MORPHOLOGICAL AND BIOCHEMICAL BASES OF RESISTANCE TO SPOTTED POD BORER, *Maruca vitrata* (FAB.) (LEPIDOPTERA: CRAMBIDAE) IN COWPEA

By

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DECLARATION

I hereby declare that the thesis entitled **"Morphological and biochemical bases of resistance to spotted pod borer, *Maruca vitrata* (Fab.) (Lepidoptera: Crambidae) in cowpea"** is a *bona fide* record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me any degree, diploma, fellowship or other similar title, of any other University or Society.

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CONTENTS

Chapter	Title	Page No
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	5
3	MATERIALS AND METHODS	18
4	RESULTS	9
5	DISCUSSION	5
6	SUMMARY	65
	REFERENCES	
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1.	Details of the cowpea accessions evaluated	20
2.	Extent of damage by *Maruca vitrata* in cowpea accessions	30
3.	Classification of the cowpea accessions based on extent of damage by *M. vitrata*	36
4.	Configuration of calyx among cowpea accessions	38
5.	Pubescence of the pods in selected accessions of cowpea	40
6.	Nature of seed testa in selected accessions of cowpea	41
7.	Morphological parameters of selected accessions of cowpea	42
8.	Biochemical parameters of selected accessions of cowpea	47
9.	Correlation between morphological parameters of cowpea and per cent damage	50
10.	Correlation between biochemical parameters of cowpea and per cent damage	52

LIST OF FIGURES

Figure No.	Title	Page No.
1.	Influence of pod wall thickness on per cent damage	57 – 58
2.	Influence of total moisture content on per cent damage	60 – 61
3.	Influence of total protein on per cent damage	60 – 61
4.	Influence of total sugar content on per cent damage	61 – 62
5.	Influence of reducing sugars on per cent damage	62 – 63
6.	Influence of peroxidase activity on per cent damage	63 – 64
7.	Influence of polyphenol oxidase on per cent damage	63 - 64

LIST OF PLATES

Plate No.	Title	Page No.
1.	View of the experimental plot	19 – 20
2.	Infestation by pod borer during different stages of cowpea	22 – 23
3.	Cowpea accessions selected for evaluation of morphological and biochemical bases of resistance	23 – 24
4.	Measurement of length of flower stalk	24 -25
5.	Measurement of trichome length	24 - 25
6.	Measurement of trichome density	24 - 25
7.	Configuration of calyx	24 - 25
8.	Configuration of calyx	24 - 25
9.	Measurement of pod wall thickness	24 - 25
10.	Measurement of pod angle	24 - 25
11.	Nature of seed testa	24 - 25
12.	Nature of seed testa	24 - 25
13.	Nature of seed testa	24 - 25

Introduction

1. INTRODUCTION

Cowpea, *Vigna unguiculata* (L.), is an important legume of the tropics and subtropics. Cowpea is a major source of dietary protein in the predominantly cereal based diet followed across the country. It is cultivated in an area of 3.9 million hectares in India, with a production of 2.21 million tonnes (Mandal *et al.*, 2009). Cowpea is used as a grain legume, vegetable and also as a fodder. It is widely cultivated in Kerala as a vegetable crop across an area of 6714 ha in Kerala (DES, 2014). However, the productivity of cowpea, at 625 kg/ha is very low, one of the reasons being infestation by a number of pests and diseases. Loss in grain yield due to pests in cowpea varied from 20 to almost 60 per cent (Singh and Allen, 1980). Among the different insect pests, legume pod borer, *Maruca vitrata* (Fab.) is the most prevalent and destructive as far as cowpea is concerned.

The legume pod borer, *M vitrata* (Fab.) (Crambidae: Lepidoptera) occurs throughout the tropics and subtropics of Central and South America, Asia and Africa. It is an important pest of tropical grain legumes because of its wide geographical distribution, host range and its ability to infest the young growing plant tips, flower buds, flowers, pods and seeds. The destructiveness at critical stages of growth *viz*, flowering and pod development constitutes a significant constraint to the productivity.

Maruca vitrata attacks cowpea during the reproductive phase. The female moth lays eggs on or near the flower buds (Sharma, 1998). The larvae feed on buds, flowers and pods. Flowers, pods and leaves are often webbed together and the infestation is indicated by the presence of frass. Being an internal feeder, management of the pest through conventional chemical means is difficult, though application of insecticides remains the primary means of management.

Given the fact that cowpea is mostly edible as a vegetable in Kerala and also that infestation occurs on the consumed parts, application of pesticides can hardly be recommended. A more integrated approach with greater emphasis on ecofriendly strategies are the need of the hour. Exploitation of host plant resistance, which is among the most, effective and ecofriendly strategies, has hardly being attempted in case of cowpea in the State.

Identification of morphological and biochemical plant characters conferring resistance to insect pests is important in breeding for resistance. Various biochemical parameters, *viz.*, total sugar, reducing sugar, non-reducing sugar, amino acids, proteins and phenols in cowpea play an important role by providing resistance to the plant against *M. vitrata* (Oghiakhe *et al.,* 1992a; Sahoo and Senapati, 2001). Anithakumari (1992) observed that parameters *viz.,* pubescence, thickness of the pod wall, configuration of calyx and thickness of testa were important morphological attributes in conferring resistance to the borer. Sunitha *et al.* (2006) reported that length as well as density of trichomes on the pods was positively correlated with pod borer resistance.

Therefore the present study titled "Morphological and biochemical bases of resistance to spotted pod borer, *Maruca vitrata* (Fab.) (Lepidoptera: Crambidae) in cowpea" was undertaken with the following objectives:

1. Evaluation of degree of resistance to spotted pod borer in selected accessions
2. Identification of morphological bases of resistance
3. Identification of biochemical bases of resistance

Review of literature

2. REVIEW OF LITERATURE

Pulses in general and cowpea in particular have been extensively screened for resistance to *Maruca vitrata.* Most of the efforts in identifying and exploiting resistance in cowpea to *M. vitrata* have focused on grain legumes and little information is available with regard to varieties/ accessions more popular for use as vegetables.

The literature pertaining to resistance in cowpea (*V. unguiculata*) to spotted pod borer, *M. vitrata* as well as morphological and biochemical bases of resistance to spotted pod borer in cowpea are reviewed here under.

2.1 SCREENING OF COWPEA AND OTHER LEGUMINOUS CROPS FOR RESISTANCE AGAINST SPOTTED POD BORER

Several studies have been carried out to identify resistant cultivars in cowpea against pod borer *M. vitrata*. Usua (1976) for instance, had reported that the stems of cowpea lines 946 and 4557 were resistant to attack by spotted pod borer, *M. vitrata,* though the pods and flowers were more susceptible.

Singh (1978) who screened 2800 accessions of cowpea at International Institute of Tropical Agriculture, Ibadan, Nigeria reported that the cultivars TVu 946 and TVu 4557 were resistant to the spotted pod borer.

Woolley and Evans (1979) screened 140 genotypes of *V. unguiculata* for resistance to *M. vitrata* and reported variation in susceptibility to the borer, with the semi wild type Wake Jaba proving to be the most resistant.

Jackai (1981) studied the onset and fluctuation of *M. vitrata* infestation on cowpea terminal shoots, flower buds, flowers and pods under field conditions at Ibadan, Nigeria. Four cowpea cultivars, Ife Brown, Vita-5, Vita-3, and TVu 946 were used in the study. Infestation started in the terminal shoots around 21 days after planting but later on spread to the reproductive parts as these was formed.

The highest infestation was found in flowers followed by flower buds, terminal shoots and pods respectively. TVu 946 consistently had the lowest number of larvae and Vita - 3 the highest.

Okeyo-Owuor *et al.* (1983) studied the influence of weather on larval populations of *M. vitrata* on cowpea in Kenya for three years from 1979 - 1981. The population was significantly lower in short rainy seasons and dry seasons than during long rainy seasons, even when the crop was maintained under irrigated conditions. Population was also lower in stands of cowpea alone than in cowpea as an intercrop with maize, especially at the 50 per cent flowering stage of cowpea. Late instar larvae were more abundant in pods than in flowers.

Macfoy *et al.* (1983) evaluated three cultivars of cowpea in screen house for resistance to *M. vitrata* and found TVu 946 as the most resistant, both in the pre-flowering and flowering stages. TVu 946 was followed by Ife Brown and Vita 1. Larval survival and development were more adversely affected on TVu 946. In a choice situation in the screen house, female moths exhibited a non-preference towards TVu 946.

Tayo (1988) reported that pod size as well as rate of pod growth were important factors in determining the susceptibility of cowpea to attack by the pod-borer.

Atachi and Ahohuendo (1989) conducted field experiments in Benin to evaluate ten cowpea varieties for resistance to the thrips, *Megalurothrips sjostedti* as well as to the legume pod borer, *M. vitrata*. Peak numbers of *M. sjostedti* generally coincided with minimum numbers of *M. vitrata* and *vice versa*. Maximum larval density of 4 – 17 larvae per 20 flowers was observed 40 and 47 days after planting. Peak numbers of both species occurred 40 and 70 days after planting.

Echendu and Akingbohungbe (1989) carried out screen house studies in Nigeria to determine the maximum infestation level as well as the appropriate period of infestation during flowering, for screening cowpea for resistance to *M. vitrata.* They reported that successful establishment of larvae was possible at the flower bud stage, but not at open flower stages.

Echendu and Akingbohungbe (1990) screened cowpea for resistance to *M. vitrata.* Varieties TVu 946, TVu 1896 AG, H 51-1 and 2 AK were identified as resistant and Ife Brown, H 144-1 and 58-185 as susceptible.

Anithakumari (1992) screened hundred accessions of cowpea for resistance to the pod borer *M. vitrata* during 1990 – 1991. The accessions were classified as highly susceptible (> 15 per cent damage), moderately susceptible (10 - 15 per cent damage) and moderately resistant (5 - 10 per cent damage), based on the field evaluation. Mannuthy local, with 5.9 per cent flower damage and 6.76 per cent pod damage was rated as moderately resistant while Pusa Komal with 23 per cent flower damage and 47.14 per cent pod damage was rated as most susceptible cultivar among the different accessions.

Veeranna *et al.* (1997) conducted a field experiment during 1992 - 1993 in Bangalore to determine the extent of infestation of *M. vitrata* in cowpea and reported that the highest infestation of terminal shoots (84.72%), flower buds (41.34%) and pods (52.30%) occurred during the 37th week while lowest infestation was found on terminal shoots (4.13%), flower buds (1.60%) and pods (0.40%) happened during the 43rd week. During summer 1993, the highest infestation was observed on terminal shoots (72.30%) during the 13th week and on flower buds (18.84%) and pods (16.85%) during the 14th week. The lowest infestation was observed on terminal shoots (21.13%), flower buds (2.63%) and pods (3.17%) during the 10th week. Infestation was highest in kharif 1993 during the 36th week on terminal shoots (45.50%), flower buds (22.71%) and during the 32nd week on pods (7.14%). The lowest infestation was observed on terminal shoots (6.14%) during the 30th week and on flower buds (3.13%) and pods (1.00%) during the 29th week. *M. vitrata* infestation was positively correlated with mean minimum and mean maximum temperatures.

Alghali (1993) conducted field studies in Nigeria to examine the effects of agrometeorological factors on *M. vitrata* population using two cowpea varieties IT84S-2246-4 and Dan ilan. Significant relationship was observed between extent of damage caused by pod borer and cumulative rainfall as well as the number of rainy days from plant emergence to 50 per cent flowering.

Gethi *et al.* (1993) conducted field studies in Kenya during 1987-88 to investigate the relative resistance of the cowpea cultivars TVu 946 and ICV 2 to *M. vitrata* in pure stands as well as when intercropped with maize. It was obtained that the resistance of TVu 946 to *M. vitrata* was reduced when intercropped with maize. This was attributed to the phenological changes, *i.e.*, a significant increase in pod and peduncle length as well as a significant reduction in the number of branches in the intercropped cowpea. Microclimatic factors also were much more favourable to the pest in intercropped situations, with less of sun light and more of temperature and relative humidity.

The biology of *M. vitrata* was studied by Oghiakhe *et al.* (1993a) on different plant parts of 18 cowpea cultivars to determine the role of antibiosis in their levels of resistance. Larvae of *M vitrata* were successfully reared from the first instar to the adult stage on floral buds, flowers and sliced pods, but not on stems, terminal shoots and unsliced pods, where there was complete larval mortality. The mean pupal weight ranged from 43.5 to 54.5 mg on floral buds, 38.5 to 58.6 mg on flowers and 42.7 to 58.6 mg on sliced pods, with highly significant differences between resistant and susceptible cultivars with respect to each part. Growth indices showed that sliced pods were the most suitable for larval growth and development, followed by flowers and floral buds.

Atachi and Djihou (1994) reported that *M. vitrata* was a polyphagous borer with 22 host species distributed in eight families of which 77 per cent were legumes. Red gram, *Cajanus cajan* (L.) Millsp and cowpea, *Vigna ungiculata* (L.) Walp. were the two most vulnerable species, with red gram being more preferred for oviposition.

Oghiakhe *et al.* (1995) screened eighteen cowpea cultivars for resistance to legume pod borer, *M. vitrata* under field conditions at two locations (Ibadan and Mokwa) in Nigeria. Based on per cent pod damage, the authors reported that TVu 946, MRx2-84F and MRx109-84F were resistant to the pod borer while IT82D-716 was highly susceptible. Yield reduction caused by *M. vitrata* at Ibadan ranged from 3.47 per cent in MRx2-84F to 49.75 per cent in IT82D-716 while the same for Mokwa ranged from 10.65 per cent for MRx54-84M to 52.23 per cent for MRx15-84F. Eight cultivars were selected as moderately resistant to *M. vitrata*, namely MRx2-84F, MRx49-84M, MRx109-84M, MRx50-84M, MRx54-84M, MRx55-84M, MRx8-84F and MRx48-84M.

Jackai *et al.* (1996) screened a large number of accessions belonging to selected wild *Vigna* sp. namely *V. unguiculata* ssp. *dekindtiana* (Harms) Verdc., *V. oblongifolia* A. Rich and *V. vexillata* (L.) A. Rich., using choice and no-choice laboratory feeding bioassays, to determine their resistance to the pod borer, *M. vitrata*. The most resistant accessions belonged to *V. vexillata*, and were followed by those from *V. oblongifolia*, with few outstanding exceptions from *V. unguiculata*.

Oghiakhe (1997) reported that highly pubescent wild *Vigna* spp. have shown good levels of resistance to pests like *M. vitrata*, *Clavigralla tomentosicollis* (Stal.) and *Callosobruchus maculatus* (Fab.).

Plant attributes *viz.*, stem and pod wall thickness, trichomes and pod forming habit were associated with resistance to *M. vitrata* (Sharma, 1998).

Vidya and Oommen (2001) conducted a field experiment during 2000 to evaluate legume pod-borer resistance in 50 accessions of yard-long bean [*Vigna unguiculata* (L.) Walp. ssp *sesquipedalis* Verd.] at Vellayani, Kerala. Evaluation based on simultaneous consideration of flower and pod damages indicated that Kottayam local, with 16 per cent pod damage as well as Palakkad local and Chengannur local, with 18 per cent pod damage were the more resistant among the cultivars evaluated.

Ganapathy (2010) studied the ecology and management of *M. vitrata* tropical food legumes and reported that spotted pod borer is one of the key insect pests of tropical food legumes damaging tender leaf axils, flower buds, flowers and pods by webbing and boring clusters of flowers or pods during cooler parts of the year on about 39 hosts. In cowpea, the damage was about 25-40 per cent and in pigeon pea, it was 9-84 per cent across the world.

Chaitanya *et al.* (2012) who carried out field studies to observe the incidence of spotted pod borer in three cultivars *viz.*, LRG 41, TRG 22 and TRG 38 revealed that the infestation started with the onset of flowering in first week of November and remained in the field till crop maturity.

Kumar *et al.* (2013) evaluated one promising variety Pusa Komal and fourteen genotypes of cowpea against legume pod borer, *M. vitrata*. The pod damage among the test cultivars varied from 22.8 to 32.56 per cent. The genotype KCP-6 which recorded 22.81 per cent damage was the least susceptible, whereas KCP-1, with 32.56 per cent damage was the most susceptible. None of the cultivars was found resistant to this pest. The varietal susceptibility to pod borer was found to be less in genotype KCP-6, Pusa Komal and RGC-5 and more in genotype KCP-1, RGC-2 and RGC-4.

Barad *et al.* (2014) conducted a field experiment involving twenty genotypes of cowpea during *Kharif* 2010. GC-706 recorded the lowest spotted pod borer population (0.06 larvae/plant) and pod damage (8.89%), while maximum larval population (1.96 larvae/plant) and pod damage (24.30%) was recorded in GC-12. Genotype GC-706 (981.48 kg/ha) also achieved higher yield of cowpea followed by genotypes/varieties GC-203, GC-521, GC-815, GC-510, RC-101, GC-723 and GC-2 recording 951.85, 914.81, 833.33, 818.52, 803.7, 792.59 and 792.59 Kg/ha respectively. Lowest yield of 511.11 Kg/ha was recorded in genotype GC-12. Based on larval population and pod damage, genotypes GC-706, GC-203, GC-510 and GC-521 were reported as resistant to the pod borer.

2.2 MORPHOLOGICAL BASES OF RESISTANCE

Morphological traits of the plants often influence the insect plant interactions. Morphological bases of resistance include factors such as colour and shape of plant that influence orientation of pest towards the plant, presence or absence of pubescence, length of flower stalk, configuration of calyx, pod angles, pod wall thickness, trichome length and density, nature of seed testa *etc.*

Halder and Srinivasan (2011) observed eight plant parameters, *viz.*, pod wall thickness, number of pods/cluster, angle between the pods, trichomes on leaves and stems, trichome length, pod length and pod width were in relation to the expression of varietal reaction towards *M. vitrata* in 11 varieties of cowpea. It was observed that highly susceptible cv. GC-9708 had least number of trichomes on stems (5.1) and leaves (4.8) as compared to highly tolerant. cv. HC-270 which had 7.5 and 9.4 trichomes/mm^2, respectively. Pod wall thickness, angle between the pods and pod width showed a negative correlation with pod damage. Further, the susceptible cv. GC-9708 recorded values for lowest pod wall thickness (0.77 mm), pod width (6.35 mm) and pod angle (40°) as compared to tolerant cv. HC-270 which recorded 0.89 mm, 7.80 mm and 85° for pod wall thickness, pod width and pod angle respectively. Similarly, highest pod length (15.55 cm) and maximum number of pods/cluster (2.8) were recorded in GC-9708 as compared to others.

2.2.1 Pod wall thickness

Tayo (1989) examined the anatomy of damaged and undamaged young stems, peduncles and young pods of three varieties of cowpea transverse sections in order to evaluate the anatomical basis of resistance to *M. vitrata.* The three varieties tested were TVu 946, a resistant variety, Vita-1, a highly susceptible variety, and ICV-2, a moderately resistant variety. It was suggested that the smaller diameter of stem and the relative abundance of strengthening tissues in the stem and peduncle of TVu 946, by limiting the total ingestible biomass, might be restricting the damage to these organs by *M. vitrata.* Pod damage, on the other hand, was concentrated on the parenchymatous tissue of the pod wall lining as well as in the inter-seed spaces. The size of this tissue were also different among the varieties tested in the order Vita-1 > ICV-2 > TVu 946, the same order of susceptibility to attack by *M. vitrata.* A strong positive correlation was observed between the size of these tissues and susceptibility.

Oghiakhe *et al.* (1992b) conducted field studies in Nigeria to determine the possibility of the involvement of pod wall toughness in the resistance of cowpea to *M. vitrata.* The toughness of non-intact and intact pod walls increased with age, but the rate of increase varied depending on the growth stages of the pod as well as between cultivars. There was a positive and significant correlation (r = 0.82**) between pod age and the amount of pressure required to penetrate the pod wall. No significant differences were observed in non-intact pod wall toughness between the resistant TVu 946 and susceptible IT82D-716 cowpea cultivars at all the growth stages tested.

2.2.2 Length of flower stalk

In one of the very few studies examining the relationship between morphological characters of flowers and pod borer damage, Khaemba (1985) identified that cowpea lines having long peduncles held upright over the plant canopy holding the pods away from each other appeared to suffer less damage by the legume pod borer, *M. vitrata.*

Anithakumari (1992) evaluated the effect of peduncle on the pest distribution for the different accessions. The accession V98 (Kuruthola) which belonged to the moderately resistant group had the shorter flower stalk. The correlation coefficient between the mean length of flower stalk of different accessions and the mean per cent of infestation of the inflorescence by the larvae was calculated, the per cent flower damage was found to be correlated positively with the mean length of flower stalk.

2.2.3 Configuration of calyx

Anithakumari (1992) observed the configuration of calyx as an important morphological attribute in conferring resistance to the spotted pod borer *M. vitrata* and reported that accessions under the moderately resistant group (V98, V30, V95, V61 and V75 possessed tight or semi tight calyx. Those accessions under the moderately susceptible group (V13, V41, V90, V89 and V2) were with partially free calyx and the calyx was free for five of the accessions under the highly susceptible group (V12, V1, V57, V86 and V100). It was thus found, that the accessions had some inherent defense mechanism at varying levels when the different groups were compared.

2.2.4 Pubescence

Pubescence is one of the most important physical characters associated with insect resistance across the plant kingdom. It is a complex character, involving several factors like the distribution of the hairs, the length of the hairs, the density of hair cover, disposition of hairs and the type of hairs (Verma and Afzal, 1940). Ovipositional non-preference, due to the presence of trichomes, has been reported to be one of the mechanisms of resistance of cowpea to *M. vitrata*.

Resistance due to pubescence has been reported in 17 crops against 32 pest species (Webster, 1975). Pubescence can affect the activity of insects by both chemical and mechanical means. The mechanical effect depends on the physical characteristics of the trichomes, including density, erectness, length and shape (Dent, 1991).

Chiang and Singh (1988) reported the role of trichomes in resistance to some pests in cowpea and other leguminous species. Resistance to *M. vitrata* in wild cowpea, *Vigna vexillata* (Acc.TVNu 72) was found to be due to presence of trichomes on pod walls.

Jackai and Oghiakhe (1989) investigated the role of pubescence in the resistance of two wild cowpea (*V. vexillata*) varieties, TVNu 72 and TVNu 73, to feeding and damage caused by *M. vitrata* and *Clavigralla tomentosicollis.* Feeding and development were deterred in both insects on pods of TVNu 72 and TVNu 73 irrespective of the presence of trichomes, as compared to that on the susceptible variety, IT84E-124. In *M. vitrata,* the same effect was observed on flowers as well. However, *M. vitrata* larvae fed and developed better on the above varieties when trichomes were removed, leading the authors to conclude that trichomes formed the first line of defense in the resistance of *V. vexillata* to *M. vitrata.*

Oghiakhe (1995) reported that pubescence in wild and cultivated cowpeas (*Vigna vexillata* and *V. unguiculata*) adversely affected oviposition, mobility, and food consumption and utilization by the *M. vitrata* in tests conducted with TVnu 72 (wild, highly resistant and highly pubescent), TVu 946 (semi-wild, moderately resistant and pubescent) and IT82D-716 (cultivated, highly susceptible and pubescent). Ovipositional activity on stipules, stem, branches, terminal shoots, adaxial and abaxial leaf surfaces of the susceptible cultivar, IT82D-716 was higher than on TVu 946 and TVnu 72. More eggs were laid on the abaxial surfaces than on the adaxial surfaces of leaves for all tests conducted. Significant negative correlations (r = -0.99** and *r* = -0.99**) were found between mean number of eggs laid and length and density of non-glandular trichomes. There was a significant negative correlation (r = -0.99**) between larval velocity and length of non-glandular trichomes on pod surface. There was a significant positive correlation (r = 0.99**) between larval penetration time on pods and length of non-glandular trichomes. Weight loss, number of feeding punctures and number of larvae on whole pods of TVnu 72 were lower and significantly different from IT82D-716 and TVu 946.

Veeranna and Hussain (1997) screened 45 cowpea genotypes for attack by *M. vitrata* in Karnataka and observed that Tvx – 7, the most resistant genotype had a high trichome density of 24.41/9 mm^2, while DPCL – 216, the most susceptible one, had a low trichome density of 12.82/9 mm^2, confirming earlier findings that trichomes are important in reducing attack by the borer.

2.3.5 Pod angle

van Emden (1989) correlated the resistance to pod borer in cowpea varieties with pod angle. Pods held widely apart on the peduncles reduced accessibility of the pods to subsequent infestation by borer larvae. The larvae reportedly penetrated the pods more successfully when pods are in contact with each other or with the foliage.

Oghiakhe *et al.* (1992c) studied the effect of pod angle on the resistance of cowpeas (*V. unguiculata*) to *M. vitrata* under field conditions in Nigeria using the susceptible cultivar IT82-D-716 and the resistant cultivar TVu 946. Three different pod angles were used in the study: a normal angle, a decreased angle and an increased angle. Negative and highly significant relationships were observed between pod angle and percentage pod damage, as well as the seed damage index in the two cowpea cultivars. Pods with wide angles (≥89°) were damaged on only one and rarely on both pods.

2.3.6 Plant architecture

Distribution of *M. vitrata* larvae is closely related to the distribution of reproductive structures which serve as the larval feeding sites and hence plant architecture is important in deciding the extent of damage.

Oghiakhe *et. al.* (1991) found that *V. unguiculata* cultivars with pods held within the leaf canopy suffered significantly more damage than cultivars with pods held above the canopy. Defoliated cultivars sustained significantly less infestation and damage than those with leaves. Relative humidity measured under the canopy was lower in defoliated cultivars, while soil and ambient temperatures were higher. Per cent pod damage and larval infestation by *M. vitrata* in flowers were positively correlated with relative humidity, and negatively correlated with temperature. Canopy structure and pod position acting together or independently, exerted profound effects on cowpea resistance to *M. vitrata*.

2.3 BIOCHEMICAL BASES OF RESISTANCE

A wide range of chemical substances including primary, secondary and intermediary metabolites present in plants play an important defensive role against herbivory.

Macfoy *et al.* (1983) studied the biochemical mechanisms of resistance in cowpea cultivars to legume pod borer *M. vitrata,* reported that the varieties resistant (TVu 946) to the pod borer had lower levels of total amino acids (stem – 11.67 μ mole/g, leaf stalk – 11.69 μ mole/g, growing parts - 18.24 μ mole/g, leaves - 17.49 μ mole/g) as compared to susceptible variety Vita – 1 (stem – 20.39 μ mole/g, leaf stalk – 14.59 μ mole/g, growing parts – 17.07 μ mole/g, leaves – 20.16 μ mole/g). Similarly, concentration of sugars in resistant variety (TVu 946) is less (stem – 12.11 mg/g, leaf stalk – 10.05 mg/g, growing parts – 9.01 mg/g, leaves – 11.49 mg/g) as compared with susceptible variety Vita – 1 (stem – 19.01 mg/g, leaf stalk – 12.79 mg/g, growing parts – 10.51 mg/g, leaves – 12.62 mg/g).

Oghiakhe *et al.* (1993b), however, reported that total sugar content in the pod wall and seeds of TVNu 72 were higher than that of IT82D-716 a susceptible genotype. Similarly, phenol content was lower in the pod wall of TVNu 72, but the reverse was true for fresh and dry seeds. This suggested that neither phenol nor total sugar was involved in the resistance of TVNu 72 to *M. vitrata.*

Oghiakhe *et al.* (1993c) conducted experiment to determine the relationship between the concentration of phenol in cowpea and field resistance to the *M. vitrata* was observed significant variation in phenol concentration between the different parts of the cultivars at the same growth stage. Phenolic content in cowpea cultivars modulated with variable levels of resistance to legume pod borer. Phenol content in flowers and pods varied considerably between cultivars. Despite the differences in phenol concentration among cultivars, correlation between phenol content and resistance parameters did not indicate any significant role of phenols in resistance to legume pod borer.

Halder *et al.* (2006) studied the biochemical parameters, viz., total sugar, reducing sugar, non-reducing sugar, amino acids, proteins and phenols in pods in relation to the expression of varietal reaction towards spotted pod borer *M. vitrata* in ten varieties of mung bean. The study revealed that highly susceptible cultivar LGG-450 had highest amount of total sugar (1.38 mg/g), reducing sugar (0.59 mg/g), non-reducing sugar (0.79 mg/g), amino acids (0.130%) and protein (23.44%) as compared to highly tolerant cultivar LGG- 497 which had 1.13 mg/g, 0.48 mg/g, 0.65 mg/g, 0.072 per cent and 18.56 per cent respectively. Whereas phenol content was higher (21.03 mg/g) in resistant cultivar LGG-497 than in susceptible cultivar LGG - 450 (20.00 mg/g).

Sunitha *et al.* (2006) reported that higher sugar content in flowers (22 per cent) and pods (10.6 per cent) as well as higher protein was responsible for the susceptibility of ICPL 88034 to pod borer as compared to the resistant genotype ICPL98003 (16.5 %).

Anithakumari (1992) observed that total sugars, amino acids, total nitrogen and crude protein were important biochemical factors in conferring resistance to the spotted pod borer *M. vitrata* and reported that accessions V98, V30, V95, V61, V75 under the moderately resistant group possessed lower total sugar content of 2.9, 2.8, 3.37, 3.2 and 3.0 per cent respectively. Highly susceptible accessions had mean total sugars ranging from 4.5 to 5.7 per cent. Higher amino acid content was recorded in case of accessions of the moderately susceptible group (V13 - 0.85 μg/g, V41 - 0.843 μg/g, V90 - 0.833 μg/g, V89 - 0.877 μg/g, V2 - 0.967 μg/g) as well as in case of accessions in the highly susceptible group, with the highest value of 1.83 μg/g being recorded in the case of V1.

Materials and methods

3. MATERIALS AND METHODS

The present investigation titled "Morphological and biochemical bases of resistance to spotted pod borer, *Maruca vitrata* (Fab.) (Lepidoptera: Crambidae) in cowpea", was carried out by a field trial at College of Horticulture, Kerala Agricultural University, Thrissur (10^0 31'N latitude and $76^0$17'E longitude at an elevation of 40 m above mean sea level) from December 2014 to June 2015. The experimental site had a mean temperature of 28.4^0 C, rain fall of 161.8 mm and relative humidity of 69 per cent. The data for the investigations were collected from two crop seasons. The details of the materials used and methods followed in the study are described in this chapter.

3.1 EXPERIMENTAL DETAILS

Forty - eight accessions of cowpea comprising of twenty - nine accessions from National Bureau of Plant Genetic Resources (ICAR - NBPGR) Regional Station, Jodhpur, Rajasthan, five accessions from University of Agricultural Sciences (UAS), Bengaluru, ten varieties released from KAU and one accession each from Vegetable and Fruits Promotion Council Keralam (VFPCK), Thiruvananthapuram, Indian Institute of Vegetable Research (ICAR - IIVR), Varanasi as well as Horticulture College and Research Institute, Periyakulam were evaluated for resistance to spotted pod borer. These genotypes constituted the treatments in the field experiment (Table 1).

3.1.1 Design and layout of pot culture experiment

The experiment was laid out in Completely Randomized Design (CRD) with 48 treatments and 10 replications, with one polybag containing one plant constituting one replication (Plate 1).

Plate 1. View of the experimental plot

3.1.2 Sowing and cultural operations

Seeds of the different accessions were sown in black polythene bags of 30 x 30 cm size and 600 gauge thickness, filled with potting mixture consisting of dry powdered cowdung, sand and soil in 1:1:1 ratio (approximately 10 kg) at rate of three seeds per bag. Agronomic practices were adopted as per the Package of Practices Recommendations of Kerala Agricultural University (KAU, 2011).

Table 1. Details of the cowpea accessions evaluated

Sl. No.	Treatment	Accessions	Source
1	T1	IC 39922	ICAR - NBPGR RS, Jodhpur
2	T2	IC 52107 A	ICAR - NBPGR RS, Jodhpur
3	T3	KM – 5	UAS, Bengaluru
4	T4	C – 152	UAS, Bengaluru
5	T5	Kanakamony	KAU, Thrissur
6	T6	PKM – 1	HC & RI, Periyakulam
7	T7	EC 100092	ICAR - NBPGR RS, Jodhpur
8	T8	IC 2196	ICAR - NBPGR RS, Jodhpur
9	T9	IC 39916	ICAR - NBPGR RS, Jodhpur
10	T10	IC 26029	ICAR - NBPGR RS, Jodhpur
11	T11	Palakkadan thandan payar	VFPCK, Thiruvananthapuram
12	T12	IC 26048	ICAR - NBPGR RS, Jodhpur
13	T13	Anaswara	KAU, Thrissur
14	T14	IC 2196	ICAR - NBPGR RS, Jodhpur
15	T15	IC 10810	ICAR - NBPGR RS, Jodhpur

Sl. No.	Treatment	Accessions	Source
16	T16	IC 39870	ICAR - NBPGR RS, Jodhpur
17	T17	TVX – 944	UAS, Bengaluru
18	T18	EC 300039	ICAR - NBPGR RS, Jodhpur
19	T19	IC 52094	ICAR - NBPGR RS, Jodhpur
20	T20	IC 39945	ICAR - NBPGR RS, Jodhpur
21	T21	IT – 3895 – 1	UAS, Bengaluru
22	T22	Vyjayanthi	KAU, Thrissur
23	T23	IC 20431	ICAR - NBPGR RS, Jodhpur
24	T24	Sreya	KAU, Thrissur
25	T25	IC 9883	ICAR - NBPGR RS, Jodhpur
26	T26	Hridya	KAU, Thrissur
27	T27	IC 20720	ICAR - NBPGR RS, Jodhpur
28	T28	IC 2918	ICAR - NBPGR RS, Jodhpur
29	T29	KBC – 2	UAS, Bengaluru
30	T30	IC 19797	ICAR - NBPGR RS, Jodhpur
31	T31	Mysore Local	IIHR, Bengaluru
32	T32	IC 7832	ICAR - NBPGR RS, Jodhpur
33	T33	IC 39921	ICAR - NBPGR RS, Jodhpur
34	T34	IC 52105	ICAR - NBPGR RS, Jodhpur
35	T35	Kashikanchan	IIVR, Varanasi
36	T36	IC 52128	ICAR - NBPGR RS, Jodhpur

Sl. No.	Treatment	Accessions	Source
37	T37	EC 98668	ICAR - NBPGR RS, Jodhpur
38	T38	IC 39947	ICAR - NBPGR RS, Jodhpur
39	T39	IC 20645	ICAR - NBPGR RS, Jodhpur
40	T40	IC 19778	ICAR - NBPGR RS, Jodhpur
41	T41	Vellayani Jyothika	KAU, Thrissur
42	T42	Malika	KAU, Thrissur
43	T43	Sharika	KAU, Thrissur
44	T44	Bhagyalakshmy	KAU, Thrissur
45	T45	EC 101216	ICAR - NBPGR RS, Jodhpur
46	T46	IC 52110	ICAR - NBPGR RS, Jodhpur
47	T47	IC 52118	ICAR - NBPGR RS, Jodhpur
48	T48	Lola	KAU, Thrissur

3.1.3 Field screening of cowpea accessions for resistance to *M. vitrata* (Fab.)

Field evaluation of forty - eight cowpea accessions was carried out by raising cowpea plants in polybags at a spacing of 30 x 15 cm, 45 x 15 cm and 2 x 2 m for bush, semi trailing and trailing types, respectively. Two weeks prior to planting, the variety Lola was sown along the border around the plot to serve as multiplication foci for the test insect, *M. vitrata.* Further, neonate larvae of *M. vitrata* were collected in large numbers from infested cowpea fields and released on the border plants at early flowering phase to ensure adequate pest population. Observations on the pod borer incidence were recorded at three days interval starting from first flowering up to two months after flowering. Five plants were selected at random from each accession and pod borer incidence on buds,

Plate 2. Infestation by pod borer during different stages of cowpea

Infestation on tender shoot

Infestation on bud

Infestation on leaf

Infestation on flower

Infestation on pods

flowers and pods were recorded (Plate 2). The per cent of damage was calculated based on the ratio of infested buds, flowers and pods to the total number of buds, flowers and pods respectively. Flower buds, flowers and pods once counted were tagged to avoid recounting.

Based on the level of infestation of buds, flowers and pods, the cowpea accessions were categorised into four groups, namely, resistant, moderately resistant, susceptible and highly susceptible. Twenty accessions, comprising of eight, four, two and nine accessions from resistant, moderately resistant, susceptible and highly susceptible groups respectively, were selected at random for detailed investigation into the morphological and biochemical bases of resistance (Plate 3).

3.1.4 Evaluation of biophysical components of the cowpea accessions on resistance to pod borer

In order to study the morphological basis of resistance in cowpea to spotted pod borer, a minimum of ten flowers and pods were selected at random per replication for each treatment and the following observations were recorded. The mean value was worked out for each observation and expressed in corresponding units.

3.1.4.1 Length of flower stalk

Length of the flower stalks was measured by using a 30 centimeter ruler for all the accessions. The mean length was calculated and expressed in centimetres (Plate 4).

3.1.4.2 Configuration of calyx

The calyx of the flowers was examined and the accessions were categorised into two groups as follows (Plate 7-8).

1. Partially free - major portion of the sepals free, the basal portion tight.
2. Semi tight - major portion of the sepals tight, only the tip free.

Plate 3. Cowpea accessions selected for evaluation of morphological and biochemical bases of resistance

C – 152

Kanakamony

PKM - 1

EC 100092

Palakkadan thandan

Anaswara

TVX – 944

EC 300039

IC 20431

Sreya

Hridya

Mysore local

IC 52105

Kashikanchan

IC 20645

Vellayani Jyothika

Malika

Bhagyalakshmy

IC 52110

Lola

3.1.4.3 Pod angles

Pod angles of different accessions were measured by using a protractor and the mean pod angle was calculated and expressed in degree (x^0) (Plate 10).

3.1.4.4 Pod wall thickness

Thickness of the pod cover of various accessions under consideration was measured at vegetable maturity, by using a screw gauge. The mean thickness was calculated and expressed in millimetres (Plate 9).

3.1.4.5 Trichome length and density

Trichome length was measured by Leica-EZ stereo microscope equipped with Leica Application Suite (LAS) image analysing software. Length of ten trichomes on pods selected at random from each accession was taken and the average was worked out and calculated in mm (Plate 5-6).

The counts of trichomes on the pod surface was made from an area of 6.25 mm^2 using a Radical Stereo Zoom microscope at 35x magnification, after marking out an area of 2.5 x 2.5 mm^2 over the excised pod. Counts were taken from three different points on each pod and the average was worked out.

Based on the density of trichomes, the accessions were classified into:

1. Glabrous - 0 to 50 trichomes per 6.25 mm^2 unit area
2. Sparsely pubescent - 51 to 100 trichomes per 6.25 mm^2 unit area
3. Pubescent - 101 to 150 trichomes per 6.25 mm^2 unit area
4. Densely pubescent - more than 151trichomes per 6.25 mm^2 unit area

3.1.4.6 Nature of seed testa

The colour and texture of the seed testa were assessed visually. According to the thickness of the seed testa, they were classified into 'thick' and 'thin' groups and based on toughness of seed testa, they were classified as 'soft' and

Plate 4. Measurement of flower stalk length

Plate 5. Measurement of trichome length

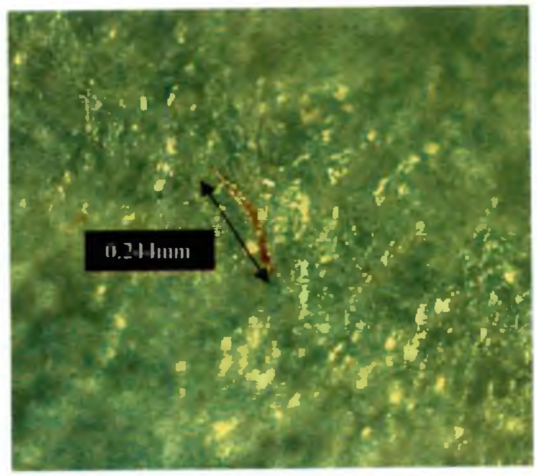


Plate 6. Measurement of trichome density

Plate 7. Configuration of calyx

Partially free

Plate 8. Configuration of calyx

Semi tight

Plate 9. Measurement of pod wall thickness

Plate 10. Measurement of pod angle

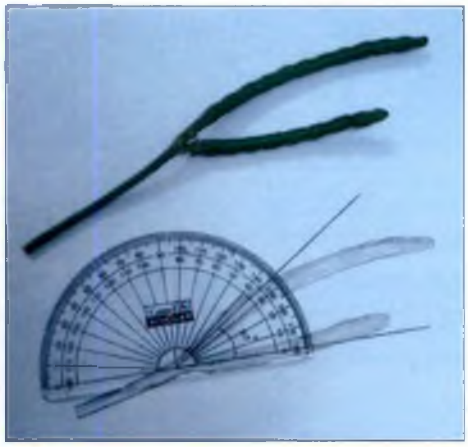


Plate 11, 12, 13. Nature of seed testa

Green

Cream

Purplish green

'rough'. Accessions were grouped into green, cream and purplish green based on colour of seed testa (Plate 11, 12 and 13).

3.1.5 Evaluation of biochemical components of the cowpea accessions on resistance to pod borer

Biochemical basis of resistance to spotted pod borer in cowpea was investigated by estimating the total protein, total moisture content, total sugar and reducing sugar contents as well as through assaying peroxidase and polyphenol oxidase enzyme activity. The studies were carried out by following standard procedure as described below and three observations were recorded. The mean value was worked out for each observation and expressed in corresponding units.

3.1.5.1 Moisture content

The moisture content in the fruit samples was determined by the hot air oven method. Ten grams of fruit was weighed and oven dried at 80° C until constant weights 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 (Ranganna, 1997).

$$\text{Moisture (\%)} = \frac{\text{Fresh weight - dry weight}}{\text{Fresh weight}} \times 100$$

3.1.5.2 Reducing sugars

Fifteen gram of fresh cowpea pod was ground using pestle and mortar and was transferred to a 250 ml volumetric flask. Then 100ml of distilled water was added to the sample followed by 2 ml pre-standardised 45 per cent neutral lead acetate, for clarification. Lead acetate was then neutralized by the addition of 2 ml pre-standardised 22 per cent potassium oxalate solution. The clarified solution was made up to the mark with distilled water. This was filtered through Whatman

No.1 filter paper. The reducing sugars were determined by titrating the clarified filtrate against standard Fehling's solution using methylene blue as an indicator (Ranganna, 1997). The reducing sugars were calculated by the following formula.

$$\text{Reducing sugar (\%)} = \frac{\text{Fehling's Factor x dilution}}{\text{Titre value x weight of sample}} \text{ X } 100$$

3.1.5.3 Total sugars

Fifty milliliters of the cowpea pod clarified filtrate was taken into a 100ml volumetric flask and 5 ml of concentrated HCl was added for hydrolyzing the sample. The hydrolysed solution was neutralized with 20 per cent NaOH by using one or two drops of phenolphthalein. Diluted HCl was added till it became colourless. Finally, the volume was made up to 100 ml and it was titrated against standard Fehling's solution using methylene blue as indicator (Ranganna, 1997). The total sugars were calculated as given below:

$$\text{Total sugar (\%)} = \frac{\text{Fehlings factor x 250 x dilution factor}}{\text{Titre value x 50 x weight of sample}} \text{ X } 100$$

3.1.5.4 Total 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}.

One gram of the fresh cowpea pods weighed and mascerated with a pestle and mortar in 10 ml phosphate buffer (0.1 M) with *p*H 7.0. This sample was centrifuged and the supernatant was used for protein estimation. Fourty microlitre of sample extract was pipetted out into the test tube and the volume was made upto 1ml. A tube with 1 ml of water served as the blank. Five ml of reagent C (50 ml of reagent A (2% sodium carbonate in 0.1N sodium hydroxide)) and 1 ml of reagent B (0.5% copper sulphate solution in 1 per cent 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. The blue colour that developed was read at 660 nm using ELICO SL 210 UV VIS Double beam spectrophotometer. Bovine Serum Albumin (BSA) (0.2 mg ml^{-1}) solution was used as standard. Standard (40 µl) was also taken and treated with the reagents as the sample was done to get a blue colour. The blue colour developed was read at 660 nm. The reagent blank was used to adjust the initial reads of absorbance as zero. Concentration of protein in the given sample was thus calculated and expressed as mg g^{-1}.

$$\left[\frac{\text{Conc. of standard}}{\text{Absorbance of standard}}\right] \text{X} \left[\frac{\text{Absorbance of the sample}}{\text{Vol. of sample pipetted out in ml}}\right] \text{X 10}$$

3.1.5.5 Peroxidase activity

Peroxidase activity was assayed by the method of Mahadevan and Sridhar (1982). Fresh pod (1 g) was homogenized in 10 ml ice-cold 0.1 M phosphate buffer (*p*H 6.0). The homogenate was centrifuged for 15 min at 10,000 rpm at 4^0 C. The supernatant was freshly used for assaying the enzyme activities. Two milliliter, of 0.1 M phosphate buffer (*p*H 7) and one ml of 20 mM guaiacol and 40 µl of supernatant were taken in a clean dry cuvette which was transferred to a spectrophotometer. In order to start the reaction 50 µ of 10 mM H_2O_2 was added to the cuvette. Initial absorbance and then change in absorbance were noted after an interval of 30 s for three min at 470 nm using ELICO SL 210 UV VIS Double beam spectrophotometer. Enzyme units are expressed in terms of change in absorbance per minutes per unit tissue weight.

$$\text{Peroxidase activity} = \frac{\text{Difference in OD per minute}}{\text{Volume of enzyme extracts}} \times \frac{\text{Total volume}}{\text{Weight of the tissue}}$$

3.1.5.6 Polyphenol oxidase activity

Polyphenol oxidase activity was assayed by the method of Esterbaner *et al.* (1977). The macerated assay mixture containing 0.5 g fresh pods, 50 mM Tris-HCl (*p*H 7.2), 0.4 M sorbitol and 10 mM NaCl (3 ml: 1 ml: 1 ml) was centrifuged at 20,000 rpm at 4^0 C for 10 minutes and supernatant was used for enzyme assay. In cuvette 2.5 ml. of 0.1 M phosphate buffer (*p*H 6.5) and 0.3 ml. of catechol solution (0.01 M) was added and the spectrophotometer, was set at 495 nm. Then 0.2 ml of enzyme extract was added in cuvette and absorbance recorded at every 30 second up to 5 min using ELICO SL 210 UV VIS Double beam spectrophotometer. One unit of catechol oxidase is defined as enzyme which transforms 1 μmol of dihydrophenol to 1 μmol of products per minute under assay condition. The activity of polyphenol oxidase was estimated by the formula given below:

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

Where, K = 0.272 (for catechol oxidase)

ΔA = Initial value of absorbance – Final value of absorbance

3.2 STATISTICAL ANALYSIS AND INTERPRETATION OF DATA

The data collected on number of buds, flowers, and pods per plant, damaged buds, flowers, pods per plant and per cent damage among the tested cowpea accessions were analyzed by one way ANOVA. The result obtained was subjected to DMRT (Duncan's Multiple Range Test). Correlation between the morphological and biochemical traits of the cowpea accessions and spotted pod borer infestation worked out using established correlation analysis technique.

Results

4. RESULTS

The results of the study on "Morphological and biochemical bases of resistance to spotted pod borer, *Maruca vitrata* (Fab.) in cowpea " carried out at College of Horticulture (Vellanikkara), Kerala Agricultural University during the period from September 2014 to June 2015 are presented here.

4.1 EVALUATION OF DEGREE OF RESISTANCE IN COWPEA ACCESSIONS TO SPOTTED POD BORER

Forty - eight accessions of cowpea were evaluated for their reaction to spotted pod borer, *M. vitrata* in the pot culture studies based on per cent damage to buds, flower and pods. The results are presented in Table 2.

4.1.1 Number of buds

The genotypes evaluated showed considerable variation in terms of total number of buds. The average number of buds varied from 13.8 per plant, recorded in case of IC 26048, to 68.6 per plant in case of EC 98668 which was followed by PKM – 1, Malika, IC 52118, IC 52128, Anaswara, Lola and Vellayani Jyothika produced 55.4, 54.2, 54, 45.6, 43.2, 42.8 and 42.4 buds per plant, respectively. IC 26048, which produced the lowest number of pods was followed by TVX – 944 (16), EC 100092 (16.2), Palakkadan thandan payar (18.2), Mysore local (20.6), IC 10810 (21.2), IC 9883 (21.8), IC 20645 (21.8), Sreya (22), IC 39921 (22), KM – 5 (22.2), IC 2815 (22.2), EC 300039 (22.6), IT – 3895-1 (22.8), Kanakamony (23.4), IC 39947 (23.6), IC 39922 (24.2), IC 20720 (25.2), IC 19778 (25.2), IC 2918 (25.8), Kashikanchan (27), IC 7832 (27.2) and IC 19797 (28). The remaining 17 accessions produced 29 to 40 buds per plant.

Table 2. Extent of damage by *Maruca vitrata* in cowpea accessions

Sl. No.	Accessions		Mean No. of buds	Mean No. of damaged buds	Mean No. of flowers	Mean No. of damaged flowers	Mean No. of pods	Mean No. of damaged pods	Total damage (%)	Total yield per plant
1	T1	IC 39922	24.20	0.00	22.20	0.00	13.00	0.20	0.34 [a]	24.31
2	T2	IC 52107 A	31.20	0.60	26.80	0.80	15.00	0.40	2.47 [a]	14.58
3	T3	KM – 5	22.20	3.40	17.40	4.00	12.40	1.00	16.15 [efghi]	25.67
4	T4	C – 152	29.60	8.00	20.60	7.60	13.60	5.80	33.54 [i]	32.16
5	T5	Kanakamony	23.40	0.40	21.00	0.40	17.00	1.40	3.58 [a]	37.54
6	T6	PKM – 1	55.40	6.60	47.20	4.60	40.00	6.00	12.06 [cdefg]	254.80
7	T7	EC 100092	16.80	0.00	15.40	0.00	12.80	0.00	0.00*	92.16
8	T8	IC 2815	22.20	0.00	16.40	0.40	16.00	0.20	1.10 [a]	22.64
9	T9	IC 39916	32.40	0.00	27.00	0.00	16.80	0.40	0.52 [a]	51.85
10	T10	IC 26029	32.40	0.60	29.40	0.80	20.40	1.20	3.16 [a]	51.33
11	T11	Palakkadan thandan payar	18.20	0.80	16.40	0.80	16.00	0.00	3.16 [a]	137.50
12	T12	IC 26048	13.80	0.20	11.60	0.40	9.00	0.00	1.74 [a]	36.72

Sl. No.	Accessions		Mean No. of buds	Mean No. of damaged buds	Mean No. of flowers	Mean No. of damaged flowers	Mean No. of pods	Mean No. of damaged pods	Total damage (%)	Total yield per plant
13	T13	Anaswara	43.20	6.40	35.80	12.90	23.20	7.40	26.13 jkl	192.51
14	T14	IC 2196	35.00	3.60	31.00	2.60	26.60	2.40	9.29 a	41.44
15	T15	IC 10810	21.20	2.00	17.80	3.00	13.20	1.80	13.03 defghi	34.17
16	T16	IC 39870	35.00	2.20	31.40	2.20	27.00	1.00	5.78 a	51.70
17	T17	TVX – 944	16.00	0.20	13.80	0.80	9.60	0.20	3.05 a	39.30
18	T18	EC 300039	22.60	0.00	20.40	0.40	17.80	0.20	0.99 a	68.35
19	T19	IC 52094	29.00	5.00	23.00	3.60	18.00	2.80	16.29 fghi	44.78
20	T20	IC 39945	30.60	0.00	26.80	0.00	20.00	0.00	0.00*	35.44
21	T21	IT – 3895 – 1	22.80	3.00	17.20	2.60	13.40	2.20	14.61 defghi	47.49
22	T22	Vyjayanthi	39.60	4.40	33.20	8.60	21.80	3.80	17.76 ghi	193.46
23	T23	IC 20431	38.00	3.40	32.40	3.20	26.60	1.40	8.25 a	52.14
24	T24	Sreya	22.00	1.80	18.20	1.20	12.80	0.80	7.17 a	39.68

Sl. No.	Accessions		Mean No. of buds	Mean No. of damaged buds	Mean No. of flowers	Mean No. of damaged flowers	Mean No. of pods	Mean No. of damaged pods	Total damage (%)	Total yield per plant
25	T25	IC 9883	21.80	0.80	19.60	2.80	15.00	0.20	6.74[a]	48.15
26	T26	Hridya	38.40	0.40	36.20	0.40	31.00	0.20	0.95[a]	39.06
27	T27	IC 20720	25.20	1.80	20.80	0.40	17.00	0.40	4.13[a]	34.92
28	T28	IC 2918	25.80	0.00	22.20	0.00	16.80	0.00	0.00*	53.59
29	T29	KBC – 2	30.80	3.60	17.20	1.60	13.40	0.00	8.47[a]	38.30
30	T30	IC 19797	28.00	4.80	22.60	4.00	17.40	1.80	15.59[efghi]	59.09
31	T31	Mysore Local	20.60	1.00	18.60	2.00	15.00	1.80	8.86[a]	43.37
32	T32	IC 7832	27.20	6.00	21.20	4.60	15.20	1.60	19.18[ghi]	32.59
33	T33	IC 39921	22.00	0.80	19.60	0.40	16.20	0.60	3.11[a]	36.83
34	T34	IC 52105	30.20	1.60	28.60	2.40	20.60	1.20	6.55[a]	30.28
35	T35	Kashikanchan	27.00	4.60	22.40	6.60	17.40	3.80	22.46[ij]	46.84
36	T36	IC 52128	45.60	1.20	41.60	0.60	36.60	0.40	1.78[a]	75.54

Sl. No.	Accessions		Mean No. of buds	Mean No. of damaged buds	Mean No. of flowers	Mean No. of damaged flowers	Mean No. of pods	Mean No. of damaged pods	Total damage (%)	Total yield per plant
37	T37	EC 98668	68.60	0.00	53.00	0.00	37.00	0.00	0.00*	69.19
38	T38	IC 39947	23.60	0.40	22.00	1.20	16.40	0.00	2.58 [a]	29.36
39	T39	IC 20645	21.80	0.60	19.60	0.20	15.80	0.00	1.39 [a]	59.85
40	T40	IC 19778	25.20	1.60	22.20	3.40	15.40	2.00	11.15 [bcdef]	35.14
41	T41	V. Jyothika	42.40	3.40	37.60	5.80	18.60	3.40	12.78 [defghi]	151.03
42	T42	Malika	54.20	9.60	42.40	8.60	24.80	6.60	20.43 [hij]	119.04
43	T43	Sharika	34.00	4.80	27.80	8.60	18.20	6.40	24.75 [jk]	176.54
44	T44	Bhagyalakshmy	40.00	13.00	25.80	13.00	14.60	7.00	41.04 [m]	57.99
45	T45	EC 101216	34.40	0.00	28.00	0.60	19.40	0.20	0.98 [a]	39.97
46	T46	IC 52110	38.80	0.00	34.00	0.00	26.20	0.00	0.00*	43.97
47	T47	IC 52118	54.00	0.20	46.80	0.40	32.00	0.00	0.45 [a]	64.70
48	T48	Lola	42.80	7.80	34.00	10.80	19.80	9.40	28.99 [kl]	218.99

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

* Not included in statistical analysis

4.1.2 Number of flowers

The average number of flowers varied from 11.6 to 53 per plant in IC 26048 and EC 98668, respectively. PKM – 1, IC 52118, Malika, IC 52128 and Vellayani Jyothika produced an average of 47.2, 46.8, 42.4, 41.6 and 37.6 flowers per plant, respectively, followed by accessions IC 26048 (11.6), TVX – 944 (13.8), EC 100092 (15.4), IC 2815 (16.4), Palakkadan thandan payar (16.4), IT – 3895 – 1 (17.2), KBC – 2 (17.2), KM – 5(17.5), IC 10810 (17.8), Sreya (18.2), Mysore local (18.6), IC 9883 (19.3), IC 39921(19.6) and IC 20645 (19.6). The remaining genotypes had 20.4 to 36.2 flowers per plant.

4.1.3 Number of pods

The average number of pods varied from nine (IC 26048) to 40 (PKM – 1). PKM – 1 was followed by IC 98668, IC 52128, IC 52118, Hridya, IC 39870, IC 20431, IC 2196 and IC 52110 with 37, 36.6, 32, 31, 27, 26.6, 26.6 and 26.2 mean pods per plant, respectively. The accessions IC 26048, TVX – 944, KM – 5, Shreya, IC 39922, IC 10810, IT – 3895 – 1, KBC – 2, C – 152, Bhagyalekshmi, IC 52107 A, IC 9883, Mysore local, IC 7832, IC 19778, IC 20645, IC 2196, Palakkadan thandan payar and IC 39921 produced fewer number of pods. The rest of the accessions produced between 16.4 and 24.8 pods per plant.

4.1.4 Per cent infestation

The accessions evaluated differed significantly in terms of borer infestation to buds, flowers and pods. Five accessions, *viz.* EC 100092, IC39945, IC2918, EC98668 and EC 101216 recorded zero damage. Twenty six accessions suffered damage between 0.34 and 9.29 per cent and were on par with each other. Among these, the lowest damage of 0.34 per cent was observed in case of IC 39922, while IC 2196 had 9.29 per cent infestation. The highest damage of 41.04 per cent was registered in case of Bhagyalakshmy. C-152, Lola, Anaswara and Sharika, with 33.54, 28.99, 26.13 and 24.75 per cent damage respectively and were on par with each other. Remaining twelve accessions had infestation ranging from 9.29 to 24.95 per cent.

Variation was also observed in infestation levels at different stages of the same accession. Ten accessions had zero bud damage. Among the remaining accessions, lowest bud damage of 0.37 per cent was observed in case of IC 52118. Seven accessions had zero flower damage. IC 52118 had the lowest mean flower damage of 0.85 per cent among the other accessions. Similarly, ten accessions had zero pod damage and among the remaining accessions, the lowest mean fruit infestation of 0.65 per cent was observed in case of Hridya. The variety Bhagyalakshmy recorded highest infestation for all the three stages, *viz.,* bud, flower and fruit, with 32.5, 50.39 and 47.95 per cent damage respectively. In all, six accessions suffered highest damage during bud stage while 21 and 16 accessions had highest infestation during flower and pod stages respectively.

The grain type accessions were found to suffer lesser infestation from the pod borer, as against the vegetable type genotypes. All the 24 accessions from ICAR - NBPGR, TVX – 944 and KBC – 2 from UAS Bengaluru as well as Shreya, Hridya and Kanakamony from KAU recorded consistently low values for bud, flower and pod damage, underscoring the above observation. Nine out of the 11 accessions which suffered highest bud, flower and pod damage belonged to vegetable type, with infestation levels varying from 3.16 to 41.04 per cent. Palakkadan thandan payar, with 3.16 per cent damage proved to be the lone exception among the vegetable type accessions with low borer infestation.

Correlation between yield and per cent infestation was not attempted, given the wide disparity in growth habit, crop duration etc. However, most of the grain type accessions had mean yield values ranging from14.58 g (IC 52107 A) to 92.16 g (EC 100092) per plant. The vegetable type accessions registered higher per plant yield, ranging from 37.54 in case of Kanakamony, a dual purpose variety, to 254.8 grams in case of PKM-1.

4.1.5 Categorisation of accessions based on extent of damage

The forty eight accessions were subjected to Duncan's Multiple Range Test (DMRT), based on per cent damage to buds, flowers and pods and were grouped as resistant, moderately resistant, susceptible and highly susceptible (Table 3). Twenty three accessions with zero to five per cent damage were categorised as resistant. Eight accessions with infestation ranging from 5 - 10 per cent were classified as moderately resistant. Five accessions with infestation ranging from 10 to 15 per cent were grouped as susceptible and twelve accessions with more than 15 per cent damage were categorised as highly susceptible.

Table 3. Classification of the cowpea accessions based on extent of damage by *M. vitrata*

Susceptibility rating	Extent of damage (%)	Accessions
Resistant	0 – 5 %	EC 100092, IC 39945, IC 2918, EC 98668, IC 52110, IC 39922, IC 52118, IC 39916, Hridya, EC 101216, EC 300039, IC 2196, IC 20645, IC 26048, IC 52128, IC 52107 A, IC 39947, TVX – 944, IC 39921, IC 26029, Palakkadan thandan, Kanakamony, IC 20720
Moderately resistant	5 – 10 %	IC 39870, IC 52105, IC 9883, Sreya, IC 20431 KBC – 2, Mysore Local, IC 2196
Susceptible	10 – 15%	IC 198778, PKM – 1, Vellayani Jyothika, IC 10810, IT – 3895 – 1
Highly susceptible	>15 %	IC 19797, KM – 5, IC 52094, Vyjayanthi, IC 7832, Malika, Kashikanchan, Sharika, Anaswara, Lola, C – 152, Bhagyalakshmy

Twenty accessions viz., Kanakamony, EC 100092, Palakkadan thandan payar, TVX – 944, EC 300039, Hridya, IC 20645 and IC 52110 from resistant category, IC 20431, Sreya, Mysore local and IC 52105 from moderately resistant group, PKM – 1 and Vellayani Jyothika from susceptible and C – 152, Anaswara, Kashikanchan, Malika, Bhagyalakshmy and Lola from highly susceptible groups were selected at random for detailed investigation into the morphological and biochemical bases of resistance.

4.2 MORPHOLOGICAL BASES OF RESISTANCE

Morphological bases of resistance were ascertained by recording the flower parameters like configuration of calyx and length of flower stalk as well as pod parameters such as pod angle, pod wall thickness, trichome length, trichome density and nature of seed testa, following the ICAR - NBPGR varietal descriptor for cowpea.

4.2.1 Length of flower stalk

The accessions exhibited marked difference in terms of the length of flower stalk (Table 7). The mean length of flower stalk varied from 3.02 cm to 13.52 cm. Mysore local, recorded the longest flower stalk of 13.52 cm length followed by Kanakamony and EC 300039 with 10.62 and 9.79 cm respectively. All these three accessions were statistically on par with each other but differed significantly from the remaining genotypes. The lowest length of flower stalk was recorded in Sreya (3.02 cm), followed by IC 20431, PKM – 1, Malika, TVX – 944 and Hridya with mean flower stalk length of 3.67, 3.72, 4.2, 4.75 and 5.12 cm respectively, all the six accessions being statistically at par. The remaining eleven accessions had mean flower stalk length between 6.27 and 9.43 cm.

4.2.2 Configuration of calyx

Categorisation of the different accessions (Table 4) on the basis of the configuration of calyx indicated that EC 100092, Palakkadan thandan payar, TVX – 944, EC 300039, IC 20645 and IC 52110 had semi tight calyx characterized by tight sepals with tips alone being free. The accessions C – 152, Kanakamony, PKM – 1, Anaswara, IC 20431, Sreya, Hridya, Mysore local, IC 52105, Kashikanchan, Vellayani Jyothika, Malika, Bhagyalakshmy and Lola had major portion of sepals free with their basal portion tight. Hence, they were grouped partially free.

Table 4. Configuration of calyx among cowpea accessions

Sl. No.	Accession	Configuration of calyx
1	C – 152	Partially free
2	Kanakamony	Partially free
3	PKM – 1	Partially free
4	EC 100092	Semi tight
5	Palakkadan thandan payar	Semi tight
6	Anaswara	Partially free
7	TVX – 944	Semi tight
8	EC 300039	Semi tight
9	IC 20431	Partially free
10	Sreya	Partially free
11	Hridya	Partially free
12	Mysore local	Partially free
13	IC 52105	Partially free
14	Kashikanchan	Partially free
15	IC 20645	Semi tight
16	Vellayani Jyothika	Partially free
17	Malika	Partially free
18	Bhagyalakshmy	Partially free
19	IC 52110	Semi tight
20	Lola	Partially free

4.2.3 Pod angles

The different accessions under study displayed wide variation in pod angles, with mean angle ranging from 18.6^0 (Kashikanchan and PKM - 1) to 86.33^0 (EC 100092) (Table 7). IC 20645, Anaswara, Palakkadan thandan payar, IC 20431 and Vellayani Jyothika recorded mean pod angle of 58.67^0, 57.0^0, 54.67^0, 48.33^0 and 48^0, respectively. All these accessions, including EC 100092 were statistically on par with each other but differed significantly from the remaining genotypes. Kashikanchan and PKM - 1 (18.67^0), followed by IC 52105, IC 52110, C – 152, Kanakamony, Mysore local and Lola had lower mean pod angles of 20.00, 22.17^0, 31.33^0, 31.83^0, 31.83 and 35.00^0, respectively. All the eight accessions were statistically on par with each other but differed significantly from the remaining genotypes. The remaining six accessions recorded pod angles varying from 36.67^0 to 43.67^0 and were on par with each other.

4.2.4 Pod wall thickness

Variation among different genotypes was also observed with regard to the thickness of pod walls (Table 7). The mean thickness ranged from 0.45 mm to 1.20 mm, with the highest value being recorded in case of in Palakkadan thandan payar, which was followed by EC 100092, PKM – 1, IC 20431, Vellayani Jyothika, TVX – 944 and IC 20645 with mean pod wall thickness of 1.20 mm, 1.03 mm, 1.03 mm and 1.01 mm respectively, all being on par. The lowest pod wall thickness of 0.45 mm was recorded in Lola, followed by C – 152 (0.47 mm), IC 52105 (0.52 mm), Hridya (0.52 mm), Mysore local (0.52 mm), IC 52110 (0.53 mm), Kashikanchan (0.54 mm), Bhagyalakshmy (0.529 mm), Malika (0.53 mm), Sreya (0.53 mm), Kanakamony (0.541 mm), Anaswara (0.541 mm) and EC 300039 (0.549 mm). All these 13 accessions were statistically on par with each other.

4.2.5 Length of trichomes

The variety Lola recorded maximum trichome length of 0.25 mm and was on par with Palakkadan thandan payar with a mean trichome length of 0.25 mm and 0.23 mm. Both the accessions were significantly superior to the remaining accessions which recorded mean trichome length of ranging from 0.04 to 0.01 mm.

4.2.6 Trichome density

The accessions varied significantly in terms of trichome density (Table 7). The number of trichomes varied from 16.34 per 6.25 mm^2 in PKM - 1 to 288.67 per 6.25 mm^2 in TVX – 944. High trichome density was also observed in

Kanakamony, Mysore local, Sreya and Anaswara, with 235.34, 212, 186 and 167.34 trichomes per 6.25 mm^2 respectively. All these accessions were statistically on par with each other but differed significantly from the remaining genotypes.

Table 5. Pubescence of the pods in selected accessions of cowpea

Sl. No.	Accession	Pubescence
1	C – 152	Pubescent
2	Kanakamony	Densely pubescent
3	PKM – 1	Glabrous
4	EC 100092	Sparsely pubescent
5	Palakkadan thandan payar	Glabrous
6	Anaswara	Densely pubescent
7	TVX – 944	Densely pubescent
8	EC 300039	Glabrous
9	Vyjayanthi	Glabrous
10	IC 20431	Glabrous
11	Sreya	Densely pubescent
12	Hridya	Pubescent
13	Mysore local	Densely pubescent
14	IC 7832	Sparsely pubescent
15	IC 20645	Glabrous
16	Vellayani Jyothika	Glabrous
17	Malika	Glabrous
18	Bhagyalakshmy	Glabrous
19	IC 52110	Pubescent
20	Lola	Glabrous

PKM - 1, which recorded the lowest trichome density of 16.34 per 6.25 mm^2 was followed by Bhagyalakshmy, Lola, IC 52105, EC 300039, IC 20645, IC 20431, Vellayani Jyothika and Palakkadan thandan payar with trichome density of 17.34, 18.34, 18.67, 27.34, 32, 33, 39.34 and 49.34 trichomes per 6.25 mm^2 respectively. All these nine accessions were statistically on par with each other but differed significantly from the remaining genotypes. The remaining accessions except Malika had trichome density ranging from 66.00 to 147.67 per 6.25 mm^2. Based on the density of trichomes, the accessions were classified into: Densely pubescent, pubescent, sparsely pubescent and glabrous (Table 5).

4.2.7 Nature of seed testa

Two varieties, namely, Vellayani Jyothika and Lola had thick seed testa while the remaining 18 genotypes had thinner testae (Table 6). All the 20 selected had soft seed testa. The seed testa had cream colour in accessions such as C – 152, Mysore local, IC 20645 and Vellayani Jyothika. The accessions Sreya and Lola had purplish green coloured seed coat and rest of the accessions had green coloured seed coat, as per ICAR - NBPGR varietal descriptor for cowpea.

Table 6. Nature of seed testa in selected accessions of cowpea

Sl. No.	Accession	Thickness	Toughness	Colour
1	C – 152	Thin	Soft	Cream
2	Kanakamony	Thin	Soft	Green
3	PKM – 1	Thin	Soft	Green
4	EC 100092	Thin	Soft	Green
5	Palakkadan thandan	Thin	Soft	Green
6	Anaswara	Thin	Soft	Green
7	TVX – 944	Thin	Soft	Green
8	EC 300039	Thin	Soft	Green
9	IC 20431	Thin	Soft	Green
10	Sreya	Thin	Soft	Purplish green
11	Hridya	Thin	Soft	Green
12	Mysore local	Thin	Soft	Cream
13	IC 52105	Thin	Soft	Green
14	Kashikanchan	Thin	Soft	Green
15	IC 20645	Thin	Soft	Cream
16	Vellayani Jyothika	Thick	Soft	Cream
17	Malika	Thin	Soft	Green
18	Bhagyalakshmy	Thin	Soft	Green
19	IC 52110	Thin	Soft	Green
20	Lola	Thick	Soft	Purplish green

Table 7. Morphological parameters of selected accessions of cowpea

Sl. No.	Accessions	Length of flower stalk (cm)	Pod angles (degree)	Pod wall thickness (mm)	Trichome length (mm)	Trichome density (No. per 6.25 mm^2)
1	C – 152	9.30^{cde}	31.33^{ef}	0.47^{de}	0.07^{b}	108.67^{gh}
2	Kanakamony	10.62^{b}	31.83^{ef}	0.54^{cd}	0.07^{b}	235.33^{b}
3	PKM – 1	3.72^{ijk}	18.67^{g}	1.03^{b}	0.07^{b}	16.33^{lm}
4	EC 100092	8.85^{cdef}	86.33^{a}	1.03^{b}	0.04^{b}	83.33^{hi}
5	Palakkadan thandan Payar	8.60^{def}	54.67^{bc}	1.20^{a}	0.23^{a}	49.33^{jk}
6	Anaswara	9.22^{cde}	57.00^{b}	0.54^{cd}	0.05^{b}	167.33^{de}
7	TVX – 944	4.75^{hi}	36.67^{de}	1.02^{b}	0.07^{b}	288.67^{a}
8	EC 300039	9.78^{bc}	41.67^{de}	0.55^{c}	0.09^{b}	27.33^{klm}
9	IC 20431	3.67^{jk}	48.33^{bcd}	1.02^{b}	0.04^{b}	33.00^{kl}
10	Sreya	3.02^{k}	40.00^{de}	0.53^{cd}	0.07^{b}	186.00^{cd}

11	Hridya	5.12^{h}	42.33^{cde}	0.52^{cde}	0.06^{b}	147.67^{ef}
12	Mysore local	13.52^{a}	31.83^{ef}	0.52^{cd}	0.046^{b}	212.00^{bc}
13	IC 52105	8.12^{f}	20.00^{fg}	0.52^{cde}	0.07^{b}	18.67^{lm}
14	Kashikanchan	8.9^{cdef}	18.67^{g}	0.54^{cd}	0.057^{b}	66.00^{ij}
15	IC 20645	8.10^{f}	58.67^{b}	1.02^{b}	0.06^{b}	32.00^{kl}
16	Vellayani Jyothika	8.30^{ef}	48.00^{bcd}	1.02^{b}	0.10^{b}	39.33^{jkl}
17	Malika	4.20^{hij}	43.67^{cde}	0.53^{cd}	-	-
18	Bhagyalakshmy	9.43^{cd}	38.00^{de}	0.53^{cd}	0.07^{b}	17.33^{lm}
19	IC 52110	9.37^{cd}	22.17^{fg}	0.526^{cd}	0.05^{b}	135.33^{fg}
20	Lola	6.27^{g}	35.00^{e}	0.45^{e}	0.25^{a}	18.33^{lm}

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

4.3 BIOCHEMICAL BASES OF RESISTANCE

Biochemical bases of resistance of the different cowpea accessions to spotted pod borer, *M. vitrata* were ascertained by estimating the biochemical parameters such as total protein content, total moisture content, total sugars, reducing sugars, peroxidase enzyme activity and poly phenol oxidase activity of the pods. The results are presented in Table 8.

4.3.1 Total moisture content

Moisture content varied significantly in all the 20 accessions and it ranged from 71.07 per cent to 91.00 per cent. Highest moisture content of 91 per cent was recorded in PKM – 1 and was followed by Lola (90.54%), Vellayani Jyothika (89.44 %), Anaswara (89.37 %), Malika (89.37 %), IC 20645 (88.87%), Kanakamony (87.61%), Sreya (86.80%), Bhagyalakshmy (86.47%) and C – 152 (85.80%). All the ten accessions were statistically on par with each other but differed significantly from the remaining ten genotypes. Mysore local had the lowest moisture content of 71.07 per cent and was followed by TVX – 944 (74.87%), EC 100092 (75.60%), IC 52105 (77.97%), IC 52110 (78.40%), EC 300039 (79.17%), Hridya (81.30%), Kashikanchan (81.43%), IC 20431 (82.27%) and Palakkadan thandan payar (83.87%). All the ten accessions being on par with each other and were at par with each other but differed significantly from the remaining genotypes.

4.3.2 Total protein content

Protein content varied significantly among the cowpea accessions evaluated. The mean protein content ranged from 20.51mg g^{-1} to 31.6 mg g^{-1} of fresh pods in different genotypes. Lola recorded the highest protein content of 31.6 mg g^{-1} and was followed by Anaswara and C – 152 with 31.04 and 30.49 mg g^{-1} of fresh pod respectively. All the three accessions were statistically on par with each other and were significantly superior to the remaining seventeen genotypes. EC 300039 had the lowest protein content of 20.51 mg g^{-1} and was followed by EC 100092 (20.62 mg g^{-1}), IC 20431 (20.63 mg g^{-1}), IC 52110 (22.29

mg g^{-1}), TVX – 944 (22.41 mg g^{-1}), Mysore local (23.25 mg g^{-1}), Palakkadan thandan payar (23.91 mg g^{-1}) and Hridya (24.38 mg g^{-1}) all being at par. The accessions Kanakamony, IC 20645, IC 52105, Sreya, PKM – 1, Kashikanchan and Vellayani Jyothika had an average protein content 25.89, 25.99, 26.46, 26.51, 26.87, 27.32 and 27.99 mg g^{-1} protein respectively and were on par with each other but differed significantly from the remaining genotypes.

4.3.3 Total sugar content

The total sugar content varied significantly among the cowpea accessions evaluated. The mean sugar content ranged from 1.39 per cent to 4.33 per cent. C – 152 with the highest total sugar content of 4.33 per cent was significantly superior followed by Lola, Bhagyalakshmy, Anaswara and Vellayani Jyothika with 3.72, 3.17, 2.62 and 2.42 per cent respectively. The lowest value of 1.39 per cent for total sugar was observed by EC 300039, followed by Sreya, EC 100092, TVX – 944, IC 20431, IC 20645, IC 52105, IC 52110, Hridya and Kanakamony with 1.44, 1.45, 1.52, 1.55, 1.66, 1.74, 1.83, 1.83 and 1.86 per cent respectively, all the ten accessions being at par. The accessions Palakkadan thandan payar, Kashikanchan, PKM – 1, Mysore local and Malika had an average of 2.10, 2.16, 2.20, 2.23 and 2.27 per cent sugar respectively and were on par with each other though they differed significantly from the remaining genotypes.

4.3.4 Reducing sugar

The reducing sugar content varied significantly among the cowpea accessions evaluated. C – 152 recorded the highest value 3.86 per cent and was followed by Lola, Bhagyalakshmy, Vellayani Jyothika, Kashikanchan and Malika with 3.07, 2.28, 1.97, 1.91 and 1.89 per cent respectively. All the six accessions were statistically on par with each other but differed significantly from the remaining genotypes. EC 100092 had the lowest reducing sugar content with 0.43 per cent, which was followed by TVX – 944, IC 20645, IC 52105, IC 52110, IC 20431, EC 300039 and Sreya with 0.455, 0.54, 0.54, 0.57, 0.72, 0.74 and 0.88 per cent respectively, all being on par with each other. The accessions Kanakamony, Mysore local, Hridya, Kanakamony, Anaswara and PKM - 1 contained, on an average, 1.09, 1.17, 1.51, 1.62, 1.76 and 1.77 per cent reducing sugars respectively and were on par with each other.

4.3.5 Peroxidase activity

Kanakamony had the highest peroxidase enzyme content of 97.81 EU g^{-1} and was significantly superior to the remaining accessions followed by Hridya, IC 52110, EC 300039, Sreya and IC 20645 with a peroxidase activity of 63.04, 48.15, 46.06, 43.06 and 39.18 EU g^{-1} respectively. They were on par with each other but differed significantly from the remaining genotypes. Lola recorded the lowest peroxidase content of 2.9 EU g^{-1} and was followed by Anaswara, Palakkadan thandan payar, IC 20431, C – 152, Bhagyalakshmy and Vellayani Jyothika with 3.30, 4.30, 4.57, 9.56, 14.18 and 14.48 EU g^{-1}. The accessions Kashikanchan, EC 100092, Mysore local, Malika, TVX – 944, PKM – 1, IC 52105 and IC 20645 had an average of 18.58, 19.33, 19.94, 20.00, 22.95, 30.58, 37.53 and 39.18 EU g^{-1} peroxodase activity respectively and were at par with each other but differed significantly from the remaining genotypes.

4.3.6 Polyphenol oxidase

Polyphenol activity of different accessions varied from 0.0034 EU g^{-1} recorded in C - 152 to 0.022 EU g^{-1} in Palakkadan thandan payar. The accessions EC 300039 (0.016), Sreya (0.016), TVX – 944 (0.015), Hridya (0.015), IC 20645 (0.015), EC 100092 (0.014), IC 52105 (0.011), IC 20431 (0.011), IC 52110 (0.010), Mysore local (0.01), Kashikanchan (0.01) Kanakamony (0.009), Malika (0.007) and PKM – 1 (0.007) had high poly phenol content and were statistically on par with each other but differed significantly from the remaining genotypes. The remaining accessions C – 152, Anaswara, Vellayani Jyothika, Lola and Bhagyalakshmy had low polyphenol oxidase enzyme activity of 0.0034, 0.0053, 0.0054, 0.0059 and 0.0061 EU g^{-1} respectively and were at par with each other.

Table 8. Biochemical parameters of selected accessions of cowpea

Sl. No.	Accession	Moisture content (%)	Total protein (mg/g)	Total sugar (%)	Reducing sugar (%)	Peroxidase (EU/g)	Polyphenol oxidase (EU/g)
1	C – 152	85.8^{cde}	30.49^{ab}	4.33^{a}	3.86^{a}	9.56^{ij}	0.003^{e}
2	Kanakamony	87.61^{abcd}	25.89^{f}	1.86^{fgh}	1.62^{gh}	97.82^{a}	0.009^{bcde}
3	PKM – 1	91.00^{a}	26.87^{def}	2.20^{e}	1.77^{ef}	30.58^{f}	0.007^{bcde}
4	EC 100092	75.60^{h}	20.62^{i}	1.45^{jk}	0.43^{m}	19.33^{gh}	0.014^{abcd}
5	Palakkadan thandan payar	83.87^{def}	23.91^{g}	2.10^{fg}	1.09^{i}	4.30^{jk}	0.022^{a}
6	Anaswara	89.37^{abc}	31.04^{a}	2.62^{d}	1.76^{fg}	3.30^{jk}	0.005^{de}
7	TVX – 944	74.87^{hi}	22.41^{h}	1.52^{ijk}	0.46^{lm}	22.95^{g}	0.015^{ab}
8	EC 300039	79.17^{gh}	20.51^{i}	1.39^{k}	0.72^{k}	46.06^{cd}	0.016^{ab}
9	IC 20431	82.27^{efg}	20.627^{i}	1.55^{hijk}	0.57^{kl}	4.34^{jk}	0.011^{bcde}
10	Sreya	86.80^{abcd}	26.51^{def}	1.44^{jk}	0.88^{j}	43.06^{cde}	0.016^{ab}

11	Hridya	81.30^{fg}	24.38^{g}	1.83^{ghi}	1.51^{h}	63.04^{b}	0.015^{abc}
12	Mysore local	71.07^{i}	23.25^{gh}	2.22^{e}	1.17^{i}	19.94^{gh}	0.010^{bcde}
13	IC 52105	77.97^{gh}	26.46^{def}	1.74^{hij}	0.54^{lm}	37.53^{ef}	0.012^{bcde}
14	Kashikanchan	81.43^{fg}	27.32^{de}	2.16^{ef}	1.91^{de}	18.58^{gh}	0.010^{bcde}
15	IC 20645	88.87^{abc}	25.99^{ef}	1.74^{hijk}	0.54^{lm}	39.18^{de}	0.015^{abc}
16	Vellayani Jyothika	89.43^{abc}	27.40^{d}	2.42^{de}	1.97^{d}	14.48^{hi}	0.005^{de}
17	Malika	89.37^{abc}	28.95^{c}	2.27^{e}	1.89^{def}	20.00^{gh}	0.007^{bcde}
18	Bhagyalakshmy	86.47^{bcde}	29.65^{bc}	3.17^{c}	2.28^{c}	14.18^{hi}	0.0061^{cde}
19	IC 52110	78.40^{gh}	22.29^{h}	1.83^{ghi}	0.54^{lm}	48.15^{c}	0.010^{bcde}
20	Lola	90.53^{ab}	31.60^{a}	3.72^{b}	3.07^{b}	2.90^{k}	0.0059^{de}

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

4.4 CORRELATION OF MORPHOLOGICAL TRAITS OF COWPEA WITH SPOTTED POD BORER INFESTATION

Analysis was carried out to identify the correlation between morphological parameters of cowpea and spotted pod borer infestation (Table 9).

4.4.1 Length of flower stalk

Length of flower stalk was positively correlated (0.067) with per cent damage due to spotted pod borer. However the correlation was not significant.

4.4.2 Pod angle

Pod angle was negatively correlated (-0.206) with per cent damage due to spotted pod borer, though the correlation was not significant.

4.4.3 Pod wall thickness

Pod wall thickness was negatively correlated (-0.447) with per cent damage with significant correlation at $p = 0.05$ level.

4.4.4 Length of trichomes

As in case of the length of flower stalk, length of the trichomes also had positive correlation (0.097) with per cent damage. However, the correlation was not significant.

4.4.5 Trichome density

Number of trichomes on pods (6.25 mm^2) was negatively correlated (-0.275) with per cent damage due to spotted pod borer, but the correlation was not significant.

Table 9. Correlation between morphological parameters of cowpea and per cent damage

	Damage	Length of flower stalk	Pod angle	Pod wall thickness	Trichome length
Length of flower stalk	0.067				
Pod angle	-0.206	0.006			
Pod wall thickness	-0.447*	-0.248	0.474*		
Trichome length	0.097	0.019	0.012	0.159	
No. of trichomes	-0.275	0.156	-0.063	-0.141	-0.202

*Correlation is significant at 0.05 level (2-tailed).

4.5 CORRELATION OF BIOCHEMICAL TRAITS OF COWPEA WITH SPOTTED POD BORER INFESTATION

4.5.1 Total protein content

The total protein content exhibited significant positive correlation (0.815) with per cent damage due to spotted pod borer (p = 0.01).

4.5.2 Moisture

A significant positive correlation (0.464) was also observed between per cent damage due to spotted pod borer (p = 0.01).

4.5.3 Total sugars

As in case of protein and moisture the total sugar content also exhibited positive correlation (0.851) with per cent damage, which was significant at p = 0.01 level.

4.5.4 Reducing sugars

Reducing sugar content also exhibited a positive correlation (0.82) with per cent damage. The correlation was significant at p = 0.01 level.

4.5.5 Peroxidase

Peroxidase activity had significant negative correlation (-0.527) with per cent damage. The correlation was significant at p = 0.05 level.

4.5.6 Polyphenol oxidase

Polyphenol oxidase content exhibited significant negative correlation (-0.734) with per cent damage by spotted pod borer at p = 0.01 level.

Table 10. Correlation between biochemical parameters of cowpea and per cent damage

Correlations						
	Damage (%)	**Total protein**	**Total moisture**	**Peroxidase**	**Polyphenol oxidase**	**Reducing sugars**
Protein	0.815**					
Moisture	0.461*	0.712**				
Peroxidase	-0.527*	-0.267	-0.069			
Polyphenol oxidase	-0.734**	-0.668**	-0.427	0.231		
Reducing sugars	0.820**	0.797**	0.540*	-0.287	-0.710**	
Total sugar	0.851**	0.772**	0.424	-0.451*	-0.700**	0.936**

*Correlation is significant at 0.05 level (2-tailed). **Correlation is significant at 0.01 level (2-tailed).

Discussion

5. DISCUSSION

A study was conducted at College of Horticulture, Vellanikkara for evaluation of resistance in 48 cowpea accessions against the spotted pod borer, *Maruca vitrata*. Investigations were also carried into the biophysical and biochemical bases of resistance to *M. vitrata*. The discussion on the results obtained in the study is presented here under.

5.1 EVALUATION OF COWPEA ACCESSIONS FOR RESISTANCE TO SPOTTED POD BORER DAMAGE

Screening of the 48 accessions for resistance to pod borer *M. vitrata* clearly brought out the significant variation among the genotypes in terms of per cent damage to buds, flowers and pods, with total damage varying from zero per cent to 41.04 per cent. Twenty three accessions which recorded 0 to 5 per cent damage, eight accessions with infestation ranging from 5 - 10 per cent, five accessions with infestation ranging from 10 to 15 per cent and twelve accessions which recorded damage of more than 15 per cent.

Variation in susceptibility to pod borer has been reported in cowpea by several workers. Singh (1978) for instance, reported that TVu 946 and TVu 4557 were resistant to *M. vitrata*; while Echendu and Akingbohungbe (1990) reported that TVu 946, TVu 1896 AG, H 51-1 and 2AK were resistant to the borer. Oghiakhe *et al.* (1995) identified TVu 946, MRx2-8F and MRx109-84F resistant genotypes, while another eight accessions were rated as moderately resistant.

In a previous study conducted by Anithakumari (1992) at the Kerala Agricultural University, 17 genotypes of cowpea were found to be moderately resistant to *M. vitrata,* with ten to fifteen per cent damage. The present study also has confirmed the prevalence of resistance to *M. vitrata* in cowpea and point to the possibility of exploiting the same for management of the spotted pod borer.

Five accessions, namely, EC 100092, IC 39945, IC 2918, EC 98668 and IC 52110 had no damage to bud, flower or pod, suggesting very high resistance, which need to be established through further studies. Once resistance has been confirmed, all the five accessions could serve as valuable sources of resistance in further breeding programmes, along with other accessions which suffered remarkably low levels of infestation.

Variation in infestation levels during different stages of the same accession was observed in the present study. Among the 43 accessions that had damage during either of the three stages, highest number of accessions suffered greater damage during flowering stage, followed by sixteen accessions, with highest damage to pods.

Highest incidence during flowering stage followed by fruiting stage observed in the present study are in agreement with the reports by Jackai (1981), Karel (1985) and Kumar *et al.* (2013). Expression of resistance is influence of the stage of the host plant on expression of resistance to insect pests have been well documented (Panda and Khush, 1995). The findings of the present study lead to similar conclusions in case of cowpea, though the exact mechanisms involved need to be elucidated.

The lower level of infestations recorded in accessions belonging to grain type of cowpea, as compared to those belonging to vegetable type is along expected lines. Vegetable varieties tend to be more succulent, with much tender pods. They also had longer crop duration, resulting in exposure to pod borer for a [illegible]od. Comparison based on parameters such as pod wall thickness, pubesc[illegible], protein and moisture content lend support to this observation. Softer tissues as well as higher nutritional value could render the vegetable varieties more attractive to a tissue borer like *M. vitrata*. The reason for the low infestation in Palakkadan thandan payar, a vegetable type, could be due to the pod wall thickness of 1.20 mm, which was the highest among all the accessions evaluated.

5.2 MORPHOLOGICAL BASES OF RESISTANCE

Morphological and anatomical attributes of host plants play a major role in mediating insect plant interactions. Factors such as trichomes and tissue thickness have been implicated to varying degrees in resistance to insect pests in a number of crops including cowpea. The present study assessed the potential role of various morphological features in resistance of cowpea to spotted pod borer in selected accessions.

5.2.1. Length of flower stalk.

The length of the flower stalk varied significantly from 13.52 cm in Mysore local to 3.02 cm in Sreya. The length of flower stalk was positively correlated with total per cent damage. However, the correlation was not significant. Elongate peduncles enable the plant to hold the pods upright above the canopy level. Infesting such pods could expose the larvae to increased risk of predation. This could be the reason for lesser infestation in accessions with longer flower stalks. Similar observations have also been made by International Institute of Tropical Agriculture (IITA) (1974) and Singh (1978) who reported that the length of the peduncle had contributed to resistance towards *M. vitrata* in cowpea.

5.2.2 Configuration of calyx

The accessions *viz.,* EC 100092, Palakkadan thandan payar, TVX – 944, EC 300039, IC 20645 and IC 52110 had semi tight calyx having tight sepals with tips alone being free. All these accessions had consistently low levels of infestation ranging from zero to 3.16 per cent. The remaining 14 accessions, which had free sepals, suffered higher borer infestation. Free sepals would provide the first instar borer larvae some extent of concealment as well as enable it to bore into the flower more easily. Tight calyx, thus, could possibly have a deterrent effect on the first instar larvae oviposition, which, however, need to be confirmed. Anithakumari (1992) also had reported that free configuration of calyx had a significant positive influence on the level of pod borer infestation.

5.2.3 Pod angles

The genotypes evaluated showed wide variation in terms of pod angle, with values ranging from 18.6^0 (EC 100092) to 86.33^0 (EC 100092). Accessions, *viz.*, EC 100092, IC 20645, Palakkadan thandan payar and IC 20431, which recorded mean pod angles of 86.33^0, 58.67^0, 54.67^0 and 48.33^0 registered lower infestation levels of 0.00, 1.39, 3.16 and 8.25 per cent, respectively. Similarly, C-152 with lower mean pod angles had significantly higher damage. However, the above relationship was not consistent across the genotypes evaluated, For instance, Anaswara and Vellayani Jyothika, which had higher mean pod angle values of 57^0 and 48^0 respectively, were among the most susceptible accessions, with infestation levels of 26.13 and 12.78 per cent respectively. Likewise, PKM-1 and Kanakamony had closely held pods, with angles of 18.67^0 and 31.33^0 respectively, but suffered only low levels of damage (12.06 and 3.58 per cent respectively). This was confirmed by the negative but no significant correlation value of (-0.206) between pod angles and per cent damage.

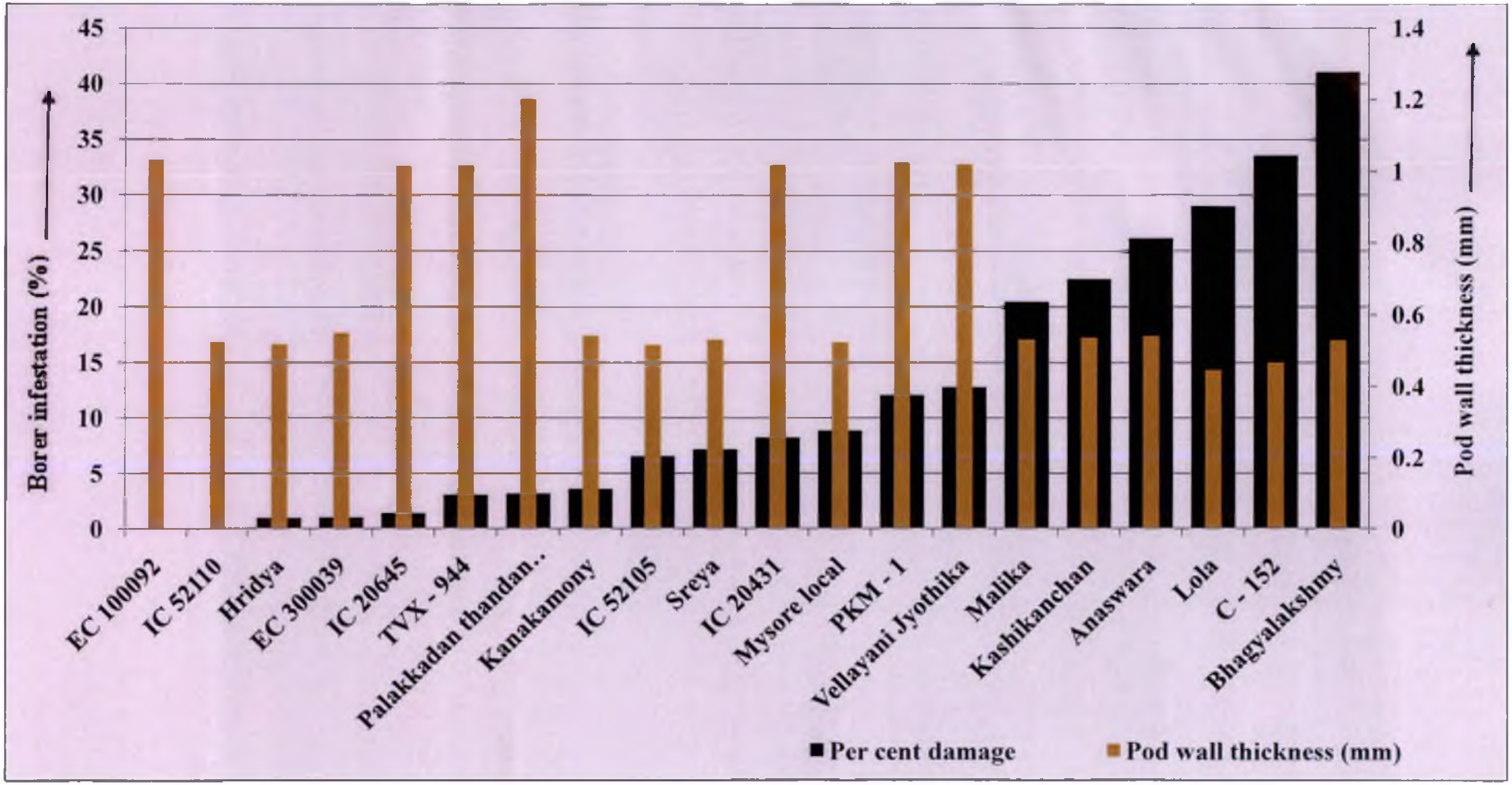
The above observations were in line with the findings of van Emden (1989) who had reported that the pod borer larvae penetrated the pods more successfully when the pod angle was low and pods were in contact with each other. A low pod angle also facilitated webbing of the pods together as well as movement from one pod to another more easily. Oghiakhe *et al.* (1991) also observed a negative relationship between pod angle and pod damage by the borer.

5.2.4 Pod wall thickness

Pods of different accessions varied in the thickness of pod walls, with mean thickness ranging from 0.45 to1.20 mm (Fig. 1). Palakkadan thandan payar,

which was followed EC 100092, PKM – 1, Vellayani Jyothika, TVX – 944 and IC 20645 had higher pod wall thickness, while thirteen accessions were observed to possess thinner pod walls of 0.45 mm to 0.56 mm thickness. Accessions with thicker pod walls, with the exception of Vellayani Jyothika, suffered lower pod borer damage ranging from 0.00 to 0.05 per cent. On the other

Figure 1. Influence of pod wall thickness on per cent damage



hand, accessions such as Lola, C-152, Bhagyalakshmy, Malika and Kashikanchan, with the thinnest pod walls among the accessions evaluated, recorded some of the highest (20.43 to 41.04 per cent) borer damage. Analysis of correlation clearly brought this out, with a significant negative correlation value (-0.447) between per cent pod borer damage and pod wall thickness.

Thickness of tissues has been considered an anatomical feature of significance in conferring resistance to insect pests, especially tissue borers, in a number of crops such as rice, sugarcane, brinjal and cotton (Srinivasan and Basheer, 1961). Pod wall thickness was the only morphological parameter to have a significant correlation with borer damage in the present study. The significant negative correlation between per cent damage and pod wall thickness was in agreement with the findings of Halder and Srinivasan (2011) who reported that pod wall thickness showed a negative correlation with pod damage.

The thicker pod walls could make it more difficult for the larvae to bore into the pods and could also lead to greater wearing off of mandibles (Panda and Khush, 1995), thus forming an important morphological basis for resistance in cowpea to *M. vitrata.*

5.2.5 Trichome length

The length of trichomes, which ranged from 0.04 mm in EC 100092 to 0.25 mm in Lola, did not show any significant variation among the different accessions.

A significant correlation between trichome length and per cent pod damage could not be observed in the study. Sunitha *et al.* (2006) however, had observed significant negative correlation (-0.097) between trichome length on pods and pod borer damage in pigeon pea.

5.2.6 Trichome density

Trichome density varied from 16.34 per 6.25 mm^2 in PKM-1 to 288.67 per 6.25 mm^2 in TVX – 944. High trichome density ranging from 167.34 to 235.34

trichomes per 6.25 mm^2 was also observed in Kanakamony, Mysore local, Shreya and Anaswara. The lowest trichome density was recorded in PKM-1 (16.34/6.25 mm^2), followed by Bhagyalekshmi, Lola, IC 52105, EC 300039, IC 20645, IC 20431, Vellayani Jyothika and Palakkadan thandan payar with 17.34, 18.34, 18.67, 27.34, 32, 33, 39.34 and 49.34 trichomes per 6.25 mm^2, respectively.

Accessions such as Kanakamony, Sreya, Hridya, Mysore local etc., with higher pubescence suffered lower pest incidence. It was also noteworthy that Bhagyalakshmy and Lola, with lowest values for trichome density also observed per cent damage 41.04 and 28.99 per cent, the highest in the present study.

Trichomes are important components of plant defense against insect attack and contribute to antixenosis in crop plants such as cotton (Dhaliwal and Arora, 2001). This was observed in the present instance also, with the number of trichomes on pods being negatively correlated (-0.275) with total damage. However, the correlation was not significant. Sunitha *et al.* (2006) reported significant negative correlation (-0.91) between trichomes on pods and pod borer damage in pigeon pea, suggesting that pubescence significantly contributed to the resistance in ICPL 98003 and ICPL 98008 to spotted pod borer in pigeon pea.

Oghiakhe (1995) also had reported that pubescence in wild and cultivated cowpea adversely affected oviposition, mobility, food consumption and utilization by the legume pod borer.

5.2.7 Nature of seed testa

Observations on thickness, softness and colour of seed testa revealed that two accessions had thick seed testa. Similarly, all selected accessions had soft seed coat. Thirteen accessions had green seed coat while three accessions, namely, C – 152, Mysore local, IC 20645 and Vellayani Jyothika had creamy seed coat. Only two varieties Sreya and Lola had purplish green seed coat.

Anithakumari (1992) also reported that the colour of seed testa had no correlation with pod borer infestation though, the susceptible accessions seemed to have thinner seed testa.

5.3 BIOCHEMICAL BASES OF RESISTANCE

Biochemical parameters *vis.,* protein, moisture, peroxidase activity, polyphenol oxidase activity, reducing sugars and total sugars in fruits have been identified as factors of significance in resistance of cowpea to spotted pod borer. The relationship between the biochemical traits of fruits and spotted pod borer infestation brought out in the present study are discussed below.

5.3.1 Moisture content

Moisture content varied significantly among the 20 accessions, ranging from 71.07 per cent in Mysore local to 91 in per cent PKM – 1 (Fig. 2). Most of the related varieties *vis.,* Lola, Vellayani Jyothika, Anaswara, Malika, IC 20645, Kanakamony, Shreya, Bhagyalekshmi and C – 152 had high moisture content. There was a significant and positive correlation (0.464) between moisture content and per cent damage, bringing out clearly the role of the former in plant herbivore interactions. Moisture, apart from directly influencing the nutritional quality of plant tissues also influences the availability of nitrogen to the herbivores. High moisture content, as in case of protein, has consistently been associated with higher infestation, which is expected as it makes the tissue more succulent. Roshni (2014) had reported a positive correlation between moisture content and melon fly infestation in bitter gourd. However, few reports linking pod borer damage to moisture content of pods are available to enable a comparison with previous studies.

5.3.2 Total protein content

The protein content in fruits of different cowpea accessions varied significantly (Fig. 3). The highest protein content of 31.596 mg g^{-1} was recorded in Lola and the lowest value of 20.51 mg g^{-1} in EC 300039.

Figure 2. Influence of total moisture content on per cent damage

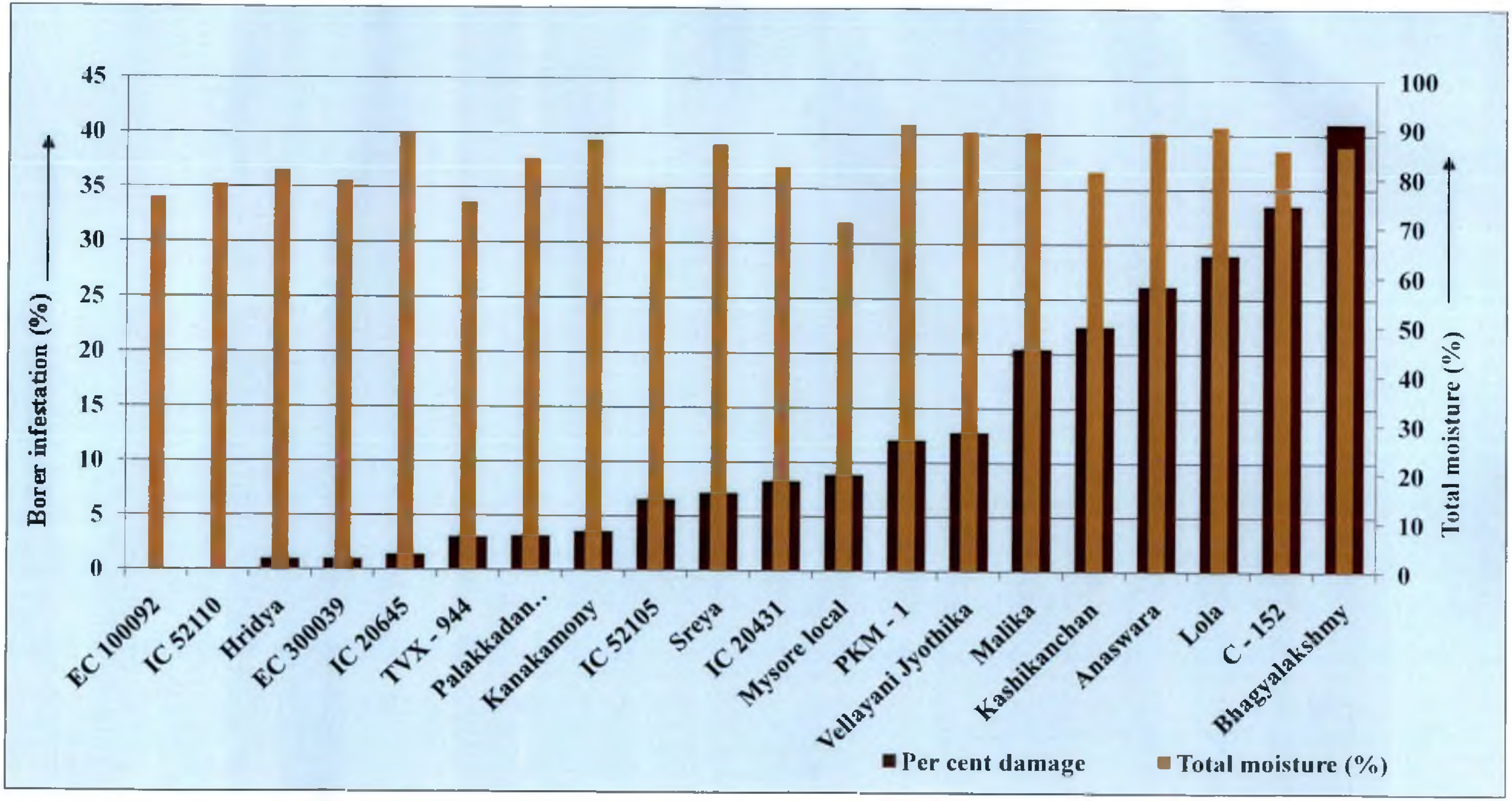
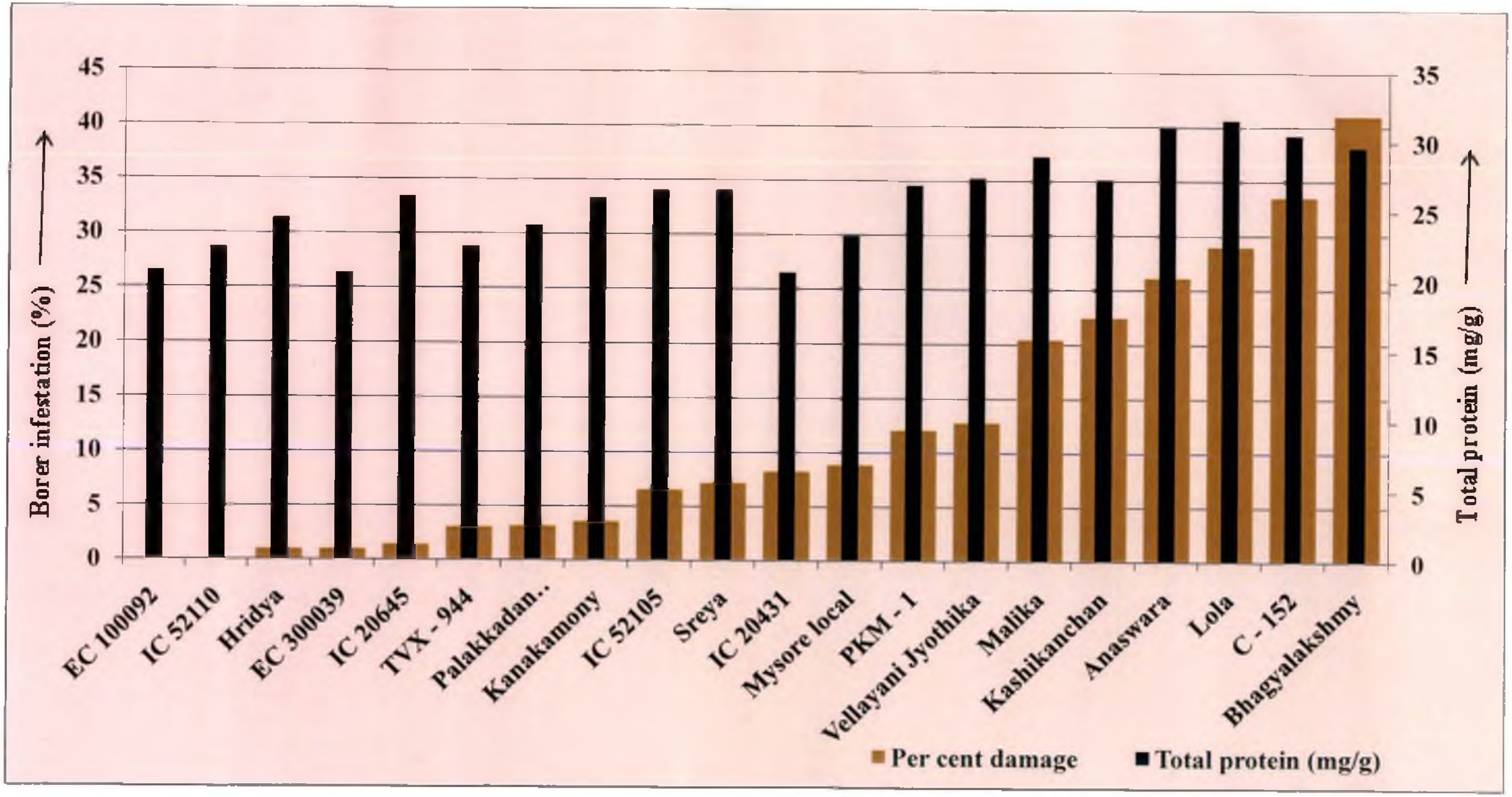


Figure 3. Influence of total protein on per cent damage



Plant proteins are one of the most important sources of dietary nitrogen for herbivores and positive correlation between total protein content and pest infestation has been established in a number of cases. The results of present study affirm the above observations. Accessions which had higher protein content consistently observed higher levels of pest infestation. Thus Lola, Anaswara and Bhagyalakshmy, with protein content of 31.6, 31.04 and 29.65 mg/g respectively, suffered total damage of 28.99, 26.13 and 41.04 per cent respectively, while accessions such as EC 300039 (20.51 mg/g), EC 100092 (20.62 mg/g) which with lower values for protein had negligible damage by borer.

Analysis of correlation between total infestation and total protein content confirmed the significant positive correlation (0.815) between the two parameters.

Several studies have reported significant positive correlation between total protein content in pods and pod borer infestation. Macfoy *et al.* (1983) observed that cowpea varieties resistant to the pod borer had lower protein content. Anithakumari (1992) also reported similar observations, with positive significant correlation between protein content and pod borer damage.

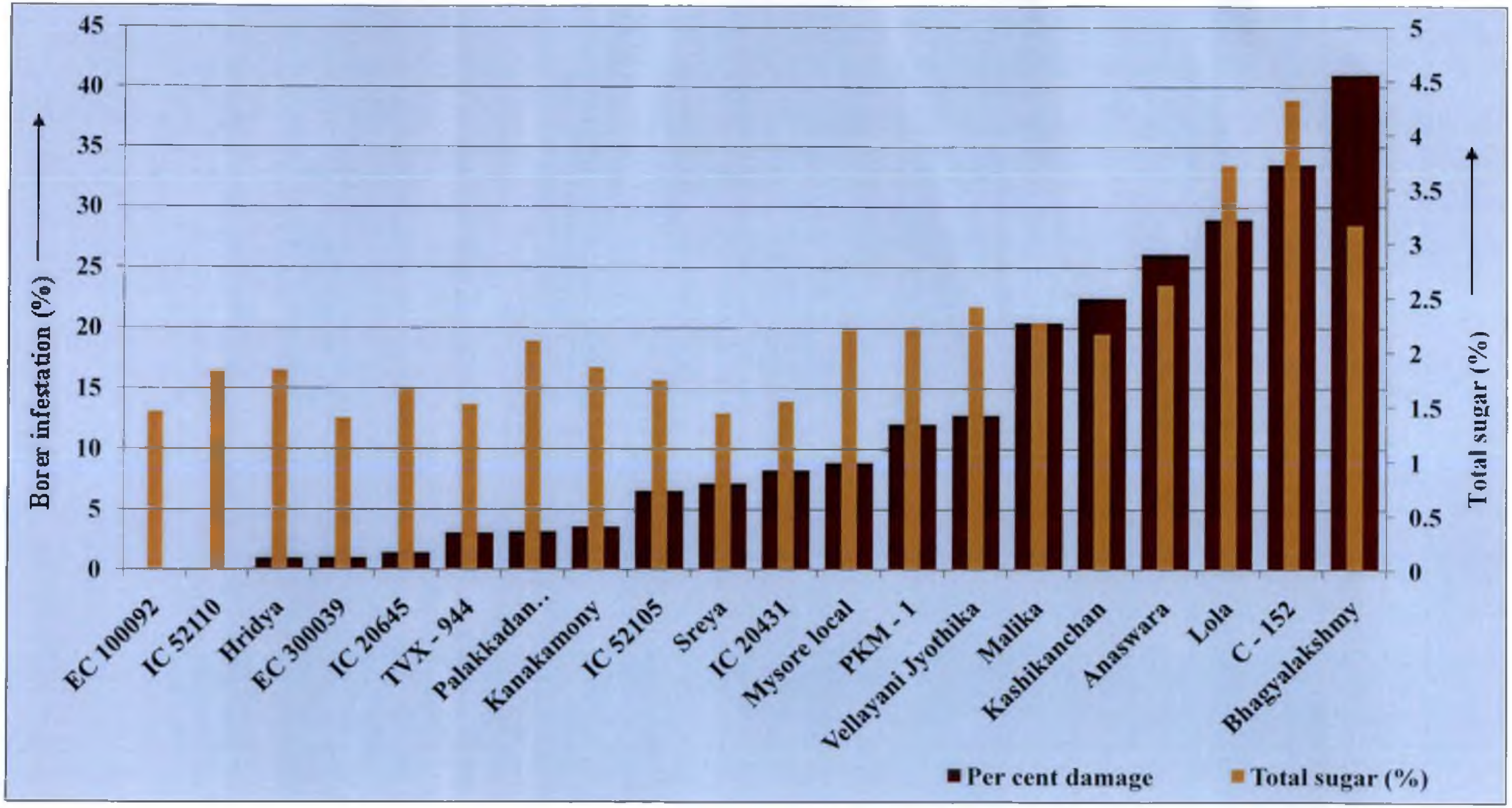
Sunitha *et al.* (2006) reported a similar positive and significant correlation of 0.86 between total protein content and per cent damage in case of pigeon pea.

The finding of the present study is in agreement with earlier reports and suggests that a higher protein content in the pods enhances the nutritional quality of the host and therefore makes it more attractive to the borer.

5.3.3 Total sugar content

Significant variation was recorded in total sugar content among the 20 genotypes evaluated (Fig. 4). C – 152 had the highest total sugar content of 4.33 per cent and was followed by Lola (3.72 %), Bhagyalekshmi (3.17%), Anaswara (2.62%) and Vellayani Jyothika (2.42%) respectively. EC 300039 recorded the lowest total sugar content of 1.39 per cent and was followed by Sreya (1.44%), EC 100092 (1.45%), TVX – 944 (1.51%).

Figure 4. Influence of total sugar content on per cent damage



As already observed in case of protein and moisture content, there was significant positive correlation (0.851) between total sugar content and per cent damage by spotted pod borer. Thus, C-152, Lola and Bhagyalakshmy, which recorded higher values for total sugar content also recorded high infestation levels of 33.54, 28.99 and 41.04 per cent respectively. Accessions such as EC 300039, Sreya and EC 100092, on the other hand, recorded infestation ranging from 0.00 - 7.7 per cent.

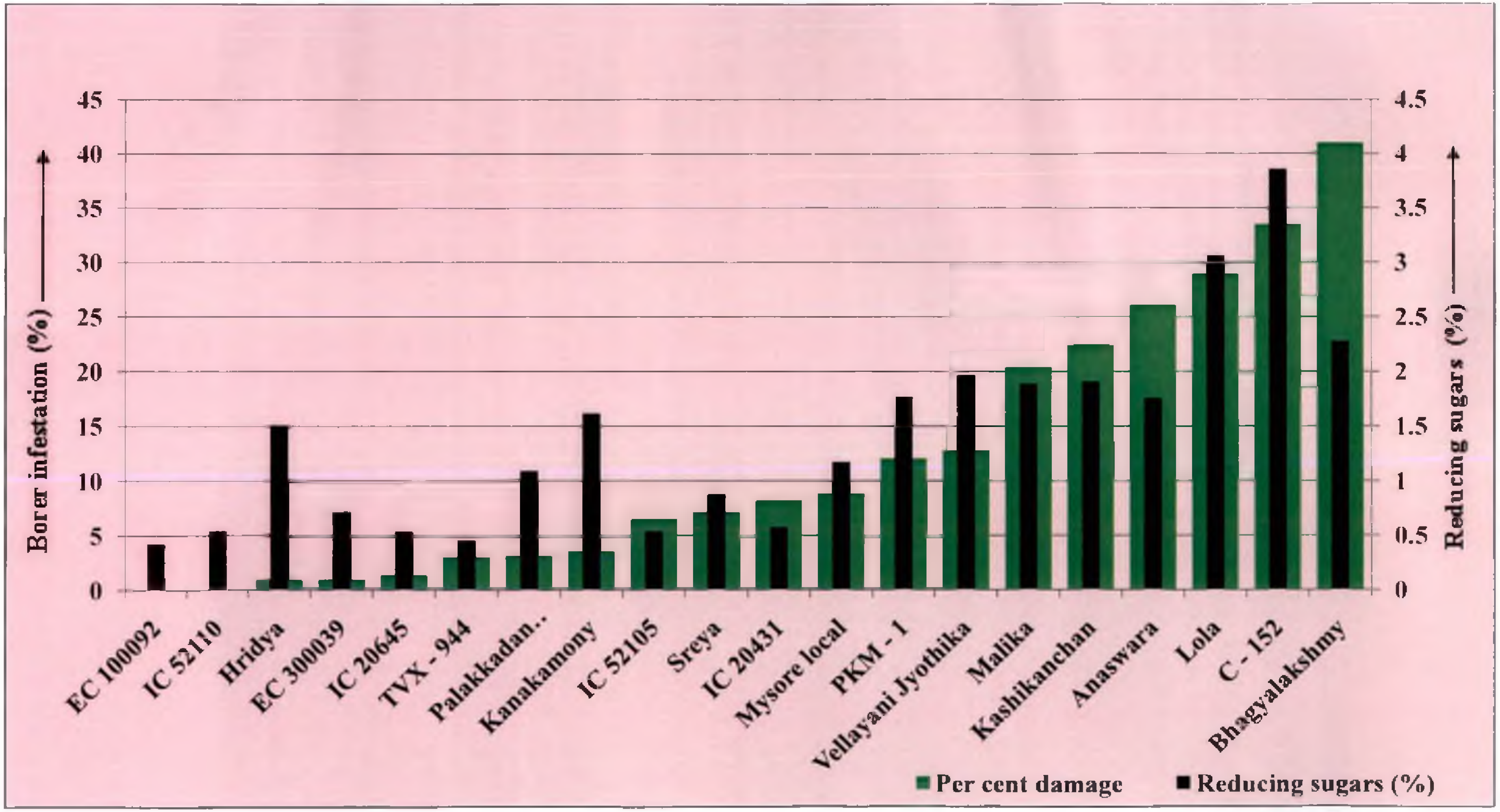
These observations were in agreement with Sahoo and Senapathi (2001) who reported lower total sugar content of 3.23 per cent in the tolerant variety S – 46 as against 4.87 per cent in the susceptible accession ICPL – 1.

5.3.4 Reducing sugars

Reducing sugar content had significant variation among different accessions of cowpea (Fig. 5). C – 152 recorded the highest value of 3.86 per cent and was followed by Lola, Bhagyalakshmy, Vellayani Jyothika, Kashikanchan and Malika with 3.07, 2.28, 1.97, 1.91 and 1.89 per cent respectively. As in case of total sugar content, lowest value of 0.43 per cent was also observed in EC 100092. A positive and significant correlation (0.820) was obtained between reducing sugars and per cent damage, with accessions like C-152, Lola and Bhagyalakshmy, which had higher reducing sugar content also registering consistently high total per cent damage. EC 100092, TVX – 944 and IC.20645, which had very low sugar content recorded pod borer infestation values of 0.00, 3.05 and 1.39 only.

Halder *et al.* (2006) reported higher content of reducing sugars (0.59 mg/g) in susceptible genotype LGG - 450 and a lower 0.48 mg/g for resistant genotype LGG – 497. Sunitha *et al.* (2006) reported significant positive correlation between sugars in pods and the pod damage by *M. vitrata* in pigeon pea. The results of the present experiment were in line with the above findings.

Figure 5. Influence of reducing sugars on per cent damage



5.3.5 Peroxidase activity

Significant variation was also recorded in peroxidase activity among the genotypes evaluated (Fig. 6). Kanakamony recorded the highest peroxidase enzyme content of 97.81 EU g^{-1} while Lola recorded the lowest peroxidase activity of 2.9 EU g^{-1}.

Enzymes *viz.*, peroxidises and polyphenol oxidases have been known to confer resistance to insect pests in a number of crops such as tomato and lettuce (Rashid *et al.*, 2012). The result of the present investigation indicated a similar role for these enzymes in cowpea as well. Kanakamony, for instance, with a value of 97.81 EU g^{-1} had 3.58 per cent damage, to be categorized as resistant. Other accessions with high peroxidase activity such as Hridya and IC 52110 also had very low levels of borer infestation. Accessions such as Lola and Anaswara which registered low levels of peroxidase activity, suffered higher borer infestation and were rated as susceptible.

The significant negative correlation (-0.527) observed between peroxidase activity and per cent damage in the study underline the above observations. 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 shoot and fruit borer infestation. Roshni (2014) reported a significant negative correlation (-0.80) between peroxidase activity with fruit damage by melon fly.

5.3.6 Polyphenol oxidase activity

Polyphenol oxidase activity of different accessions ranged from 0.0034 EU g^{-1} in C - 152 to 0.022 EU g^{-1}, in Palakkadan thandan payar (Fig. 7).

As observed in case of peroxidase, polyphenol oxidae activity was also significantly and negatively correlated (-0.734) with total pod borer damage. The per cent damage was consistently low in case of accessions like Palakkadan thandan payar, EC 300039 and Sreya, all of which had high polyphenol oxidase

Figure 6. Influence of peroxidase activity on per cent damage

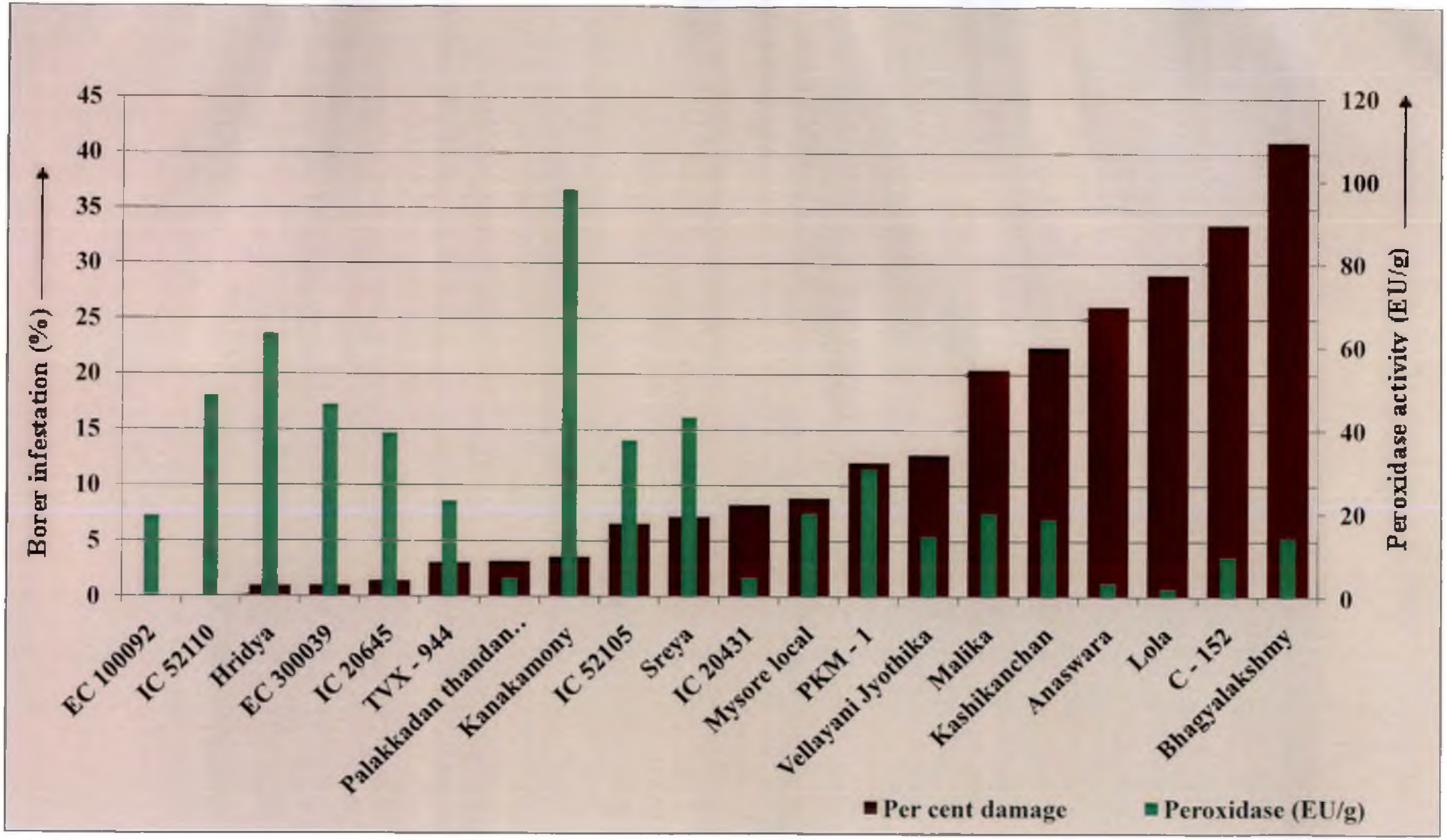
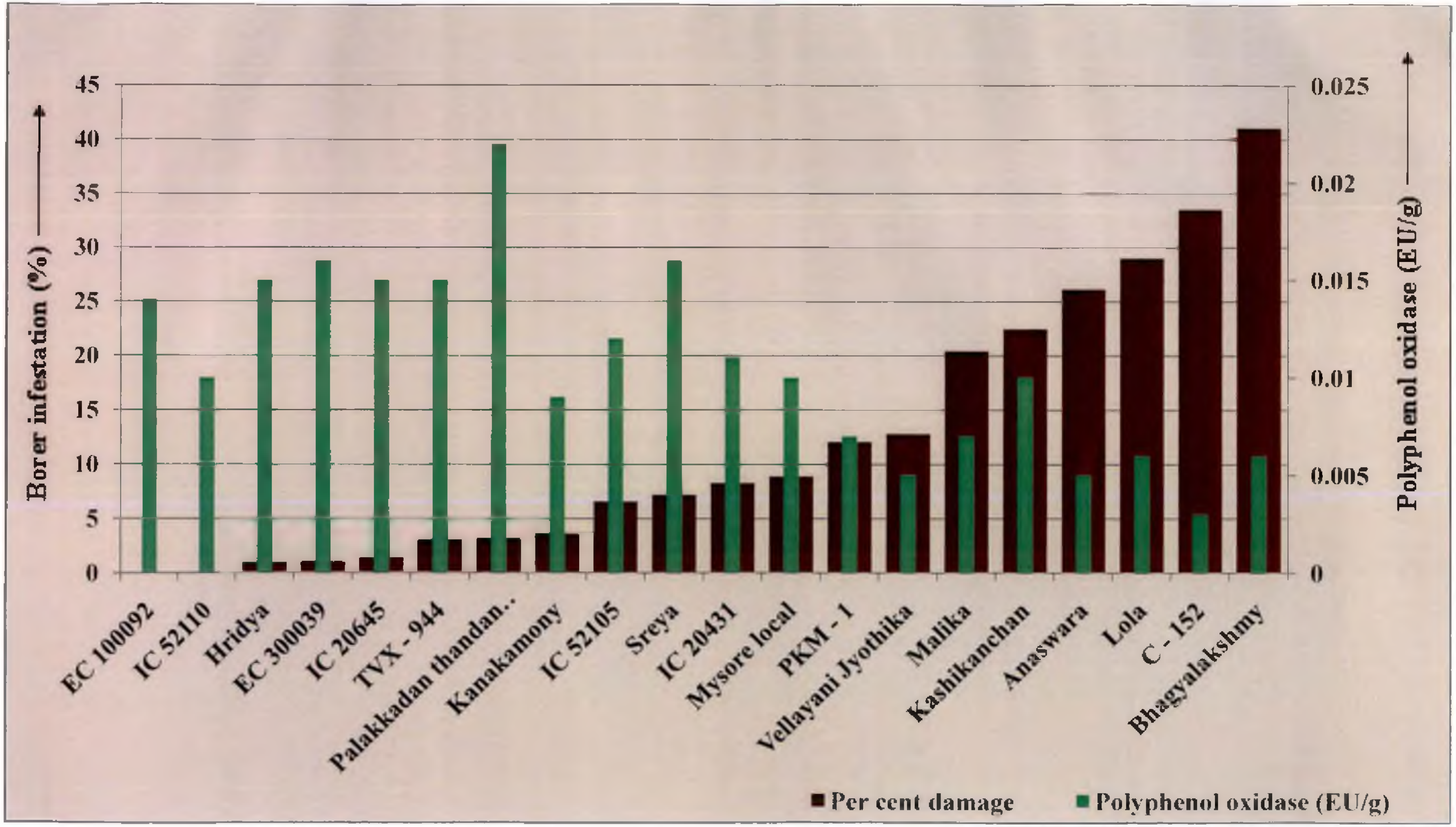


Figure 7. Influence of polyphenol oxidase on per cent damage



activity while accessions having low polyphenol oxidase content were susceptible, with ranging from 26.13 to 41.04 per cent.

Polyphenol oxidases, considered as a biochemical constituent of plant defence, along with peroxidase could be involved in conferring resistance to cowpea genotypes evaluated. Polyphenol mediated resistance have been hardly been reported in case of cowpea against any pests. However, these observations are in agreement with Sunitha *et al.* (2006) who observed significant negative correlation between total phenol content in pods (-0.63) and pod borer damage in pigeon pea.

Summary

7. SUMMARY

The present study titled "Morphological and biochemical bases of resistance to spotted pod borer, *Maruca vitrata* (Fab.) (Lepidoptera: Crambidae) in cowpea" was carried out in the Department of Agricultural Entomology, COH, KAU, Thrissur from September 2014 to June 2015. The objectives of the experiment were to evaluate degree of resistance to pod borer in selected accessions of cowpea as well as to identify the morphological and biochemical bases of resistance in cowpea to pod borer.

An experiment was carried out in CRD with 48 treatments and ten replications. The resistance of different accessions were evaluated in terms of per cent damage to flower buds, flowers and pods.

Forty - eight accessions, obtained from different sources, were evaluated for resistance to the spotted pod borer, *M. vitrata.* Significant variation was observed between the genotypes for bud, flower and fruit damage.

Ten accessions namely IC 39922, EC 100092, IC 2196, IC 39916, EC 300039, IC 39945, IC 2918, EC 98668, EC 101216 and IC 52110 were free from bud damage. The remaining genotypes recorded bud damage ranging from 0.37 per cent to 32.5 per cent. The highest bud damage of 32.5 per cent was recorded in case of the variety Bhagyalekshmi.

The extent of borer infestation in flowers was zero in IC 39922, EC 100092, IC 39916, IC 39945, IC 2918, EC 98668 and IC 52110. The accessions IC 20645, Kanakamony, EC 300039, Hridya, IC 20720, IC 39921, IC 52118, IC 52128 and EC 101216 also recorded low levels of (0.85 to 2.1 %) of damage to flowers and were on par with the above accessions. Bhagyalekshmi recorded the highest per cent of damaged flowers (50.39 per cent).

The genotypes EC 100092, Palakkadan thandan payar, IC 26048, IC 39945, IC 2815, KBC – 2, EC 98668, IC 39947, IC 20645, IC 52110 and IC 52118 recorded no damage to pods. The accessions IC 20720, TVX – 944, IC

39922, IC 9883, IC 2815, IC 52128, EC 300039, EC 101216 and Hridya also recorded low per cent of damaged pods and were statistically on par with above accessions. The highest extent of pod damage (47.95%) was recorded in case of Lola.

The 48 accessions were subjected to Duncan's Multiple Range Test (DMRT), based on per cent damage and were grouped as resistant, moderately resistant, susceptible and highly susceptible. Twenty three accessions with zero to five per cent damage were categorised as resistant. Eight accessions with infestation ranging from 5 - 10 per cent were classified as moderately resistant. Five accessions with infestation ranging from 10 to 15 per cent were grouped as susceptible and twelve accessions with more than 15 per cent damage were categorised as highly susceptible.

Analysis of morphological bases of resistance to pod borer indicated that pod wall thickness was significantly and negatively correlated (-0.447) with per cent damage. Similarly trichome density was also negatively correlated (-0.307) with pod damage, though the correlation was not significant.

Length of flower stalk as well as length of trichomes was positively correlated with borer damage but the correlation was not significant.

Positive and significant correlation was observed between per cent damage and biochemical parameters *viz.* total protein content (0.815), moisture content (0.461), total sugars (0.851) and reducing sugars0.820).

Borer infestation was correlated negatively and significantly with peroxidase (-0.51) as well as for polyphenol oxidase (-0.734).

The study clearly brought out the need for further efforts to exploit the resistance in cowpea to the spotted pod borer, *M. vitrata.*

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MORPHOLOGICAL AND BIOCHEMICAL BASES OF RESISTANCE TO SPOTTED POD BORER, *Maruca vitrata* (FAB.) (LEPIDOPTERA: CRAMBIDAE) IN COWPEA

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ABSTRACT OF THE THESIS

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ABSTRACT

Cowpea, *Vigna unguiculata* (L.), is an important legume of the tropics and subtropics. It is used as a grain, vegetable and fodder crop. Cowpea is cultivated as a vegetable crop across an area of 6714 ha in Kerala (DES, 2014). However, the productivity of cowpea, at 625 kg ha^{-1} is very low, one of the reasons being infestation by insect pests. Among the different insect pests, the legume pod borer, *Maruca vitrata* (Fab.) is the most important one causing loss of up to 60 per cent in cowpea (Singh and van Emden, 1979).

The larvae of the spotted pod borer, as the name implies, bore into buds, flowers and pods and feed on internal tissues. Their concealed nature protects the larvae from natural enemies as well as application of insecticides to a considerable extent, making management of the pest difficult. Exploitation of host plant resistance, which is one of the most effective and eco friendly pest management strategies, has hardly been attempted in case of cowpea.

It was in this context that the present study 'Morphological and biochemical bases of resistance to spotted pod borer, *Maruca vitrata* (Fab.) (Lepidoptera: Crambidae) in cowpea' was undertaken in the Department of Agricultural Entomology, College of Horticulture, Kerala Agricultural University, Thrissur during 2014 - 2015. The objectives of the experiment were to evaluate degree of resistance to pod borer in selected accessions of cowpea, as well as to identify the morphological and biochemical bases of resistance in cowpea to pod borer.

Forty eight accessions, obtained from different sources, were evaluated for resistance to the spotted pod borer, *M. vitrata.* Significant variation was observed between the genotypes to pod borer damage at bud, flower and pod stages.

The extent of borer infestation varied from zero to 41.04 per cent. Five accessions, *viz.*, EC 100092, IC 39945, IC 2918, EC 98668 and IC 52110 recorded zero per cent damage at all the three stages. Twenty - six accessions recorded damage varying from 0.1 to 9.29 per cent and were on par with each other. All the above accessions were rated as resistant.

Seventeen accessions with borer damage ranging from 11.15 to 41.04 per cent were rated as susceptible. The variety Bhagyalakshmy recorded the highest damage of 32.5, 50.39 and 47.95 per cent for bud, flower and pod respectively.

Analysis of morphological basis of resistance to pod borer indicated that pod wall thickness was significantly and negatively correlated with per cent damage. Similarly trichome density was also negatively correlated with pod damage, though the correlation was not significant. Length of flower stalk as well as length of trichomes was positively correlated with borer damage but the correlation was not significant.

Significant positive correlation was observed between per cent damage and total protein content, moisture content, total sugars and reducing sugars. However, significant negative correlation was observed in peroxidase and polyphenol oxidase activity with per cent damage.

The study confirmed variability among cowpea genotypes in response to pod borer infestation, indicating the potential for utilization of host plant resistance in the management of pod borer in cowpea. It also indicated that the resistance in cowpea to *Maruca vitrata* could be related to biochemical constituents of the plant, which, however, need to be confirmed.