

**MORPHOLOGICAL AND BIOCHEMICAL BASES
OF RESISTANCE TO MELON FRUIT FLY,
[*Bactrocera cucurbitae* (Coquillett) (Diptera:
Tephritidae) IN BITTER GOURD (*Momordica
charantia* L.)]**

By
MANJU ROSHNI K
(2012-11-137)

THESIS

submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture

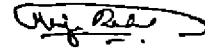
**Faculty of Agriculture
Kerala Agricultural University**

**DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR-680 656
KERALA, INDIA
2014**

DECLARATION

I hereby declare that the thesis entitled “**Morphological and biochemical bases of resistance to melon fruit fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) in bitter gourd(*Momordica charantia* L.)**” is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

Vellanikkara,
30.09.2014

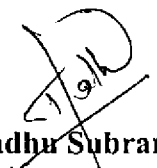


Manju Roshni K
(2012-11-137)

CERTIFICATE


Certified that the thesis entitled “**Morphological and biochemical bases of resistance to melon fruit fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) in bitter gourd (*Momordica charantia* L.)**” is a record of research work done independently by Ms. **Manju Roshni K (2012-11-137)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

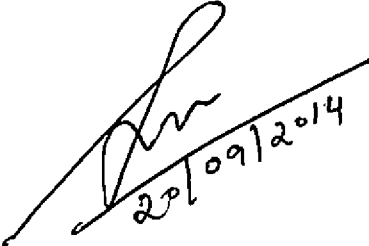
Vellanikkara
36.09.2014

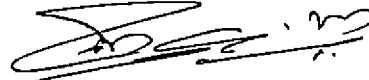

Dr. Madhu Subramanian
(Chairman, Advisory Committee)
Assistant Professor
Department of Agricultural Entomology
College of Horticulture,
Vellanikkara

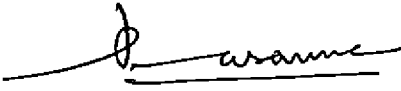
CERTIFICATE

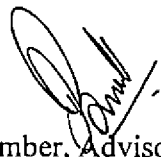
We, the undersigned members of the advisory committee of Ms. **Manju Roshni K. (2012-11-137)** a candidate for the degree of Master of Science in Agriculture, with major field in Agricultural Entomology, agree that the thesis entitled “**Morphological and biochemical bases of resistance to melon fruit fly, (Momordica charantia L.)**” may be submitted by Ms. **Manju Roshni K. (2012-11-137)**, in partial fulfillment of the requirement for the degree.

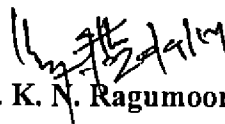

Dr. Madhu Subramanian
(Chairman, Advisory Committee)
Assistant Professor
Department of Agricultural
Entomology College of Horticulture,
Vellanikkara


Dr. Sosamma Jacob
(Member, Advisory Committee)
Professor and Head
Department of Agricultural Entomology
College of Horticulture,
Vellanikkara


Dr. Mani Chellappan
(Member, Advisory Committee)
Associate Professor (Ag. Entomology)
AINP on Agrl. Ornithology
College of Horticulture,
Vellanikkara


Dr. K. P. Prasanna
(Member, Advisory Committee)
Professor
Department of Olericulture
College of Horticulture,
Vellanikkara


Dr. C. Beena
(Member, Advisory Committee)
Associate Professor (Biochemistry)
AICRP on Medicinal and Aromatic Plants
College of Horticulture,
Vellanikkara


Dr. K. N. Ragumoorthi
(External Examiner)
Professor (Agrl. Entomology)
Directorate of Research
Tamil Nadu Agricultural University
Coimbatore

ACKNOWLEDGEMENT

First of all, I bow my head before Lord Krishna whose blessings enabled me to undertake this venture successfully.

It is with great respect and devotion, I place on record my deep sense of gratitude and indebtedness to my chairperson Dr. Madhu Subramanian, Assistant Professor, Department of Agricultural Entomology for his sustained and valuable guidance, constructive suggestions, unfailing patience, friendly approach, constant support and encouragement during the conduct of this research work and preparation of the thesis. I gratefully remember his knowledge and wisdom which nurtured this research project in right direction without which fulfillment of this endeavor would not have been possible.

No words can truly represent my profound gratitude and indebtedness to Dr. Sosamma Jacob, Professor and Head, Department of Agricultural Entomology, member of my Advisory Committee for her unstinted support and guidance in the conduct of field experiments, constructive criticism, valuable suggestions and critical scrutiny of the manuscript.

I am deeply obliged to Dr. Mani Chellappan, Department of Agricultural Entomology and member of my Advisory Committee for the generous and timely help he has always accorded to me during the course of this study.

I am deeply indebted to Dr. K. P. Prasanna, Professor, Department of Olericulture and member of my Advisory Committee for her unbounded support and valuable suggestions.

With ineffable gratitude, I thank Dr. C. Beena, Associate Professor, Department of medicinal and aromatic plants, for her technical guidance rendered at every stage of work and meticulous personal care.

I consider it is a privilege to express my sincere thanks to Dr. Susannamma Kurien, Dr. Ushakumari, Dr. Maicykutty P. Mathew, Dr. Haseena Bhaskar, Dr. Babu

Philip, Dr. K. R. Lyla, Smt. Sreeja S. and Smt. Vidya C. V. Department of Agricultural Entomology for the valuable help and advice rendered during the course of my study.

I am extremely delighted to place on record my profound sense of gratitude to Dr. S. Krishnan, Assistant Professor, Department of Agricultural Statistics, for his support, critical comments and valuable advice during the preparation of this manuscript.

I wish to express my sincere gratitude to Mr. Kesavan, Mr. Vijayakumar, Miss Celine, Miss Rintu, Miss Niji, Mrs. Sindhu and all labourers at Instructional Farm, Thrissur for the sincere help, timely suggestions and encouragement.

Words fall short as I place on record my indebtedness to my seniors, classmates, juniors and friends, Uma, Ashwathy Krishna, Subha, Lakshmi, Surya, Sandhya, Deepak, Rubi, Aparna, Mahsu, Teena, Santhi, Sajeera and Minnu for their prompt help and cooperation for the entire period of study.

It is my fortune to gratefully acknowledge the support of two individuals Anila and Eldo who were always beside me during the happy and hard moments in my life to push and motivate me.

Special thanks to Mr. Aravind, students computer club, COH, for rendering necessary help whenever needed.

The award of junior research fellowship of the KAV is also thankfully acknowledged

Family is the compass that guides us, inspiration to reach great heights and our comfort when we occasionally falter. I would like to thank Achan, Amma and Ettan for their selfless sacrifice, boundless patience and unflinching interest throughout the period of my course. I owe everything to them.

It is a pleasant task to express my thanks to all those who contributed in many ways to the success of this study and made it an unforgettable experience for me.



Manju Roshini K

CONTENTS

Chapter	Title	Page No.
1	INTRODUCTION	1-3
2	REVIEW OF LITERATURE	4-18
3	MATERIALS AND METHODS	19-26
4	RESULTS	27-41
5	DISCUSSION	42-51
6	SUMMARY	52-55
7	REFERENCES	i-ix
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1	Details of the bitter gourd accessions evaluated	20
2	Fruit infestation by melon fly and larval density in bitter gourd accessions	28
3	Fruit colour and fruit spine form among bitter gourd accessions	30
4	Morphological features of fruits of bitter gourd accessions	32
5	Biochemical parameters of bitter gourd accessions	34
6	The correlation analysis of morphological and biochemical traits of bitter gourd accessions with per cent fruit infestation and larval density per fruit	37
7	Number of visits and time spent by melon flies on fruit extracts of bitter gourd accessions	40
8	Susceptibility rating scale of bitter gourd accessions based on fruit damage	44
9	Influence of fruit colour on per cent damage	45

LIST OF FIGURES

Fig. No.	Title	Between pages
1	Lay out of the field experiment	19&20
2	Per cent fruit infestation in bitter gourd accessions	42&43
3	Larval density in bitter gourd accessions	43&44
4	Influence of spine density on per cent damage	46&47
5	Influence of spine length on per cent damage	46&47
6	Influence of Peroxidase activity on per cent damage	48&49

LIST OF PLATES

Plate No.	Title	Between pages
1	View of the experimental plot	19&20
2	Bitter gourd accessions used for evaluation	20&21
3	Bitter gourd accessions used for evaluation	20&21
4	Bitter gourd accessions used for evaluation	20&21
5	Colour variation among fruits in bitter gourd accessions	21&22
6	Spine forms of bitter gourd fruits	21&22
7	Measuring flesh thickness using Vernier calipers	21&22
8	Measuring fruit width using Vernier calipers	21&22
9	Measuring spine length using Vernier calipers	22&23
10	Paper window used for counting number of spines in bitter gourd accessions	22&23
11	Fibre glass box used to study the olfactory response of melon fly	26&27
12	Melon fly visiting filter paper disc treated with fruit extract	26&27

INTRODUCTION

1. INTRODUCTION

Bitter gourd (*Momordica charantia* L.), is a tropical vegetable crop belonging to the family Cucurbitaceae. Bitter gourd is of old world origin and is a native of tropical Asia, particularly in the Indo Burma region. It is widely grown in India, Indonesia, Malaysia, China and tropical Africa. It is an important vegetable in South Indian states, particularly in Kerala and is grown for its immature tuberculate fruits which have a unique bitter taste. Bitter gourd fruits are considered as a rich source of vitamins and minerals. Fruits are used after cooking and delicious preparations are made after stuffing and frying. Bitter gourd fruits have medicinal value and are used for treating diabetes, asthma, blood diseases and rheumatism (Gopalakrishnan, 2007).

Bitter gourd is attacked by a number of pests. One of the most important among them is the melon fruit fly *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). The melon fruit fly is distributed all over the world, though it is considered to be a native of India. It is known to infest over seventy species of host plants, amongst which members of Cucurbitaceae family such as bitter gourd (*Momordica charantia*), muskmelon (*Cucumis melo* L.), snap melon (*Cucumis melo* var. *momordica* L.) and snake gourd (*Trichosanthes anguina* L.) are the most preferred (Doharey, 1983).

The melon fly attacks the fruits of the crop and often inflicts yield loss ranging from 30 to 100 per cent depending upon cucurbit species and the season (Dhillon *et al.*, 2005a). In case of bitter gourd, melon fruit fly damage is the major limiting factor in obtaining good quality fruits and high yield, often causing damage in excess of 50 per cent (Rabindranath and Pillai, 1986).

Female fruit flies prefer young, green and tender fruits for egg laying. They deposit the eggs in the fruit pulp at 2 to 4 mm depth after puncturing the fruit with ovipositor. After hatching, the maggots bore into the pulp tissue and feed. The fruit

subsequently rots or becomes malformed. Young larvae leave the rotten area and move to healthy tissue, where they often introduce various pathogens and hasten fruit decay. The fruits attacked in early stages fail to develop properly and drop or rot on the plant (Dhillon *et al.*, 2005a). Infested fruits become completely non consumable, leading to significant reduction in the total yield.

Almost all the commercially grown varieties of bitter gourd are susceptible to melon fruit fly. As the fly oviposits in the fruit pulp and the maggots remain inside the fruits, it is difficult to control this pest with insecticides. Moreover, application of insecticides during fruiting stage cannot be recommended. Viable alternative management measures are hardly available against the melon fly. Hence there is a need to develop eco-friendly management options against melon fly. Thus host plant resistance, which is an important component of integrated pest management, is yet to be fully exploited in the case of bitter gourd, in spite of the considerable genetic variability reported in the crop.

The pest resistance in plants has often been attributed to morphological as well as biochemical features of the host plants. Therefore, in order to develop resistant varieties, it is important to understand the morphological and biochemical bases of resistance.

Thakur *et al.* (1996) reported that melon fly infestation in bitter gourd was positively correlated with flesh thickness, fruit diameter and fruit length. Flesh thickness and fruit diameter together explaining for 93 per cent and flesh thickness and fruit length explained for 76.3 per cent of total variation to fruit fly infestation and larval density per fruit respectively. In addition, a number of biochemical factors such as solasodine, polyphenol oxidase and peroxidase have been reported to be associated with insect resistance in crop plants and at times could be more significant than morphological factors in conferring resistance (Prabhu *et al.*, 2009).

In this background the present study titled “Morphological and biochemical bases of resistance to melon fruit fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) in bitter gourd (*Momordica charantia* L.)” was undertaken with the following objectives:

1. Evaluation of degree of resistance to melon fly in selected accessions
2. Identification of morphological and biochemical bases of resistance
3. Assessment of the olfactory response of melon fly to extracts of selected accessions

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The literature pertaining to the variation in resistance of bitter gourd accessions to melon fruit fly, *Bactrocera cucurbitae*, morphological and biochemical bases of resistance to fruit fly in bitter gourd and studies related to the olfactory responses in melon fruit fly are reviewed here.

2.1 SCREENING OF BITTER GOURD ACCESSIONS FOR RESISTANCE AGAINST MELON FRUIT FLY

Several workers have screened bitter gourd genotypes for resistance to melon fruit fly over the last four decades. Singh *et al.* (1977) screened various cultivars of bitter gourd for resistance to melon fly and found all the cultivars to be equally susceptible. The per cent fruit infestation ranged from 29.4 to 48.7 with lowest damage in BG-12 and the highest in BG-9 as well as BG-11 cultivars.

Sixty six accessions of bitter gourd were screened for fruit fly resistance by Padmanabhan (1989) who classified them into highly susceptible, moderately susceptible and moderately resistant types. Bitter gourd varieties 'Green Rough', 'White Rough' and 'White Smooth' reportedly showed resistance to fruit fly.

In Ludhiana (Punjab) twenty eight genotypes of bitter gourd were evaluated for their resistance to fruit fly and none of the genotypes were free from attack of the fruit fly. However, six cultivars *viz.* AC-23 (10.6%), ACC-23-5 (11.2 %), ACC-25 (12.6%), ACC-28 (11.8%) and ACC-33 (12.9%) showed moderate resistance. Two cultivars, ACC-16-3 and Kalyanpur Sona were found to be highly susceptible with more than 20 per cent damage (Thakur *et al.*, 1992).

Thakur *et al.* (1994) screened ten cultivars of bitter gourd and recorded lowest fruit infestation of 9.9 per cent in C-96 which was significantly lower than that

of Kalyanpur Sona which recorded the highest value of 17.0 per cent. Peter (1998) reported that more prickly variety 'Phule BG 4' was comparatively resistant to fruit fly.

Pareek and Kavadia (1994)³ studied the relative preference of melon fruit fly for different cucurbits and reported that musk melon, round melon, bitter gourd and long melon were the most preferred hosts, with 83.12-84.71, 76.62-85.50, 73.46-78.77 and 67.41-75.95 per cent infestation respectively. In comparison, ridge gourd, sponge gourd, cucumber, pumpkin and bottle gourd were found to be less preferred, with 2.83-7.95, 7.82-13.41, 9.71-14.70, 11.88-23.83 and 13.08-17.52 per cent infestation respectively.

Gupta and Verma (1995) observed slower rate of development rate, lower fecundity and high mortality of melon flies reared on sponge gourd as against those reared on bitter gourd and cucumber.

An experiment carried out by Singh *et al.* (2000) to study the host preference of melon fly for various cucurbits revealed that bitter gourd was the most preferred host (31.27% infestation) compared to pumpkin (21.61%), cucumber (21.46%), and long melon (21.18%). Water melon (28.55%), bottle gourd (25.30%) and musk melon (23.95%) were moderately preferred hosts.

Choubey and Yadav (2000) screened twelve cucurbits including bitter gourd, to assess their tolerance to melon fruit fly. Musk melon and long melon recorded the highest per cent of fruit infestation whereas little gourd and snake gourd recorded significantly lower fruit infestation than other cucurbits. Summer squash suffered the highest (86.48%) reduction in fruit weight, while snake gourd suffered the lowest weight loss (11.42%).

In an experiment conducted in Nepal to analyze the response of bitter gourd varieties to fruit fly, the variety Creeper was found to be superior to the Green Long in terms of resistance to melon fly. Variety Green Long was found more prone to damage by fruit fly (68%) as compared to Creeper (42%) (GC, Y.D. 2001).

Rajpoot *et al.* (2002) evaluated the response of melon fruit fly to thirteen cucurbits *viz.*, cucumber, melon, water melon, round gourd, bottle gourd, smooth gourd, ridge gourd, bitter gourd, pumpkin, long melon, ash gourd, snake gourd and pointed gourd. Maximum emergence of fruit flies was observed in melon as well as bottle gourd and minimum in round gourd. The long melon was recorded as the most preferred host, bitter gourd as the preferred host, melon and round gourd as moderately preferred hosts and cucumber, bottle gourd and snake gourd were recorded as the least preferred hosts.

Kutty and Dharmatti (2005) screened forty genotypes of bitter gourd for fruit fly infestation. Among these, BLG-1 and DWD-2 recorded a cluster mean of 10.05 while IC-85619 recorded cluster mean of 7.60 for fruit fly infestation.

Seventeen bitter gourd genotypes were evaluated for their resistance to melon fruit fly during the rainy season of 2001 and summer season of 2002 in Hisar, Haryana. Genotypes IC 213311, IC 248282, IC 256110, IC 248254, IC 248281, IC 248292 and IC 68314-B showed resistance to the melon fruit fly in both rainy and summer seasons. Melon fruit fly infestation was significantly lower in IC 256185 (9.4%) and IC 248256 (10.2%) compared to 82.1 per cent infestation in the susceptible variety, Pusa Do Mausmi. The genotypes with lower infestation levels had fewer number of maggots in their fruits indicating a positive correlation between fruit infestation and number of larvae per fruit (Dhillon *et al.*, 2005a).

Nath and Bhushan (2006) screened thirteen cucurbits against melon fruit fly for two seasons during 2001 and 2002 and reported that none of the cucurbits were

free from the fruit fly attack in either of the seasons. The damage during summer season was highest in bitter gourd (26.11% and 31.96%) and minimum in pumpkin (2.78% and 1.39%). Similarly damage during rainy season was again maximum in bitter gourd (46.8% and 45.3%) and minimum in pumpkin (7.4% and 11.1%).

Screening of thirteen bitter gourd cultivars for resistance against melon fly carried out by Gogi *et al.* (2009) in Faisalabad (Pakistan) revealed that the per cent fruit infestation and larval density per fruit varied significantly in all tested genotypes. Based on per cent fruit infestation (<20%) and larval density per fruit, the genotypes COL-II and FSD-long with 17.7 to 19.65 per cent fruit infestation and one to two larvae per fruit were categorized as resistant while the genotypes COL – Nankana, Sahib, COL-I and GS-51, with 20 to 50 per cent fruit infestation and three to six larvae per fruit, were categorized as moderately resistant. COL-III, COL-Multan, COL-Vehari, Chamman, Sunder-F1, Janpuri, F1-484 and F1-485 with 50 to 80 per cent fruit infestation and six to ten larvae per fruit were categorized as susceptible genotypes. They also reported positive correlation between larval density per fruit and per cent fruit infestation.

Praveena (2010) screened forty eight bitter gourd genotypes for resistance against melon fruit fly. The genotypes Madurai Local and Changanassery Local-1 were categorized as moderately resistant under field screening and highly resistant under laboratory screening.

In yet another study, forty eight genotypes of bitter gourd were screened against melon fruit fly under field conditions. The average fruit infestation and number of larvae per fruit ranged from 11.05 per cent (IC-213311) to 76.22 per cent (IC-85619-A) and 3.01 (IC-213311) to 9.24 (IC-44410) respectively. None of the genotypes were found to be highly resistant, while six genotypes *viz.*, IC-213311, IC-256185, IC-248256, IC-248282, MC-58 and HK-127 were categorized as resistant. Thirty-five genotypes were moderately resistant, six genotypes were susceptible and

genotype IC-85619-A was categorized as highly susceptible. Less oviposition (12.8-21.0 eggs/fruit) was observed in fruits of resistant genotype as compared to susceptible ones (61.1-75.2 eggs/fruit) in no choice tests. Less number of eggs were deposited by the flies in fruit of resistant genotypes (2.6-14.9) than in susceptible one (67.6-97.0) under multiple choice tests as well (Singh *et al.*, 2010).

Panday *et al.* (2012) studied the varietal resistance of bitter gourd against melon fruit fly at Varanasi, Uttar Pradesh. The results of the trial revealed that, IC 248282 with 13.64 per cent fruit infestation, followed by Kerala collection - 1 (15.68%) and IC 68314 (18.11%) were found to be the least infested. The variety Pusa Do Mausami with 81.57 per cent fruit infestation was the most susceptible, followed by Arka Harit (78.17%) and Jaunpuri (76.21%). They reported that, out of the 74 genotypes screened, five genotypes i.e., VRBT-4, DRAR-1, IC-248282, IC-68314 and Kerala collection -1 were resistant, with melon fruit fly infestation in the range of 11- 20 per cent. The moderately resistant category (21-50%) included 61 genotypes. Five genotypes viz. VRBT- 21, VRBT - 22, VRBT- 38, VRBT- 93 and VRBT - 175 were found to be susceptible and three varieties viz. Jaunpuri, Arka Harit and Pusa Do Mausami were reported as highly susceptible.

2.2 SCREENING OF OTHER CUCURBITS FOR RESISTANCE AGAINST MELON FLY

In one of the earliest studies on resistance to melon fruit fly, Nath (1966) evaluated thirty cultivars of pumpkin, bottle gourd, sponge gourd and ridge gourd and observed that although no entry showed true resistance, they exhibited varied degree of susceptibility. Sponge gourd variety Pusa Chikni showed high susceptibility, and NS-14 showed moderate resistance. The ridge gourd varieties NR-2, NR-5 and NR-7 showed moderate degree of resistance and variety Pusa Nasdar exhibited low susceptibility. Eighty two lines of pumpkin were screened for resistance and the lines

IHR-35, 40, 79-2, 83 and 86 were found to have high resistance combined with high yield. (Nath, 1966).

Gupta and Verma (1978) screened eleven cucurbit crops for their relative susceptibility to the melon fruit fly, which revealed highest per cent incidence in snap melon (57%) and lowest in summer squash. The order of susceptibility of remaining cucurbits was bitter gourd, mush melon, water melon, citron smooth gourd, ridge gourd, bottle gourd, squash melon and pumpkin.

Pal *et al.* (1983), who screened fifty indigenous as well as exotic musk melon collections against melon fruit fly for three consecutive years revealed that the insect damage was upto 20 per cent in the first year. During second year, the collections 2, 9, 11, 41, 42, 43 and 44 retained high level of resistance, however in third year only No. 19 showed high level of resistance while No. 41 and 44 remained in the resistant class.

Shivarkar and Dumber (1985) screened ten cultivars of water melon against melon fly infestation and reported that none of the varieties were resistant.

Pareek and Kavadia (1994)^b carried out field studies at Udaipur, Rajasthan to evaluate varieties of musk melon for their resistance to melon fruit fly and reported that none of the varieties were free from attack of the pest. However, significant variation was observed in the degree of infestation among the varieties. The maximum damage was recorded on Durgapura Madhu (83.12-84.71%) followed by Punjab Hybrid (75.74-80.07%). The varieties Hara Gola (73.48-74.72%), Pusa Sharbati (69.20-71.97%), Arka Rajhans (71.18-75.19%), Hira Madhu (72.49-74.18%) and Punjab Sunhari (69.50-72.87%) also recorded high levels of damage. The rest of the varieties showed less than 60 per cent infestation and were categorized as susceptible.

An experiment was conducted in Jabalpur, Madhya Pradesh, during 1991 to evaluate the response of watermelon cultivars Arka Jyoti, MHW-4, MHW-5, MHW-6, MHW-11, MHWHM-101 and Madhu against melon fly among which Arka Jyoti was reported as the least susceptible (38.81%) under Jabalpur conditions (Choubey *et al.*, 2000).

Twenty cucumber genotypes were screened against melon fruit fly in Himachal Pradesh and none of the genotypes exhibited resistance to melon fly. However, infestation varied among the genotypes. Three genotypes were found to be moderately resistant, eight genotypes susceptible and nine as highly susceptible. The fruits of susceptible and highly susceptible genotypes contained higher amounts of total free amino acid and total sugars but lower amount of phenols than moderately resistant genotypes (Ingoley *et al.*, 2005).

Gichimu *et al.* (2008) screened four commercial watermelon cultivars available in Kenya and one local landrace viz., Sugarbaby, Crimson Sweet, Charleston Gray, Yellow Crimson and GBK-043014 for resistance against melon fruit fly. Among them, Yellow Crimson demonstrated resistance against the pest probably because of its hard rind. The Kakamega landrace was completely resistant to melon fly with no signs of infestation even on a single fruit.

In another study conducted at Arabhavi, Karnataka, reaction of bottle gourd genotypes to fruit fly infestation under natural conditions evaluated 25 genotypes which comprised of six open pollinated varieties and 19 hybrids. The genotype Anand Bottlegourd - 1 recorded the highest per cent (83.33) incidence of fruit fly followed by Sarika (72.72%), NS-443 (63.40%), Super Dhana (62.50 %), INDAM-204 (60.00%), Bio Gaurav (60.00%) and Anand (55.56 %). There was no incidence of fruit fly in the genotype Elina, whereas, low incidence was reported in US-15 (8.33%), Sharada (9.09 %), Arka Bahar (11.76 %), Champion (12.50 %) and NBBL-52 (16.66 %) (Harika *et al.*, 2012).

Amin *et al.* (2011) conducted a field experiment with a view to understand the fruit infestation by fruit fly as well as its life history on different cucurbits under laboratory conditions. The highest (71.5%) and lowest (21.0%) fruit infestation were observed on sweet gourd and ridge gourd respectively. The highest fecundity and hatching were observed on sweet gourd (56.2 per female and 53.5%) and the lowest on ridge gourd (36.7 per female and 34.8%). The highest infestation as well as shortest pre-mating, pre-oviposition, incubation, larval and pupal periods on sweet gourd indicated that it was the most favorable host for *B. cucurbitae* among the crops studied.

Shivananda *et al.* (2012) screened fifty seven pumpkin genotypes for resistance against melon fruit fly under natural epiphytotic conditions on the bases of per cent fruit infestation. The genotypes KP-3, KP-19, KP-32 and KP-38 showed 10-20 per cent fruit infestation and were categorized as resistant genotypes. They observed that these genotypes could be used as source of resistance for developing pumpkin varieties resistant to melon fruit flies.

2.3 MORPHOLOGICAL BASES OF RESITANCE

Insect plant interactions are often influenced by morphological and biochemical traits of the plants. Morphological factors interfere with feeding and oviposition by the insects (Jaiswal *et al.*, 1990). The effect of such morphological characters of bitter gourd fruits on the infestation by melon fruit fly has been studied by a number of workers. Thakur *et al.* (1996) had reported that melon fruit fly infestation was negatively correlated with fruits per plant and total marketable yield. There was, however, significant and positive correlation ($r= 0.96$) between per cent fruit infestation and larval density per fruit. These two were, in turn, positively correlated with flesh thickness, fruit diameter and fruit length. Flesh thickness and

fruit diameter together explained 93 per cent while flesh thickness and fruit length together explained 76.3 per cent of total variation to fruit fly infestation and larval density per fruit respectively.

Chelliah and Sambandam (1971) observed that egg laying by the melon fruit fly was lower (17.8%) in fruits having tough rind, such as wild melon as compared to susceptible variety (87.33%), Delta Gold which had soft rind. Jaiswal *et al.*, (1990) reported that per cent fruit infestation increased with an increase in fruit length and diameter in bitter gourd.

Boller and Prokopy (1976) reported that host preference of cucurbit fruit fly was potentially regulated by different morphological factors of the host plants such as hairiness, color, smell, fruit structure etc. Hairiness, color and smell of the host plants interfere with feeding and oviposition by the insects. Characters such as fruit length, fruit diameter, roughness of the fruit surface, flesh thickness, number of ribs per fruit, and depth of fruits were positively correlated with oviposition, incubation, larval as well as pupal duration and total life span of the insect.

Evaluation of seventeen accessions of bitter gourd was carried out to identify the traits associated with resistance to melon fruit fly (Dhillon *et al.*, 2005c). There was a significant and positive correlation ($r = 0.96$) between per cent fruit infestation and larval density per fruit. Both parameters were positively correlated with depth of ribs, flesh thickness, fruit diameter and fruit length and negatively associated with fruit toughness.

Gichimu *et al.* (2008), who evaluated five accessions of water melon for resistance against melon fruit fly reported that the Kakamega landrace was completely resistant to melon fly primarily due to the hairy nature of ovary and fruitlets that deterred the adult fly from laying eggs in the fruitlets and also due to its hard rind that inhibited penetration by ovipositor.

Gogi *et al.* (2009) studied the bio-physical bases of antixenosis in bitter gourd against melon fruit fly. They reported that the larval density per fruit had a significant positive correlation ($r=0.992$) with per cent fruit infestation. The fruit-length, fruit-diameter, number of longitudinal ribs per fruit and number of small ridges per square cm area, had a significant positive correlation with the per cent fruit infestation and larval-density per fruit. However, fruit toughness, height of small ridges, height of longitudinal ribs and pericarp thickness, had a significant negative correlation with the per cent fruit infestation and larval density per fruit.

The authors further observed that morphological traits explained 100 per cent of the total variation in fruit infestation and larval density per fruit. The fruit length, fruit diameter, fruit toughness and number of longitudinal ribs accounted for 95.49 per cent of the total variation in fruit fly infestation and 99.67 per cent of the total variation in the larval density per fruit. The maximum variation, in fruit infestation and larval density per fruit was explained by fruit toughness (63.4 and 49.2 per cent respectively) followed by fruit diameter (23.22 and 22.34 per cent respectively) and number of longitudinal ribs (8.23 and 11.57 per cent respectively). They opined that these traits could be used as markers to identify resistance against melon fruit fly in bitter gourd.

Singh *et al.* (2010) conducted a study on twelve genotypes of bitter gourd viz., IC-213311, IC-256185, IC-248256, IC-248282, HK-127, MC-58, HK-112, HK-156, BL-237, Jaunpuri, IC-44410 and IC-85619-A to identify the different physico-chemical traits associated with resistance to melon fruit fly and their influence on pest multiplication. Per cent fruit infestation and larval density were significantly and positively correlated with fruit diameter and negatively correlated with fruit toughness.

Laskar and Chatterjee (2013) conducted an experiment on ten bitter gourd cultivars including open pollinated varieties, hybrids and local accessions to study the

effect of morphological traits on fruit infestation and larval density of melon fly. They reported that significant variation in per cent fruit infestation and larval density per infested fruit in different test cultivars and were correlated positively ($r=0.48$). Positive correlation of per cent fruit infestation and larval density per fruit were derived with fruit weight ($r=0.76$ and 0.75), length ($r=0.71$ and 0.72) and diameter ($r=0.68$ and 0.60). On the contrary, negative correlation were observed with ribs density ($r=-0.78$ and -0.73), ribs depth ($r=-0.24$ and -0.18) and skin toughness ($r=-0.80$ and -0.84) of fruits.

2.4 BIOCHEMICAL BASES OF RESISTANCE

Biochemical traits of the host plants play a potential role in the host-fly interaction as well as host suitability. Chelliah and Sambandam (2001) suggested that perception of chemical stimuli was well developed in *B. cucurbitae*. Robinson (1992) reported that hard rind and biochemical components, possibly including high cucurbitacin and phenol content as well as low concentration of sugars, organic acids, and minerals were some of the factors contributing to resistance against melon fly.

Tewatia *et al.* (1998) opined that chemical factors such as moisture level, ascorbic acid, reducing, non-reducing and total sugars, nitrogen, protein, phosphorus and potassium contents were responsible for the variation in levels of infestations by melon fruit fly.

Dhillion *et al.* (2005b) reported that protein, reducing sugars, non-reducing sugars and total sugars were negatively correlated with fruit fly infestation. However, the moisture content showed a positive association with fruit fly infestation and larval density per fruit. Moisture, potassium and reducing sugar content explained 97.4 per cent of the total variation in fruit infestation, while moisture, phosphorus, protein, reducing and total sugars explained 85.7 per cent variation for larval density per fruit.

Gichimu *et al.* (2008) evaluated five bitter gourd cultivars and found that a local cultivar called 'Kakamega land race' was completely resistant to melon fruit fly. They suggested that the resistance could be attributed to the high level of cucurbitacin in this accession.

Singh *et al.* (2010) observed that protein, total phenol, reducing sugar, non-reducing sugar and total sugar were significantly and negatively correlated with fruit fly infestation ($r=-0.85$ to -0.98) and larval density per fruit, while moisture content was positively associated with fruit fly infestation ($r=0.89$) and larval density per fruit ($r=0.89$).

Praveena (2010) who screened forty eight bitter gourd genotypes reported that correlation analysis between fruit fly infestation and biochemical traits revealed significant and positive phenotypic correlations for both protein content and moisture content with fruit fly infestation.

Haldhar *et al.* (2013) evaluated eleven genotypes of muskmelon for resistance against melon fly under field conditions. Significant differences were found in tested genotypes for fruit infestation and larval density per fruit. The authors reported that there was significant positive correlation ($r = 0.97$) between per cent fruit infestation and larval density per fruit. Total sugars, reducing sugar, non-reducing sugar and pH were consistently low in resistant genotypes but were high in susceptible ones. On the other hand, tannins, phenols, alkaloids and flavinoid contents were high in resistant lines but were low in susceptible genotypes. Total alkaloid and pH explained 97.96 per cent of the total variation in fruit fly infestation and 92.83 per cent of the total variation in larval density per fruit.

2.5 ROLE OF PLANT VOLATILES IN HOST SELECTION BY MELON FLY

Metcalf *et al.* (1983) studied the response of male melon flies to the olfactory stimulant, raspberry ketone. The nature of the response of the species to the compounds present in the stimulant were orientation, searching, and compulsive feeding. It has been suggested that host plant odours act as stimulants for the Tephritidae to bring the sexes together in the environment of suitable host plants (Bush, 1966). The raspberry ketone in picogram quantities act as attractants and compulsive feeding stimulants for male *Dacus* sp. and, under some circumstances, as oviposition stimulants for female *Dacus* sp. The strong odorant activity of phloretic acid and its methyl ester to *D. cucurbitae* males, present in a variety of plants especially in *Cucurbita pepo* L., suggests an important role for these as kairomones for host selection. The authors further observed that apart from the kairomone blend, other factors such as color, texture, and nutritional status of the potential host plants must also be important.

Pinero *et al.* (2006) evaluated the response of sexually mature host seeking female melon flies to different types of visual and chemical host-associated stimuli with the main aim of developing a monitoring device for females. Females were particularly attracted to objects of spherical shape coloured either yellow, white, or orange, the three pigments with the highest reflectance values. They reported that cucumber odour was more attractive to females than the odours of four other cultivated host fruits *viz.*, zucchini, papaya, tomato or of ivy gourd. A combination of both visual and olfactory stimuli was needed to elicit high levels of response compared to each stimulus offered alone.

Siderhurst and Jang (2010) studied the attractiveness of cucumber volatile blend to melon fruit fly. Thirty one compounds, among the volatiles collected from the puréed cucumber, elicited consistent electroantennogram responses from melon

fly females in GC-EAD runs. The identified compounds were mostly alcohols and aldehydes, many of which are commonly produced by fruits and flowers of various plants. A number of the compounds (e.g. AA, 6:al, (E)-2-hexenal (E2-6:al), 6:1-ol, 1-8:3-ol, (E)-2-octen-1-ol, linalool, and methyl geranate) that elicited EAD responses from female melon flies are also known to stimulate responses in other fruit fly species, such as *Anastrepha ludens* (Malo *et al.* 2005) and *B. dorsalis* (Lee *et al.* 1998). The predominant volatile isolated from fresh blended cucumber is E2Z6-9:al, which was also the most important compound in producing the characteristic cucumber smell perceived by humans (Schieberle *et al.* 1990). Several close analogs had also been implicated previously in melon fly attraction. One of these, (E)-6-nonenyl acetate, attracts female melon flies (Jacobson *et al.* 1971) as well as stimulates oviposition (Keiser *et al.* 1973).

Volatile components of bitter melon such as myrtenol, *cis*-hex-3-enol, benzyl alcohol, pent-1-en-3-ol, *cis*-pent-2-enol and *trans*-hex-2-enal were reported as attractant to melon fruit fly by Binder *et al.* (1989).

Pareek and Kavadia (1994)^b evaluated musk melon varieties and concluded that the variation in degree of infestation of the melon fly might be attributed to the olfactory stimuli emitted by fruits of different varieties. Varieties having strong odour registered higher infestation than varieties with weak odour. It was suggested that odouriferous differences might be due to the varied concentration of the total soluble sugars in different varieties.

Jang *et al.* (1997) tested the attractiveness of male and female oriental fruit flies *Bactrocera dorsalis* to the fresh whole leaves and leaf extracts of the hedgerow plant panax (*Polyscias guilfoylei*) in laboratory flight tunnel and cage olfactometer bioassays. Fresh, mature whole panax leaves were found to be attractive to mated female oriental fruit flies in the flight tunnel. Response of males and virgin females

was low and in most instances not significantly different from controls. The results suggested that volatile semiochemicals from this non host plant were attractive to mated female oriental fruit flies.

Siderhurst and Jang (2006) evaluated the response of fruit flies to the extracts of the tropical almond fruit, *Terminalia catappa* L., a preferred host of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Tephritidae: Dacini). It was tested for the attractance with both male and female flies in three separate bioassays. Multi-choice laboratory olfactometer tests showed female biased responses to an ethanol extract of *T. catappa*. These results were confirmed by laboratory wind tunnel experiments, with mature mated females responding significantly more to the ethanol extract than males, immature, or virgin females. The male activity was presumably due to the presence of methyl eugenol, in the *T. catappa* extracts by GC/MS analysis. These results suggest that while the compound(s) responsible for the attraction of *B. dorsalis* to *T. catappa* were largely unknown, methyl eugenol may play an important role in the interaction of fly and host.

Hany *et al.* (2013) evaluated the olfactory preference for egg laying in *Citrus* sp. by *Drosophila melanogaster*. Flies reportedly detected terpenes characteristic of these fruits via a single class of olfactory sensory neurons, expressing odorant receptor *Or19a*. Results indicated that a single dedicated olfactory pathway determined oviposition fruit substrate choice.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present investigation titled “Morphological and biochemical bases of resistance to melon fruit fly, *Bactroceracucurbitae*(Coquillett) (Diptera: Tephritidae) in bitter gourd(*Momordicacharantia* L)”, was carried out by a field trial at the Instructional Farm, College of Horticulture, Kerala Agricultural University, Thrissur (10° 31’N latitude and 76°17’E longitude and at an elevation of 40 m above mean sea level) during October 2013 to March 2014. The experimental site had a well-drained sandy loam soil and experienced a warm humid tropical climate. The details of the materials used and the methods followed in the study are described in this chapter.

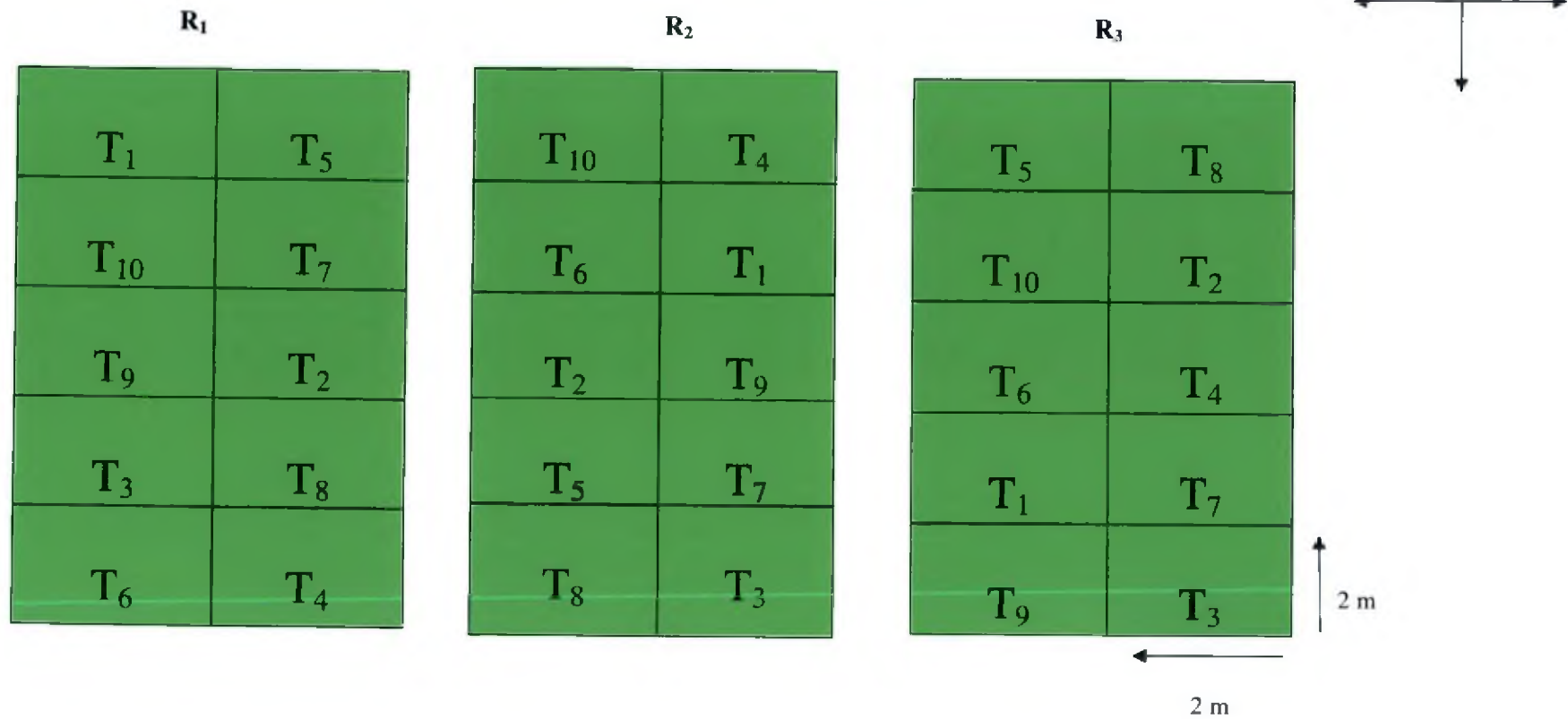
3.1 EXPERIMENTAL DETAILS

Ten accessions of bitter gourd comprising of three accessions from National Bureau of Plant Genetic Resources (NBPGR) Regional Station, Thrissur, three varieties released from Kerala Agricultural University, three accessions obtained from All India Co-ordinated Resesarch Project on Vegetables, College of Horticulture, Vellanikkara and one variety released from Tamil Nadu Agricultural University were evaluated for resistance to melon fruit fly. These genotypes constituted the treatments in the field experiment. The details of the treatments are furnished in Table 1.

3.1.1 Design and layout of field trial

The field experiment was laid out in randomized block design with ten treatments and three replications. Each replication consisted of four pits at 2x2m spacing with two plants per pit.(Figure 1 and Plate 1).

Fig. 1. Lay out of the field experiment



Treatments

T ₁	Priya	T ₆	VKB-130
T ₂	Preethi	T ₇	VKB-196
T ₃	Priyanka	T ₈	IC-0596980
T ₄	CO-1	T ₉	IC-0596981
T ₅	VKB- 136	T ₁₀	IC-0596983

Plate 1. View of the experimental plot



3.1.2 Sowing and cultural operations

Seeds of the ten genotypes were first sown in polybags and the seven day old seedlings were transplanted to the field. Agronomic practices were adopted as per the package of practices recommended by Kerala Agricultural University (KAU, 2011).

Table 1. Details of the bitter gourd accessions evaluated

Treatment	Accessions	Source
T ₁	Priya	KAU
T ₂	Preethi	KAU
T ₃	Priyanka	KAU
T ₄	CO 1	TNAU
T ₅	VKB-136	AICRP on Vegetables
T ₆	VKB-130	AICRP on Vegetables
T ₇	VKB-196	AICRP on Vegetables
T ₈	IC-0596980	NBPGR
T ₉	IC-0596981	NBPGR
T ₁₀	IC-0596983	NBPGR

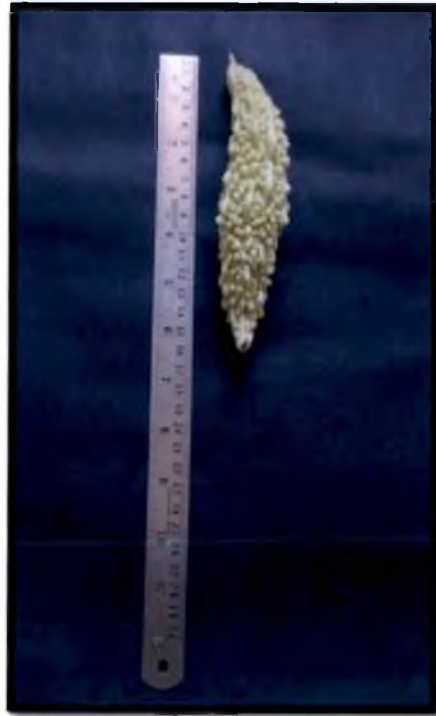
3.1.3 Observations for evaluation of degree of resistance

Individual fruits from each accession were counted and harvested as and when they were mature (Plate 2,3 and 4). The fruits were examined for infestation at three days interval and the number of infested fruits were recorded. The damaged fruits were harvested and the number of melon fly maggots in each damaged fruit were also recorded and expressed as mean larval density. Melon fruit fly incidence was calculated as given below.

Plate 2. Bitter gourd accessions used for evaluation



PRIYA



PREETHI



PRIYANKA

Plate 3. Bitter gourd accessions used for evaluation



CO-1



VKB-136



VKB-130

Plate 4. Bitter gourd accessions used for evaluation



VKB-196



IC-0596980



IC-0596981



IC-0596983

$$\text{Percentage of melon fly incidence (\%)} = \frac{\text{Number of fruits damaged per plant}}{\text{Total number of fruits per plant}} \times 100$$

3.1.4 Biometric observations

In order to study the morphological basis of resistance in bitter gourd to melon fruit fly, ten fruits were selected at random per replication for each treatment and the following observations were recorded. For each observation the mean value was worked out and expressed in corresponding units.

a) Colour of fruit

The fruit colour at the marketable stagewas recorded as per the varietal descriptor for bitter gourd by NBPGR, as follows (Plate 5):

- 1) White
- 2) Milky white
- 3) Light green
- 4) Green
- 5) Dark green

b) Flesh thickness

After recording the colour, the fruits were cut at the middle with a knife and the thickness of the flesh was measured using Vernier calipers and expressed in millimeters (Plate 7).

c) Fruit length

The length of fruit was measured from the base of the fruit to the tip and the mean length was expressed in centimeters.

Plate 5. Colour variation among fruits in bitter gourd accessions

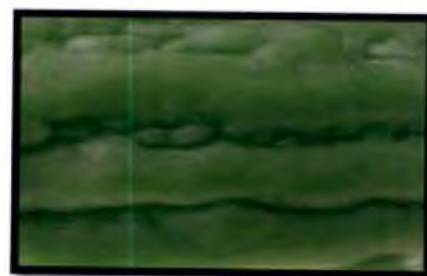


(1) Milky White (2) White (3) Light Green (4) Green (5) Dark Green

Plate 6. Spine forms of bitter gourd fruits



Pointed



Round

Plate 7. Measuring flesh thickness using Vernier calipers

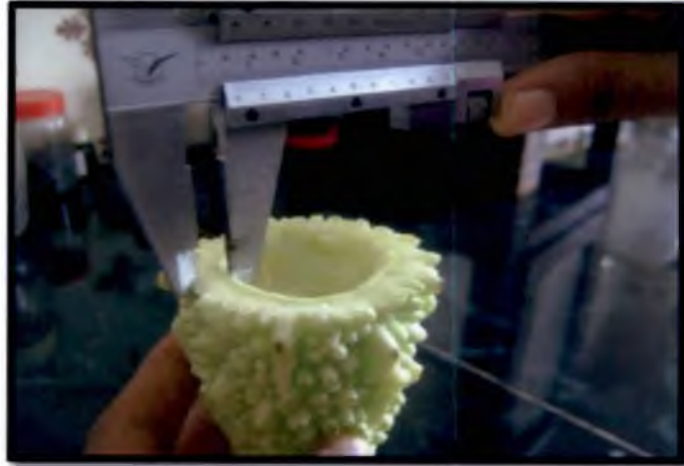


Plate 8. Measuring fruit width using Vernier calipers



d) Fruit width

Fruit width was measured using Vernier calipers and the mean width was expressed in centimeters (Plate 8).

e) Spine density

The spine density was measured by cutting a one centimeter square window on a card board sheet and placing the window over the fruits (Plate 10). The number of spines within the window was counted. The spine density at the basal, middle and apical portions of each fruit was recorded and the average of the three observations was worked out for each fruit.

f) Spine length and form

The length of the spine from the base to the tip was recorded for three spines at random, at the widest portion of the fruit. The spine form was recorded as pointed or round as per the varietal descriptor for bitter gourd by NBPGR (Plate 9 and Plate 6).

3.1.5 Biochemical characters

Biochemical traits of resistance to melon fruit fly in bitter gourd was investigated by estimating the average protein and moisture content as well as through assaying peroxidase and polyphenol oxidase enzyme activity. The studies were carried out by using three fruits per accession, following standard procedure as described below.

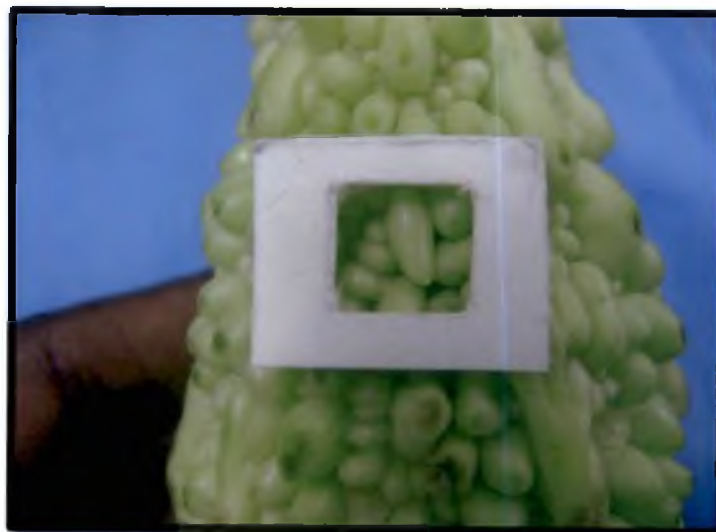
3.1.5.1 Protein content

Protein content of fruits was estimated as per the following procedure described by Lowry *et al.*, (1951) and was expressed as mg g^{-1}

Plate 9. Measuring spine length using Vernier calipers



Plate 10. Paper window used for counting number of spines in bitter gourd accessions



Five hundred milligram of the bitter gourd sample was weighed and mascerated with a pestle and mortar in 10 ml phosphate buffer (0.1 M) with pH 7.0. This sample was centrifuged and the supernatant was used for protein estimation. Hundred microlitres each of standard (0.2 mg/ml bovine serum albumin) and sample extract were pipetted out into the individual test tubes and the volume was made up to 1 ml. A tube with 1 ml of water served as the blank. Five millilitres of reagent C (50 ml of reagent A (2% sodium carbonate in 0.1 N sodium hydroxide)) and 1 ml of reagent B mixed (0.5 % copper sulphate solution in 1% sodium potassium tartarate)) was added to each tube including the blank. It was mixed well and allowed to stand for 10 minutes. Then 0.5 ml of reagent D (Folin – Ciocalteu reagent) was added, mixed well and incubated at room temperature in the dark for 30 minutes. A blue color is developed and the readings were taken at 660 nm using ELICO SL 210 UV VIS Double beam spectrophotometer.

Concentration of protein in a given sample =

$\frac{\text{Reading of test} \times \text{concentration of standard} \times 10 \text{ ml} \times 1000}{\text{Reading of standard} \times \text{weight of sample}}$

3.1.5.2 Moisture content

The moisture content in the fruit samples was determined by the hot air oven method. Ten gram of fruit was weighed and oven dried at 80° C until consistent values were obtained. The reduction in weight represented the weight of moisture lost due to drying. The moisture content was calculated as given below and expressed as per cent.

$$\text{Moisture (\%)} = \frac{W_1 - W_2}{W_1} \times 100$$

where, W_1 and W_2 represented the weight before and after drying respectively.

3.1.5.3 Peroxidase assay

Peroxidase activity in fruits was assayed as per the procedure described by Malik and Singh (1980).

One gram fruit of each accession was mascerated in 1 ml of 0.1 M phosphate buffer with pH 7.0 in a pre-cooled mortar and pestle. This homogenate was centrifuged at 18,000 rpm at 5°C for 15 minutes. The supernatant was used as the enzyme source. Three milliliters of buffer solution, 0.05 ml of guaiacol solution, 0.1 ml of enzyme extract and 0.03 ml of hydrogen peroxide solution were pipetted out into a cuvette. Readings were taken at 436 nm in ELICO SL 210 UV VIS Double beam spectrophotometer such that the absorbance was increased by 0.05. The time required in minutes (Δt) for increase in the absorbance by 0.1 was noted with the help of a stop-watch.

$$\text{Enzyme activity units/litre} = \frac{3.18 \times 0.1 \times 1000}{6.39 \times 1 \times \Delta t \times 0.1} = \frac{500}{\Delta t}$$

3.1.5.4 Poly phenol oxidase assay

Poly phenol oxidase activity in fruits was assayed as per the procedure described by Sarvesh and Reddy (1988).

The enzyme extract was prepared by grinding 5g of fresh fruit with a mortar and pestle in about 20ml medium containing 50mM Tris-HCl (pH 7.2), 0.4M Sorbitol and 10mM NaCl. This homogenate was centrifuged at 20,000 rpm for 10 minutes and the supernatant was used for assay. 0.2 ml of the extract was taken in a cuvette and mixed with 2.5 ml of 0.1M phosphate buffer (pH 6.5) and 0.3ml of catechol solution (0.01M). Readings were recorded using ELICO SL 210 UV VIS Double

beam spectrophotometer at 495 nm as the change in absorbance for every 30 seconds up to 5 minutes.

$$\text{Enzymatic units in the test} = K \times (\Delta A / \text{min})$$

where K is 0.272 for catechol oxidase and ΔA was the decrease in absorbance.

3.1.2.6 OLFACTORY RESPONSE OF FRUIT FLIES TO SELECTED ACCESSIONS OF BITTER GOURD

3.1.2.6.1 Rearing of melon fruit fly

Infested fruits containing melon fly maggots were collected from field for rearing the flies. These fruits were kept in plastic trays of size 30 x 20cm filled with soil to a depth of 4 cm for pupation of maggots. The trays were placed in rearing cages of size 0.5 x 0.5 x 0.5m³ and moistened by sprinkling water at regular intervals to prevent drying up. The trays were examined regularly for the emergence of adult flies. Freshly emerged adults were transferred to small plastic jars (250 ml) and were provided with a swab of cotton dipped in diluted honey enriched with vitamin E. Both male and female flies, identified by the presence of ovipositor, were kept in the same jar to facilitate mating. Healthy, gravid females were separated after 6-7 days of pre oviposition period and were used for the study.

3.1.2.6.2 Preparation of fruit extracts

Bittergourd fruits from each accession were harvested at the marketable stage. From each accession, 10 g of the fruit was weighed and transferred into a 100 ml glass bottle. Ten millilitres of hexane was added to the fruit sample and was stored at room temperature overnight. The extract was then filtered out using Whatman No.1 filter paper. Aqueous extract of the fruit samples were prepared by grinding 10 g of fruit sample in five ml of distilled water using a pestle and mortar followed by filtering through a Whatman No. 1 filter paper kept in a glass funnel.

3.1.2.6.3 Olfactometer studies

A non odourous fibre glass box of size 0.3 x 0.2 x 0.2 m³ with a 2 cm opening at the top was used as the test arena (Plate 11). Twenty microlitre each of aqueous as well as hexane extract was applied on to 2cm filter paper discs (Whatman No.1) using a micropipette. A third disc treated with an equal volume of distilled water was used as control. The treated filter paper discs were placed on the floor of the arena so as to form the three corners of an equilateral triangle (Plate 12). A single gravid female fly was released into the box through the opening at the top and was allowed to move freely in the box for 30 minutes. The number of visits by the fly to each disc as well as the time spent by the fly on each disc was recorded. The experiment was replicated thrice for each accession.

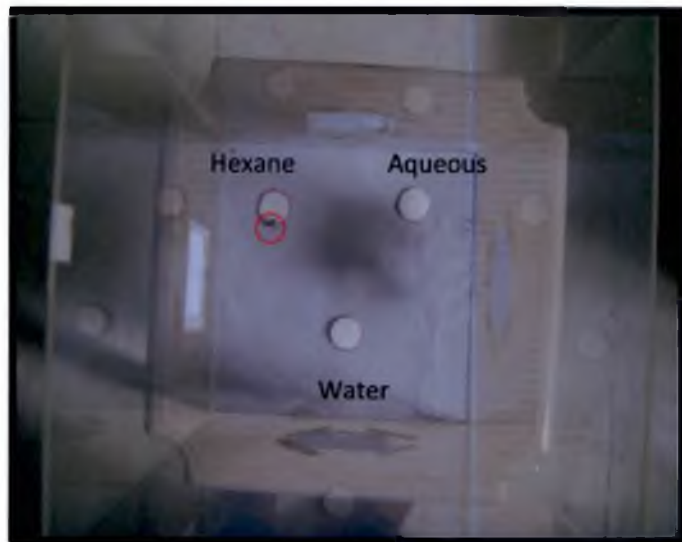
3.2 STATISTICAL ANALYSIS AND INTERPRETATION OF DATA

The data collected on number of fruits per vine, damaged fruits per vine, per cent damage and larval density among the tested bitter gourd genotypes were analyzed by one way ANOVA. The data on morphological characters and biochemical parameters were tested by Analysis of covariance (ANOCOVA), taking the number of plants for each accession as covariate. The result obtained was subjected to DMRT (Duncan's Multiple Range Test). Correlation between the morphological and biochemical traits of the bitter gourd genotypes and melon fly infestation as well as larval density were also worked out using established correlation analysis technique.

Plate 11. Fibre glass box used to study the olfactory response of melon fly



Plate 12. Melon fly visiting filter paper disc treated with fruit extract



RESULTS

4. RESULTS

The results of the investigation on “Morphological and biochemical bases of resistance to melon fruit fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) in bitter gourd (*Momordica charantia* L.)” are presented here.

4.1 EVALUATION OF DEGREE OF RESISTANCE IN BITTER GOURD ACCESSIONS TO MELON FRUIT FLY

Ten accessions of bitter gourd were evaluated for the degree of resistance based on the observations, total number of damaged fruits, number of melon fly maggots per fruit (larval density) and per cent damage, The results are presented in Table 2.

4.1.1 Mean number of fruits

The total number of fruits showed significant variation among the genotypes. The average number of fruits varied from 6.05 to 14.5. Preethi recorded the maximum number of 14.5 fruits, followed by CO 1 and VKB-196 producing 12.41 and 11.5 fruits per vine respectively. All the three accessions were statistically on par with each other but differed significantly from the remaining seven genotypes. The accessions Priyanka and VKB- 130 yielded 9.75 and 9.33 fruits per vine respectively and were at par with each other. Both IC-0596980 and IC-0596981 yielded an average of 8.58 fruits followed by VKB- 136 with 8.25 fruits. The lowest number of 6.05 fruits was recorded in the accession IC-059698.

4.1.2 Total number of damaged fruits

Considerable variation was observed among the genotypes in the number of damaged fruits (Table 2). The mean number of damaged fruits ranged from 9.41 in Preethi to 1.66 in CO 1. VKB-196 (2.33) and IC-0596981 (2.33) also recorded low number of damaged fruits, and were on par with CO 1. Priya, Priyanka and VKB-130

Table 2. Fruit infestation by melon fly and larval density in bitter gourd accessions

Sl. No.	Accessions	Mean no. of fruits	Mean no. of damaged fruits	Number of melon fly maggots/fruit	Per cent damage
1	T ₁ - Priya	10.08 ^{bcd}	6.08 ^b	18.3 ^{ab}	49.08 ^{ab}
2	T ₂ - Preethi	14.5 ^a	9.41 ^a	20.4 ^a	64.56 ^a
3	T ₃ - Priyanka	9.75 ^{cd}	5.33 ^{bc}	14 ^{bc}	50.99 ^{ab}
4	T ₄ - CO 1	12.41 ^{ab}	1.66 ^d	6.9 ^{de}	10.17 ^e
5	T ₅ - VKB-136	8.25 ^{de}	2.74 ^{cd}	10.9 ^{cde}	33.27 ^{bcd}
6	T ₆ - VKB-130	9.33 ^{cd}	3.72 ^{bcd}	15 ^{abc}	53.14 ^{ab}
7	T ₇ - VKB-196	11.5 ^{bc}	2.33 ^d	5.2 ^e	9.13 ^e
8	T ₈ - IC-0596980	8.58 ^d	2.66 ^{cd}	11.5 ^{cde}	35.67 ^{bc}
9	T ₉ - IC-0596981	8.58 ^d	2.33 ^d	12 ^{bcd}	24.57 ^{cde}
10	T ₁₀ - IC-0596983	6.05 ^e	2.83 ^{cd}	7.4 ^{de}	14.82 ^{de}

Mean values in each column followed by a common letter are not significantly different by DMRT (P = 0.05)

recorded 6.08, 5.33 and 3.72 damaged fruits respectively and were statistically on par.

4.1.3 Per cent fruit infestation

The per cent fruit infestation varied significantly in the bitter gourd genotypes evaluated. The mean value of fruit infestation ranged from 9.13 to 64.56 per cent. The lowest damage of 9.13 per cent was recorded in VKB-196 followed by CO 1 10.17 per cent which were at par. They were followed by IC-0596983 (14.82%), IC-0596981 (24.57 %), VKB-136 (33.27 %) and IC-0596980 (35.67%). Four accessions, viz. Priya (49.08 %), Priyanka (50.99 %), VKB-130 (53.14 %) and Preethi (64.56 %) were found to be the most infested accessions and were statistically on par with each other.

4.1.4 Number of maggots per fruit

The genotypes evaluated varied significantly with respect to number of maggots per fruit (Table 2). The highest number of maggots were recorded in Preethi (20.4) and the lowest in VKB-196 (5.2). The mean number of maggots in CO 1 and IC-0596983 were 6.9 and 7.4 respectively and were on par with each other. Similarly, VKB-136 and IC-0596980 recorded values of 10.9 and 11.5 maggots per fruit respectively. They were followed by IC-0596981 (12), Priyanka (14), VKB-130 (15) and Priya (18.3)

4.2 MORPHOLOGICAL BASES OF RESISTANCE

Morphological bases of resistance were ascertained by recording the fruit parameters such as colour, length, width, weight, thickness, spine length, spine density and spine form, following the NBPGR varietal descriptor for bitter gourd.

4.2.1 Fruit colour

The ten accessions exhibited marked difference in terms of the colour of the fruits (Table 3). CO 1, VKB-196 and IC-0596983 had dark green fruits while Priya, VKB-136, IC-0596980 and IC-0596981 had green fruits and Preethi, Priyanka and VKB-130 had white coloured fruits.

4.2.2 Spine form

Spine form was observed to be pointed in all the genotypes evaluated except in VKB-130 which had rounded spines (Table 3).

Table 3. Fruit colour and fruit spine form among bitter gourd accessions

Sl. No.	Accessions	Fruit colour *	Fruit spine form *
1	T ₁ - Priya	Green	Pointed
2	T ₂ - Preethi	White	Pointed
3	T ₃ - Priyanka	White	Pointed
4	T ₄ - CO 1	Dark green	Pointed
5	T ₅ - VKB-136	Green	Pointed
6	T ₆ - VKB-130	White	Round
7	T ₇ - VKB-196	Dark green	Pointed
8	T ₈ - IC-0596980	Green	Pointed
9	T ₉ - IC-0596981	Green	Pointed
10	T ₁₀ - IC-0596983	Dark green	Pointed

* Described as per Varietal descriptors for bitter gourd by NBPGR

4.2.3 Fruit weight

Weight of fruit at harvest varied from 71.63g in VKB-130 to 117.31g in CO 1 (Table 4). However, there was no significant difference in fruit weight among the genotypes in terms of fruit weight.

4.2.4 Fruit length

Length of fruit showed significant variation among the accessions (Table 4). The mean value ranged from 13.85 cm to 22.94 cm. Fruit length was highest in CO 1 and lowest in VKB-130. Genotypes Priya, VKB-196, Priyanka and VKB-136, with fruit lengths of 19.13, 18.10, 17.79 and 17.52 cm respectively were on par with each other. This was followed by Preethi (16.51 cm), IC-0596980 (15.83 cm) and IC-0596981 (14.50 cm).

4.2.5 Fruit width

The mean fruit width ranged from 2.87 cm in VKB-130 to 3.69 cm in VKB-196 (Table 4). However, all the accessions were statistically on par with each other.

4.2.6 Flesh thickness

The accessions evaluated exhibited difference in the flesh thickness which varied from 0.26 cm in VKB-130 to 0.52 cm in VKB-196. The difference, however, was not statistically significant.

4.2.7 Spine length

Spine length exhibited significant difference among the accessions (Table 4). The mean value varied from 0.21 cm to 0.57 cm in the genotypes. VKB-196 and CO 1 recorded the highest values of 0.57 cm and 0.52 cm respectively, which were at par. Genotypes Preethi, Priyanka, VKB-136, IC-0596980, IC-0596981 and IC-0596983, with spine lengths of 0.42 cm, 0.40 cm, 0.36 cm, 0.35 cm, 0.41 cm and 0.41cm

Table 4. Morphological features of fruits of bitter gourd accessions

Sl No.	Accessions	Fruit weight (g)	Fruit length (cm)	Flesh thickness (cm)	Fruit width (cm)	Spine length (cm)	Spine density (No./cm ²)
1	T ₁ - Priya	104.59 ^a	19.13 ^b	0.29 ^a	3.25 ^a	0.33 ^c	6.43 ^c
2	T ₂ - Preethi	111.68 ^a	16.51 ^{cde}	0.32 ^a	3.60 ^a	0.42 ^b	7.03 ^c
3	T ₃ - Priyanka	104.64 ^a	17.79 ^{bcd}	0.35 ^a	3.24 ^a	0.40 ^{bc}	6.50 ^c
4	T ₄ - CO 1	117.31 ^a	22.94 ^a	0.43 ^a	3.45 ^a	0.52 ^a	9.33 ^{ab}
5	T ₅ - VKB-136	105.98 ^a	17.52 ^{bcd}	0.32 ^a	3.32 ^a	0.36 ^{bc}	7.18 ^c
6	T ₆ - VKB-130	71.63 ^a	13.85 ^f	0.26 ^a	2.87 ^a	0.21 ^d	4.81 ^d
7	T ₇ - VKB-196	114.75 ^a	18.10 ^{bc}	0.52 ^a	3.69 ^a	0.57 ^a	9.83 ^a
8	T ₈ - IC-0596980	88.00 ^a	15.83 ^{def}	0.30 ^a	3.21 ^a	0.35 ^{bc}	7.12 ^c
9	T ₉ - IC-0596981	92.25 ^a	14.50 ^{ef}	0.34 ^a	3.41 ^a	0.41 ^b	7.25 ^c
10	T ₁₀ - IC-0596983	79.71 ^a	13.96 ^f	0.33 ^a	3.27 ^a	0.41 ^b	8.46 ^b

Mean values in each column followed by a common letter are not significantly different by DMRT (P= 0.05)

respectively were on par with each other. VKB-196 recorded the lowest value of 0.21 cm which was significantly lower than all other genotypes.

4.2.8 Spine density

Spine density showed significant variation between different accessions (Table 4). The number of spines varied from 4.81/cm² to 9.83/cm² among the accessions. CO 1 and VKB-196 recorded the highest values of 9.33/cm² and 9.83/cm² respectively, which were on par. They were followed by IC-0596983 which recorded spine density of 8.46/cm². Genotypes Priya, Preethi, Priyanka, VKB-136, IC-0596980 and IC-0596981 recorded 6.43, 7.03, 6.50, 7.18, 7.12 and 7.25 spine /cm² and were on par with each other. VKB-130 recorded the lowest value of 4.81/cm² which was significantly lower than other genotypes.

4.3 BIOCHEMICAL BASES OF RESISTANCE

Biochemical bases of resistance of the different bitter gourd accessions to melon fly were ascertained by estimating the biochemical parameters such as protein content, moisture content, peroxidase enzyme activity and polyphenol oxidase activity of the fruits. The results are presented in Table 5.

4.3.1 Protein content

Protein content varied significantly among the bitter gourd genotypes evaluated. The mean protein content ranged from 5.78 mg g⁻¹ to 13.30 mg g⁻¹ in different genotypes. IC-0596980 recorded the highest protein content of 13.30 mg g⁻¹ and was followed by Preethi, with 11.0 mg g⁻¹. Both the genotypes were significantly different from each other as well as from other genotypes. Genotypes Priyanka, VKB-130, VKB-136, Priya and IC-0596981 with protein content of 8.52, 8.75, 8.04, 7.74 and 7.70 mg g⁻¹ respectively were on par with each other. This was followed by IC-0596983 which recorded a value of 7.12 mg g⁻¹. CO 1 recorded the lowest value

Table 5. Biochemical parameters of bitter gourd accessions

Sl. No.	Accessions	Protein (mg gm ⁻¹)	Moisture content (%)	Peroxidase (EU/l)	Polyphenol oxidase (EU/l)
1	T ₁ - Priya	7.74 ^{cd}	91.81 ^d	228.31 ^b	4.62 ^a
2	T ₂ - Preethi	11.0 ^b	92.73 ^{bc}	236.96 ^f	3.81 ^a
3	T ₃ - Priyanka	8.52 ^c	92.46 ^c	235.84 ^f	1.77 ^a
4	T ₄ - CO 1	5.78 ^f	90.57 ^f	328.94 ^c	1.90 ^a
5	T ₅ - VKB-136	8.75 ^c	92.74 ^{bc}	245.09 ^e	3.26 ^a
6	T ₆ - VKB-130	8.04 ^{cd}	91.85 ^d	204.08 ^h	3.53 ^a
7	T ₇ - VKB-196	6.57 ^{ef}	91.42 ^{de}	490.19 ^a	1.90 ^a
8	T ₈ - IC-0596980	13.3 ^a	92.95 ^{ab}	243.90 ^e	3.53 ^a
9	T ₉ - IC-0596981	7.70 ^{cd}	93.21 ^a	297.61 ^d	4.76 ^a
10	T ₁₀ - IC-0596983	7.12 ^{de}	91.28 ^e	337.83 ^b	5.30 ^a

Mean values in each column followed by a common letter are not significantly different by DMRT (P= 0.05)

of 5.78 mg g⁻¹ which was significantly lower than that of other genotypes including 6.57 mg g⁻¹ recorded in case of VKB-196 (Table 5).

4.3.2 Moisture content

Moisture content varied significantly in all the ten accessions and it ranged from 90.57 per cent to 93.21 per cent. IC-0596981 and IC-05969830 recorded the highest values of 93.21 per cent and 92.95 per cent respectively which were on par with each other. They were followed by Preethi and VKB-136, with moisture content of 92.73 per cent and 92.74 per cent respectively which were also on par with each other. Priyanka recorded a value of 92.46 per cent and whereas the genotypes Priya, VKB-130, VKB-196 with moisture per cent 91.81, 91.85 and 91.42 per cent respectively were at par with each other. CO 1 recorded the lowest value of 90.57 per cent, which was significantly lower than that of other genotypes, including 91.28 per cent recorded in case of IC-0596983 (Table 5).

4.3.3 Peroxidase activity

VKB-196 recorded the highest enzyme content of 490.19 (EU/l) and this was followed by CO 1 and IC-596983 with a peroxidase activity of 328.94 (EU/l) and 337.83 (EU/l) respectively. IC-0596981 recorded a value of 297.61 (EU/l) while the accessions IC-0596980 and VKB-136 with of 243.90 and 245.09 (EU/l) peroxidase activity, were at par with each other. This was followed by Preethi and Priyanka with 236.96 and 235.84 (EU/l) which were on par with each other. VKB-130 recorded the lowest value of 204.08 (EU/l) which was significantly lower than that of other accessions including 228.31 (EU/l) recorded in case of Priya.

4.3.4 Polyphenol oxidase activity

Polyphenol activity was estimated in the bitter gourd accessions. Results showed values ranging from 1.77 (EU/l) to 5.30 (EU/l) among the accessions. The

maximum value was recorded in IC-0596983 and the minimum was recorded in Priyanka. However, all the accessions were statistically on par with each other.

4.4 CORRELATION OF MORPHOLOGICAL TRAITS OF BITTER GOURD FRUITS WITH FRUIT INFESTATION AND LARVAL DENSITY

Analysis was carried out to identify the correlation between morphological parameters of bitter gourd fruits and melon fly infestation. The results are presented in Table 6.

4.4.1 Fruit weight

Fruit weight was negatively correlated with per cent damage (-0.13) and the mean number of maggots per fruit (-0.06). However, the correlation was not significant.

4.4.2 Fruit length

Length of the fruit showed negative correlation with per cent fruit infestation (-0.25) and larval density (-0.19) though the correlation was not significant.

4.4.3 Fruit width

Fruit width was negatively correlated with per cent damage (-0.36) and larval density per fruit (-0.19). As in case of fruit length, correlation with per cent damage and larval density was not significant (Table 6).

4.4.4 Flesh thickness

Flesh thickness was negatively correlated with per cent damage (-0.695) and larval density (-0.692). The correlation was significant at $p=0.5$ level.

Table 6. The correlation analysis of morphological and biochemical traits of bitter gourd accessions with per cent fruit infestation and larval density per fruit.

	FWT	FT	FL	FW	SL	SD	TF	DF	PD	PC	MC	POD	PPO
FT	0.63*												
FL	0.80**	0.51 ^{ns}											
FW	0.80**	0.74*	0.41 ^{ns}										
SL	0.73*	0.91**	0.54 ^{ns}	0.89**									
SD	0.56*	0.87**	0.48 ^{ns}	0.77**	0.93**								
TF	0.69*	0.35 ^{ns}	0.52 ^{ns}	0.56 ^{ns}	0.42 ^{ns}	0.19 ^{ns}							
DF	0.19 ^{ns}	-0.37 ^{ns}	-0.07 ^{ns}	0.12 ^{ns}	-0.18 ^{ns}	-0.39 ^{ns}	0.53 ^{ns}						
PD	-0.13 ^{ns}	-0.69*	-0.25 ^{ns}	-0.36 ^{ns}	-0.62 ^{ns}	-0.81**	0.27 ^{ns}	0.82**					
PC	-0.18 ^{ns}	-0.50 ^{ns}	-0.34 ^{ns}	-0.14 ^{ns}	-0.38 ^{ns}	-0.40 ^{ns}	0.02 ^{ns}	0.37 ^{ns}	0.54 ^{ns}				
MC	-0.41 ^{ns}	-0.47 ^{ns}	-0.49 ^{ns}	-0.08 ^{ns}	-0.38 ^{ns}	-0.51 ^{ns}	-0.13 ^{ns}	0.28 ^{ns}	0.50 ^{ns}	0.70*			
POD	0.34 ^{ns}	0.90**	0.20 ^{ns}	0.65*	0.81**	0.87**	0.08 ^{ns}	-0.46 ^{ns}	0.80**	-0.50 ^{ns}	-0.47 ^{ns}		
PPO	-0.57 ^{ns}	-0.62 ^{ns}	-0.61 ^{ns}	-0.61 ^{ns}	-0.45 ^{ns}	-0.30 ^{ns}	-0.47 ^{ns}	0.15 ^{ns}	0.13 ^{ns}	0.15 ^{ns}	0.26 ^{ns}	-0.28 ^{ns}	
LD	-0.06 ^{ns}	-0.69*	-0.19 ^{ns}	-0.19 ^{ns}	-0.55 ^{ns}	-0.74*	0.32 ^{ns}	0.86**	0.94**	0.46 ^{ns}	0.49 ^{ns}	-0.77**	0.38 ^{ns}

*Correlation is significant at 0.05 level (2-tailed). **Correlation is significant at 0.01 level (2-tailed). ^{ns}Correlation is non-significant at 0.05 level (2-tailed)

FWT: Fruit weight; **FT:** Fruit thickness; **FL:** Fruit length; **FW:** Fruit width; **SL:** Spine length; **SD:** Spine density; **TF:** Total number of fruits per vine; **DF:** Damaged fruits per vine; **PD:** Per cent damage; **PC:** Protein content; **MC:** Moisture content; **POD:** Peroxidase; **PPO:** Poly phenol oxidase; **LD:** Larval density

4.4.5 Spine length

Spine length of the fruit showed negative correlation with per cent fruit infestation (-0.62) and larval density (-0.55). However the correlation was not significant.

4.4.6 Spine density

Spine density exhibited significant and negative correlation with per cent damage (-0.816) and number of maggots per fruit (-0.742).

4.4.7 Mean number of damaged fruits

Damaged fruits showed significant and positive correlation with per cent damage (0.82) as well as number of maggots per fruit (0.86).

4.4.8 Per cent fruit infestation

Per cent fruit infestation was positively and significantly correlated with number of maggots per fruit (0.94).

4.5 CORRELATION OF BIOCHEMICAL TRAITS OF BITTER GOURD FRUITS TO FRUIT INFESTATION AND LARVAL DENSITY

4.5.1 Protein content

Protein content exhibited positive correlation with per cent fruit infestation (0.54) and larval density (0.46) (Table 6). However the correlation was not significant.

4.5.2 Moisture content

Moisture content exhibited positive correlation with per cent fruit infestation (0.50) and larval density (0.49) though the correlation was not significant (Table 6).

4.5.3 Peroxidase activity

Peroxidase activity had significant and negative correlation with maggots per fruit (-0.77) as well as per cent fruit damage (0.80) (Table 6).

When the relative damage of ten accessions was calculated with respect to peroxidase activity and per cent damage, the total coefficient of variation was recorded as 64.38. When this was calculated as two separate groups based on higher and lower per cent damage, significant variation was noted in the coefficient of variation of these two groups. Mean of the three accessions VKB-196, CO 1 and IC-0596983 (with low per cent damage) showed higher coefficient of variation (47.41) than the other seven accessions (32.76).

4.5.4 Polyphenol oxidase activity

Polyphenol oxidase activity showed low correlation with per cent damage (0.13) and larval density (0.38) (Table 6).

4.6 OLFACTORY RESPONSE OF MELON FLIES TO FRUIT VOLATILES OF BITER GOURD ACCESSIONS

Response of female melon fly to fruit extracts showed variation, based on nature of extract as well as on the genotype evaluated (Table 7).

Melon fly response, in terms of number of visits as well as time spent was consistently higher in case of filter paper discs treated with hexane extracts as against discs treated with aqueous extracts. The number of visits to hexane extract treated discs varied from 4 to 13. However, the number of visits in case of discs treated with aqueous extract ranged from 1-2, recorded in case of VKB-130, IC-0596981 and IC-0596983. Discs treated with aqueous extract of four accessions, namely, CO 1, VKB-136, VKB-196 and IC-0596980, recorded no visits by the female flies.

Table 7. Number of visits and time spent by melon flies on fruit extracts of bitter gourd accessions

Sl. No.	No. of visits				Time spent (s)		
	Accessions	Hexane extract	Aqueous extract	Water	Hexane extract	Aqueous extract	Water
1	Priya	6-8	1	0	62-126	12	0
2	Preethi	7-13	1	0	76-188	19	0
3	Priyanka	9-10	1	0	78-129	20	0
4	CO 1	4-6	0	0	45-101	0	0
5	VKB-136	7-6	0	0	80-191	0	0
6	VKB-130	8-10	2	0	135-164	46-51	0
7	VKB-196	4-7	0	0	41-62	0	0
8	IC-596980	4-7	0	0	28-81	0	0
9	IC-596981	6-8	2	0	73-134	35-57	0
10	IC-596983	4-7	2	0	58-104	32-48	0

Similarly, adult flies spent more time on hexane extract treated discs when compared to filter paper discs treated with aqueous extracts of fruits. Melon flies spent 28 to 191 seconds on hexane extract treated discs, but spent only 12 to 57 seconds on discs treated with aqueous extracts.

Variation in response of adult melon flies to fruit extracts of different accessions was also observed.

The lowest number of visits was recorded in case of CO 1, VKB-196, IC-0596980 and IC-0596983, all the four recording 4 visits to hexane extract treated discs. Highest number of visits was recorded in case of Preethi (13 visits), followed by Priyanka and VKB-130, which recorded 10 visits each, again on hexane extract treated discs.

Fruit extracts of CO 1, VKB-136, VKB-196 and IC-0596980 in water elicited no response from the melon fly. One visit each was recorded in discs treated with aqueous fruit extracts of Priya, Preethi and Priyanka while the corresponding figure for VKB-130, IC-0596981 and IC-0596983 was two.

The hexane extracts of IC-0596980 recorded the lowest cumulative time of 28 seconds and was followed by VKB-196 and CO 1, recording 41 and 45 seconds, respectively. The maximum time spent by the melon fly on the treated discs was in case of VKB-136, at 191 seconds. This was followed by Preethi, which recorded 188 seconds. Melon fly females spent a maximum of 57 seconds on water extracts of the fruits of IC-596981, followed by IC-596983 (48 seconds) and VKB-130 (51 seconds). The lowest time spent on any filter paper discs treated with aqueous extracts was 12 seconds in case of Priya.

DISCUSSION

5. DISCUSSION

A study was conducted at College of Horticulture, Vellanikkara using ten bitter gourd accessions for evaluation of resistance against melon fly. The discussion on the results obtained in the study is presented hereunder.

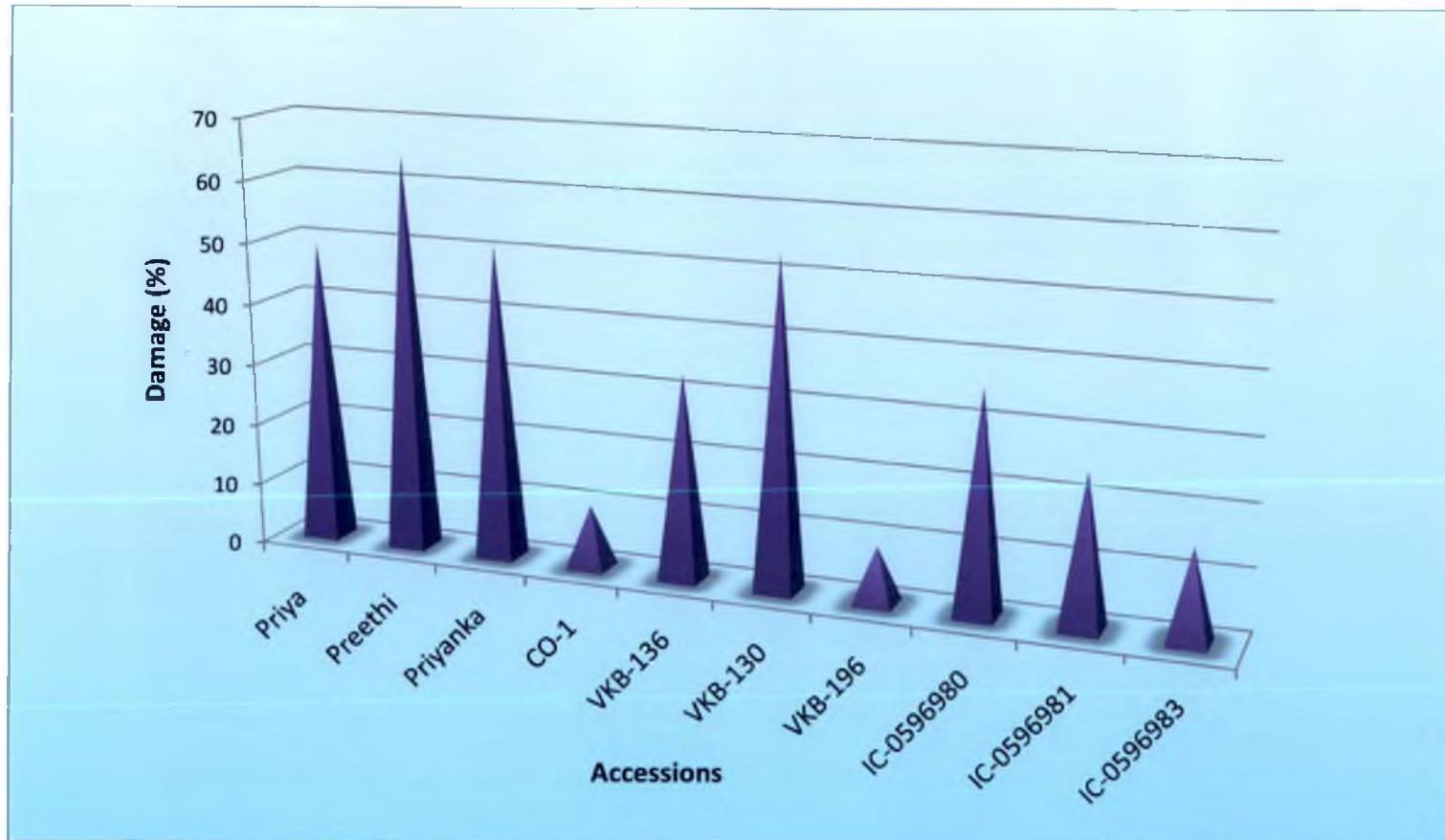
5.1 EVALUATION OF DEGREE OF RESISTANCE IN BITTER GOURD ACCESSIONS TO MELON FRUIT FLY

Screening of the ten bitter gourd accessions done at Instructional Farm, Vellanikkara showed significant variation in fruit infestation by melon fly, which ranged from 9.13 to 64.56 per cent (Figure 2). The lowest damage of (9.13%) was recorded in VKB-196 which was followed by CO 1 (10.17%), both being on par with each other. They were followed by IC-0596983 (14.82%), IC-0596981 (24.57 %), VKB-136 (33.27 %) and IC-0596980 (35.67). Four varieties, viz. Priya (49.08 %), Priyanka (50.99 %), VKB-130 (53.14 %) and Preethi (64.56 %) were found to be on par with each other, registering higher levels of infestation, by melon fly.

The above results confirm the variability in fruit infestation in bitter gourd by melon fly, as have been reported by several workers. Dhillon *et al.*, (2005c) had reported that resistant accessions recorded 8.3-12.6 per cent and susceptible accessions had 65.5-69 per cent fruit infestation. Similarly Gogi *et al.* (2009) reported that Col-II and Faisalabad-Long showed 17.7 and 19.65 per cent fruit infestation respectively and ranked them as resistant genotypes. While Col-Nankana Sahib, Col-I and GS-51, with 35-48.35 per cent fruit infestation, were reported as moderately resistant to melon flies, Col-III, Col-Multan, Col-Vehari, Chaman, Sunder-F1, Janpuri, F1-484 and F1-485, with 54.3-74.4 per cent fruit infestation were ranked as susceptible. Panday *et al.* (2012) also observed variability in fruit infestation ranging from 13.64 per cent to 81.57 per cent in screening trials against melon fly. The highest mean per cent fruit infestation was recorded in Pusa Do Mausami (81.57 %) followed by Arka Harit (78.17 %), Jaunpuri (76.21 %), and VRBT-175 (65.54 %). Fruit infestation was low in case of IC-248282 (13.64 %) and Kerala collection-1 (15.68 %).

Screening studies have identified a number of sources of resistance to melon fly. For instance, Short Green Kerali (Lall and Sinha, 1974), IIHR – 89 and IIHR – 213 (Pal *et al.*, 1983),

Fig. 2. Per cent fruit infestation in bitter gourd accessions



Hisar II, Acc.3 and Ghoti (Srinivasan, 1991), Acc. 23 and Acc. 33 (Thakur *et al.*, 1992), C 96 and NBTI 1 (Thakur *et al.*, 1994 and 1996), Kerala collection 1 and Faizabad collection 17 (Tewatia *et al.*, 1997), IC-256185, IC-248256, IC-213311, IC-248282, IC-256110, IC-248281, IC- 68314(b) (Dhillon *et al.*, 2005a), Col-I and FSD-long (Gogi *et al.*, 2009), VRBT-4, DRAR-1, IC-248282, IC-68314 and Kerala collection-1 (Panday *et al.*, 2012) have been reported as resistant to melon fly.

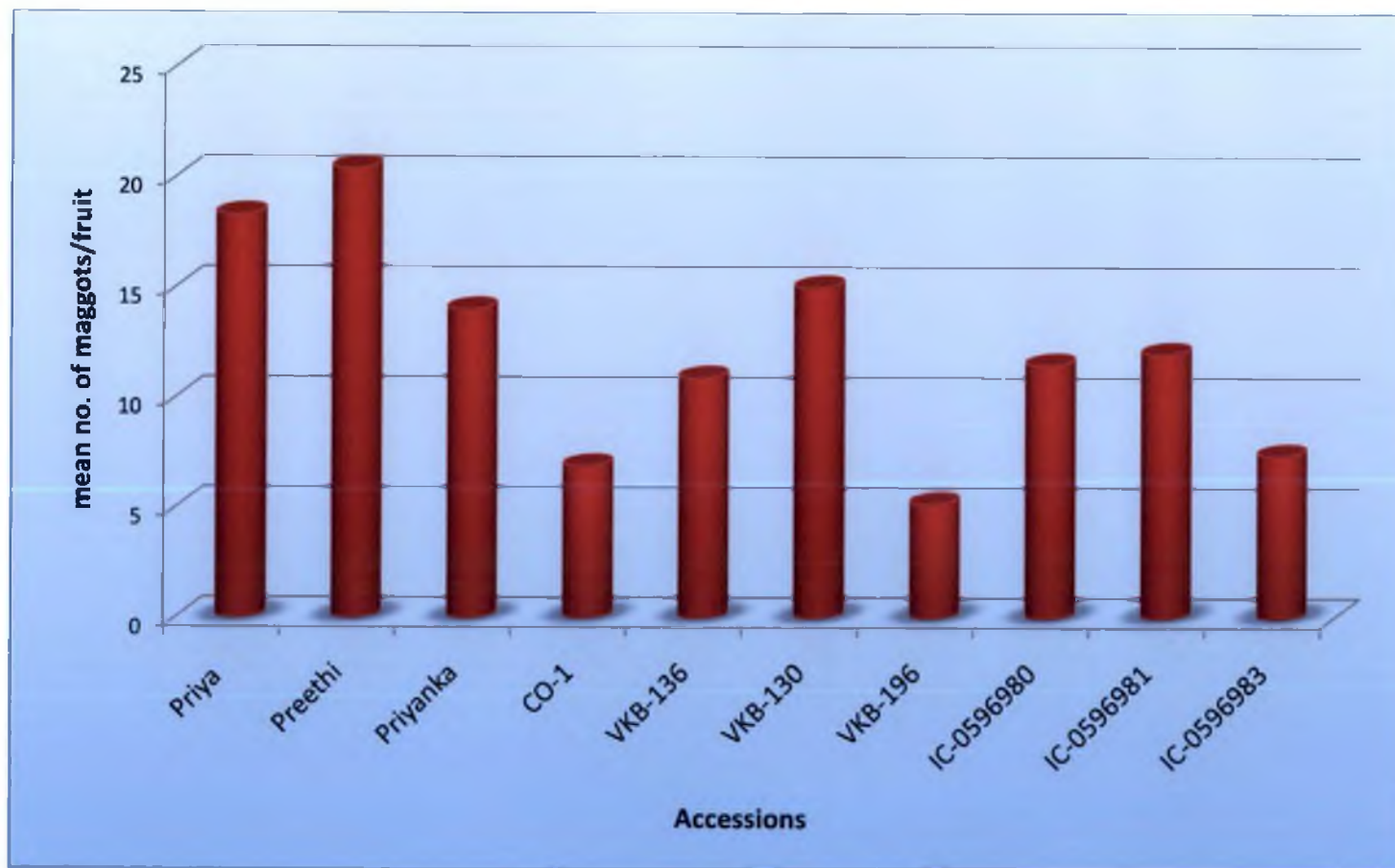
Larval density also showed significant variation in the present study, with values ranging from 5.2 to 20.4 per fruit (Figure 3). The lowest number of 5.2 maggots per fruit was recorded in VKB-196. The mean number of maggots in CO 1 and IC-0596983 were 6.9 and 7.4 respectively and were on par with each other. Similarly, VKB-136 and IC-0596980 recorded values of 10.9 and 11.5 maggots per fruit respectively. They were followed by IC-0596981 (12), Priyanka (14), VKB-130 (15) and Priya (18.3). The highest number of maggots (20.4) was recorded in Preethi.

The above results are in agreement with of Dhillon *et al.*, (2005a), who evaluated seventeen bitter gourd cultivars including six wild and eleven cultivated genotypes of bitter gourd for fruit-infestation and larval-density per fruit. The resistant accessions recorded 3.8 to 5.1 larvae per fruit and susceptible accessions showed 7.8 to 8.5 larvae per fruit. Gogi *et al.*, (2009) also had reported that the larval density (2.4 to 9.35 larvae per fruit) were significantly lower in resistant genotypes as compared to susceptible genotypes.

Correlation analysis showed significant positive correlation (0.94) between fruit infestation and number of maggots per fruit. Gogi *et al.*, (2009), had reported a similar positive correlation of 0.992 between fruit infestation and larval density. A significant positive correlation (0.96) between fruit infestation and number of larvae per fruit was also reported by Dhillon *et al.* (2005a) as well as Haldhar *et al.* (2013) who reported a significant positive correlation of 0.971 between fruit infestation and larval density per fruit.

The result of the present screening trials establishes the high degree of variability in per cent fruit infestation as well as mean larval density per fruit in bitter gourd. It also confirms the significant positive correlation between per cent fruit infestation and larval density, reported by a

Fig. 3. Larval density in bitter gourd accessions



number of workers. Nath (1966) had classified bitter gourd genotypes into various categories based on fruit infestation levels (Table 8).

Table 8. Susceptibility rating scale of bitter gourd accessions based on fruit damage

Sl No.	Fruit damage (%)	Rating
1.	No fruit damage	Immune
2.	1-10 % fruit damage	Highly resistance
3.	11-20 % fruit damage	Resistance
4.	21-50 % fruit damage	Moderately resistance
5.	51-75 % fruit damage	Susceptible
6.	76-100 % fruit damage	Highly susceptible

Result of the present investigation indicated that VKB-196 and CO 1 could be rated as highly resistant to melon fly, IC-0596983 as resistant, IC-0596981, VKB-136, IC-0596980 and Priya as moderately resistant and VKB-130, Preethi and Priyanka as susceptible.

However, confirmation of the resistance requires further trials over different seasons, as the above two parameters could vary from season to season. Laskar and Chatterjee (2013) reported that while larval density of melon fly among different bitter gourd varieties ranged from 6.60 ('Pundibari local') to 11.97 ('Green long') during summer season, the same was 300 to 400 per cent higher among the same varieties during rainy season.

5.2 MORPHOLOGICAL BASES OF RESISTANCE

Morphological parameters of fruits such as colour, shape, fruit weight and spine characteristics have been implicated in bitter gourd resistance to melon fly. The relationship observed between the fruit traits and melon fly infestation brought out in the study are discussed below.

CO 1, VKB-196 and IC-0596983 had dark green fruits while Priya, VKB-136, IC-0596980 and IC-0596981 had green fruits and Preethi, Priyanka and VKB-130 had white coloured fruits (Table 9).

Table 9. Influence of fruit colour on per cent damage

Sl. No.	Accessions	Colour of fruit	Per cent damage
1	T ₂ - Preethi	White	64.56 ^a
2	T ₃ - Priyanka	White	50.99 ^{ab}
3	T ₆ - VKB-130	White	53.14 ^{ab}
4	T ₁ - Priya	Green	49.08 ^{ab}
5	T ₅ - VKB-136	Green	33.27 ^{bcd}
6	T ₈ - IC-0596980	Green	35.67 ^{bc}
7	T ₉ - IC-0596981	Green	24.57 ^{cde}
8	T ₄ - CO 1	Dark green	10.17 ^e
9	T ₇ - VKB-196	Dark green	9.13 ^e
10	T ₁₀ - IC-0596983	Dark green	14.82 ^{de}

Mean values in each column followed by a common letter are not significantly different by DMRT (P= 0.05)

Fruit infestation ranging from 9.13 per cent to 14.82 per cent, was relatively low in genotypes with dark green fruits such as VKB-196, CO 1 and IC-0596983. Moderate infestation (24.57% to 49.08%) was recorded in accessions with the green coloured fruits *viz.* Priya, VKB-136, IC-0596980 and IC-0596981 while highest infestation of 50.99 per cent to 64.56 per cent was recorded in case of accessions with white fruits namely Priyanka, VKB-130 and Preethi. Experiments conducted by several authors (Drew *et al.*, 2003; Katsoyannos *et al.*, 1985; Katsoyannos and Kouloussis 2001) have indicated that *Bactrocera* species were able to discriminate colours and that some visual characteristics of host colour play an important role on oviposition site selection. Sharma and Singh (2010) reported that less preference was shown to dark green coloured fruits in brinjal fruit borer (*Leucinodes orbonalis* Guenee). Boller and Prokopy (1976) as well as Prokopy and Owens (1983) reported that physical properties such as size, shape and colour of fruits were the initial stimuli that elicited locomotory response of fruit

flies leading to host location and orientation to the potential ovipositional site. A similar relationship between fruit colour and fruit infestation could hold good in case of melon fly as well.

Spine on the bitter gourd fruits were pointed in all genotypes except in VKB-130 which had rounded spines. However, there was no relationship between spine form and fruit infestation, as both VKB-196 which recorded the lowest infestation as well as Preethi, which recorded the highest infestation had pointed spines. This indicated that the shape of the spine had little influence on the host selection by melon fly.

Variation in fruit weight among the different cultivars of bitter gourd was observed in the present study. Fruit weight varied from 71.63 g in VKB-130 to 117.31 g in CO 1. However, there was no significant difference between the genotypes in terms of fruit weight. Likewise no significant correlation was obtained between fruit weight and per cent damage as well as larval density. This is at variance with the study of Laskar and Chatterjee (2013) who reported a positive correlation between fruit weight and per cent fruit fly infestation as well as larval density in different cultivars.

Bitter gourd fruit length showed significant variation among the accessions. The longest fruits were recorded in CO 1 (22.94 cm) and the shortest fruits were recorded in VKB-130 (13.85 cm). Fruit infestation and larval density in different cultivars though were negatively correlated with fruit length, the correlation was not significant. The results of the present investigation is at variance with as earlier study by Gogi *et al.*, (2009) who had reported a significant positive correlation between fruit fly infestation and larval density on one hand and fruit length on the other.

Variation among the accessions was also observed in the flesh thickness of the fruits (Figure 4). It varied from 0.26 cm to 0.52 cm among the different accessions screened. However, there was no significant difference between the genotypes in terms of flesh thickness. Fruit fly infestation and larval density in different cultivars showed significant negative correlation with flesh thickness (-0.69 and -0.69 respectively at $p = 0.05$). Dhillon *et al.* (2005b) reported that flesh thickness was higher in resistant genotypes, which is consistent with the results of the

Fig. 4. Influence of flesh thickness on per cent damage

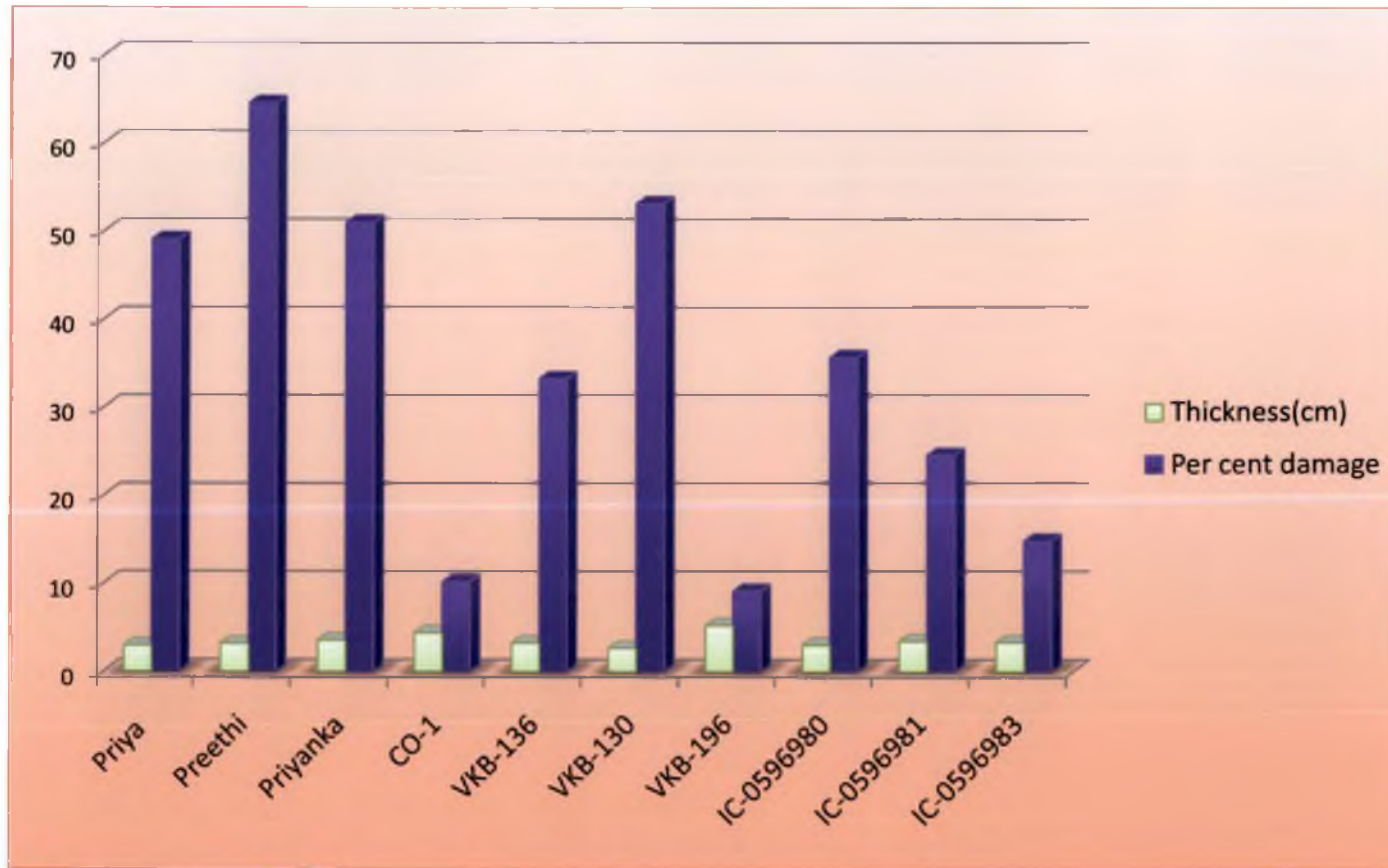


Fig. 5. Influence of spine length on per cent damage

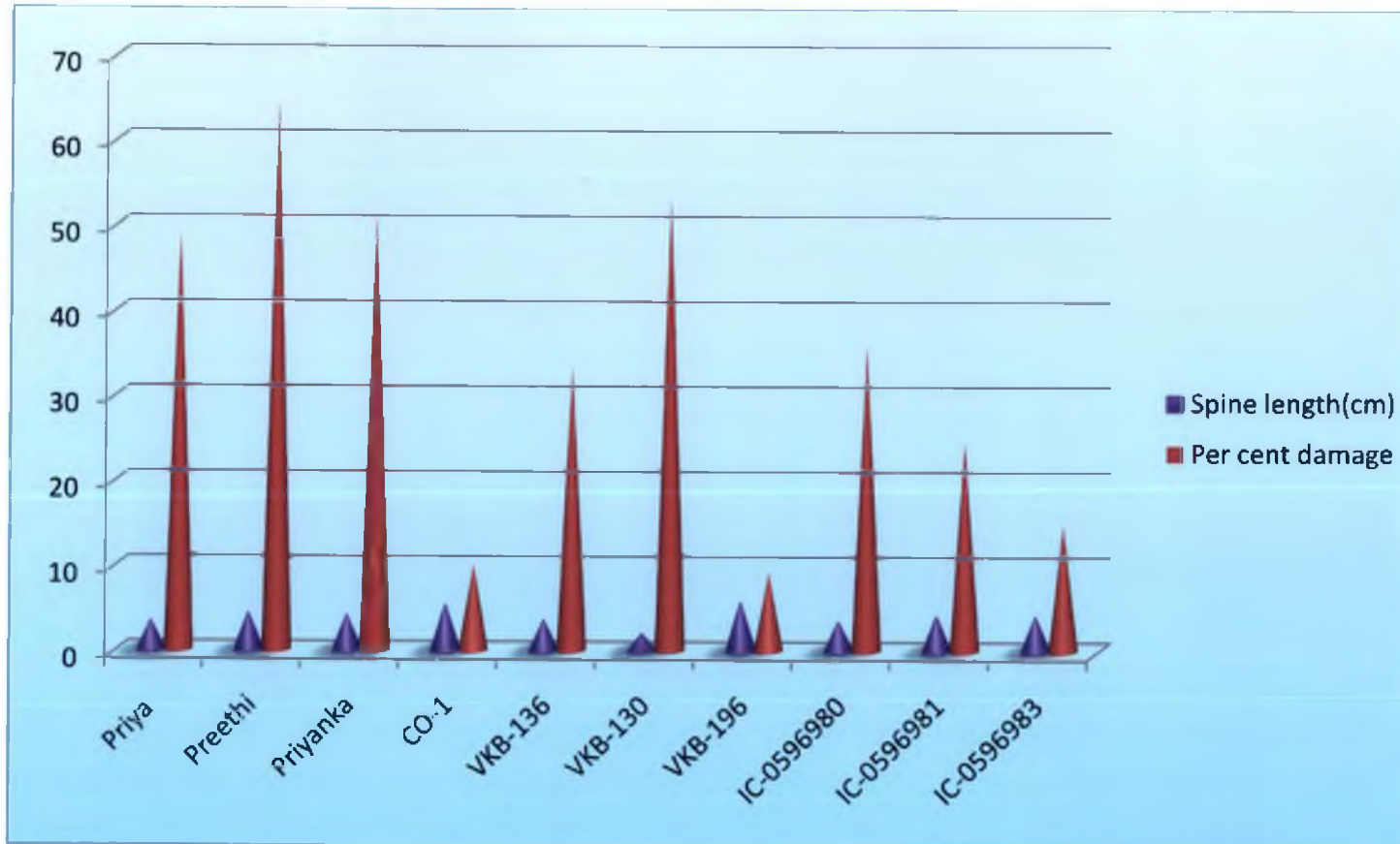
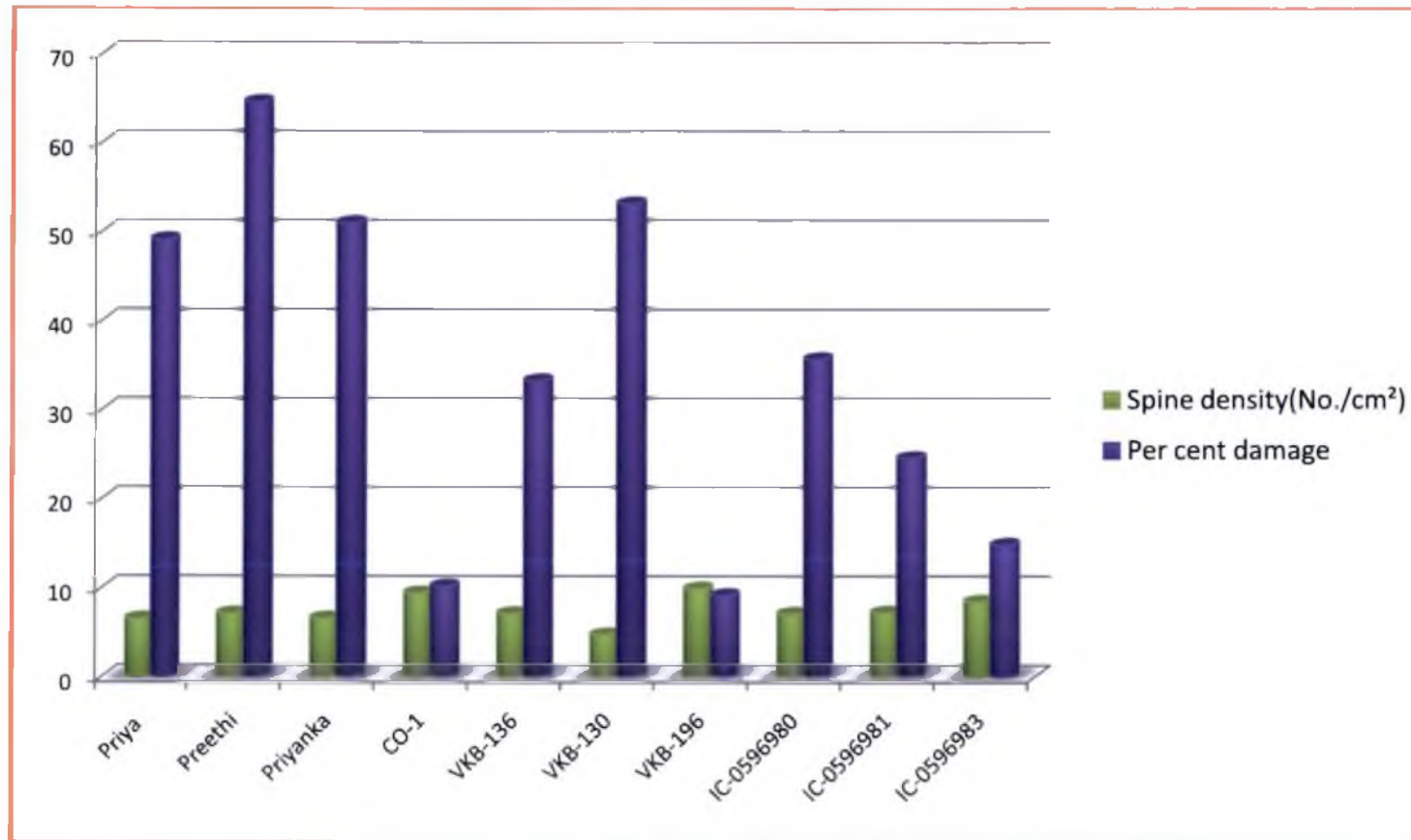


Fig. 6. Influence of spine density on per cent damage



present study. In *Cucumis callosus*, Chelliah and Sambandam (1971), observed that egg laying by the melon fruit fly was 17.77 per cent in fruits having tough rind as compared to 87.33 per cent in fruits with soft rind. Similar results have been reported by Pal *et al.*, (1983), who found thick and tough rind fruits of IHR 89 and IHR 213 genotypes to be resistant to melon fruit fly. Laskar and Chatterjee (2013) also recorded that fruit toughness was associated negatively both with per cent fruit infestation and larval density.

The mean fruit width ranged from 2.87 cm in VKB-130 to 3.69 cm in VKB-196, though the genotypes did not differ significantly in terms of fruit width. There was no significant correlation between fruit width and per cent fruit infestation as well as larval density.

Spine length recorded significant difference among the genotypes evaluated (Figure 5). VKB-196 and CO 1 recorded the highest values of 0.57 cm and 0.52 cm respectively, which were on par with each other. Genotypes Preethi, Priyanka, VKB-136, IC-0596980, IC-0596981 and IC-0596983 with spine lengths of 0.42 cm, 0.40 cm, 0.36 cm, 0.35 cm, 0.41 cm and 0.41cm respectively were at par with each other. VKB-196 recorded the lowest spine length of 0.21 cm which was significantly lower than that of other genotypes. Fruit infestation and larval density though were negatively correlated with spine length of the fruit, the correlation was not significant. Laskar and Chatterjee (2013) also had reported that negative but not significant correlation between spine length and fruit infestation as well as larval density.

Spine density showed significant variance between different accessions, with mean values varying from 4.81 to 9.83spines/cm² among the lines tested (Figure 6). VKB-196 and CO 1 recorded the highest values of 9.33/cm² and 9.83/cm² respectively, which were on par with each other. This was followed by IC-0596983 with spine density of 8.46/cm². Genotypes Priya, Preethi, Priyanka, VKB-136, IC-0596980 and IC-0596981 with spine density of 6.43, 7.03, 6.50, 7.18, 7.12 and 7.25 spines/cm² were at par with each other. VKB-130 recorded the lowest value of 4.81 which was significantly lower than that of other genotypes. Fruit infestation was negatively and significantly correlated (-0.81 at p=0.01) with spine density of the fruit. Similarly, significant negative correlation existed between spine density and larval density (-0.74 at p=0.05). Dhillon *et al.* (2005b) reported findings in agreement with the present results. They

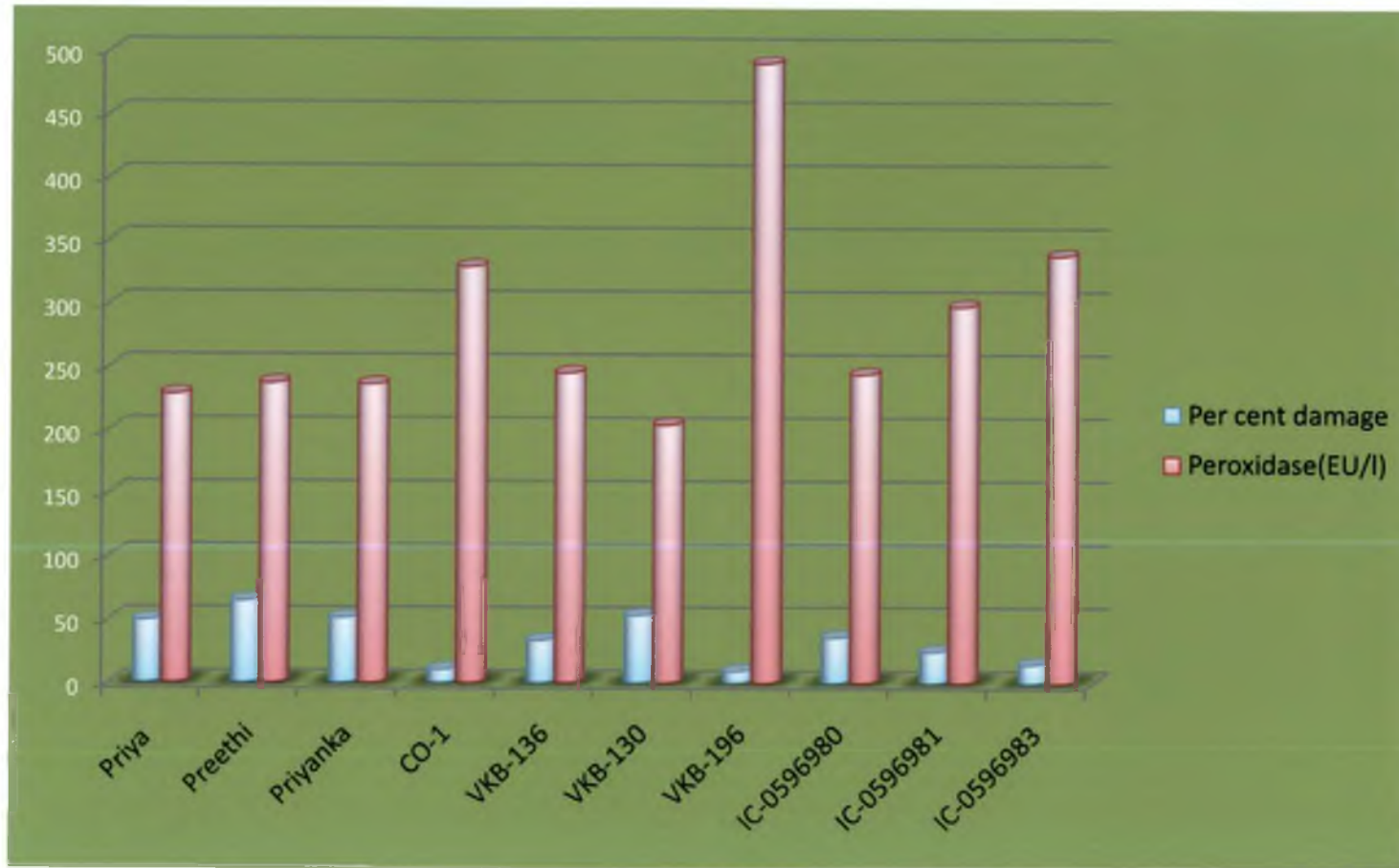
opined that greater the number of spines per unit area, lesser will be the fruit infestation and larval density per fruit. Laskar and Chatterjee (2013) also had reported a negative correlation of spine density with fruit infestation and larval density and had opined that as the number of deep spines per unit area of fruit surface increased, the adult fly might be hindered in puncturing the fruit surface with ovipositor and laying eggs, leading to ovipositional antixenosis. Results of the present study confirm the observations of earlier workers. However, while spine density appears to be more important in mediating the ovipositional behaviour of melon fly, the spine length also could be a factor as neither too few long spines nor too many small spines might serve as a physical deterrent to ovipositing flies.

5.3 BIOCHEMICAL BASES OF RESISTANCE

Biochemical parameters such as protein, moisture, peroxidase activity and poly phenol oxidase activity in fruits have been implicated in bitter gourd resistance to melon fly. The relationship between the biochemical fruit traits and melon fly infestation brought out in the study are discussed below.

The protein content in fruits of different bitter gourd genotypes varied significantly. The highest protein content of 13.3 mg g^{-1} was recorded in IC-0596980 and the lowest value of 5.78 mg g^{-1} was recorded in CO 1. Protein content was positively correlated with per cent fruit infestation (0.54) and larval density (0.46) but the correlation was not significant, suggesting that protein content had little influence on either fruit infestation or mean larval density. This explains the fact that while IC-0596980 had the highest value for protein content, the per cent fruit infestation was only 10.17, while Priya, with relatively low protein content had the highest value for fruit infestation. Similarly Preethi (11.0 mg g^{-1}) and Priya (7.74 mg g^{-1}) differed significantly in terms of protein content but the larval density, at 20.4 and 18.3 respectively, were at par with each other. Tewatia *et al.* (1998) had observed that chemical factors such as protein were also responsible for the variation in infestation by melon fruit fly. Praveena (2010) reported positive correlation between fruit fly infestation and protein content. The results of the present study are inconsistent with the above observations. Further investigations may be required to bring out the influence of protein content on melon fly infestation in bitter gourd.

Fig. 7. Influence of Peroxidase activity on per cent damage



Moisture content in all the ten accessions varied significantly and it ranged from 90.57 per cent to 93.21 per cent. IC-0596981 and IC-0596983 recorded the highest moisture content of values of 93.21 per cent and 92.95 per cent respectively which were on par with each other. This was followed by Preethi and VKB-136 with moisture content of 92.73 per cent and 92.74 per cent respectively which were also on par with each other. Priyanka recorded a value of 92.46 per cent whereas the genotypes Priya, VKB-130 and VKB-196, with of 91.81, 91.85 and 91.42 per cent moisture respectively, were at par with each other. CO 1 recorded the lowest moisture content of 90.57, which was significantly lower than that of other genotypes. There was a positive correlation between moisture content and per cent fruit infestation (0.50) as well as larval density (0.49). However the correlation was not significant. Dhillon *et al.*, (2005a) reported that moisture content had positive and significant association with fruit fly infestation and larval density. Singh *et al.* (2010) also observed that moisture content had significant positive correlation with fruit fly infestation ($r=0.89$) and larval density ($r=0.89$). Praveena (2010) reported highly significant, positive correlation for moisture content with fruit fly infestation. The results of the present study agree broadly with the above findings, appear to differ with the above findings, though not at a significant level.

Significant variation was also recorded in peroxidase activity among the genotypes evaluated (Figure 7). VKB-196 recorded the highest enzyme content of 490.19 (EU/l) and this was followed by CO 1 and IC-596983 with a peroxidase activity of 328.94 (EU/l) and 337.83 (EU/l) respectively. IC-0596981 recorded a value of 291.67 (EU/l) while the accessions IC-0596980 and VKB-136 with of 243.90 and 245.09 (EU/l) peroxidase activity, were at par with each other. This was followed by Preethi and Priyanka with 236.96 and 235.84 (EU/l) which were on par with each other. VKB-130 recorded the lowest value of 204.08 (EU/l) which was significantly lower than that of other accessions including 228.31 (EU/l) recorded in case of Priya. Peroxidase activity showed significant negative correlation with number of maggots per fruit (-0.72) as well as per cent fruit damage (-0.80). Higher peroxidase activity had consistently been linked with resistance in a number of crops, though studies linking biochemical constituents and host plant resistance were very scarce in case of cucurbits. Prabhu *et al.*, (2009) reported that the brinjal genotypes with a high or moderate level of the biochemical constituents such as

peroxidase and solasodine suffered less damage from against shoot and fruit borer infestation in brinjal.

Polyphenol activity assay showed that it varied from 1.77 (EU/l) to 5.30 (EU/l) among the accessions. The maximum value was recorded in IC-0596983 and the minimum was recorded in Priyanka. Analysis indicated that polyphenol activity was not correlated with fruit infestation and larval density. Polyphenol oxidases, though as a biochemical constituent of plant defense, appear not to be of significance at least in the bitter gourd genotypes evaluated. Phenol mediated responses studies have hardly been studied in case of bitter gourd against any pest.

5.4 OLFACTORY RESPONSE OF MELON FLIES TO FRUIT VOLATILES OF BITTER GOURD ACCESSIONS

Response of gravid female melon flies to extracts of the fruits of different accessions varied, based on genotype as well as method of extraction. The number of visits by the fruit fly ranged from 4-13 in case of filter paper discs treated with hexane extract of the fruits. The lowest number of four visits was recorded in CO 1, VKB-196, IC-0596981 and IC-0596983. The highest number of 13 visits was recorded in case of Preethi. The time spent on treated discs also varied among the accessions evaluated. The female melon fly showed more attraction by spending 191 seconds on discs treated with fruit extract of VKB-136. This was followed by Preethi, with the female fly recording 188 seconds of retention time. The corresponding value, at 28 seconds was considerably low in case of IC-0596980. The number of visits by the melon fly (1-2) was negligible in case of aqueous extracts. The time spent on treated patches varied from 12 in case of Priya to 57 seconds in case of IC-596981. In general, the melon fly females showed greater response to hexane extracts.

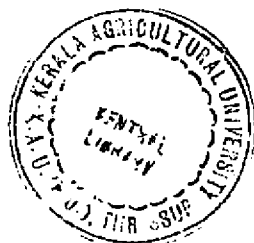
The greater number of visits as well as the higher retention on filter paper discs treated with hexane extract indicates the presence of volatiles that are soluble in organic solvents, but are insoluble in water. The number of visits as well as time spent on treated discs was greater in case of genotypes which recorded higher fruit infestation. Accessions CO 1, VKB-196 and IC-0596983 appeared to be less attractive to the female melon flies. It might be premature to comment on the exact nature of volatiles as to whether they serve as attractants or repellents based on the present study. However the presence of volatiles capable of mediating response of

gravid melon fly females was strongly indicated by the response of the flies to the hexane extract of the fruits.

These results are in agreement with that of Padmanabhan (1989), who reported that the number of flies alighting on the fruit extract ranged from 1.83 to 5.3 and that the number of ovipositional punctures varied from 12.5 to 34.16 in the filter paper discs impregnated with fruit extracts within a time period of sixty minutes. Pinero *et al.* (2006) also had reported that both visual and olfactory stimuli are very important in the process of host finding behaviour in female melon flies.

The variations in fruit infestation and larval density can be correlated with the biophysical and biochemical fruit traits (De Ponti, 1977). In the present study, the per cent fruit infestation and larval density were found to vary significantly in the ten bitter gourd genotypes evaluated. Morphological parameters like flesh thickness and spine density were significantly and positively correlated with per cent fruit infestation and larval density. Peroxidase activity was negatively and significantly correlated with fruit infestation and larval density. The olfactometer studies revealed the presence of compounds, which were soluble in organic solvents and had influenced the attractiveness of bitter gourd accessions to female melon flies

It may be concluded that considerable variation exists in bitter gourd genotypes in terms of response to melon fruit fly infestation that could be mediated by morphological and biochemical characters of particular genotype. Further studies are required to confirm the resistance, to understand the mechanisms of resistance and to develop fruit fly resistant bitter gourd accessions.



173413

SUMMARY

6. SUMMARY

The present study titled “Morphological and biochemical bases of resistance to melon fruit fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) in bitter gourd (*Momordica charantia* L)”, was carried out by a field trial at the Instructional Farm, College of Horticulture, Kerala Agricultural University, Thrissur during 2013-2014. The experiment was used to evaluate the degree of resistance in bitter gourd accessions against melon fruit fly. The objectives of the experiment were: evaluation of the degree of resistance to melon fruit fly in selected accessions of bitter gourd, identification of morphological and biochemical bases of resistance and assessment of the olfactory response of melon fruit flies to fruit extracts of bitter gourd accessions.

A field experiment was carried out in Randomised Block Design with ten treatments and three replications at Instructional Farm, Vellanikkara, Thrissur. The resistance of bitter gourd accessions to melon fly was evaluated in terms of per cent fruit damage and larval density.

- ✓ Ten bitter gourd accessions viz. accessions Priya, Preethi, Priyanka, CO-1, VKB-136, VKB-130, VKB-196, IC-0596980, IC-0596981 and IC-0596983 were used for evaluating the resistance against melon fly.
- ✓ The results showed significant variation in fruit infestation among the accessions evaluated. The lowest damage of 9.13 per cent was recorded in VKB-196 followed by CO-1 at 10.17 per cent, both being on par. Four accessions, including Priya (49.08 %), Priyanka (50.99 %), VKB-130 (53.14 %) and Preethi (64.56 %) recorded higher levels of infestation and were on par.

- ✓ Melon fly damage was found to be influenced by the fruit characters of different accessions. Dark green coloured fruits were preferred least by melon fly and the accessions yielding white fruits were found to be infested more. Accessions yielding green fruits recorded intermediate infestation.
- ✓ Correlation of infestation with fruit characters such as colour, weight, length, width, flesh thickness, spine length, spine form and spine density revealed that spine density and flesh thickness were negatively and significantly correlated with per cent fruit damage (-0.81 and -0.69 respectively) and larval density (-0.74 and -0.69 respectively).
- ✓ VKB-196, which recorded the highest values for flesh thickness and spine density (0.52 cm and 9.83/cm², respectively) recorded the lowest pest incidence of 9.13 per cent while VKB-130 with flesh thickness of 0.32cm and spine density of 4.81 cm² recorded higher damage of 53.14 per cent.
- ✓ Analysis of parameters like protein content, moisture content, peroxidase activity and polyphenol oxidase activity showed significant variation among the accessions evaluated. Negative and significant correlation of peroxidase activity was recorded with fruit fly infestation as well as larval density.
- ✓ Studies on olfactory responses of gravid female melon fly to fruit extracts recorded higher response to hexane fruit extract as against aqueous extract. Similarly melon flies showed greater attraction to those accessions which had recorded higher per cent damage, suggesting presence of volatile chemicals which might have mediated response of melon fly towards the accessions.
- ✓ Based on present investigations, VKB-196 and CO-1 may be rated as highly resistant to melon fly, IC-0596983 as resistant, IC-0596981, VKB-136, IC-

0596980 and Priya as moderately resistant and VKB-130, Preethi and Priyanka as susceptible.

- ✓ The studies also show that resistance could be mediated by fruit characters like flesh thickness, spine density and peroxidase activity. Sustained efforts could lead to development of melon fly resistant bitter gourd varieties, providing the much needed edge to melon fly management in bitter gourd.

REFERENCES

6. REFERENCES

- Amin, R. M., Sarkar, T. and Chun I. J. 2011. Comparison of host plants infestation level and life history of fruit fly (*Bactrocera cucurbitae* Coquillett) on cucurbitaceous crops. *Hort. Environ. Biotech.* **52**(5): 541-545.
- Binder, R. G., Flath, R. A. and Richard Mon, T. 1989. Volatile components of bitter melon. *J. Agric. Food Chem.* **37**(2): 418-420.
- Boller, E. F. and Prokopy, R. J. 1976. Bionomics and management of *Rhagoletis*. *Annu. Rev. Ent.* **21**: 511-512.
- Bush, G. L. 1966. *The taxonomy, cytology and evolution of the genus Rhagoletis in North America (Diptera: Tephritidae)*. Museum of Comparative Zoology, Cambridge, Mass., U.S.A., 562p.
- Chelliah, S. and Sambandam, C. N., 1971. Role of certain mechanical factors in *Cucumis callosus* (Rottl.) Cogn. in imparting resistance to *Dacus cucurbitae*. *Auaru* **3**: 48-53.
- Chelliah, S. and Sambandam, C. N. 2001. Mechanisms of resistance in *Cucumis callosus* (Rottl.) Cogn. to the fruit fly, *Dacus cucurbitae* Coq. (Diptera; Tephritidae). *Indian J. Entomol.* **36**: 98-102.
- Choubey, P.K. and Yadav, H. S. 2000. Screening of different cucurbits against melon fruit fly. *JNKVV Res. J.* **33**(1): 17-21.
- Choubey, P. K., Yadav, H. S. and Pandey, R. P. 2002. Screening of different varieties of water melon against melon fruit fly. *JNKVV Res. J.* **35**(1): 91-92.

- De Ponti, O. M. B. 1977. Resistance in *Cucumis sativus* L. to *Tetranychus urticae* Koch. The role of plant breeding in integrated control. *Euphytica* 26: 633.
- Dhillon, M. K., Singh, R., Naresh, J. S. and Sharma, N. K. 2005a. Evaluation of bitter gourd (*Momordica charantia* L.) genotypes to melon fruit fly, *Bactrocera cucurbitae* (Coquillett). *Indian J. Pl. Prot.* 33: 55-59.
- Dhillon, M. K., Singh, R., Naresh, J. S. and Sharma, N. K. 2005b. Influence of physico-chemical traits of bitter gourd, *Momordica charantia* L. on larval density and resistance to melon fruit fly, *Bactrocera cucurbitae* (Coquillett). *J. Applied Ent.* 129 (7): 395-399.
- Dhillon, M. K., Singh, R., Naresh, J. S. and Sharma, H. C. 2005c. The melon fruit fly, *B. cucurbitae*: A review of its biology and management. *J. Insect Sci.* 5: 40-60.
- Doharey, K.L. 1983. Bionomics of fruit flies (*Dacus* spp.) on some fruits. *Indian J. Ent.* 45: 406-413.
- Drew, R., Prokopy, R. J. and Romig, M. C. 2003. Attraction of fruit flies of the genus *Bactrocera* to colored mimics of host fruit. *Ent. Exp. Appl.* 107: 39-45.
- GC, Y. D. 2001. Performance of bitter gourd varieties to cucurbit fruit fly in Chitwan condition. *J. Inst. Agric. Anim. Sci.* 21: 251-252.
- Gichimu B. M., Owuor B. O. and Dida, M. M. 2008. Assessment of four commercial watermelon cultivars and one local landrace for their response to naturally occurring diseases, pests and non-pathogenic disorders in sub-humid tropical conditions. *J. Agri. Bio. Sci.* 3: 237-242.

- Gogi, M. D., Ashfaq, M., Arif, M. J. and Khan, M. A. 2009. Screening of bitter gourd (*Momordica charantia* L.) germplasm for sources of resistance against melon fruit fly *Bactrocera cucurbitae* (Coquillett) in Pakistan. *Int. J. Agric. Bio.*, **11**(6): 746-750.
- Gopalakrishnan, T. R. 2007. *Vegetable crops*. New India Publishing, New Delhi, 343p.
- Gupta, D. and Verma A. K. 1995. Host specific demographic studies of the melon fruit fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae). *J. Insect Sci.* **8**: 87-89.
- Gupta, J. N. and Verma, A. N. 1978. Screening of different cucurbit crops for the attack of the melon fruit fly, *Dacus cucurbitae* Coq. (Diptera: Tephritidae). *Haryana J. Hort. Sci.* **7**: 78-82.
- Haldhar, M. Shraavan, Bhargava, R. Choudhary, B. R., Pal Garima and Kumar Suresh. 2013. Allelochemical resistance traits of muskmelon (*Cucumis melo*) against the fruit fly (*Bactrocera cucurbitae*) in a hot arid region of India. *Phytoparasitica.* **41**: 473-481.
- Hany, K. M. D., Shima, A. M. E., Kromann, S., Bown, D., Hillbur, Y., Sachse, S., Bill, S. H. and Marcus C. S. 2013. Olfactory preference for egg laying. *Curr. Bio.* **23**: 2472-2480.
- Harika, M., Gasti, V. D., Kulkarni, M. S., Mulge, R., Mastiholi, A. B., Shirol, A. M. and Shantappa, T. 2012. Reaction of bottle gourd genotypes against fruit fly and downey mildew under natural epiphytotic conditions. *Karnataka J. Agric. Sci.* **25**(2) : 296-297.
- Ingole, P., Mehta, P. K., Chauhan, Y. S., Singh, N. and Awasthi, C. P. 2005. Evaluation of cucumber genotype for resistance to fruit fly under mid hill condition of Himachal Pradesh. *J. Ent. Res.* **29**(1): 57-60.

- Jacobson, M., Keiser I., Chambers, D. L., Miyashita, D. H. and Harding, C. 1971. Synthetic nonenyl acetates as attractants for female melon flies. *J. Med. Chem.* **14**: 236–239.
- Jaiswal, R. C., Kumar, S., Raghav, M. and Singh, D. K. 1990. Variation in quality traits of bitter gourd (*Momordica charantia* L.) cultivars. *Veg. Sci.* **17**: 186-190.
- Jang, E. B., Carvalho, L. A. and Stark, J. D. 1997. Attraction of female oriental fruit fly, *Bactrocera dorsalis*, to volatile semiochemicals from leaves and extracts of a non host plant, panax (*Polyscias guilfoylei*) in laboratory and olfactometer assays. *J. Chem. Ecol.* **23**(5): 1389-1401.
- Katsoyannos, B. I., Patsouras G. and Vrekoussi, M. 1985. Effect of color hue and brightness of artificial oviposition substrates on the selection of oviposition site by *Dacus oleae*. *Ent. Exp. Appl.* **12**: 205-214.
- Katsoyannos, B. I. and Kouloussis, N. A. 2001. Captures of the olive fruit fly *Bactrocera oleae* on spheres of different colours. *Ent. Exp. Appl.* **100**: 165-172.
- Keiser, I., Kobayashi, R. M., Miyashita, D. H., Jacobson, M., Harris, E. J. and Chambers, D. L. 1973. trans-6-Non en-1-ol acetate: An ovipositional attractant and stimulant of the melon fly. *J. Econ. Entomol.* **66**: 1355–1356.
- KAU [Kerala Agricultural University]. 2011. Package of Practice Recommendations: Crops (14th Ed.). Kerala Agricultural University, Thrissur, 267p.
- Kutty, M. S. and Dharmatti, P. R. 2005. Genetic divergence in bitter gourd. *Karnataka J. Agri. Sci.* **18**(3): 740-745.

- Lall, B. S. and Sinha, R. P. 1974. Reaction of different cucurbit varieties to invasion by melon fruit fly, *Dacus cucurbitae* Coq. *Proc. Bihar Acad. Agri. Sci.* **22/23**: 100-103.
- Laskar, N. and Chatterjee, H. 2013. Fruit infestation and larval density of melon fly, *Bactrocera cucurbitae* (Coq.) as influenced by morphological traits of bitter melon (*Momordica charantia* L.). *Int. J. Bio. Stress Manage.* **4**(1): 54-57.
- Lee, K. C., Lin J. T., and Wu C. Y. 1998. Electroantennogram of the Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera:Tephritidae) to some short-chain organic acids. *Chinese J. Entomol.* **18**: 1-12.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall R. J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
- Malik, C. P. and Singh, M. B. 1980. *Plant enzymology and histoenzymology*, Kalyani Publishers, Ludhiana, 431p.
- Malo, E. A., Cruz-López, L., Toledo, J., Del Mazo A., Virgen, A. and Rojas, J. C. 2005. Behavioral and electrophysiological responses of the mexican fruit fly (Diptera: Tephritidae) to guava volatiles. *Fla. Entomol.* **88**: 364-371.
- Metcalf, R. L., Mitcheltt, W. C. and Metcalf, E. R. 1983. Olfactory receptors in the melon fly *Dacus cucurbitae* and the oriental fruit fly *Dacus dorsalis*. *Proc. Natl. Acad. Sci. USA.* **80**: 3143-3147.
- Nath, P. 1966. Varietal resistance of gourds to the fruit fly. *Indian J. Horti.* **23**: 69-78.
- Nath, P. and Bhushan, S. 2006. Screening of cucurbit crops against fruit fly. *Ann. Plant Pro. Sci.* **14**(2): 472- 473.

- Padmanabhan, V. 1989. Host resistance studies in bitter gourd to the infestation by the fruit fly (*Dacus cucurbitae* Coquillett). M. Sc. (Ag) thesis. Kerala Agricultural University, Thrissur. 80 p.
- Pal, A. B., Srinivasan, K. and Doijode, S. D. 1983. Sources of resistance to melon fruit fly in bittergourd and possible mechanisms of resistance. *Sabrao J.* **16**: 57-69.
- Panday, A. K., Nath, P., Kumar, A. and Pai, A. B. 2012. Reaction of different bitter gourd genotypes against infestation of fruit fly (*Bactrocera cucurbitae* Coquillett). *Prog. Hort.* **44**(2): 304-306.
- Pareek, B. L. and Kavadia, V. S. 1994a. Relative preference of fruit fly, *Dacus cucurbitae* Coquillett on different cucurbits. *Indian J. Ent.* **56**: 72-75.
- Pareek, B. L. and Kavadia, V. S. 1994b. Screening of muskmelon varieties against melon fruit fly, *Dacus cucurbitae* Coquillett under field conditions. *Indian J. Ent.* **57**: 417-420.
- Peter, K.V. 1998. *Genetics and breeding of vegetables*. ICAR, New Delhi. 333 p.
- Pinero, J. C., Jacome, I., Vargas, R. and Prokopy, R. J. 2006. Response of female melon fly, *Bactrocera cucurbitae*, to host associated visual and olfactory stimuli. *Ent. Expt. Appl.* **121**(3): 261-269.
- Prabhu, M., Natarajan, S., Veeraragavathatham, D. and Pugalendhi, L. 2009. The biochemical basis of shoot and fruit borer resistance in interspecific progenies of brinjal (*Solanum melongena*). *Eur. Asia J. Bio. Sci.* **3**: 50-57.

- Praveena, V. S. 2010. Genetic variability studies for yield and fruit fly resistance in bitter gourd (*Momordica charantia* L.) M. Sc. (Ag) thesis. Kerala Agricultural University, Thrissur. 162 p.
- Prokopy, R. J. and Owens, E. D. 1983. Visual detection of plants by herbivorous insects. *Ann. Rev. Ent.* **28**: 337-364.
- Rabindranath, K. and Pillai, K. S. 1986. Control of fruit fly of bitter gourd using synthetic pyrethroids. *Entomon* **11**: 269-272.
- Rajpoot, S. K. S., Ali, S. and Rizvi, S. M. A. 2002. Relative population and host preference of fruit fly *Bactrocera dorsalis* on cucurbits. *Annals Pl. Prot. Sci.* **10**(1): 62-64.
- Robinson, R.W. 1992. Genetic resistance in the Cucurbitaceae to insect and spider mites. *Plant Breed. Rev.* **10**: 309-360.
- Sarvesh, A. and Reddy, T. P. 1988. Peroxidase, polyphenol oxidase, acid phosphatase and alkaline inorganic pyrophosphatase activities during leaf senescence in varieties of castor (*Ricinus communis* L.) *Indian J. Expl. Biol.*, **26**: 133-136.
- Schieberle, P., Ofner, S. and Grosch, W. 1990. Evaluation of potent odorants in cucumbers (*Cucumis sativus*) and muskmelons (*Cucumis melo*) by aroma extract dilution analysis. *J. Food Sci.* **55**:193-195.
- Sharma, B. N. and Singh, S. 2010. Biophysical and biochemical factors of resistance in brinjal against shoot and fruit borer. *Indian J. Entomol.* **72**: 212-216.
- Shivarkar, D. T. and Dumber, S. K. 1985. Bionomics and chemical control of melon fly, *J. Maharashtra Agric. Univ.* **10**(3): 298-300.

- Shivananda, M. M., Madalageri, M.B., Srinivas, S., Mohankumar, A. B. and Yathiraj, K. 2012. Screening of pumpkin (*Cucurbita* sp.) germplasm for sources of resistance against melon fruit fly (*Bactrocera cucurbitae*). *Int. J. Plant Prot.* 5(2): 446-447.
- Siderhurst, M. S. and Jang, E. B. 2006. Attraction of female oriental fruit fly, *Bactrocera dorsalis*, to *Terminalia catappa* fruit extracts in wind tunnel and olfactometer tests. *Formosan Entomol.* 26: 45-55.
- Siderhurst, M. S. and Jang, E. B. 2010. Cucumber volatile blend attractive to female melon fly, *Bactrocera cucurbitae* (Coquillett). *J. Chem. Ecol.* 36: 699-708.
- Singh, H. N., Srivastava, J. P. and Prasad, R. 1977. Genetic variability and correlation studies in bitter gourd. *Indian J. Agri. Sci.* 47(12): 604-607.
- Singh, S. V., Alok Mishra, Bisen, R. S. and Malik, Y. P. 2000. Host preference of red pumpkin beetle *Aulacophora foveicollis* and melon fruit fly, *Dacus cucurbitae*. *Indian J. Entomol.* 62(3): 64-66.
- Singh, V., Chillar, B. S. and Singh, R. 2010. Antibiosis and antixenosis mechanisms of resistance in bitter gourd (*Momordica charantia* L.) to melon fruit fly, *Bactrocera cucurbitae* (Coquillett). *Res. Crops* 11(1): 99-105.
- Srinivasan, K. 1991. Pest management in cucurbits – An overview of work done under AICVIP. Group Discussion of Entomologists Working in the Coordinated Projects of Horticultural Crops, 28-29 January 1991. Lucknow, Uttar Pradesh, India: Central Institute of Horticulture for Northern Plains, 44-52.
- Tewatia, A. S., Dhankhar, B. S. and Dhankhar, S. K. 1997. Growth and yield characteristics of melon fruit fly resistant and highly susceptible genotypes of bitter gourd- a note. *Haryana J. Hort. Sci.* 25: 233-255.

- Tewatia, A. S., Dhankhar, B. S. and Singh, R. 1998. Evaluation of bitter gourd (*Momordica charantia* L.) cultivars for resistance to melon fruit fly, *Bactrocera cucurbitae* (Coquillett). *Haryana J. Hort. Sci.* **27**: 266-271.
- Thakur, J. C., Khattra, A. S. and Brar, K. S. 1992. Comparative resistance to fruit fly in bitter gourd. *Haryana J. Hort. Sci.* **21**: 285-288.
- Thakur, J. C., Khattra, A. S. and Brar, K. S. 1994. Stability analysis for economic traits and infestation of melon fruit fly (*Dacus cucurbitae*) in bitter gourd (*Momordica charantia*). *Indian J. Agric. Sci.* **64**: 378-381.
- Thakur, J. C., Khattra, A. S. and Brar, K. S. 1996. Correlation studies between economic traits, fruit fly infestation and yield in bitter gourd. *Punjab Veg. Grow.* **31**: 37-40.

**MORPHOLOGICAL AND BIOCHEMICAL BASES OF
RESISTANCE TO MELON FRUIT FLY, [*Bactrocera
cucurbitae* (Coquillett)(Diptera: Tephritidae) IN BITTER
GOURD (*Momordica charantia* L.)]**

By
MANJU ROSHNI K
(2012-11-137)

ABSTRACT OF THE THESIS

submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture

**Faculty of Agriculture
Kerala Agricultural University**

**DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR-680 656
KERALA, INDIA**

2014

ABSTRACT

A study titled “Morphological and biochemical bases of resistance to melon fruit fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) in bitter gourd (*Momordica charantia* L.)” was undertaken at College of Horticulture, Vellanikkara during 2013-2014 with the objectives of evaluation of the degree of resistance to melon fruit fly in selected accessions of bitter gourd, identification of morphological and biochemical bases of resistance and assessment of the olfactory response of melon fruit flies to fruit extracts of bitter gourd accessions.

A field experiment was carried out in Randomised Block Design with ten treatments and three replications at Instructional Farm, Vellanikkara. The treatments consisted of accessions Priya, Preethi, Priyanka, CO-1, VKB-136, VKB-130, VKB-196, IC-0596980, IC-0596981 and IC-0596983. The resistance of bitter gourd accessions to melon fly was evaluated in terms of per cent fruit damage and larval density. The results showed significant variation in fruit infestation among the accessions evaluated. The lowest damage of 9.13 per cent was recorded in VKB-196 followed by CO-1 at 10.17 per cent, both being on par. Four accessions, including Priya (49.08 %), Priyanka (50.99 %), VKB-130 (53.14 %) and Preethi (64.56 %) recorded higher levels of infestation and were on par.

Melon fly damage was found to be influenced by the fruit characters of different accessions. Correlation of infestation with fruit characters such as colour, weight, length, width, flesh thickness, spine length, spine form and spine density revealed that spine density and flesh thickness were negatively and significantly correlated with per cent fruit damage (-0.81 and -0.69 respectively) and larval density (-0.74 and -0.69 respectively). VKB-196, which recorded the highest values for flesh thickness and spine density (0.52 cm and 9.83/cm², respectively) recorded the lowest incidence of 9.13 per cent while VKB- 130 with flesh thickness of 0.32cm and spine density of 4.81 cm² recorded higher damage of 53.14 per cent.

Analysis of parameters like protein content, moisture content, peroxidase activity and polyphenol oxidase activity showed a negative and significant correlation of peroxidase activity with fruit fly infestation.

Studies on olfactory responses of gravid female melon fly to fruit extracts recorded higher response to hexane fruit extract as against aqueous extract. Similarly melon flies showed greater attraction to those accessions which had recorded higher per cent damage, suggesting presence of volatile chemicals which might have mediated response of melon fly towards the accessions.

Based on present investigations, VKB-196 and CO-1 may be rated as highly resistant to melon fly, IC-0596983 as resistant, IC-0596981, VKB-136, IC-0596980 and Priya as moderately resistant and VKB-130, Preethi and Priyanka as susceptible. The studies also show that resistance could be mediated by fruit characters like flesh thickness, spine density and peroxidase activity. Sustained efforts could lead to development of melon fly resistant bitter gourd varieties, providing the much needed edge to melon fly management in bitter gourd.

173413

