Phenolics as chemical barriers to female fruit fly, *Bactrocera dorsalis* (Hendel) in mango

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A study was conducted to know if phenolics play a defensive role in preventing herbivory of fruit fly *Bactrocera dorsalis* (Tephritidae: Diptera) in mango. Results showed lower phenolics between 6.06 and 13.56 mg/g in peels of susceptible varieties (Banganapalli, Alphonso and Totapuri), whereas in resistant varieties (Langra, EC-95862) it was higher between 42.37 and 53.12 mg/g. The trend was the same for phenolics in pulp. In the susceptible varieties, the phenolic content was <0.60 mg/g and in resistant varieties, it was 2.33–2.36 mg/g. The results show that phenolics play a defensive role in preventing fruit fly herbivory in mango. Susceptible varieties under field conditions had infestation of 22–64%, whereas in resistant varieties no infestation was seen. It was shown earlier that phenolics have been responsible for plant defence against insect herbivory and the same has been found true in the present study. This may be a useful index in large-scale screening of mango germplasm for fruit fly resistance and to breed resistant commercial varieties of mango.

Keywords: *Bactrocera dorsalis*, herbivory, fruit fly, India, mango, phenolics.

PHYTOPHAGOUS insects use chemical cues emanating from plants to orient to their food hosts while plants ward off herbivory again through use of chemicals. This interplay between chemicals is an interesting area of chemical ecology. Secondary metabolites that occur constituently in plants act as chemical barriers to insects and many protect plants against attack by a wide range of potential pests[1,2]. Among secondary metabolites, phenolic compounds have been repeatedly shown to play a vital role in plant resistance and protect fruits and vegetables against pests[3]. A key role was proposed for phenolics in resistance of dates (*Phoenix dactylifera*) to insect infestation during storage[4,5]. Earlier studies on different parts of mango have revealed that peel, pulp and seed kernel are the main sources of phenolics with quantities varying in different parts. Peel constitutes about 15–20% of the fruit[6]. Higher amount of phenolics is reported in peel than in pulp of mango[7] and guava[8]. Based on the findings from the literature, it was hypothesized that phenolics may inhibit fruit fly herbivory. Fruit fly herbivory leads to loss of fruit due to infestation. As a first step, the objective of the study was to investigate in five varieties of mango with known differential infestation levels, the probable role of phenolics in imparting resistance through warding off fruit fly herbivory.

Mango (*Mangifera indica* L.; Anacardiacae) is one of the most important tropical fruits, globally. Though India ranks first in world production, its optimum production is restricted by many insect pests, especially fruit flies (Tephritidae: Diptera), that develop in ripe and unripe fruits. The Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Tephritidae: Diptera) is polyphagous and infests more than 250 host plants, including many types of commercial fruits[9–11]. This species attacks mango and causes serious loss ranging from 5 to 80% (refs 12 and 13). Studies on plant–insect interaction suggest the effects of plant chemicals on host selection behaviour of insects[14,15]. Host plant resistance is one of the most effective tools for reducing insect damage. This has also been found true for fruit flies in mango[16]. A study[17] found cv. Langra to be the least susceptible commercial variety which could be used for breeding. Hybrids with Banganapalli parentage were susceptible[16].

The study was undertaken at the Indian Institute of Horticultural Research, Bangalore (12°58′N; 77°35′E), south India. The five varieties selected for the present study (Table 1) were grouped as susceptible (Banganapalli, Alphonso and Totapuri) and resistant (Langra and EC-95862) to fruit fly based on earlier studies[16,18]. The trees were approximately 15 years old. Four healthy fruits/variety at harvest stage were randomly selected from four random trees in the orchards of each variety. Harvested fruits were brought to laboratory and extracted on the same day. Each fruit was divided into four sections; peel and pulp were extracted from all the four sections to avoid bias. The peel was removed gently using a sharp knife and underlying pulp was also removed. The peel and pulp thus obtained were homogenized in a blender separately. There were four replications of peel and pulp of each variety from which further two subreplications of the extract of each from the main replication

Table 1. Phenolics in peel and pulp and percentage of infestation

Mean phenolic level in peel and pulp			Infestation (%) Mean fruit fly infestation
Variety	Peel (mg/g)	Pulp (mg/g)	At harvest (%)
Banganapalli	13.56	0.53	62
Alphonso	13.19	0.6	24
Totapuri	6.06	0.48	22
Langra	53.13	2.36	0
EC-95862	42.38	2.33	0

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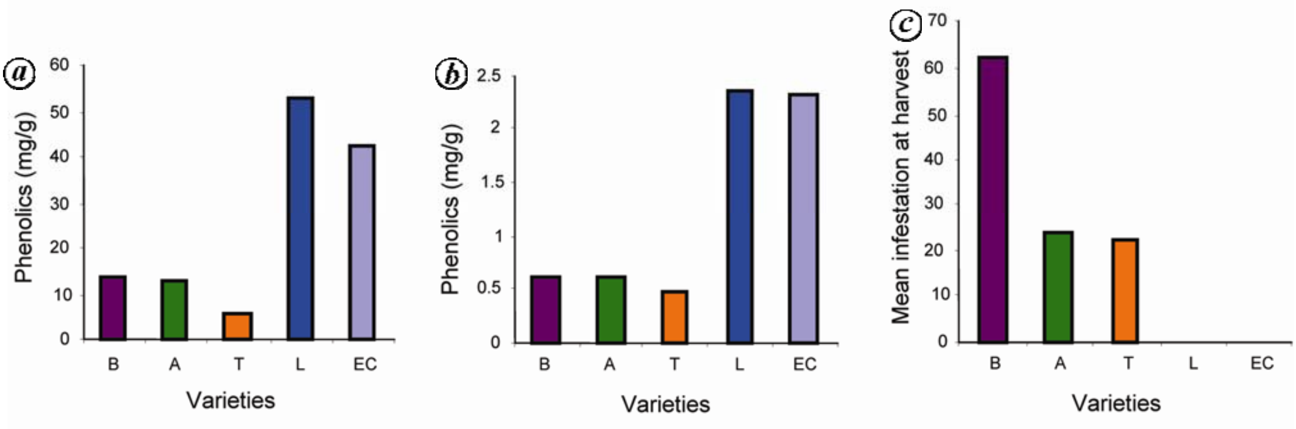

Figure 1. ***a***, Concentration of phenolics in peels of Banganapalli (B ■), Alphonso (A ■), Totapuri (T ■), Langra (L ■) and EC-95862 (EC ■). ***b***, Concentration of phenolics in pulp. ***c***, Percentage of infestation.

were used for analysis. The samples were kept in 80% methanol for 48 h and later extracted after grinding using a pestle and mortar. The final volume was made up to 50 ml and refrigerated at 4°C for 24 h to obtain a clear supernatant for analysis. 0.1 ml aliquot of this extract was used for quantification of total phenolics. The peel and pulp were assayed separately. The total phenolics were quantified spectrophotometrically by Folin–Ciocalteau method as described by Singleton and Rossi[19]. The blue-coloured phosphomolybdic–phosphotungstic–phenol complex formed in alkaline solution was stirred and allowed to stand for 30 min. A blank sample containing water and reagent was used as reference. The absorbance at lambda max of 700 nm was measured spectrophotometrically. All the chemicals and solvents used were analytical grade with 99% purity from Merck Company. Aqueous solutions used were prepared in double distilled water. The concentration of total phenol was determined using a standard calibration curve of gallic acid and expressed as gallic acid equivalent/g of sample.

For confirming the extent of herbivory or infestation status of the selected varieties, 50 randomly selected fruits from insecticide-free orchards were harvested and brought to laboratory and kept in cages for ripening. They were dissected on full ripening and those with maggots were recorded. The percentage of infestation was calculated. The data were subjected to analysis of variance (ANOVA), with critical difference (CD) ($P = 0.05$) as the test criterion[20]. The results were subjected to a regression analysis to test the validity of the hypothesis that phenolics as independent variable influence infestation with R^2 as the test criterion[20].

The results are presented in Table 1 and Figures 1 and 2. Distribution and composition of phenolic phytochemicals are affected by maturity, cultivars, horticultural practices, geographic origin and growing season[21–24]. Data presented in Table 1 show the phenolic profile in both peel and pulp of the five varieties to be varying, mainly due to varietal nature, than other factors as all the varieties were grown under uniform field conditions in a geographic place.

Susceptibility and resistance were on expected lines with Banganapalli, Alphonso and Totapuri showing infestation whereas the reported resistant varieties Langra and EC-95862 were free of infestations (Table 1). The general trend suggest that higher the phenolic content, lower the infestation level. Preharvest studies showed significant infestation rate in all the three susceptible varieties when compared to Langra and EC-95862. Phenolic content was high in resistant varieties Langra and EC-95862 in which no infestation was found, whereas all the susceptible varieties Banganapalli, Alphonso and Totapuri had less phenolic content with infestations of 62%, 24% and 22% respectively. The studies indicate that lower phenolic content of the fruit makes it more vulnerable to fruit fly herbivory and infestation. Langra and EC-95862 had high phenolic content characterizing resistant trends.

From the data, peel phenolics of 13.56 mg/g or less at harvest render the fruit susceptible to fruit fly attack. Phenolic content in the pulp indicated that phenolics less than 0.60 mg/g at harvest made the fruit prone to fruit fly attack, whereas resistant varieties had a phenolic content >2.30 mg/g. Low concentration of phenolics may not inhibit fruit fly and provide favourable media for larval development. Under forced no-choice conditions, Jayanthi and Verghese[18] have shown that female fruit fly oviposit Langra and EC, but maggots which hatched fail to develop. They found that in EC, 92.5% maggots died at different stages of their development till formation of pupa. In Totapuri, a susceptible variety, mortality was 0% in a no-choice bioassay. The models confirm the hypothesis that phenolics inhibits or reduce infestation or insect herbivory, irrespective of varieties.

Phenolics in peel and pulp account for 50% and 58% variability inhibiting fruit fly infestation respectively (Figure 2). A study on susceptible varieties, it was

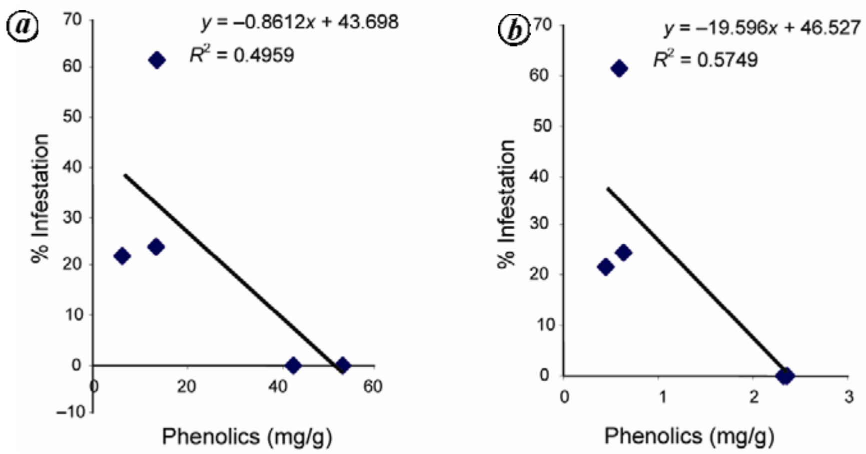

Figure 2. Models showing relationship between infestation and phenolics in (***a***) peel and (***b***) pulp.

shown that every fruit on the tree has equal probability of being selected by the fruit fly (*B. dorsalis*) for oviposition[25]. In the present study, the resistance may be possibly attributed to antibiosis (which is prevention of development of the organism in the host[9,26–28]) due to higher concentration of phenolics in EC-95862 and Langra, as phenolics are known to impart resistance. This is an important lead in fruit fly resistance breeding as use of resistant varieties is an important tool for environment-friendly pest control.

Phenolic compounds play a vital role in plant defense against insect herbivory[3,4] and infection by pathogens[29] in fruits and vegetables. Phenolics have also been associated extensively with the chemical defence of plants against higher herbivores[30]. Several associations have been reported between phenolics and the resistance of plants to insect damage[31]. It is to be explored whether phenolics affect gustatory or olfactory sense or act as an ovicidal ovipositional deterrent.

Mango fruit fly (*B. dorsalis*) is a major herbivore on mango worldwide. The present study has shown high incidence of fruit fly in susceptible varieties, Banganapalli, Alphonso and Totapuri, whereas in resistant varieties, Langra and EC-95862, herbivory or infestation was not observed (Table 1). The secondary metabolite, total phenolics, varied among the varieties. The study indicated the probable role of phenolics in fruit fly herbivory infestation patterns across the selected varieties. In all the varieties, it is the peel that had much higher phenolic content than the pulp. The resistant/tolerant varieties showed higher levels of phenolics compared to that of the susceptible varieties. Phenolic compounds have the ability to form insoluble complexes with proteins, act as enzymes inhibitors or are oxidized to toxic quinones. Further studies will be undertaken to evaluate other important secondary metabolites which can bring a greater understanding of the resistant or susceptible nature of the different varieties of mango and interaction between mango (primary producer) and fruit fly (primary consumer/herbivore).

Studies on phenolics as a direct or indirect chemical barrier should be taken forward to breed varieties with higher content and acceptable commercial traits. Langra as an acceptable commercial variety, with higher phenolics, tops the bill as an ideal parent, also suggesting that phenolics can enhance commercial value while affording resistance. This would obviate the need to use insecticides which will render fruits residue-free as fruit flies attack mango during preharvest phase.

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