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Biochemical Basis of *Bactrocera* spp. (Diptera: Tephritidae) Resistance in some Genotypes and Improved Varieties of *Cucumis sativus* L.

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ABSTRACT: Studies were conducted at farmer's field, Marhun, Bara (Hamirpur) in Himachal Pradesh to evaluate the potential of biochemical basis of host plant resistance in the management of melon fruit flies of cucumber (*Cucumis sativus* L.). None of the improved varieties tested showed significant and consistent resistance to this devastating pest. The genotype IC-430026 however, supported relatively less maggot population and thus suffered significantly lower damage (infested fruits) under no insecticide protection than the improved varieties. The different levels of biochemical constituents namely total sugars, total free amino acids and total phenols were observed in genotypes and improved varieties. The higher levels of total sugars and total free amino acids were observed in improved varieties KCU-006 and Mohani-5300. There was a clear significantly positive correlation between total sugars and melon fruit flies incidence.

Keywords: Cucurbits, host plant resistance, *Bactrocera* spp., antibiosis, cucurbits.

INTRODUCTION

Tephritid fruit flies belong to a large group of insects in Diptera: Tephritidae and are widely distributed throughout the world, with more than 500 genera and 5000 species (Scolari *et al*., 2021). Among them, harmful species are mainly occurs in six genera, Anastrepha, Bactrocera, Ceratitis, Zeugodacus and Rhagoletis (Doorenweerd *et al*., 2018). However, 250 species are of economic importance and distributed widely in temperate, sub-tropical and tropical regions of the world (Christenson and Foote 1960). They cause damage not only to hosts but also to the development of related industries and economic income (He *et al*., 2023). They also comprise important invasive and quarantine species that threaten the world fruit and vegetables production (Diller *et al*., 2023). In India, fruit flies are identified as one of the ten most serious problems of the entire agriculture and because of their polyphagous nature, many species cause high economic losses in fruits and vegetables. Of 207 species of fruit flies in India, nine are identified to be the major and economically important (Sardana *et al*., 2005). The fruit flies constitute an important group of pests infesting cucurbit vegetables. Two species namely *Bactrocera cucurbitae* (Coquillett) and *Bactrocera tau* (Walker) commonly called as melon fruit flies are the major species found infesting cucurbits. Besides, they have been found feeding on solanaceous crops like tomato and brinjal (Kapoor and Agarwal 1983). Another fruit fly species, *Bactrocera scutellaris* (Bizzi) has also been recorded on cucurbits (Sunandita and Gupta 2007). The extent of losses caused by *B. cucurbitae* varies from 30 to 100 per cent depending on the cucurbit species and season (Dhillon *et al.*, 2005a). It has been reported to infest 95 per cent bitter gourd fruits in New Guinea and 90 per cent snake gourd and 60 to 87 per cent pumpkin fruits in Solomon Islands (Hollingsworth *et al.*, 1997). However, in India fruit infestation of 31.27 per cent and 28.55 per cent was reported on bitter gourd and water melon, respectively (Singh *et al.*, 2000). About 50 per cent of cucurbits are partially or completely damaged by fruit flies in India (Gupta and Verma 1992). The melon fruit flies have more than 80 hosts and their economic importance cannot be evaluated entirely from the standpoint of the direct damage to the various crops affected. Quarantine laws aimed at preventing the entry and establishment of melon flies reduce the export potential of crop produce.

Generally, the female fruit flies puncture the soft and tender fruits by their sharp ovipositor and lay the eggs under fruit tissues and watery fluid oozes from the puncture. Sometimes pseudo-punctures (punctures without eggs) have also been reported on fruit skin, which reduces the market value of the produce. The eggs are also reported to be laid into unopened flowers and the maggots successfully develop in the taproots, stems and leaf stalks (Weems and Heppner 2001). Due to their reproductive capacity and ability to rapidly multiply, fruit flies may cause severe economic damage to plantations, destroying up to 100% of the produce without control (Qin *et al*., 2015). After hatching, the maggots feed inside the fruit on pulp by making galleries and pupate in the soil, thereby remaining unaffected even with the frequent application of insecticides. It is imperative to explore the alternative methods for the control of this pest. Different host fruits are known to vary in their suitability for fruit flies to complete their life cycle (Follett *et al*., 2021). These studies were therefore; conducted to screen out the available cucumber genotypes and some improved varieties for identifying potential biochemical constituent basis resistance sources against melon fruit flies in mid-hill Himalayas of Himachal Pradesh.

MATERIALS AND METHODS

Twelve varieties/genotypes *viz*., Khira Paprola, Sikkim Cucumber, Mohini-5300, Nepal Local, KCU-006, IC-355960, IC-469811, IC-479979, IC-430026, IC-4698, IC-429994 and check Khira-90 were evaluated for their relative resistance against melon fruit flies under natural field conditions. The cucumber genotypes for the present investigation were procured from the Department of Vegetable Science and Floriculture, College of Agriculture, CSKHPAU, Palampur and NBPGR, Regional Station, Bhowali Niglat, Nainital, Uttrakhand.

The seeds of each cucumber genotype were sown in polythene bags containing mixture of soil, sand and FYM and raised under polyhouse conditions at Krishi Vigyan Kender, at Bara (CSKHPAU, Palampur). The seedlings were transplanted during third week of February at farmer's field, Marhun, Bara (Hamirpur). The experiment was laid out in Randomized Block Design with plot size of 4.5 × 4.0 meter. The row to row and plant to plant distance was maintained as 1.5 meter and 60 cm, respectively. All the recommended agronomic practices were carried out, except the pest management practices to screen the varietal resistance of tested cucumber genotypes against melon fruit flies.

The marketable size fruits irrespective of healthy and infested were picked at weekly intervals. A total of the fruit pickings was five in each tested genotype. The infested fruits were sorted out based on the oviposition punctures, brownish pin hole size pseudo-punctures (without eggs) and healthy. The percentage of fruit infestation data was recorded on the basis of cumulative damage in all the pickings. After sorting out, ten randomly selected infested fruits were placed in polythene bags from each genotype and kept in the laboratory. After 1 to 2 days, each infested fruit was cut open and then observed with the help of hand lens (10 X) to record the number of maggots per infested fruit. The genotypes were grouped by following the rating system given by Nath (1966) for the fruit damage.

Biochemical traits. Some important biochemical components like total sugars, free amino acids and phenols were estimated in fruits of each screened genotypes for their relative resistance to melon fruit flies.

Preparation of samples. The healthy fruits of cucumber varieties/genotypes were cleaned and freed of any extraneous substances and dried in an electric oven at 55±5ºC. The dried samples were ground in a grinder and stored in polythene air tight bags. These samples were properly labeled and kept at room temperature for further analysis. The total sugars content was determined by the method of Dubois *et al*. (1956). The total phenols were estimated by the method suggested by Makkar *et al.* (1993) and total free amino acids were estimated by the method given by Jayaraman (1981). Correlation coefficients were worked out to assess the relationship between biochemical components and pest incidence. The data was subjected to analysis by using CPCS-1 software SPSS.

RESULTS AND DISCUSSION

Twelve cucumber genotypes including check Khira-90 were tested for their relative resistance to fruit flies attacking cucurbits in Himachal Pradesh. The observations were recorded on the basis of per cent fruit infestation and number of maggots per infested fruit at each fruit picking throughout the fruiting period. Finally, genotypes were categorized on the basis of mean per cent fruit infestation as immune, highly resistant, resistant, moderately resistant, susceptible and highly susceptible (Table 1).

None of the genotype was rated as immune and highly resistant to fruit infestation by fruit flies. One genotype IC-430026 was rated resistant and nine genotypes K-Paprola, Sikkim Cucumber, Nepal Local, IC-355960, IC-469811, IC-479979, IC-4698, IC-429994 and check Khira-90 were rated moderately resistant. The improved varieties Mohini-5300 and KCU-006 were proved to be susceptible. The mean per cent fruit infestation was significantly low in resistant genotype followed by moderately resistant genotypes and higher in susceptible genotypes. Most of the genotypes showed moderate resistance, but there was a significant difference among them ranging from 28.67 to 48.67 per cent. Development of varieties resistant to pests provides a suitable and desirable means of pest management without any specific change in the cultural practices at field level. The success of such attempts depends up on the extent of variability in the germplasm. Host plant selection by insects is either expressed by the occurrence of population insects on plant in nature or by feeding, oviposition, or utilization of the plant for complete offspring development (Rafiq *et al.*, 2008). In the present study none of the screened genotype was found free from the attack of fruit flies during both the years, however, significant difference was observed in the rate of infestation among the tested genotypes. The maximum damage and maggot density was recorded in susceptible genotypes Mohini-5300 and KCU-006. The fruit infestation and maggot density ranged from 19.00 to 66.93 per cent and 19.75 to 54.40 per infested fruit, respectively. The significantly lowest fruit infestation and maggot density was observed in genotype IC-430026 followed by Nepal Local and IC-355960 during both the study years. However, mean per cent fruit infestation and maggot density were found to be varied from year to year, as it was slightly high during second study year than that of previous year in most of the genotypes. These differences could be attributed to the variations in pest population size and plant genotypes either due to the environmental stress or genetic makeup posses physiological and biochemical differences which alter the nutritional value for herbivores (Siemens *et al*., 2002). These results are also confirmed by the findings of Gogi *et al.* (2009) who reported that population build up of melon fruit fly was directly or indirectly influenced by the year wise variations in the abiotic factors. Su (1986) and Lee *et al.* (1992) documented the similar reason for the fluctuation in population density of *B. cucurbitae* in Taiwan. There was a significant positive correlation between per cent fruit infestation and mean number of maggots per infested fruit. Gogi *et al.* (2010) also found significant positive correlation between fruit infestation and maggot density per fruit in bitter gourd screened genotypes. The percentage fruit infestation and maggot population was significantly lower in resistant genotype followed by moderately resistant genotypes and higher in susceptible genotypes. However, both fruit infestation and maggot density was also varied among the tested genotypes. Numerous studies have shown that genotypes of the same species could significantly differ in their resistance to insect pests (Sarfraz *et al*. 2006; 2007). Our results are also in agreement with the findings of Dhillon *et al.* (2005b) who reported a lower infestation and maggot population per fruit in wild genotypes (resistant) and higher in cultivated genotypes (susceptible) and Gogi *et al.* (2010) also observed lower fruit infestation and maggot density in resistant genotypes than in their susceptible counterparts of bitter gourd.

The total sugar content in fruits of the screened genotypes ranged from 2.25 to 13.20 mg/g with values significantly lower in resistant and moderately resistant genotypes than susceptible genotypes. The resistant genotype IC-430026 had lowest total sugar content (2.25 mg/g) and was statistically at par with IC-355960 (2.57 mg/g) which proved moderately resistant to fruit flies. The significantly highest total sugar content was observed in susceptible genotypes KCU-006 and Mohini-5300 (13.20 and 10.47 mg/g, respectively). Among the moderately resistant genotypes, the maximum total sugar content was recorded in the genotype IC-429994 (9.40 mg/g) which was at par with IC-469811 (8.20 mg/g), both also showed more fruit infestation and relatively higher maggot density than that of other moderately resistant genotypes (Table 2). The per cent fruit infestation and maggot population per infested fruit were found significantly and positively correlated with total sugar content (Table 3).

It can be revealed from the results that the total free amino acid content in the fruits of resistant genotype IC-430026 was 7.00 mg/g which was at par with susceptible genotype Mohini-5300 (7.37 mg/g), KCU-006 (7.50 mg/g) and moderately resistant genotype Nepal Local and IC-4698 (8.12 and 6.57 mg/g, respectively). The minimum total free amino acid content was observed in the genotype Sikkim Cucumber (3.17 mg/g) which proved moderately resistant to fruit infestation by fruit flies and was statistically at par with Khira Paprola (4.77 mg/g) and check Khira-90 (4.23 mg/g). However, maximum total free amino acid content was also observed in the moderately genotype IC-429994 (9.37 mg/g) which was also found significantly more susceptible, registering highest fruit infestation as well as maggot density among all the moderately genotypes (Table 2). The per cent fruit infestation as well as maggot population was found to be positively correlated with total free amino acid content, however the relationship was non-significant (Table 3).

Higher total phenols were recorded in resistant and moderately resistant genotypes. The total phenols ranged from 1.67 to 11.00 mg/g. The significantly highest total phenol content was found in the fruit of resistant genotype IC-430026 (11.00 mg/g), showed lowest per cent fruit infestation and maggot population. The significantly lowest total phenol content was recorded in susceptible genotypes Mohini-5300 and KCU-006 (1.67 and 2.17 mg/g, respectively), as a resulting in registered significantly highest per cent fruit infestation and maggot population per infested fruit. The moderately resistant genotypes showed also significant variation in the total phenol content, being highest in the genotype IC-355960 (9.23 mg/g) followed by Nepal Local and IC-479979 (8.43 and 7.37 mg/g, respectively) and lowest in IC-469811 and IC-429994 (2.40 and 3.30 mg/g, respectively) (Table 2). The amount of total phenols in the fruits of tested genotypes exhibited strong significant negative correlation with per cent fruit infestation and maggot density per infested fruit (Table 3).

Fruit fly infestation and length of ovary pubescence, rind thickness, flavonoid content, ascorbic acid, free amino acid, tannins content, and phenols content were the reliable variables for characterization of resistance (Haldhar *et al*., 2015). During the present study, the total content of biochemical traits like total sugars, total free amino acids and total phenols varied significantly among the screened cucumber genotypes. Numerous studies have shown that the genotypes of the same species could significantly differ in their resistance to insect pests (Sarfraz *et al.*, 2006; 2007) and it is caused by biophysical and/or biochemical compounds of plants (Gogi *et al.*, 2010). Similar to our results, higher content of total sugars was recorded in susceptible genotypes as compared to resistant and moderately resistant genotypes. It is evident from the results that the higher content of total sugars in fruits was generally associated with higher fruit infestation and maggot density per infested fruit. There was also significant variation in the total sugar content among the resistant and moderately resistant genotypes. The lowest sugar content was observed in resistant genotype IC-430026 (2.25 mg/g), as a result proved least susceptible to fruit flies attack. Per cent fruit infestation and maggot population significantly and positively correlated with total sugars. The total free amino acid content was varied among the different genotypes ranged from 3.17 to 9.37 mg/g. The studies indicated that in most of the moderately resistant genotypes, the total free amino acids was lower than susceptible genotypes. The results revealed the positive correlation of per cent fruit infestation and maggot population per infested fruit with total free amino acids, but the relationship was non-significant. The total phenols differed significantly among the tested cucumber genotypes. The resistant and moderately resistant genotypes contained significantly higher phenols than susceptible genotypes. The significantly highest total phenols were observed in the genotype IC-430026 (11.00 mg/g), which proved to be resistant to fruit flies attack. The total phenols had a significant and negative correlation with per cent fruit infestation and maggot density. These results are supported by the findings of Pareek and Kavadia (1994) reported that the high degree of infestation among muskmelon genotypes by melon fruit fly might be attributed to the olfactory stimuli emitted by fruits which varied with the concentration of the total soluble sugars among different cultivars. Ingoley *et al.* (2005) who also observed higher amount of total sugars, total free amino acids and lower amount of phenols in highly susceptible genotypes of cucumber. Gogi *et al*. (2010) also found significant negative correlation of phenols, tannin, flavanol, ash and silica content with the per cent fruit infestation and maggot density per fruit in the different bitter gourd genotypes. The non-reducing, reducing, total sugars, total phenols, silica and ash content had significant impact on the fruit damage and showed significant negative correlation with fruit fly infestation in bitter gourd and maggot density (Nath *et al*., 1966). Sambandam and Chelliah (1972) also observed lower quantity of essential nutrients like amino acids and sugars in musk melon genotypes resistant to fruit flies.

Table 1: Categorization of cucumber genotypes on the basis of fruit infestation by fruit flies.

Infestation (%)	Category	Genotypes
No damage	Immune	—
1-10	Highly resistant	—
11-20	Resistant	IC-430026
21-50	Moderately resistant	Khira Paprola, Sikkim Cucumber, Nepal Local, IC-355960, IC-469811, IC-479979, IC-4698, IC-429994, Khira-90
51-75	Susceptible	Mohini-5300, KCU-006
76-100	Highly Susceptible	—

Based on mean of two years study

Table 2: Biochemical compounds and relative susceptibility of cucumber genotypes in relation to melon fruit flies, *Bactrocera* spp. at Bara (Hamirpur).

Genotype	1st year		2nd year		Mean		Biochemical compounds		
	Mean fruit infestation (%)#	Mean no. of maggots /fruit##	Mean fruit infestation (%)	Mean no. of maggots /fruit	Fruit infestation (%)	No. of maggots fruit	Total sugars (mg/g)	Total amino acids (mg/g)	Total phenols (mg/g)
Khira Paprola	36.00 (36.57)	34.95 (5.90)	37.59 (37.69)	36.45 (6.05)	36.80	35.70	4.72	4.77	5.77
Sikkim Cucumber	30.66 (33.29)	30.95 (5.54)	34.00 (35.43)	32.85 (5.73)	32.33	31.90	4.60	3.17	6.60
Mohini-5300	48.00 (43.83)	45.40 (6.69)	62.00 (52.11)	50.10 (7.09)	55.00	47.75	10.47	7.37	1.67
Nepal Local	27.33 (31.10)	27.60 (5.29)	30.00 (33.03)	28.00 (5.35)	28.67	27.80	3.93	8.12	8.43
KCU-006	56.66 (49.36)	49.75 (6.99)	66.93 (51.83)	54.40 (7.36)	61.80	52.08	13.20	7.50	2.17
IC-355960	27.33 (31.34)	25.20 (5.11)	31.33 (33.98)	27.05 (5.28)	29.33	26.13	2.57	5.67	9.23
IC-469811	40.66 (39.41)	37.50 (6.11)	43.33 (41.04)	39.20 (6.28)	41.00	38.35	8.20	5.47	2.40
IC-479979	28.66 (32.13)	26.90 (5.23)	31.33 (33.87)	29.20 (5.45)	30.00	28.05	3.43	5.10	7.37
IC-430026	19.00 (27.38)	19.75 (4.46)	21.66 (28.40)	21.50 4.60)	20.33	20.63	2.25	7.00	11.00
IC-4698	38.00 (37.87	36.95 (6.08)	40.66 (39.48)	37.30 (6.13)	39.33	37.13	6.87	6.57	4.60
IC-429994	45.33 (42.26)	43.30 (6.57)	52.00 (46.21)	43.60 (6.58)	48.67	43.45	9.40	9.37	3.30
Check Khira-90	30.13 (32.66)	29.21 (5.37)	36.27 (36.55)	33. 56 (5.76)	33.20	31.39	6.45	4.23	6.53
CD (P= 0.05)	3.71	0.56	3.17	0.46			1.24	1.30	0.71

Figures in the parentheses are arc sine transformed values ; ## Figures in the parentheses are square root transformed values

Table 3: Correlation co-efficient between melon fruit flies incidence and biochemical compounds.

Characters	Biochemical compounds		
	Total sugars (mg/g)	Total amino acids (mg/g)	Total phenols (mg/g)
Percent fruit infestation	+0.973**	+0.421	-0.909**
Mean number of maggots per infested fruit	+0.965**	+0.357	-0.946**
Between fruit infestation and number of maggots per infested fruit	+0.992**		
** Significant at 1% level of significance			

CONCLUSIONS

It is concluded that the genotype, IC-430026 seems to hold some promise and thus deserve closer study to establish more accurately the levels and mechanisms of resistance. This cucumber genotype would provide the much needed base for breeding varieties resistant to melon fruit flies. There is also the need to explore more germplasm and materials from various sources to identify those that are less susceptible to this key pest. Though difficult to find, insect pest resistant varieties should form the basis for developing sustainable IPM systems for cucumber. Breeding for resistance is a powerful tool in pest management and once it is achieved, it will not only reduce the cost of production but also substantially prevents the environmental pollution.

FUTURE SCOPE

The promising genotypes identified in this study can be exploited commercially to breeding for development of resistant varieties against fruit flies.

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Conflict of Interest. None.

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