitha *et al*., 2015a), which was aggressively attacked by *Odoiporus longicollis* (Olivier) and if control measures are not properly applied, 70% crop loss will be certain (Padmanabhan and Sundararaju, 1999; Alagesan *et al*., 2016). Interaction made with traditional farmers of various districts in Kerala has resulted in the identification of many indigenous *Nendran* cultivars, and each of them is unique to a particular locality of Kerala. Recently, an indigenous *Nendran* cultivar from Guruvayoor of Thrissur District, Kerala has got GI tag and it was named as *Kazhchakkula*, a famous item in worship of Guruvayoor temple. In association with many indigenous *Nendran* cultivars, Agricultural Department of Kerala has introduced many exotic/ hybrid *Nendran* cultivars, which could not get wide appreciation from farmers of Kerala.

Presence of secondary metabolites in host plants is a major determining factor which influences herbivory (Harborne, 1982). All the *Nendran* cultivars, both indigenous and exotic are highly vulnerable to infestation by *O. longicollis* and since the destructive larvae are purely endophytic, farmers adopt systemic insecticides and also injection of Monocrotophos to control the pest. Interestingly, many indigenous *Musa* cultivars of Kerala are showing extreme degree of pest resistance through allelopathic interaction in the larvae of *O. longicollis* (Kavitha *et al*., 2015a, b). A comparative study on the content of total phenols, flavonoids and related enzymes in pest susceptible, indigenous or exotic/hybrid *Nendran* cultivars with a few pest resistant *Musa* cultivars and the mechanism of allelopathy induced by the pest resistant *Musa* cultivar on *O. longicollis* larvae form the subject matter of this communication.

MATERIALS AND METHODS

Indigenous *Nendran* cultivars were collected from Malappuram district of Kerala, India, where a traditional farmer is maintaining different indigenous *Nendran* cultivars in his sprawling fields. The exotic/hybrid cultivars of *Nendran* were collected from the Agriculture Department, Govt. of Kerala, at Kazhakuttom, Thiruvananthapuram district of Kerala, which supplies tissue cultured cultivars of *Nendran* types. *Ottamungili*, an indigenous *Nendran* cultivar was collected from Kottur, under Neyyar forest division Thiruvananthapuram district. *Yangambi* cultivar of *Musa* was collected from Agrifarm, a *Musa* diversity centre under the Agriculture Department, Peringammala, Thiruvananthapuram District, and Govt. of Kerala. The cultivars (suckers) brought from different sites were planted in the campus of University College and were provided with leaf litter as organic manure. *Changanassery Nendran*, *Chengazhikkodan, Manjeri Nendran, Mettupalayan, Swarnamukhi* and *Trichi manjeri* are the indigenous *Nendran* cultivars. *Ottamungili* is not cultivated in the any agroecosystems by farmers. All other cultivars are either exotic or hybrids.

Leaf sample collection: Tender cigar leaf, of 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 of host plants: 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 (Hammerschmidth *et al*., 1982). Activity of enzymes was expressed as units/mg protein.

Rearing of *O. longicollis* larvae in *Musa* cultivars: *Yangambi*, a *Musa* cultivar which never showed infestation by *O. longicollis* under the field condition and which possessed very high activity of PAL, PO, PPO and bearing very high content of TP and TF was used for studying allelopathy in larvae. 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 cultivar with pseudostem of 25 to 30 cm circumference, whose crown was chopped down at a height of 100 cm above the ground. A small depression was made on the free cut end of live pseudostem and seven *O. longicollis* fourth instar larvae were released to it since they 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 was 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 in which larvae showed no mortality were designated as Susceptible (S) (Kavitha *et al*., 2015a).

Study of haemocytes: The larvae were separated from pseudostem, washed in distilled water, blotted in filter paper, were used. Larvae were placed on a glass plate, kept on ice cubes and a sharp cut was given on the ventral side, without cutting the gut. The hemolymph was analysed for total count in standard counting chamber and differential count after staining by Giemsa stain.

Biochemical analysis of larval fat body and haemolymph: Larvae maintained in any of the susceptible *Nendran* cultivar (control) and *Yangambi* for four days were used for this experiment. Fat bodies of larvae were carefully separated. 100 mg fat body was weighed and homogenized in appropriate buffers under ice cold condition and used for estimating glycogen. 100 µl of hemolymph was centrifuged in a micro centrifuge and the supernatant was used for estimation of enzymes and biomolecules; glucose (Glucose Oxidase Peroxidase method, Trinder, 1969), trehalose (Roe, 1955), trehalase (Friedman, 1966), glycogen (Dubois *et al*., 1956), glycogen phosphorylase (Singh *et al*., 1961), lactic acid (Baker and Summerson, 1941) and lactic acid dehydrogenase (Queen, 1972).

The data collected from five leaf samples from each cultivar types was statistically analysed by one way analysis of variance (ANOVA) at pÃ0.05 level of significance.

RESULTS

The content of TP was very low in all the indigenous *Nendran* cultivars. Exotic/ hybrid cultivars possessed a slightly elevated TP and *Yangambi* possessed a very high content of TP compared to all other cultivars (Fig.1). *Ottamungili* is a commercially non viable cultivar which possessed only one or three fruits in the whole bunch, each fruit possessed a length of 35-40 cm. No flower bud could be located after one or two tier of fruits. It could not survive in the agroecosystem unless great care was provided. In Kottur forest, *Ottamungili* never showed symptoms of pest attack by *O. longicollis*. The content of TP in *Ottamungili* was low compared to that of exotic *Nendran* cultivars. The exotic cultivar, *Popaulu* showed slightly high content of TP than indigenous cultivars (Fig.1). Another group of secondary metabolites is flavonoids, the content of which was also very low in *Nendran* cultivars. Among the *Nendran* cultivars *Ottamungili*, *Changanassery Nendran*, and *Trichi Manjeri* possessed the lowest amount of TP and *Popaulu* the highest amount, which was almost of one third of the amount of TF in *Yangambi* (Fig.1)

Activity of PAL was very low in all the indigenous *Nendran* cultivars, (Fig.2). Some of the exotic cultivars such as *Popaulu* and *Mysore Ethan* showed a preferably good activity of PAL, almost one third of the activity of PAL in *Yangambi* cultivar. Another related enzyme PPO was also very low in all the different *Nendran* cultivars and it was least in *Changanassery Nendran* (Fig.2). All the exotic *Nendran* cultivars have maintained slightly elevated activity of PPO than to indigenous cultivars. Activity of PPO in *Yangambi* was several times higher than *Nendran* cultivars (Fig.2). Activity of PO was also low in all the *Nendran* cultivars, compared to *Yangambi*. Activity of PO in *Popaulu* and *Mysore Ethan* was almost one third to that of the activity of PO in *Yangambi* (Fig.2).

Rearing of 4th instar larvae of *O. longicollis* in either indigenous or exotic/hybrid cultivar of *Nendran* did not result any mortality or adverse effects in larvae. The larvae survived well in all the indigenous and exotic *Nendran* cultivars. All the larvae of *O. longicollis* maintained in *Yangambi* died between 5th and 6th day of their maintenance. The hemolymph of the larvae on the third day of maintenance in *Yangambi* cultivar showed sharp hemocytopaenia (Table 1), together with significant change in the differential hemocyte count (Fig.3). Population of granulocytes have undergone a sharp increase, together with sharp decrease in the population of plasmatocytes. Wide spread cytopathological changes were observed in larvae maintained in *Yangambi* cultivar. Lack of cell membrane integrity, lack of nuclear membrane integrity and enucleation were observed in hemocytes (Fig.4 a&b).

The amount of glucose in the hemolymph of healthy larvae was very much lower than the fasting blood sugar of healthy human and it was 21.34±1.20, which became sharply decreased in larvae reared in *Yangambi* cultivar (Table 1). The amount of hemolymph trehalose was very much (15 times) higher than the amount of glucose and it became sharply reduced through the elevated activity of Trehalase under the influence of allelopathy by*Yangamb*i cultivar (Table 1). The amount of fat body glycogen was significantly elevated in larvae reared in *Yangambi* and the enzyme glycogen phosphorylase was inhibited. Amount of lactic acid and its enzyme lactic acid dehydrogenase in the hemolymph was sharply elevated in larvae reared in *Yangambi* (Table 1).

DISCUSSION

TP and TF are phenolic compounds give bitter taste to the plants and host plants effectively used these compounds to get rid of the herbivorous insect pests (Georgima *et al*., 2015). In all the *Nendran* cultivars studied, the contents of TP and TF were low quantity when compared to *Yangambi*, a pest resistant *Musa* cultivar. The cultivar Yangambi was reported to be resistant to infestation by nematodes (Fogain, 1996; Valette *et al*., 1997). *Musa* cultivars exhibited high variation in the distribution of phenolic compounds in (Alfredo and Stalin, 2017) and phenolic compounds are acting as allelochemicals and have significant role in plant defense against herbivory (Usha Ravi and Ravibabu, 2011). Flavonoids are more bitter than phenols and also has significant role in pest defence (Joseph *et al*., 2004).

Activity of enzymes which are very much related to the formation of phenolic compounds such as PAL, PO and PPO were very low in all the *Nendran* cultivars when compared with *Yangambi*. It has been reported that PAL is a very important enzyme involved in the plant defence mechanism, which is evolved into phenyl propanoid pathway which imparts resistance against various types of pests (Ramesh kumar *et al*., 2012). Many investigators have reported the importance of PAL, PO and PPO in many crop plants including *Musa* cultivars and these enzymes showed elevated activity under the infestation of pests (Felipe Otalvaria *et al*., 2002; Valette *et al*., 1998). The mechanism of defence seen in plants against their insect enemies is through excessive synthesis of phenols and flavonoids and enzymes such as PAL, PO and PPO are key enzymes behind these secondary metabolites (Sung Kim and Hwang, 2014; Abdul *et al*., 2012).

Yangambi is not a CVC of *Musa* and farmers did not show interest in cultivating this cultivar because the ripe fruit bunch is small and attain weight of 8-10 Kg and in our experience, the ripe fruits are not so delicious and palatable and is slightl bitter , which may be due to the presence of excess of TP and TF. Under the field condition this cultivar was not attacked by *O. longicollis* but all the *Nendran* cultivars were attacked by this pest. *Yangambi* did not show any symptoms of attack by this pest such as small bore holes on the pseudostem with exudation of viscous fluid through the holes or breakage of pseudostem which are common symptoms of attack by *O. longicollis* (Kavitha *et.al*., 2015a,b). Rearing of this larva in *Yangambi* cultivars has resulted 100% mortality of 4th instar larvae in one week. Wide spread changes in the hemolymph which resulted hemocytopaenia together with cytopathological change in the hemocytes. Similar observations were reported in the hemolymph of *Dysdercus cingulatus* (Pandey

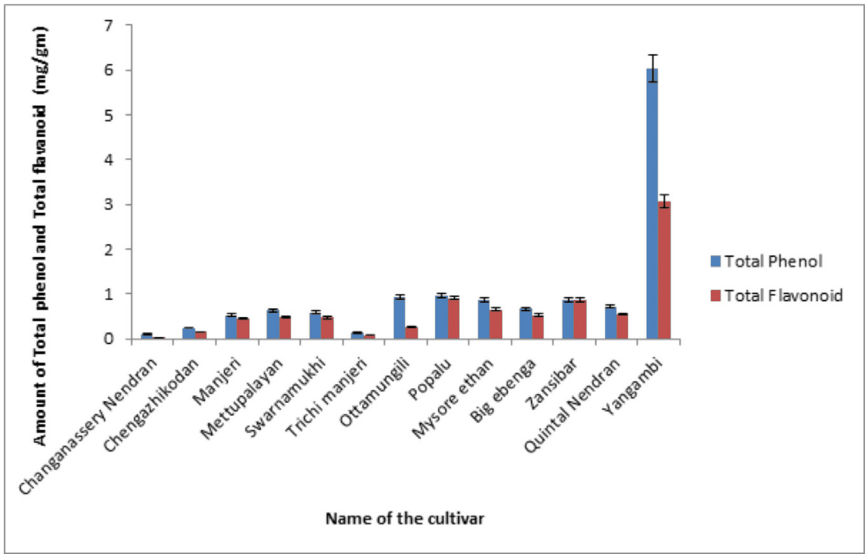

Fig.1. Amount of total phenols and flavonoids in different cultivars of *Musa*

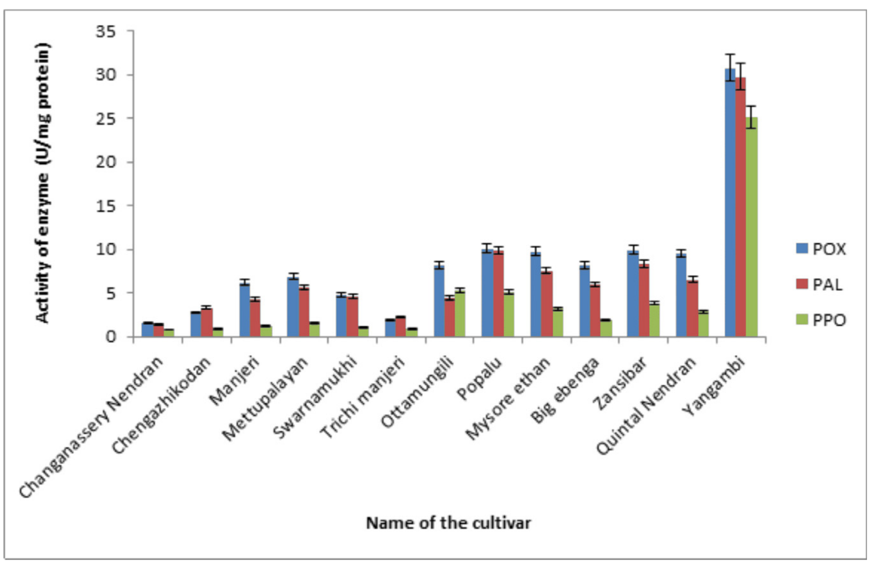

Fig. 2. Activities of three enzymes related to the production of secondary metabolites in different *Musa* cultivars

and Tiwari, 2011) and *Papilio demoelus* (Pandey *et al.,* 2012) under toxicity by extracts of insecticidal plants and in *Oryctes rhinoceros* larvae experimentally injected by *Bacillus thuringienesis* (Adhira *et al*., 2010, Adhira and Evans, 2011).

Cytopathological changes observed in *O. longicollis* larvae, reared in *Yangambi* cultivar indicated that the live pseudostem of this cultivar possessed toxic compounds. Differential hemocyte count of the larvae reared in *Yangambi* cultivar showed selective elevation of granulocytes and selective decrease in the population of plasmatocytes. Similar type of observation was also observed in *O. rhinoceros* larvae infected by *B.thuringienesis* (Adhira *et al*., 2010). Lack of membrane integrity of hemocytes of the *O. longicollis* larvae reared in *Yangambi* is indicated that this cultivar has cytotoxic molecule in the pseudostem. Cytopathological changes were observed mostly in plasmatocytes and granulocytes are are the main hemocytes concerned with the

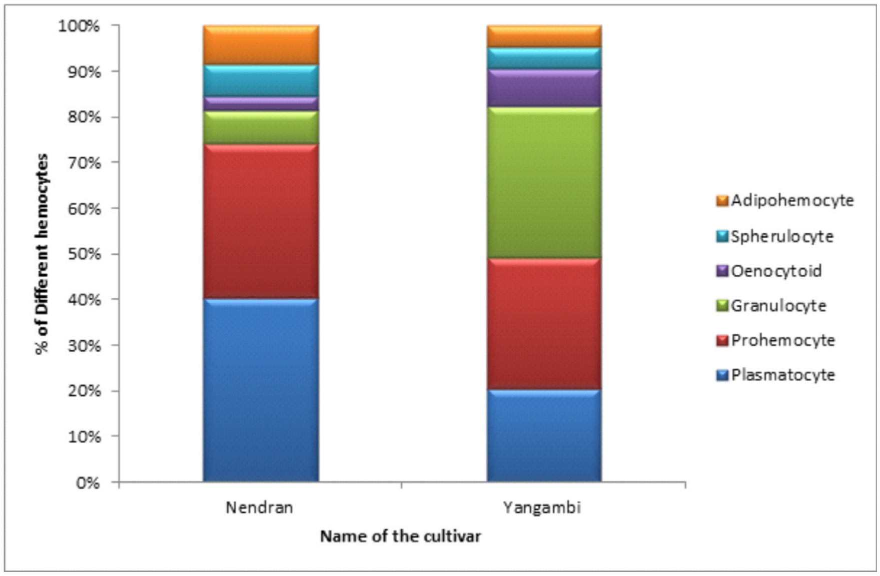

Fig.3. Allelopathic reactions of *Yangambi* cultivar on differential hemocyte count

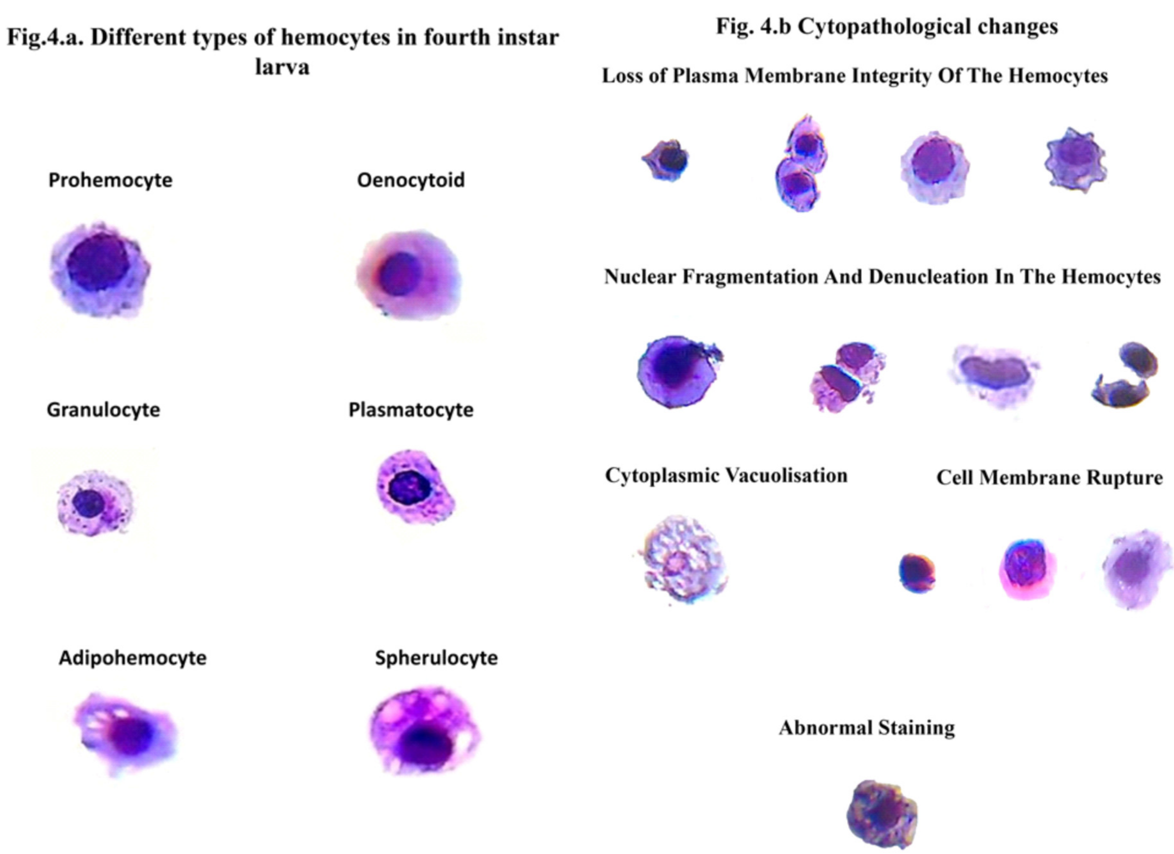

Fig.4. Normal hemocytes (4a) and Cytopathological changes (4b) induced by *Yangambi* cultivar in the hemocytes of *O. longicollis*

Table. 1. Allelopathic reactions induced in larvae by *Yangambi* cultivar during the third day of existence in the pseudostem

Sl. No.	Biochemical/Cellular parameters	Control (*Nendran* cultivar)	Test (*Yangambi*)
1	Glucose	21.34±1.20	14.42±0.96
2	Trehalose	318.36±18.50	248.56±12.8
3	Trehalase	42.43±2.52	58.35±3.15
4	Glycogen	354.76±20.16	404.12±19.54
5	Glycogen phosphorylase	365.52±18.50	274.92±16.52
6	Lactic acid	249.17±11.80	439.12±22.52
7	Lactic acid dehydrogenase	106.44±8.96	297.04± 20.7
8	Total hemocyte count	4438± 202	2328±102

All values are mean I SD, n=6, $p \geq 0.05$ with respect to corresponding control values.

1. Amount of glucose is expressed as mg/100ml hemolymph.
2. Trehalose is expressed as glucose units in mg/100ml hemolymph.
3. Activity of trehalase is expressed as amount of glucose in micromoles liberated/minutes/mg protein.
4. Glycogen content of fat body is expressed as microgram of glucose equivalent/100mg tissue.
5. Glycogen phosphorylase activity is given as micromoles of organic phosphate liberated/minutes/mg protein.
6. Lactic acid in microgram/ml of hemolymph.
7. Activity of lactic acid dehydrogenase is expressed as micromoles of lactic acid liberated/minutes/mg protein.

immunity of insects and the cells are phagocytic in function and act against pathogens entering in to the body of the insect. Plasmatocytes are more involved in phagocytosis of non self cells whereas granulocytes are apparently the only hemocytes that engulf the dead cells (Ling and Yu, 2006; Amral *et al*., 2009).

The content of glucose in the hemolymph of the healthy larvae of *O. longicollis*, very much agreed with the observation in *O. rhinoceros* larvae and in larvae of *Oecophylla smaragdina*. The glucose level of larval hemolymph of these insects was very low when compared to that of fasting blood of healthy humans (Adhira, 2015; Vidhu, 2015). In *O. longicollis*, the content of Trehalose was very much higher than that of the amount in glucose which is also agreeable with the observation in *O. rhinoceros* (Adhira, 2015). Rearing the *O. longicollis* has resulted sharp decrease in the content of trehalose which may be due to elevated activity of trehalase, under the influence of *Yangambi* cultivar. The storage polysaccharide glycogen became significantly elevated in larvae reared in *Yangambi* and such elevation may be through the inhibition of glycogen phosphorylase. In *O. rhinoceros*, experimental infection of *Bacillus thuringiensis* and exposure to cold shock also resulted similar changes in glycogen phosphorylase and glycogen content (Adhira and Evans, 2014). The content of lactic acid became greatly increased in larvae maintained in *Yangambi* cultivar which very well attested the weak, lethargic appearance of larvae which were reared in *Yangambi* cultivar and the elevated activity of lactic acid dehydrogenase substantiated the increase in lactic acid content.

Development of resistance against a serious pest of *Musa* by a natural *Musa* cultivar may be through years of evolution. Modern agricultural practices aimed only on commercial gains are not interested in commercially non-viable and pest resistant cultivars. So these types of *Musa* cultivars require special conservation efforts to keep their germplasm healthy and viable for studying the molecular mechanism of pest resistance.

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REFERENCE

Abdul R.W., Paul Raj M., Tariq A., Ahamed A.B., Savarimuthu., Ignacimuth and Sharma A.C. (2012) Mechanism of plant defence against insect herbivores. Plant Signalling and Behaviour 7: 306-1320.

Adhira M. Nayar., Symala Devi G and Evans D.A. (2010) Effects of *Bacillus thuringiensis* infection on the biochemical and haematological profile of hemolymph in the larvae of *Oryctes rhinoceros*. Entomon 35: 241-245.

Adhira M. Nayar and Evans D.A. (2011) Effect of some selected physical, chemical and biological stressors on the protein metabolism of *Oryctes rhinoceros* [L] grubs. Entomon 36: 119-129.

Adhira M. Nayar and Evans D.A. (2014) Influence of various stressors on the energy metabolism of *Oryctes rhinoceros*. International seminar on insect pest management, ICAR Bangalore, India. p 32.

Adhira M.Nayar (2015) Cytopathological and biochemical effects on the hemolymph of *Oryctes rhinoceros* grub in response to various stressors. Ph.D thesis, University of Kerala. pp 70-98.

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

Alfredo E and Stalin S. (2017) Phenolic compounds of *Musa* Cavendish, *Musa accuminata* and *Musa cavandaish*. Revisita Politenia-Enero 38: 102, 1-5.

Amral I., Rodrigues M., Neto J., Felipe M., Pereria G.B and Carlos U.V. (2009) Circulating hemocytes from honey bee larva *Melipona scutellaris*, cell types and their role in phagocytosis. Micron 30:15-18.

Baker S.B and Summerson W.H. (1941) The colorimetric determination of lactic acid in biological chemistry. Journal of biological chemistry 138:53-554.

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

Dubois M., Gillies K.A., Hamilton J.K., Rebers P.A and Smith F. (1956) Colorimetric estimation of sugars and related substances. Analytical Bio-chemistry 28: 350-356.

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 Horticulture 540: 215-224.

Friedman S. (1966) Trehalase from insects, Methods in Enzmology, Academic press, New York.18:600-620.

Georgina N., Diaz N and Sara M P. (2015) Bioinsecticidal effect of flavanoids pinocembrin and quercetin against *Spodoptera frugiperda*. Journal of Pest Science 88: 629-635.

Hammerschmidt 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.

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

Josesph C.O., Jennifer L., Marget Y and Martin E.A. (2004) Effect of flavanoid in feeding preference and development of the Crucifer pest *Mamestra configurata* Waller. Journal of Chemical Ecology 30: 109-124.

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 *Odoiporus 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 *Odoiporus longicollis*. Entomon 40: 209-220.

Kavitha K.J., Ajitha T., Shabith Raj K and Evans D.A. (2017) Diversity, genome constitution, pest status and commercial viability of *Musa* cultivar in Kerala. Proceedings of the third National biodiversity conference Thiruvannathapuram 30-40. Published by The Kerala State Bio-diversity Board.

Ling E and Yu X.Q. (2006) Hemocytes from the Tobacco horn worm *Manducaa sexta* have distinct function in phagocytosis of foreign particles and dead cells. Development and Comparative Immunology 30: 309.

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.

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

Pandey J.P and Tiwari R.K. (2011) Neem based insecticides on the development and fecundity of *Dysdercus cingulatus*. International Journal of Agricultural Research 6: 335-346.

Pandey S., Pandey J.P and Tiwari R.K. (2012) Effect of some botanicals on the hemocytes and moulting of *Papilio demoleus* larvae. Journal of Entomology 9: 23-31.

Queen M .1972. Inhibition and activation of human LDL by urea. Annls of clinical biochemistry **11**(3):70-80.

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

Roe J.H. (1955) Determination of Sugar in body fluids with anthrone reagent. Journal Biological Chemistry 212: 335-343.

Singh V.N., Venkitasubramanian R and Viswanathan R. (1961) The glycolytic enzymes of guinea pig lung in experimental *bugassosis*. Journal of Biochemistry 78:728-732.

Sung Kim D and Hwang B.K. (2014) An important role of PAL- gene in Sialic acid- dependent signalling of the defense response to microbial pathogens. Journal of Experimental Botany 65(9): 2295-2306.

Trinder P. (1969) Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromagen. Journal of clinical pathology 22: 158-161.

Usha Ravi T and Ravibabu M.V. (2011) Allelochemicals in Castor *Ricinis commuris* and their impact on pest defence and anti herbivore defence. Allelopathy Journal 27: 1-11.

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

Valette C., Andary C., Gerger J P., Sarah J L and Nicole M. (1998) Histochemical and cytochemical investigations of phenols in roots of banana infected by the burrowing nematode *Radopholus simils*. Phtopathology 88:1141-1148.

Vidhu V.V. (2015) Biology of *Oecophylla smaragdina* (Fab.) with special reference to formic acid profile and ethno entomological practices. Ph.D. thesis, University of Kerala.

Whetten R.W and Sederoff R.R. (1992) Pheylalanine Ammonia Lyase from lobololly pine. Purification of the enzyme and isolation of complementary DNA clones. Plant Physiology 98: 380-386.

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