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Does change in tannin content in mango (*Mangifera indica)* fruits influence the extent of fruit fly (*Bactrocera dorsalis* Hendel) herbivory?

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Abstract
The present study was undertaken to determine the defensive role played by tannins present in mango peel in the prevention of *B. dorsalis* infestation. The results showed significant differences in the tannin contents, *i.e*. lower tannin levels between 8.17 and 9.93 mg/g dry weight in peels of susceptible varieties (Totapuri, Alphonso and Banganapalli), 10.67 and 11.71 mg/g in moderately resistant varieties (Dasheri and Mylepellian), whereas in resistant varieties (Langra, EC-95862) it was higher – between 13.08 and 13.66 mg/g. At 50% maturity all the varieties had same tannin levels; however, as maturity progressed (50-100%) the levels of tannin reduced in all except for the resistant varieties. The presence of high tannin content in mango peel at critical stages of infestation played an important role in deterring or preventing fruit fly damage in mango. The results obtained will be useful in host plant resistance and chemical ecology studies.

Keywords: *Bactrocera dorsalis*, infestation, fruit fly, mango, tannins, secondary metabolites.

1. Introduction

In India, the Oriental Fruit Fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is a major polyphagous pest with diversified host range (more than 100 fruits and vegetables) and geographic distribution [1]. The most economically important host of *B. dorsalis* is mango (*Mangifera indica* Linnaeus: Anacardiaceae). In India the fruit is cultivated in 2,312 thousand hectares with a production of around 15.03 million tons, contributing 40.48% of the total world production [2]. Though India is the chief mango producer, Indian mangoes are grossly inadequate in the international market as the export of fresh fruits is less than 5% mainly due to the damage by fruit flies [3]. The Oriental fruit fly is a major pest of mangoes, particularly in the more commercially valuable varieties. The yield loss due to *B. dorsalis* in India ranges from 1 to 31% with a mean of 16% [1].

There is a constant struggle between the plants and the herbivores and in this process of co-evolution plants have evolved mechanisms to overcome biotic and abiotic stress. Plants produce an array of secondary metabolites to defend themselves from attack by insects and pathogens [4]. One such metabolite is tannin in Mango fruits. Their levels vary in different varieties of mango at different maturity levels. These are found to affect fruit fly infestation in mango, *i.e*., fruit fly oviposition and maggot behavior [5]. These chemical relationships will be vital cues for fruit fly foraging and oviposition, which in turn will help in management [6].

Tannins are the phenolic compounds of high molecular weight ranging from 500 Da to more than 3000 Da. They are present in leaves, bark, fruit, wood and roots located basically in the vacuoles and are associated with plant defense mechanisms against insect herbivores [7, 8]. They cause a sharp, astringent sensation in the mouth due to their binding to salivary proteins. Defensive tannins decrease the quality of the plant tissue by combining with two important groups of proteins [9]. First, they combine with plant protein, which reduces the ability of proteases such as trypsin to digest them into simpler polypeptides for N metabolism in the herbivore. Second, they bind to the digestive enzymes of the phytophage, thereby reducing their ability to breakdown plant proteins (and other enzymatically digested compounds) [9]. This non-selective cross-linkage of proteins (both hosts and herbivores) can reduce the N availability (and hence quality) of the plant [11-14]. With this background, this study was initiated on seven mango varieties representing resistant, moderately resistant and susceptible varieties to mango fruit fly with the following objectives.

First to study the levels of tannins at different maturity levels of fruit and second its influence on the infestation.

2. Materials and methods

Plant material

The present study was undertaken in the experimental mango orchard at the Indian Institute of Horticultural Research, Bangalore (12°58′N; 77°35′E) during march 2013-14. In the present study, seven mango varieties were selected and grouped into three groups as susceptible (cvs. Banganapalli, Alphonso and Totapuri), moderately resistant (cvs. Mylupilian and Dusheri) and resistant (cvs. Langra and EC-95862) to fruit flybased on the earlier studies of Verghese *et al*. [5] and Jayanthi and Verghese, [15]. All the mango trees were approximately 15 years old.

Four trees of each cultivar were selected for study purpose; these selected trees were regularly monitored for flowering and fruiting. At fruit set more than 100 inflorescence were tagged on each selected tree. Once the fruit setting was stabilized nearly 50 marble sized fruits (~2-2.5cm). Pea sized fruits were considered as day -1 fruits and they were monitored for maturity. The tagged fruits were harvested at different maturity levels, namely 50% maturity, 75% maturity and 100% maturity in order to obtain variability in tannin levels in the fruits and variability was further increased by using fruits unknown to be susceptible, moderately resistant and resistant. Maturity was calculated based on the protocol standardized by Verghese *et al.* [5].

Five apparently healthy fruits were randomly selected and harvested along with a long stalk (3-4cm) to avoid oozing of the sap from fruits and prepared for further analysis on the same day. Each maturity class fruits were separately grated using grater/peeler to get exocarp (Peel) which is the greenest part of the fruit. Peeling was done till the whitish or yellowish pulp was visible. The remaining part except seed was considered as mesocarp (Pulp).

Peel and pulp of each fruit was divided into four sections representing different portions of the fruit and sample was collected from all the sections and mixed together to avoid bias. Dry samples were prepared by drying 50g of peel and 50g of pulp of all varieties (five replications each) in glass Petri plates in hot air oven at 65ºC for 48 hours or the period was extended until the samples had completely dried. This was then blended in the mixer for 4-6 min to get a fine powder of the sample. Further two sub replications of the extract from each main replication were used for analysis (five main replications; ten sub replications).Powdered samples were stored in butter paper covers with proper labeling at room temperature till further analysis.

Measurements of biochemical parameters

Tannin: Tannin determination was done according to the method of AOAC [16] with some modifications. One-fifth gram (200mg) of the sample was added to 20 ml of 50% methanol. This was shaken thoroughly and placed in a water bath at 80ºC for 1 hr to ensure a uniform mixing. The extract was filtered into a 100 ml volumetric flask, followed by adding 20 ml of distilled water, 2.5 ml of Folin-Denis reagent and 10 ml of 17% aq. Na_2CO_3 and thoroughly mixed by shaking. The mixture was made up to 100 ml with distilled water, mixed thoroughly and allowed to stand for 20 minutes. The absorbance of the tannic acid standard solutions as well as sample was measured after colour development at 760 nm using the AJI-C03 UV-VIS spectrophotometer. The amount of tannin in the sample was calculated from a standard curve and expressed in mg tannic acid equivalents g^{-1} DW.

Percentage of infestation

Extent of herbivory or infestation status of the seven varieties was recorded at the three maturity levels. Randomly selected fruits (n=50 of each variety at each maturity level) from insecticide-free orchards of the seven varieties were harvested at three maturity levels and brought to laboratory and kept in cages for symptom development. They were dissected on full ripening and those with maggots were recorded as infested. The percentage of infestation was calculated.

Statistical Analysis

The data on biochemical components at three different maturity levels were subjected to one way Analysis of Variance (ANOVA) using Microsoft corporation version 12.0. Further, possible influence of biochemical factors on infestation by fruit fly were subjected to correlation and linear regression analysis (Minitab Inc. version 16.1.1).

3. Results and discussion

The levels of tannins varied with time/temporal, maturity and varieties/ cultivars and the variations occurred within the fruit parts.

Tannin content in peel and pulp

Data recorded showed that the peel recorded 125 to155% more tannin compared to pulp, among the varieties. It varied between 8 to 14 mg in peel among the varieties, whereas in pulp it was 3.5 to 10.6mg (Table 1).Towards harvest the susceptible varieties peel and pulp recorded less tannin content which supports oviposition and the growth of the fruit fly maggots rendering the fruit infested. Similarly in cowpea, tannins contribute to resistance against infestation by insects such as the cowpea weevil *Callosobruchus maculatus* (F.) Lattanzio *et al*., [17] reported that the seed coat tannin content was 13 times higher in undamaged seeds than in infested seeds of *Vigna unguiculata* resistant and susceptible cultivars respectively.

Tannin content among varieties

Langra recorded highest tannin in both peel (14mg to 13.6mg) and pulp (10.6mg to 7.3mg) at all maturity levels followed by EC 95862 (peel 13.4 to 13mg, pulp 9.7 to 7.2mg). In susceptible varieties the tannin content was at low in peel 14 to 9.9, 12.7 to 8.6, 13 to 8mg and pulp 7.2 to 5.7, 6.7 to 4.8, 5.2 to 3.6mg respectively. Thus it was evident that, lower the tannin content more susceptible the variety for fruit fly. Peel tannin less than 13mg/g at harvest leaves the fruit susceptible to fruit fly attack. Jayanthi and Verghese [15] have shown that under forced no-choice tests, female fruit fly oviposit Langra and EC, but maggots which hatched fail to develop. Further they observed that in EC 92.5% maggots died at different stages of their life cycle. In Totapuri, a susceptible variety, mortality was 0% in a no-choice bioassay.

At different Maturity levels

The tannin content in different varieties at 50%, 75% and 100% maturity levels are given in table 1. Tannin levels were high in both peel and pulp of immature fruits (50% maturity) followed by 75% and 100% in maturity levels. The tannin content was more at 50% maturity level in all varieties, it declined with advancement of maturity. There was reduction in the tannin content with advance of age. Langra and EC retained same level of tannin content in the peel at all

maturity levels (13.42 to 14.17 and 13.08 to 13.26 respectively). Whereas, in susceptible varieties the reduction in tannin content was highest (29 to 37% reduction in peel, 20 to 29% in pulp) in moderately resistant varieties it was 8 to 22% and 27 to 34% in peel and pulp. The data on the varying tannin content across the maturity is in line with the infestation percentage recorded in field, as the tannin content was high in the mangoes irrespective of the varieties at 50% maturity which recorded no (0%) or very less (0.6%) infestation. With the progress of maturity towards ripening there is a drastic reduction in the tannin content in the susceptible varieties which favors the development of the fruit fly [15]. Whereas the resistant varieties; Langra and EC retain the high levels of tannins which impart resistance to the fruit fly infestation. Even in case of *Quercus emoryi* (Fagaceae) the amount of herbivory was significantly and negatively correlated with tannin content. Where the tannin content was at its peak during bud break period and rapidly declines to remain at constant levels for the remainder of the growing season [18].

Tannin content Vs. infestation

The negative relationship between tannin levels and insect herbivory was demonstrated in the present study. The regression analysis was carried out to look for the variability in fruit fly infestation caused by the independent variables tannin content in peel and pulp. Tannins in peel and pulp account for 72% variability inhibiting fruit fly infestation (Fig.1 a, b, c). The Langra with higher tannin content recorded 0% fruit fly infestation followed by EC 95862 (1.4% infestation at 13.1 mg). Varieties with lowest tannin content recorded 55 to 69% of infestation. Moderately resistant varieties recorded 24 to 42% infestation at 11 to 10 mg of tannin. Tannin is an index of fruit fly herbivory in *Mangifera indica*. They act as feeding deterrents against many insects such as, *Lymantria dispar* (L.), *Euproctis chrysorrhoea* (L.) and *Operophtera brumata*, *Aphis craccivora* (Koch). In the present study adult fruit flies seemed deterred by the tannins in the peel. Especially, during exploratory oviposition a prick which causes vacuole ruptures and release of tannins. The tannins may be perceived by flies in their pursuit for yeast on surface of the peels, which gustatoryliy may deter them from egg laying. Adult females will select fruit where maximum larval fitness and survival is ensured. The instinctive response of the Langra and EC 95862's plant tissue provides a signal presumed to be the astringent taste (mouth puckering)of tannins which females immediately recognize and avoid.

Infestation was inversely related to the amount of tannin in fruits. Among the varieties, Banganpalli (69.20%) had significantly higher infestation and EC 95862 (1.40%) and Langra (0.00%) were on par having lower infestation percentages during 100 per cent maturity. In EC 95862 there was one percent increase in tannin over maturity compared to 13 to 30 percent reduction in susceptible varieties. This marginal reduction can increase foraging of fruit flies. Decrease of tannin content in peel is reflected in pulp which favours maggots.

Table 1: Total tannin content in peel and pulp at 50, 75 and 100% maturity

Total tannin content (mg /g DW)									
Maturity levels	**50%**			**75%**			**100%**		
Mango Variety	**Peel**	**Pulp**	**Infestation**	**Peel**	**Peel**	**Infestation**	**Pulp**	**Pulp**	**Infestation**
Totapuri	13.062^{d}	5.122^{f}	0.48	11.603^{c}	5.059^{c}	24.90	8.171^{g}	3.624^{e}	62.50
Alphonso	12.787^{e}	6.736^{d}	0.55	12.594^{b}	5.110^{c}	20.80	8.604^{f}	4.893^{c}	55.80
Banganpalli	14.077^{a}	7.278^{c}	0.60	12.132^{b}	6.192^{b}	34.50	9.930^{e}	5.775^{b}	69.20
EC 95862	13.265^{c}	9.717^{b}	0.00	13.485^{a}	8.167^{a}	0.10	13.082^{b}	7.213^{a}	1.40
Langra	14.170^{a}	10.631^{a}	0.00	13.427^{a}	8.245^{a}	1.88	13.661^{a}	7.332^{a}	0.00
Dusheri	13.715^{b}	5.802^{e}	0.10	12.299^{b}	5.152^{c}	29.80	10.670^{d}	4.229^{d}	42.80
Mylupilian	12.754^{e}	7.414^{c}	0.20	12.261^{b}	6.166^{b}	21.80	11.711^{c}	4.859^{c}	24.00
CD (p=0.01)	0.113	0.482	NS	0.490	0.123	1.82	0.153	0.167	2.10

DW: Dry Weight For a particular maturity level, means followed by different alphabets in the same column is significantly different (ANOVA, $p < 0.01$).

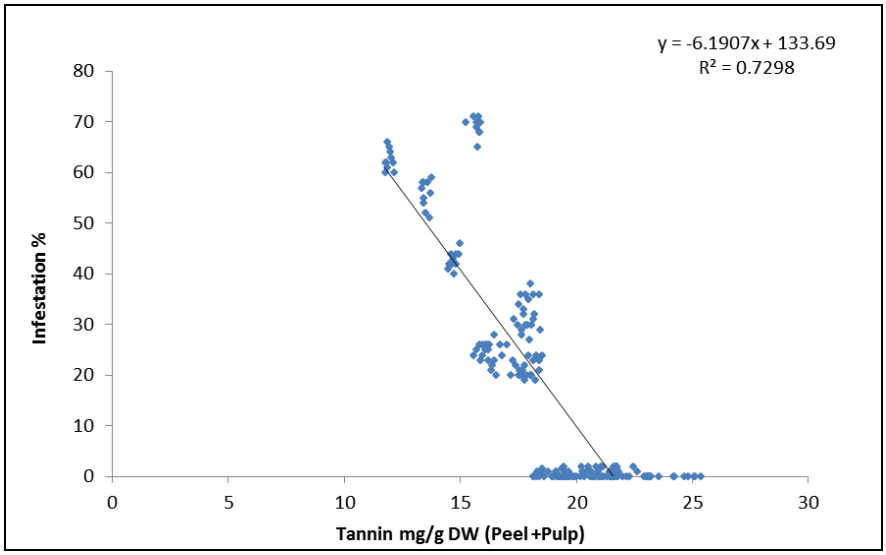

Fig 1 a): Influence of tannins Peel +Pulp (mg/g DW) on the percentage infestation by *B. dorsalis* (No of samples, N=210)

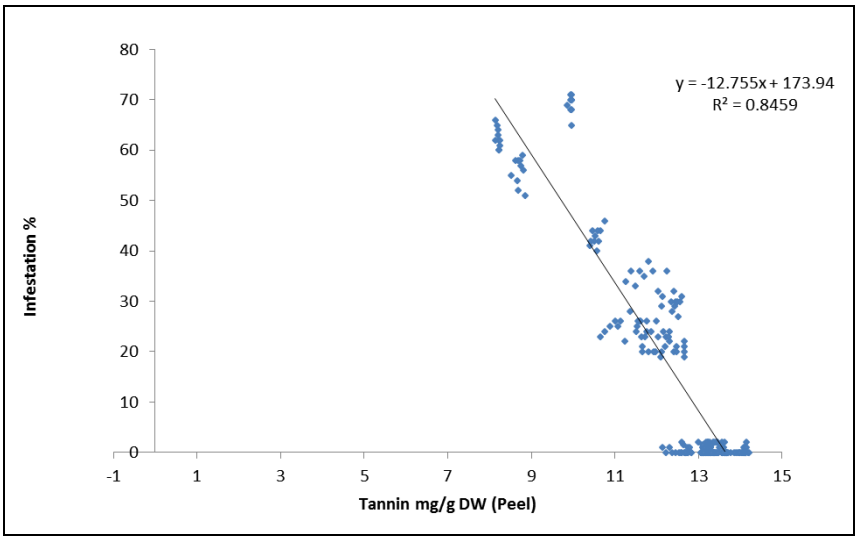

Fig 1b): Influence of tannins in Peel (mg/g DW) on the percentage of infestation by *B. dorsalis* (No of samples, N=210)

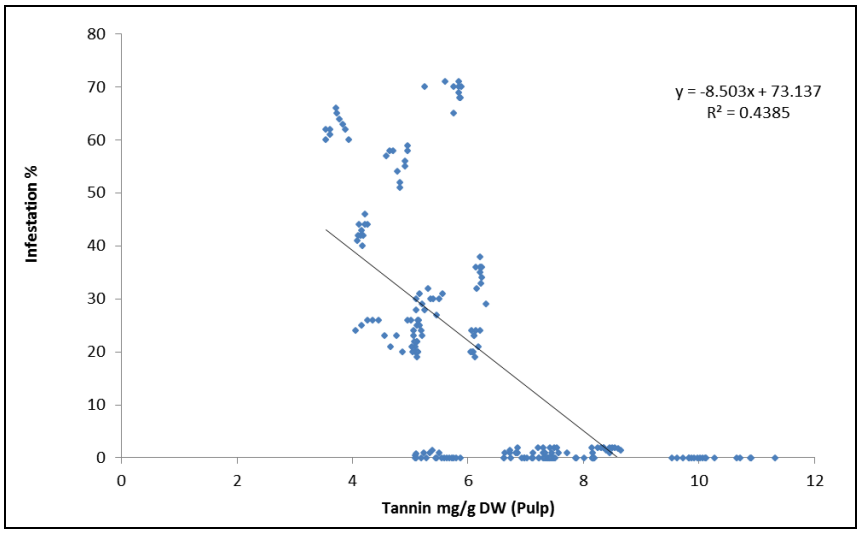

Fig 1c): Influence of tannins in Pulp (mg/g DW) on the percentage of infestation by *B. dorsalis* (No of samples, N=210)

4. Conclusion

Therefore from the present study, the changing cycles of tannin according to varieties and time is an index of infestation of fruits. The defensive mechanism exhibited by the tannins in resistant varieties is evident from the results and confirmed through regression. The signal start by Langra and EC 95862 via., tannins may therefore deter females from ovipositing. If commercial varieties like Alphonso and Banganpalli have to be made resistant to fruit flies the peels need to be augmented with tannins with modern breeding tools or gene transfers. From the knowledge of the present studies, semiochemical lures, attractants and repellents can be developed for tephritids and future research should not only continue the mix of basic and applied research on fruit flies but also explore multimodal communication systems such as sound, vision and gustation in order to improve the application of chemical ecology to solve real-world pest problems.

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