# INSECTICIDE RESISTANCE IN SPOTTED POD BORER, *Maruca vitrata* (Fabricius) ON VEGETABLE COWPEA AND ITS MANAGEMENT

***by***

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**THESIS**

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# DECLARATION

I, hereby declare that this thesis entitled **"INSECTICIDE RESISTANCE IN SPOTTED POD BORER *Maruca vitrata* (Fabricius) ON VEGETABLE COWPEA AND ITS MANAGEMENT"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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22-07-2014. (2012-11-189)

# CERTIFICATE

Certified that this thesis entitled **"INSECTICIDE RESISTANCE IN SPOTTED POD BORER *Maruca vitrata* (Fabricius) ON VEGETABLE COWPEA AND ITS MANAGEMENT"** is a record of research work done independently by Ms. Pattapu Sreelakshmi under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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*Pattapu Sreelakshmi*

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| % | Per cent |
| @ | At the rate of |
| a.i. | Active ingredient |
| BDL | Below detectable level |
| C.D. | Critical Difference |
| cm | Centimetre(s) |
| DAP | Days after planting |
| DAS | Days after spraying |
| EC | Emulsifiable Concentrate |
| *et al* | And others |
| fig. | Figure |
| g | Gram |
| $ha^{-1}$ | per hectare |
| ml | Millilitre |
| mm | Millimetre |
| ppm | Parts per million |
| SC | Suspension Concentrate |
| SD | Standard deviation |
| SG | Soluble Granule |
| spp. | Species |
| *viz*., | Namely |

# *Introduction*

# 1. INTRODUCTION

Cowpea (*Vigna unguiculata* subsp. s*esquipedalis* (L.) Verdc.) more commonly termed as yard long bean is a popular vegetable grown in tropical and sub-tropical countries. This drought tolerant crop is cultivated in an area of 12.50 million hectares, with an annual production of over 3 million tons in the tropics (Feleke *et al*., 2006). The immature pods are used widely as fresh vegetable and are a rich source of proteins, vitamins and minerals. Among the various bottlenecks, pests and disease incidence contribute to the key share of the crop loss. Though an array of pests attack this crop, the major loss is inflicted by the spotted pod borer *Maruca vitrata* Fabricius (Lepidoptera: Crambidae), the larvae of which damage both vegetative and reproductive stages of the crop. It is reported that the loss due to pod damage alone goes 42 to 80 per cent (Halder and Srinivasan, 2011).

The occurrence of spotted pod borer or legume pod borer, *M. vitrata* was first reported on "Katjan" (bean) from Indonesia (Dietz, 1914). In India, it was first reported on dwarf pigeon pea by Srivastava in 1964. In order to manage and alleviate the losses due to this pest, farmers often resort to chemical interventions involving conventional organophosphorus and synthetic pyrethroid molecules. Recent studies on monitoring of pesticide residues in cowpea under the Department of Agriculture and Co-operation (DAC) funded project entitled "Monitoring of pesticide residues at National Level indicated frequent occurrence of chlorpyriphos, acephate, ethion, profenophos and lambda-cyhalothrin residues in samples collected from local markets in Kerala. Observations made in recent years indicate that *M. vitrata* has acquired reduced susceptibility to insecticides that previously have been effective in appropriate doses indicating the development of insecticide resistance. Occurrence of resistance of *M. vitrata* to insecticides like cypermethrin, dimethoate and endosulfan in Nigeria was reported earlier by Ekesi in 1999. It is one of the most important phenomenons that threaten sustainable pest management programmes. Hence, it is important to detect resistance at its budding level and monitor its increase and further spread so as to implement appropriate measures to restrain its increase. The information available on insecticide resistance against this pest under Indian condition is so scanty in spite of development of insecticidal resistance in *M. vitrata* globally. However, no study on insecticidal resistance in *M. vitrata* has been carried out in Kerala. Hence, this investigation is proposed to assess the extent of insecticide resistance in *M. vitrata* and to suggest measures for resistance management towards framing Good Agricultural Practices.

In the above perspective, the present study was undertaken with the following objectives.

- To conduct a preliminary survey among cowpea growers to gather information on pesticide use and incidence/ development of insecticide resistance.
- To assess the insecticide resistance in field population of spotted pod borer, *M. vitrata*.
- To evaluate the efficacy of new generation insecticides against resistant population of *M. vitrata.*
- To determine the harvest time residues in cowpea pods.

# *Review of literature*

# REVIEW OF LITERATURE

Spotted pod borer, *M. vitrata* is the most important post flowering pest of cowpea in the tropics, which act as a major limiting factor in cowpea cultivation. The pod damage of 40 - 70 per cent was recorded from cowpea due to the infestation of spotted pod borers (Halder and Srinivasan, 2011; Srinivasan, 2008). Farmers usually adopt frequent sprays of chemical insecticides for containing the infestation of spotted pod borers in the field. The repeated use of these chemicals resulted in the development of insecticide resistance and observations made in recent years indicate that the pest has acquired reduced susceptibility to insecticides which previously have been effective. The literature pertaining to the biology, mass rearing of *M. vitrata*, development of insecticide resistance in the population of *M. vitrata* and their management are reviewed and presented under the following heads.

## 2.1 BIOLOGY AND NATURE OF DAMAGE OF *M. VITRATA*

### 2.1.1 Biology

Wolcott (1933) made initial studies on biology of *M.vitrata* on lima beans in Puerto Rico. Later the biology of this pest on cowpea was studied by Booker (1965), Taylor (1967), Akinfenwa (1975), Okeyo-Owuor and Ochieng (1981), Jackai and Singh (1983), Ramasubramanian and Sundara Babu (1989), Naveen *et al.* (2009), Sonune *et al.* (2010) and Fousseni *et al*. (2013).

#### 2.1.1.1 *Hosts of M. vitrata*

The literature on the details of the hosts of *M. vitrata* is presented in Table 1.

#### 2.1.1.2 *Life stages of M. vitrata*

The review of research work related to the details of total larval period of *M. vitrata* are presented in Table 2.

Table 1. Host range of cowpea pod borer, *Maruca vitrata* (F)

| Place | Host | Reference |
|---|---|---|
| Puerto Rico | Common beans | Wolcott, 1933; Scott, 1940 |
| USA | Common beans, cowpea, black gram, green gram | Williamson, 1943 |
| The Philippines | Cowpea | Djamin, 1961 |
| India | Dwarf pigeonpea | Srivastava, 1964 |
| Taiwan | All grain legumes | Lee, 1965 |
| Australia | Navy bean | Passlow, 1968 |
| Asia | Common beans, vegetable cowpea | Barroga, 1969; Das and Islam, 1985 |
| Fiji Islands, Indonesia | Pigeon pea, soybean, cowpea, black gram, green gram | Oei-Dharma, 1969 |
| Papua, New Guinea | Winged beans | Lamb, 1978 |
| Sri Lanka | Pigeon pea | Subasinghe and Fellowes, 1978 |
| East, west and South Africa | Cowpea | Akinfenwa, 1975; Taylor, 1978 |
| Brazil | Soybean | Smith, 1978 |
| Nigeria | *Crotalaria juncea. C.retusa, C.mucronata, C.usaramoensis* | Jackai and Singh, 1983 |

Table 2. Total larval period of cowpea pod borer, *Maruca vitrata* (F) on different host plants

| Host | Duration of larval period (days) | Reference |
|---|---|---|
| Pigeon pea | 12.65 | Okeyo-Owuor and Ochieng, 1981 |
| Cowpea | 7.30 | Jackai and Singh, 1983 |
| Pigeon pea | 16.40 | Jackai and Singh, 1983 |
| *Crotalaria juncea* | 21 | Jackai and Singh, 1983 |
| *C. miserensiensis* | 19.90 | Jackai and Singh, 1983 |
| *C. mucronata* | 16.90 | Jackai and Singh, 1983 |
| Artificial diet | 13.5-14.3 | Ochieng and Bungu, 1983 |
| Cowpea | 10.00 | Ramdas Rai, 1983 |
| Cowpea | 13.90 | Ramasubramanian and Sundara Babu, 1989 |
| Cowpea | 11.10 | Arulmozhi, 1990 |
| Cowpea flour diet | 16.50 | Arulmozhi, 1990 |
| Soybean flour diet | 14.40 | Arulmozhi, 1990 |
| Cowpea | 11.90 | Ganapathy, 1996 |
| Pigeon pea | 14.70 | Ganapathy, 1996 |
| Blackgram | 16.50 | Ganapathy, 1996 |
| Greengram | 15.40 | Ganapathy, 1996 |
| Cowpea | 11.00 | Veeranna *et al.*,1999 |
| Blackgram | 14.04 | Sonune *et al.*, 2010 |

Ganapathy (1996) observed the pre-pupal and pupal durations of *M. vitrata* as 1.60 and 1.95 - 2.15 respectively, whereas, Naveen *et al.* (2009) reported these as 6.60 and 7.30 days respectively on cowpea. Sonune *et al.* (2010) reported pupal duration as 10.84 days on black gram.

The average life-span of adult of both sexes varied on different hosts. On artificial diet, the longevity was reduced to 7.10 days in females and 6.30 days in males (Ochieng and Bungu, 1983). Ganapathy (1996) found that females and males lived for about 8.50 and 6.30 days respectively on cowpea. Naveen *et al.* (2009) reported that male moths lived for 9.50 - 11.19 days whereas female survived for 10.40 - 13.10 days on cowpea. Longevity and average life span of female moth were 8.06 and 32.04 days respectively while in the case of male moth it was 6.24 and 26.52 days respectively on black gram (Sonune *et al.*, 2010).

#### 2.1.1.3 *Mass rearing of M. vitrata*

Ochieng *et al*. (1981) developed a procedure for mass rearing of *M. vitrata* on natural food and reported that average life span of female and male were 7.70 and 9.50 days respectively which allow production of over 75000 eggs per month. Jackai and Raulston (1988) attempted rearing of *M. vitrata* on a soybean flour and cowpea flour based artificial diet and reported that the growth index value was decreasing after 10 generations. However, in 1983 Ochieng and Bungu attempted a mass rearing technique for *M. vitrata* and prepared a diet based on chickpea and found a decline in the survival of larval population and failed to obtain adequate numbers of mated pairs of *M. vitrata* after a number of successive generations. Onyango and Ochieng - Odero (1993) developed a semi - synthetic diet composed of soybean flour and cowpea flower powder as main ingredients for *M. vitrata* on which the fecundity of the females increased around 70 – 90 per cent with advancing generations.

Fousseni *et al.* (2013) reared *M. vitrata* on cowpea flowers and reported that the larvae that fed on the reproductive organs of the flower had significantly shorter development span as compared to the larvae that fed on other flower components.

### 2.1.2 Nature of damage and extent of loss

*M. vitrata* larvae feed on flowers, buds and pods by webbing them. This typical feeding protects the larvae from natural enemies and other adverse factors, including insecticides. Moths prefer to oviposit at the flower bud stage. Larvae move from one flower to another and each may consume 4 - 6 flowers before larval development is completed. Third to fifth instar larvae are capable of boring into the pods and occasionally into peduncle and stems (Singh and Allen, 1980; Vijayasree, 2013).

Infestation starts in the terminal shoots (21 days after planting) and spreads to the reproductive parts (Jackai, 1981). Karel (1985) also observed more larvae (52.30 %) on flowers than on pods (37.80 %) and leaves (9.90 %). In Sri Lanka, Dharmasena *et al.* (1992) reported about 84 per cent pod borer damage in pigeon pea. Ganapathy (1996) estimated a loss of nearly 50.00 per cent and flower drop damage ranging from 9.40 to 12.70 per cent in short, medium and long duration pigeon pea cultivars in Tamil Nadu. In pigeon pea, third instar larvae prefer pods compared to flowers and leaves, and flowers over leaves (Sharma, 1998). Arulmozhi (1990) fixed the Economic Injury Level (EIL) for *M. testulalis* in cowpea and a threshold of 40 per cent larval infestation in flowers was established by Ogunwolu, 1990 in cowpea. Ganapathy (1996) enumerated an EIL of 3.0 larvae per plant and a combined threshold of 2 pairs each of *M. testulalis* per plant at 50 per cent flowering stage when both occurred together on pigeon pea.

## 2.2 DEVELOPMENT OF INSECTICIDE RESISTANCE

Development of insecticide resistance in insects especially lepidopterans to most commonly used insecticides belonging to different groups has become a common phenomenon across the world.

### 2.2.1 History of development of insecticide resistance

The first report of resistance was published by Melander (1914) who described the resistance of sanjose scale to sulfur - lime, a compound typical of the inorganic chemicals used for pest management. In India, resistance was first furnished by Pradhan *et al*. (1963) near Delhi province in Singhara beetle, *Gallerucella birmanica* (Jacoby) which is a pest on water nut to DDT and BHC.

### 2.2.2 Resistance against conventional insecticides

#### 2.2.2.1 *Studies across the world*

A maiden research work conducted by Ekesi (1999) in three different locations of Nigeria on insecticide resistance in *M. vitrata* revealed that, the insect developed resistance of 17 - 35 fold against cypermethrin, 27 - 60 fold against dimethoate and 15 - 35 fold against endosulfan. This may be the only report work available on insecticide resistance in *M. vitrata*. Hence, the studies on insecticide resistance in other lepidopteran pests are reviewed here. Hama (1990) reported the declining efficacy of various insecticides against diamondback moth in Japan. In Florida, high levels of resistance to synthetic insecticides were reported in a single field population of *Plutella xylostella* (L.) (Yu and Nguyen, 1992). However, in North America the highest degree of resistance was reported against methomyl followed by permethrin and methamidophos against diamondback moth (Shelton *et al.,* 1993). Yu and Nguyen (1996) reported 20 to 73 fold resistance to five organophosphates against the populations of diamondback moth collected from Florida, USA as compared to the laboratory strain.

Dunley and Welter (2000) found out that cross resistance was positively correlated with azinophosmethyl, organophosphates, DDT and few pyrethroids like esfenvelarate and fenpropathrin in tests against field and laboratory population of codling moth. Negatively correlated cross resistance was identified between azinophosmethyl, chlorpyriphos and methyl parathion. They suggested the use of chemicals with negatively correlated cross resistance as tools for resistance management.

Populations of diamondback moth in Australia found to be resistant to synthetic pyrethroids and diamondback moth from canola crops in Northern Agricultural Region of Western Australia had a moderate level of resistance to synthetic pyrethroids (Cook *et al.,* 2000). Whereas in 2001, Bouvier and his co-workers studied deltamethrin resistance and its inheritance in codling moth *Cydia pomonella* (L). They crossed the resistant and susceptible strain of codling moth from population collected from South - Eastern France and proved that deltamethrin resistance was suspected to be under the control of kdr - type allele and enhanced mixed function oxidase (MFO).

Torres - Villa and co-workers in 2002 studied the development of insecticide resistance in *H. armigera* in different locations of Spain against endosulfan, carbamates and organophosphates. Chemicals tested include carbaryl, methomyl, thiodicarb, chlorpyriphos, acephate, monocrotophos etc., and 97 per cent of insecticides tested were susceptible with resistance factor ≤ 1. Carbamates showed moderate levels of resistance with resistance factor 2 - 10. They concluded that the low levels of resistance in *H. armigera* may be due to insect migration and cropping structure leading to existence of refugia.

Qu Mingjing *et al*. (2003) reported the development of resistance in rice stem borer, *Chilo suppressalis* Walker against triazophos. They reported that field populations of rice stem borer with 203.30 fold resistance to triazophos developed a 787.20 fold resistance after eight generations. They also observed the cross - resistance with other organophosphorous compounds and reported that esterase activity and insensitivity of AchE may involve in triazophos resistance mechanism.

Diamondback moth showed nearly 331 hundred fold resistance to chlorpyriphos and 45, 200 fold resistance to bifenthrin in the field population of Multan, Pakistan (Attique *et al.*, 2006). Munir Ahmad and his co-workers (2007) studied the genetics and mechanisms of resistance to deltamethrin in a field population of *Spodoptera litura*. They reported the increase of resistance against deltamethrin about 63 - fold after 4 generations of continuous selection.

Another study conducted by Saleem *et al*. (2008) revealed that *S. litura* population collected from Pakistan developed resistance for pyrethroids, carbamates, chlorcyclodienes and organophosphates tested which included 5 - 11 fold for cypermethrin, 2 - 98 fold for deltamethrin, 7 - 86 fold for beta - cyfluthrin, 16 - 200 fold for thiodocarb, 10 - 389 fold for methomyl, 10 - 92 fold to endosulfan, 3 - 169 fold to profenophos, 18 - 421 fold to chlorpyriphos, and 3 - 160 fold to quinalphos. They also reported the occurrence of cross - resistance among several insecticides tested.

Botwe *et al*. (2012) observed that, *P. xylostella* population collected in Opeibea farm from Ghana region exhibited the highest level of resistance of 62.4 - fold and 10.5 - fold for lambda - cyhalothrin and emamectin benzoate. A monitoring study on resistance of *S. exigua* by Ishitaq *et al.* (2012) from four districts of Southern Punjab and Pakistan against the synthetic pyrethroids and organophosphate compounds revealed the development of resistance in deltamethrin, cypermethrin as 7 - 105, 12 - 136 fold and chlorpyriphos and profenophos as 20 - 134 fold and 37 - 143 fold respectively when compared with laboratory susceptible strain. They suggested to rotate the insecticides with new molecules with different modes of action to prevent the resistance development.

A study conducted by Hong Tong *et al*. (2013) on *S. litura* from five districts of Hunan province in China revealed a resistance of 12 - 227 fold for deltamethrin and bifenthrin and 14 - 229 fold for organophosphates like chlorpyriphos and profenophos.

#### 2.2.2.2 *Studies across India*

The studies on the insecticide resistance of lepidopteran pests against conventional insecticides in India are presented in Table 3.

### 2.2.3 Resistance against new generation insecticides

No research report is available on insecticide resistance in *M. vitrata* against new generation insecticides. However, development of insecticide resistance against new generation insecticides in other lepidopterans is reviewed here.

#### 2.2.3.1 *Studies across the world*

A population of diamond back moth collected from Hawaii showed high levels of resistance against indoxacarb (Mau and Gusukuma - Minuto, 2004). Feng *et al*. (2005) reported that the development of resistance to avermectins and microbial insecticide, *Bacillus thuringiensis* (Berliner) in the laboratory and field populations of DBM for at least eight years. In 2006, Zhao and his co-workers in Georgia reported the development of resistance in diamondback moth against spinosad, indoxacarb and emamectin benzoate. In Multan (Pakistan) 1800, 11 and 5600 fold resistance was reported to emamectin benzoate, spinosad and indoxacarb in field population of diamond back moth respectively (Attique *et al.,* 2006).

Munir Ahmad *et al*. (2008) from Rawalpindi, Pakistan reported the field level development of resistance in *S. litura* against new generation insecticides and they found out that *S.litura* has developed 7 - 122 fold resistance against spinosad, 3 - 95 fold against indoxacarb, 4 - 186 fold for abamectin, 2 - 77 fold for

Table 3. Development of insecticide resistance in the lepidopteran pests in India

| Common name | Scientific name | Resistance to | References |
|---|---|---|---|
| Tobacco caterpillar | *Spodoptera litura* (Fab.) | Chlorpyriphos, Fenvalerate | Niranjan Kumar and Regupathy, 2001 |
| Spotted bollworm | *Earias vittella* (Fabricius) | Carbamates | Kranthi *et al.*, 2001 |
| Rice leaf folder | *Cnaphalocrocis medinalis* (Guenee) | Chlorpyriphos, Quinalphos | Anbalagan, 2001 |
| American bollworm | *Helicoverpa armigera* (Hubner) | Organophosphates, Carbamates | Ren *et al.*, 2002 |
| American bollworm | *Helicoverpa armigera* (Hubner) | Chlorpyriphos, Endosulfan<br>Cypermethrin | Kranthi *et al.*, 2002 |
| Tobacco caterpillar | *Spodoptera litura* (Fab.) | Cypermethrin, chlorpyriphos | Kranthi *et al.*, 2002 |
| Army caterpillar | *Spodoptera exigua* (Fab.) | Chlorpyriphos, Profenophos<br>Deltamethrin, Cypermethrin | Ishitaq *et al.*, 2012 |

emamectin benzoate, 13 - 224 fold for fipronil, 2 - 66 fold against lufenuron, 8 - 56 fold against diflubenuron, and 2 - 153 fold for methoxyfenzoile. However, in 2008, Sayyed and co-workers reported that field populations of *S. litura* collected from Multan of Pakistan showed the resistance ratio of 15, 23, 37 and 16 - fold for indoxacarb, spinosad, abamectin and emamectin benzoate respectively compared to laboratory susceptible population.

Populations of *P. xylostella* developed resistance to spinosad, avermectins (abamectin), emamectin benzoate, indoxacarb and bio pesticide *Bacillus thuringiensis* Cry toxins in the field (Pu *et al.,* 2010).

Sarfraz *et al.* (2010) conducted an experiment on cross resistance, mode of inheritance and stability of resistance to emamectin benzoate in *S. litura*. They found an increase of resistance from 80 to 730 - fold at third generation when compared with a laboratory susceptible strain with 13 - fold resistance.

A study conducted by Bartek *et al.* (2012) on resistance to diamide compounds in diamond back moth in Philippians and Thailand revealed the development of 200 - fold resistance against chlorantraniliprole and flubendiamide when compared to susceptible strain.

In China, among the field populations of diamond back moth collected from 29 locations revealed that, one population showed the highest level of resistance to indoxacarb (110 fold) and other 28 populations showed 5 - 58 fold resistance (Khakame *et al.,* 2013). However, in Southern China, Wang *et al.* (2013) reported that three field populations of *P. xylostella* showed high levels of cross - resistance between chlorantraniliprole (18 - 1150 fold) and flubendiamide (15 - 800 fold) when compared with susceptible reference strain.

#### 2.2.3.2 *Studies across India*

Among the new generation insecticides, extensive use of fipronil against diamondback moth led to resistance problems in India (Mohan and Gujar, 2003; Sayyed and Wright, 2004)

Cheema (2009) reported the effectiveness of chlorantraniliprole against the resistant populations of *S. litura*. Whereas, in 2013, Kishore reported 22 fold resistance in diamond back moth against chlorantraniliprole in Tamil Nadu.

## 2.3 MANAGEMENT OF SPOTTED BORER, *M. VITRATA*

Several studies have been conducted in different parts of the world on the efficacy of insecticides including the conventional ones like organophosphates, synthetic pyrethroids and the newer classes like neonicotinoids, diamides, phenyl pyrrazoles, thiourea compounds, oxadiazienes, microbial insecticides etc. against the flower and pod borer of cowpea *M. vitrata*. However, the reports on the efficacy of insecticides against resistant population of *M. vitrata* are meagre.

The studies on the management of *M. vitrata* in different hosts by conventional and new generation insecticides are reviewed and presented in Table. 4 and 5.

## 2.4 RESIDUAL TOXICITY OF INSECTICIDES IN COWPEA PODS

Constant monitoring of pesticide residues in agricultural commodity is needed for ensuring food safety in agricultural commodities. Data generated by All India Network Project on Pesticide Residues [AINP (PR)], Kerala Agricultural University, revealed that 44.44 per cent of cowpea were contaminated with pesticide residues (Nair, 2013). The literature related to the persistence and degradation of new generation insecticide residues were reviewed here.

Table 4. Management of *Maruca vitrata* (F) by conventional insecticides

| Crop | Insecticides | Reference |
|---|---|---|
| Redgram | Endosulfan- 0.07 %, Carbaryl- 0.10 %,Chlordane- 0.07 % | Srivastava, 1980 |
| Green gram | Fenvalerate- 0.01% + miraculan (plant growth stimulator) | Venkataria and Vyas, 1985 |
| Pigeon pea | Monocrotophos- 0.05 %, Endosulfan- 0.07 % | Somalo and Patnaik, 1986 |
| Pigeon pea | Deltamethrin- 0.0025 %, Fluvalinate- 0.03 % | Bhalani and Prasana, 1987 |
| Pigeon pea | Cypermethrin- 0.008 % | Rahaman and Rahaman, 1988 |
| Pigeon pea | Cypermethrin- 0.0045 %, Deltamethrin- 0.0025 %, Fenvalerate- 0.005 %,Endosulfan- 0.05 % | Sontakke and Mishra, 1991 |
| Pigeon pea | Lambda-cyhalothrin (25 g a.i $ha^{-1}$), Profenophos (100 ml/ha) | Durairaj and Ganapathy. 1998 |
| Cowpea | Chlorpyriphos- 0.05 %, Acephate- 0.05 %, Acetamiprid- 0.002 %,Profenophos- 0.05 %,Diafenthiuron- 0.05 % | Betty Varghese, 2003 |
| Cowpea | Profenophos- 0.05 + DDVP- 0.5 ml/L | Gopali *et al.*, 2010 |
| Cowpea | Cypermethrin- 0.005 %, Acetamiprid- 0.002 %,Deltamethrin- 0.003 %<br>Lambda-cyhalothrin- 0.0025 % | Soliman, 2011 |
| Cowpea | Imidacloprid- 0.003% + cypermethrin- 0.005% | Ajeigbe *et al.*, 2012 |

Table 5. Management of *Maruca vitrata* (F) with new generation insecticides

| Crop | Insecticide | Reference |
|---|---|---|
| Pigeon pea | Spinosad 2.5 % SC | Bhoyar *et al.*, 2004 |
| Chick pea | Spinosad 48 SC – 0.01 % | Ladaji, 2004 |
| Black gram | Novaluron- 2.5 ml/L | Srinivasan, 2008 |
| Black gram | Indoxacarb 30 WDG- 0.008 % | Ashok Kumar and Shivaraju, 2009. |
| Black gram | Indoxacarb 30 WDG- 0.008 %<br>Spinosad -0.009 % | Sonune *et al.*, 2010 |
| Black gram | Flubendiamide 480 SC @ 48 g a.i $ha^{-1}$ | Patil *et al.*, 2008<br>Dey *et al.*, 2012 |
| Cowpea | Indoxacarb 14.5 SC – 0.007 % | Patel *et al.*, 2012 |
| Cowpea | Chlorantraniliprole 18.5 SC @ 75 g a.i $ha^{-1}$<br>Indoxacarb 14.5 SC @60 g a.i. $ha^{-1}$<br>Emamectin benzoate 5 G @ 10 g a.i $ha^{-1}$ | Vijayasree, 2013. |

Soliman (2011) reported the initial deposit of acetamiprid in cowpea was 6.57 ppm and residues dissipated to 1.76 ppm after one week of application. Vijayasree (2013) conducted a dissipation study of emamectin benzoate (10 g a.i. ha $^{-1}$), indoxacarb (60 g a.i. ha $^{-1}$) and spinosad (75 g a.i. ha $^{-1}$) in cowpea at College of Agriculture, Vellayani. She reported that the residues dissipated to 0.07 mg kg $^{-1}$, 0.56 mg kg $^{-1}$ and 0.94 mg kg $^{-1}$ in the fruits two hours after spraying. She also reported the half - life of emamectin benzoate, indoxacarb and spinosad were 1.25, 1.08 and 0.92 days respectively and their waiting periods were 2.99, 5.33 and zero days respectively.

# *Materials and Methods*

# 3. MATERIALS AND METHODS

The present study “Insecticide resistance in spotted pod borer, *M. vitrata* on vegetable cowpea and its management” aims to assess the insecticide resistance in field population of *M. vitrata*, evaluate the efficacy of new generation insecticides against this resistant population and to determine the insecticide residues in cowpea pods at harvest. Survey in connection with the present study was conducted among the farmers in Kalliyoor and Venganoor panchayats and monitoring of insecticide resistance in field population of *M. vitrata* were taken up from Kalliyoor and Venganoor panchayats and the Instructional Farm, Vellayani. Laboratory experiments were conducted at the Department of Entomology, College of Agriculture, Vellayani and the field evaluation on bio efficacy was conducted in a farmers’ field at Kalliyoor panchayat. The materials used and the methods adopted are detailed here under.

## 3.1 PRELIMINARY SURVEY TO GATHER THE INFORMATION REGARDING THE PESTICIDE USE AND THE DEVELOPMENT OF INSECTICIDE RESISTANCE

A detailed survey was conducted to study the consumption and use pattern of pesticides and awareness regarding insecticide resistance among cowpea farmers in Kalliyoor and Venganoor panchayats during 2012-13. A total of 50 farmers were selected randomly from both the locations and each of them was interviewed separately and information pertaining to major pests, rate, time and frequency of pesticide application, awareness regarding insecticide resistance, source of pesticide use, and source of technical information etc., were recorded. A suitable questionnaire was prepared for collecting the required information (Appendix-I).

## 3.2 LAORATORY REARING OF SPOTTED POD BORER, *M. VITRATA* IN DIFFERENT DIETS

To maintain a large population of *M. vitrata* in the laboratory for bioassay studies, a suitable laboratory rearing technique was standardized by suitably modifying the artificial diet. The culture was maintained in fresh pods of cowpea and lab-lab bean also.

### 3.2.1 Mass rearing on artificial and natural diets

#### 3.2.1.1 *Artificial diet*

The egg and first instar larvae of *M. vitrata* were collected from the infested cowpea (Variety- Vellayani Jyothika) grown in the Instructional Farm, Vellayani for rearing using artificial diet. Initially, the diet (Diet-1) was prepared based on the procedure described by Ochieng and Bungu, 1983 (Table 6). Further two more diets (Diet 2 and Diet 3) were prepared by slightly modifying the composition (Table 7 and 8). Procedure for the preparation was same for all the three diets. The ingredients in fraction A of each diet (Table 6-8) were mixed separately in a blender for 3 minutes. The agar-agar from fraction B was heated in 80 ml distilled water to boiling. The agar was allowed to cool to $60^0$C. The melted agar was poured to the prepared fraction ‘A’ in a blender and mixed thoroughly for 3 minutes. The formaldehyde (0.4ml) was added to this mixture and blended further for 1.5 minute. The whole ingredients were transferred into a sterilized sandwich box (Plate 1a) covered with lid and left over night for solidification. When properly solidified, the diet is cut into small plugs of size 2 x 2 cm (Plate 1b and 1c) and introduced to the plastic rearing trough. The first instar larvae were introduced to the diet plug in the trough by using camel hair brush. The same procedure was used to rear the larvae using diet 2 and 3 also. Based on long shelf life and good palatability, the best diet was selected for further rearing of *M.vitrata.*

Table 6. Ingredients of artificial diet (diet-1) for rearing *Maruca vitrata* (F)

| Sl. No. | Ingredients | Quantity |
|---|---|---|
| | Fraction-A | |
| 1 | Water | 48ml |
| 2 | Yeast | 1.60 g |
| 3 | Ascorbic acid | 0.44 g |
| 4 | Kabuli gram powder | 14 g |
| 5 | Cowpea flower powder | 2 g |
| 6 | Vitamin E | 0.16 g |
| | Fraction B | |
| 7 | Agar- agar | 2.04 g |
| 8 | Water | 80 ml |
| 9 | Formaldehyde | 0.40 ml |

Table 7. Ingredients of artificial diet (diet-2) for rearing *Maruca vitrata* (F)

| Sl.no | Ingredients | Quantity |
|---|---|---|
| | Fraction-A | |
| 1 | Water | 48ml |
| 2 | Yeast | 1.60 g |
| 3 | Ascorbic acid | 0.44 g |
| 4 | Cowpea seed powder | 14 g |
| 5 | Vitamin E | 0.16 g |
| | Fraction B | |
| 6 | Agar- agar | 2.04 g |
| 7 | Water | 80 ml |
| 8 | Formaldehyde | 0.40 ml |

Table 8. Ingredients of artificial diet (diet-3) for rearing *Maruca vitrata* (F)

| Sl.no | Ingredients | Quantity |
|---|---|---|
| | Fraction-A | |
| 1 | Water | 40 ml |
| 2 | Yeast | 1.30 g |
| 3 | Ascorbic acid | 0.37 g |
| 4 | Cowpea seed powder | 12 g |
| | Fraction-B | |
| 5 | Agar- agar | 1.20 g |
| 6 | Water | 66 ml |
| 7 | Formaldehyde | 0.40 ml |
| 8 | Inositol | 7 g |
| 9 | ABDEC (Vitamin) | 0.20g |

a

b

c

Plate 1. Artficial diet (diet-2) prepared for mass rearing of *Maruca vitrata* (F)

### 3.2.1.2 *Mass rearing of M. vitrata on natural diet - cowpea pod and lab-lab bean*

The egg and first instar larvae of *M. vitrata* collected from the infested cowpea were released on to fresh cowpea pods and lab-lab bean pods of 4 -5 cm length kept in polyvinyl containers of 9 cm height and 11 cm diameter. The open end of the containers were closed with muslin cloth for providing aeration and secured tightly with a rubber band to prevent escape of larvae. Dried pods were removed periodically by using forceps and larvae present inside the dried pods were transferred carefully onto fresh pods by using camel hair brush. The pods were replaced with fresh ones every alternate day to ensure their suitability for the larvae. All these containers with larvae were kept in a rectangular iron rat proof cage (Plate 2 a). Containers and cage were cleaned every day to maintain sanitation.

The larvae pupated periodically on different dates were collected by using a camel hair brush and kept in glass cylindrical jars of 20 cm height and 8cm diameter with multi-layered tissue paper bed at the base for adult emergence and mating (Plate 2 b). Cotton buds soaked in ten per cent honey solution were provided as food source to the adult moths (Plate 2 c). Pieces of muslin cloth were placed inside to facilitate oviposition. Fresh and tender cowpea flowers were introduced into the containers for neonates to feed. The processes were repeated as described above to get adequate number of second instar larvae for conducting the experiments. The data on the time taken by the different stages of *M. vitrata* were recorded and documented.

### 3.2.2 Comparison between natural diet and artificial diet

The data on time taken to complete different life stages of *M. vitrata* reared on artificial and natural diet were compared and documented.

a

b

c

Plate 2. Mass rearing of *Maruca vitrata* (F)

## 3.3 ASSESSMENT OF INSECTICIDE RESISTANCE IN FIELD POPULATIONS OF *M. VITRATA*

The egg and first instar larvae of *M. vitrata* collected from the infested cowpea (Variety - Vellayani Jyothika) grown in the three different locations *viz.* Venganoor Panchayath with no previous history of pesticide application, Instructional Farm attached to the College of Agriculture, Vellayani where no control failures had been observed and the third from Kalliyoor Panchayat with known reported control failures (Table. 9). These three populations were reared under the laboratory condition as described in expt. 3.2. The susceptibility/ resistance of the population were tested using the two insecticides selected based on the preliminary survey conducted in the region, the details of which are furnished in Table.10.

Table 9. Locations from where populations of *Maruca vitrata* (F) were collected

| Sl.no | Locations | Details of locations |
|---|---|---|
| 1 | Location-I, Venganoor | Field with no history of pesticide application |
| 2 | Location-II, College of Agriculure, Vellayani | Field with no control failures |
| 3 | Location-III, Kalliyoor | Field with control failures |

### 3.3.1 Study on the toxicity of insecticides to *M. vitrata* collected from different Locations

The bioassay was conducted by the procedures described by Elzen *et al.* (1992). The details of the study are given below,

Table 10. Insecticides used for resistance study

| Sl.no | Details of insecticides | | | | | |
|---|---|---|---|---|---|---|
| | Chemical name | Trade name | Chemical group | Mode of action as per IRAC, 2014 | Dosage (g a.i.ha$^{-1}$) | Target pest |
| 1 | Chlorpyriphos | Classic 20 EC | Organo phosphates | Acetyl choline esterase inhibitors | 600 | Bean pod borer |
| 2 | Lambda-cyhalothrin | Karate 5 EC | Synthetic pyrethroids | Sodium channel modulators | 25 | Pulse pod borer |

Design- CRD

Replications-3

Treatments- 15

$T_1$- chlorpyriphos 0.02 %

$T_2$- chlorpyriphos 0.03 %

$T_3$- chlorpyriphos 0.04 %

$T_4$- chlorpyriphos 0.05 %

$T_5$- chlorpyriphos 0.06 %

$T_6$- chlorpyriphos 0.07 %

$T_7$- chlorpyriphos 0.08 %

$T_8$- lambda-cyhalothrin 0.0005 %

$T_9$- lambda-cyhalothrin 0.001 %

$T_{10}$- lambda-cyhalothrin 0.002 %

$T_{11}$- lambda-cyhalothrin 0.003 %

$T_{12}$- lambda-cyhalothrin 0.004 %

$T_{13}$- lambda-cyhalothrin 0.005 %

$T_{14}$- lambda-cyhalothrin 0.006 %

$T_{15}$- control (water spray)

Newly moulted second instar larvae from laboratory reared culture collected from the three locations were used for the study. The fresh flowers collected from the unsprayed field were split opened and rinsed with distilled water and allowed to dry. The insecticide solutions were prepared by dissolving required quantity of insecticides in water.

The washed and air dried flowers of cowpea were dipped into the test insecticide solutions for 25 seconds with gentle agitation. The excess moisture was removed from the flowers by using filter paper. Each treated flower was placed in a separate container. Ten newly moulted second instar larvae were transferred to each treated flower in the container and this formed one replication. Three such replications were kept for each treatment. Mortality was recorded at 12, 24 and 48 hours after treatment. Corrected percentage mortality was calculated using Abbot's formula (Abbot, 1925).

Corrected mortality =

$$\frac{\text{Observed mortality in treatment} - \text{observed mortality in control}}{100 - \text{Observed mortality in control}} \times 100$$

The data were subjected to statistical analysis and the respective $LC_{50}$ value for each individual case was arrived at by using the logarithmic model.

Mortality percentage = $a \times x^b$

$$LC_{50} = \frac{\exp(\log_{50} - a)}{b}$$

x = concentration of insecticide

a = intercept

b = regression coefficient

Fiducial limits were computed using

$b \pm t\,[SE\,(b)]$

The population of *M. vitrata* found to be resistant was taken for further study.

## 3.4 EVALUATION OF EFFICACY OF NEW GENERATION INSECTICIDES AGAINST THE RESISTANT POPULATION OF *M. VITRATA*

The resistant strains identified from the experiment 3.3 were utilized for this laboratory study. The population of *M. vitrata* taken from location- II and III were selected for the evaluation of efficacy of new generation insecticides. The following eight new generation insecticides mentioned in Table. 11 were tested at their recommended doses under laboratory conditions to evaluate the comparative efficacy against the resistant population of *M. vitrata*.

Design-RBD

Replication- 3

Treatments -9 (8+1)

$T_1$- Novaluron - 0.015%

$T_2$- Flubendiamide - 0.01%

$T_3$- Spinosad – 0.015 %

$T_4$- Emamectin benzoate – 0.002%

Table 11. New generation insecticides used for the management of resistant population of *Maruca vitrata* (F)

| Sl. No | Details of insecticides | | | | | |
|---|---|---|---|---|---|---|
| | Chemical name | Trade name | Chemical group | Mode of action as per IRAC, 2014 | Dosage (g a.i.ha$^{-1)}$ | Target pest |
| 1 | Novaluron | Rimon 10 EC | Benzoylureas | Inhibitors of chitin biosynthesis (Type-0) | 600 | Bean pod borer |
| 2 | Flubendiamide | Fame 480 SC | Diamides | Ryanodine receptor modulators | 25 | Pulse pod borer |
| 3 | Spinosad | Tracer 45 SC | Spinosyns | Nicotinic Acetylcholine receptor (allosteric) activators | 75 | Red gram pod borer |
| 4 | Emamectin Benzoate | Proclaim 5 SG | Avermectins | Chloride channel activators | 10 | Red gram and chickpea pod borers |
| 5 | Indoxacarb | Indoxacarb 14.5 SC | Oxadiezenes | Voltage-dependent sodium channel blockers | 60 | Pigeon pea pod borer complex |
| 6 | Chlorantraniliprole | Coragen 18.5 SC | Diamides | Ryanodine receptor modulators | 30 | Cotton boll worms |
| 7 | Indoxacarb+acetamiprid | Caeser | Oxadiezenes and Neonicotinoids | Voltage-dependent sodium channel blockers and Nicotinic acetylcholinen receptor agonists. | 100 | Cotton boll worm |
| 8 | Acephate+imidacloprid | - | Organophosphates and Neonicotinoids | Acetylcholinesterase inhibitors and Nicotinic acetylcholinen receptor agonists. | 518 | Boll worms |

$T_5$- Indoxacarb - 0.10 %

$T_6$- Chlorantraniliprole-0.03%

$T_7$- Indoxacarb 14.5 % + Acetamiprid 7.7 % SC - 0.09%

$T_8$- Acephate 50% + Imidacloprid 1.8% SP - 0.20%

$T_9$- Chlorpyriphos - 0.05%

$T_{10}$- Water spray (Control)

The laboratory evaluation was done based on the procedure adopted by Ekesi, 1999 as in expt. 3.3. Corrected percentage mortality was calculated using Abbot's formula (Abbot, 1925).

## 3.5 FIELD EVALUATION OF SELECTED NEW GENERATION INSECTICIDES AGAINST RESISTANT POPULATION OF *M. VITRATA*

The promising three insecticides found effective in expt. 3.4 were further evaluated in field for their efficacy in controlling the resistant population of *M. vitrata*

The experiment was laid out in RBD with 5 replications in location-III where the populations of *M. vitrata* were found to be more resistant (Plate 3). The details of treatments given in Table-12. The treatments were given when 5-10 per cent of pod damage was observed. Two to three day old unopened flowers (25 numbers) were selected at random from each plot in one, two, three, five, seven, ten and fifteen days after spraying and the number of flowers with larvae of *M. vitrata* were recorded and the per cent flower damage was worked out. Similarly, ten cowpea pods were randomly picked from each plot after one, two, three, five, seven, ten and fifteen days of spraying and per cent fruit damage was calculated.

Table 12. Insecticides selected for field evaluation

| Treatment No. | Chemical name | Trade name | Dosage (g a.i.ha$^{-1}$) |
|---|---|---|---|
| $T_1$ | Emamectin benzoate | Proclaim 5 %SG | 10 |
| $T_2$ | Indoxacarb + acetamiprid | Caeser 22.2 %SC | 100 |
| $T_3$ | Spinosad | Tracer 45 %SC | 75 |
| $T_4$ | Untreated control | | |

## 3.6 ESTIMATION OF HARVEST TIME RESIDUES OF INSECTICIDES IN COWPEA PODS

Mature cowpea pods from different plots in Expt. No. 3.5 (field evaluation) were collected for the determination of harvest time pesticide residues. The estimation of insecticide residues at harvest was done in the Pesticide Residue Research and Analytical laboratory, AINP on Pesticide Residues, College of Agriculture, Vellayani using LC-MS/MS (Applied Biosystems API-3200 triple quadrupole MS-MS with electro spray ionisation (ESI) in the positive mode coupled to a Waters LC (Acquity UPLC). Validation parameters *viz.*, Limit of Detection, Limit of Quantification, Linearity, Recovery and Repeatability (Zanella *et al.*, 2000) were evaluated.

### 3.6.1 Fortification and Recovery Experiment

Cowpea fruits (500 g) harvested from control plots were chopped and ground to a fine paste. Five replicates of 25 g representative samples of the fruits were taken in 50 ml centrifuge tubes and spiked at 0.05 mg kg$^{-1}$, 0.25 mg kg$^{-1}$ and


Plate 3. View of the experimental plot

0.5 mg $kg^{-1}$ levels. The extraction and clean-up was done following the QuEChERS method (AOAC, 2012) and quantified using UPLC-MS/MS under optimized conditions.

Insecticide sprayed harvestable fruits of cowpea were collected from each treated plot on 7th and 15th days after spraying and brought to the laboratory in polythene bags and processed immediately for residue analysis.

A sub- sample of 500 g cowpea was taken from each of the treatment plot by quartering and comminuting. The blended sample (25g) was taken from each replicate, homogenized at 14,000 rpm for 2 min. after adding 50 ml acetonitrile. The samples was shaken for 1 minute and 10 g sodium chloride were added. The sample was centrifuged for 5 min. at 2500 rpm. A 16 mL supernatant was transferred in to 50 mL centrifuge tube containing 6 g anhyd. $Na_2SO_4$ and mixed well using high speed vortex shaker for 2 min. A 12 ml extract was transferred to a 15 mL centrifuge tube containing 0.2 ± 0.01 g PSA sorbent and 1.2 ± 0.01 g anhyd. $MgSO_{4.}$ The sample was shaken and centrifuged for about 3 min at 2500 rpm. 5ml of supernatant was evaporated in turbovap and made up with 2 ml using methanol for LC-MS/MS analysis.

The chromatographic separation was achieved using Waters Acquity UPCL system equipped with a reversed phase Atlantis C-18 (2.1 × 100 mm, 5 micron particle size) column. A gradient system involving the following two eluent components: A: 10 per cent methanol in water + 0.1 per cent formic acid + 50 mM ammonium acetate; B: 10 per cent water in methanol + 0.10 per cent formic acid + 50 mM ammonium acetate was used as mobile phase for the separation of residues. The gradient elution was as follows: 0 min isocratic 20 per cent B, 0.0-4.0 min linear from 20 to 90 per cent B, 4.0-5 min linear from 90 to 95 per cent B, and 5-6.6 min linear from 95 to 100 per cent B, with 6.6-7 min for initial conditions of 20 per cent B. the flow rate remained constant at 0.8 ml $min^{-1}$ and injection volume was 10 µl. The column temperature was maintained at $40^oC$. The effluent from the LC system was introduced into Triple quadrapole API 3200

Table 13. Retention time and Multiple Reaction Monitoring (MRM) transitions of the insecticides

| Name of the compound | | RT (min) | MRM transitions | | Declustering Potential | Entrance Potential | Collision Cell Entrance Potential | Collision Energy | Collision Cell Exit Potential |
|---|---|---|---|---|---|---|---|---|---|
| | | | Quantitative ion pair | Qualitative ion pair | | | | | |
| Emamectin benzoate | Emamectin B1b1 | 4.5 | 872.5→158.2 | 872.5→126.2 | 70 | 10 | 51 | 50 | 4 |
| | Emamectin B1a1 | 4.6 | 886.6→158.2 | 886.6→126.2 | 70 | 10 | 52 | 50 | 4 |
| Spinosad | Spinosyn A | 4.2 | 732.6→142.2 | 732.6→98.2 | 70 | 10 | 46 | 50 | 4 |
| | Spinosyn D | 4.38 | 746.5→142.2 | 746.5→98.2 | 70 | 10 | 46 | 50 | 4 |
| Indoxacarb | | 4.32 | 528→203 | 528→150 | 70 | 10 | 38 | 50 | 4 |
| Acetamiprid | | 1.29 | 223.1→126 | 223.1→99 | 46 | 9 | 19 | 29 | 1 |
| | | 1.29 | 223.1→126 | 223.1→99 | 46 | 9 | 19 | 54 | 1 |

MS/MS system equipped with an electrospray ionization interface (ESI) operating in the positive ion mode. The source parameters were temperature 600$^{o}$C; ion gas (GSI) 50 psi, ion gas (GS2) 60 psi, ion spray voltage 5,500 V, curtain gas 13 psi. The Retention Time, MRM transitions and the optimized compound dependent parameters, used for the estimation of compounds in LC-MS/MS are given in Table 13.

Based on the peak area of the chromatogram obtained for various insecticides, the quantity of residue was determined as detailed below.

Pesticide Residue (mg kg$^{-1}$) = Concentration obtained from chromatogram by using calibration curve × Dilution factor

$$\text{Dilution factor} = \frac{\text{Volume of the solvent added} \times \text{Final volume of extract}}{\text{Weight of sample (g)} \times \text{Volume of extract taken for concentration}}$$

The Limit of Quantification (LOQ) of this method was 0.05 mg kg$^{-1}$.

# *Results*

# 4. RESULTS

## 4.1 PRELIMINARY SURVEY TO GATHER THE INFORMATION ON THE PESTICIDE USE AND THE DEVELOPMENT OF INSECTICIDE RESISTANCE

Results of the survey on consumption and use pattern of pesticides and awareness regarding insecticide resistance among cowpea farmers in Kalliyoor and Venganoor panchayaths are presented in Tables 14 and 15.

### 4.1.1 Details of pests, effectiveness of plant protection chemicals and awareness of insecticide resistance

Information on details of pests, effectiveness of plant protection chemicals and awareness of insecticide resistance among cowpea farmers in Kalliyoor and Venganoor panchayaths are presented in Table 14. Among the farmers, 44 per cent responded that pod borers are the major pest affecting cowpea. However, 30 and 26 per cent of farmers opined that aphids and bugs respectively are the major pests of cowpea. In case of insecticide consumption, 34 per cent farmers revealed that lambda-cyhalothrin is the widely used insecticide against cowpea pests while 30 per cent responded chlorpyriphos is the widely used insecticide and 24 and 10 per cent farmers revealed that fenvalerate and quinalphos respectively are the widely used insecticides.

The frequency of pesticide application against pod borers of cowpea ranged from 2-21 days. Among surveyed farmers, 50 per cent sprayed insecticides in two days interval, 30 per cent within 5 days and 15 per cent at interval of 7-14 days. Only 5 per cent farmers kept an interval of 14-21 days between sprays.

Majority of farmers (80 %) agreed that control failures are observed by the continuous application of single insecticide. However, 20 per cent responded that no control failures are reported by the continuous application of insecticides.

Table 14. Knowledge on details of pests and effectiveness of plant protection chemicals among cowpea farmers in Kalliyoor and Venganoor panchayats of Thiruvananthapuram district (January 2013 to March 2013).

| Particulars | | | Farmers (%) |
|---|---|---|---|
| Major pests attacking cowpea | a) | Pod borers | 44 |
| | b) | Bugs | 26 |
| | c) | Aphids | 30 |
| Insecticides widely used against cowpea pests | a) | Lambda-cyhalothrin | 34 |
| | b) | Chlorpyriphos | 30 |
| | c) | Fenvalerate | 24 |
| | d) | Quinalphos | 10 |
| Frequency of application | a) | 2 days | 50 |
| | b) | 5 days | 30 |
| | c) | 7-14 days | 15 |
| | d) | 14-21 days | 5 |
| Control failures reported by the continuous application of one insecticide | a) | Yes | 80 |
| | b) | No | 20 |
| Pests difficult to control | a) | Pod borers | 60 |
| | b) | Bugs | 32 |
| | c) | Aphids | 8 |
| Against which pest control failures more prominent | a) | Pod borers | 80 |
| | b) | Bugs | 14 |
| | c) | Aphids | 6 |
| Which insecticide against control failures obtained | a) | Lambda-cyhalothrin | 41 |
| | b) | Chlorpyriphos | 31 |
| | c) | Fenvalerate | 25 |
| | d) | Quinalphos | 3 |
| Awareness regarding insecticide resistance | a) | Aware | 20 |
| | b) | Unaware | 80 |
| Source of information on insecticide resistance | a) | Training classes | 34 |
| | b) | Media | 0 |
| | c) | Other farmers | 66 |

The survey revealed that pod borers, bugs and aphids are the pests difficult to control. Among the farmers, 60 per cent responded that pod borers are the most difficult to control, whereas 32 and 8 per cent of farmers revealed that bugs and aphids respectively are very difficult ones. Regarding the pest against which control failures are more prominent, 80 per cent observed failure in the case of pod borers, 14 per cent in the case of bugs and 6 per cent in the case of aphids.

According to 41 per cent farmers, lambda-cyhalothrin is the insecticide having the highest control failures, while 31, 25 and 3 per cent farmers revealed that the control failures are higher in area where chlorpyriphos, fenvalerate and quinalphos respectively are sprayed.

Among the surveyed farmers, 80 per cent are unaware of the development of insecticide resistance whereas, 20 per cent are aware about the development of resistance in insects. Majority of farmers collected the information on insecticide resistance from other farmers (66 %) whereas 34 per cent gathered the information during various training classes. However, none of the farmers collected information on insecticide resistance from media.

### 4.1.2 Information on pesticide use

The data on the information of pesticide use among surveyed farmers are presented in Table 15. Regarding the source of technical information of pesticides, 40 per cent of farmers gathered information from other progressive farmers whereas, 26 per cent was taken their own decisions on technical matters without any consultation. Twenty per cent farmers gathered information from Agricultural Officers and 10 and 4 per cent farmers collected the information from pesticide shops and media respectively.

Cent per cent farmers purchased insecticides from the pesticide shops itself. Considering the application of insecticides, 68 per cent farmers applied insecticides as prophylactic while 32 per cent sprayed insecticides as and when required. Among the surveyed farmers, 48 per cent of farmers following the

Table 15. Pesticide use among cowpea farmers in Kalliyoor and Venganoor panchayats of Thiruvananthapuram district. (January 2013 to March 2013).

| Particulars | | Farmers (%) |
|---|---|---|
| Source of technical information on pesticides | a) Agricultural officers | 20 |
| | b) Pesticide company | 10 |
| | c) Other progressive farmers | 40 |
| | d) Own decisions | 26 |
| | e) Media | 4 |
| Source of insecticides | a) Pesticide shops | 100 |
| | b) Directly from companies | 0 |
| Prophylactic use of insecticides | a) Yes | 68 |
| | b) No | 32 |
| Manual mixing of different insecticides | a) Yes | 48 |
| | b) No | 52 |
| Attention towards labels on pesticide bottles before use | a) Yes | 70 |
| | b) No | 30 |
| Source of information on dose of pesticides | a) Agricultural officers | 5 |
| | b) Pesticide shops | 5 |
| | c) Other progressive farmers | 40 |
| | d) Own decisions | 50 |
| | e) Media | 0 |
| Time of pesticide application | a) Early morning | 32 |
| | b) Morning | 36 |
| | c) Afternoon | 16 |
| | d) Evening | 16 |
| Adoption of IPM strategies | a) Yes | 30 |
| | b) No | 70 |

practice of manual mixing of different insecticides for spraying against various pests, whereas 52 per cent farmers do not follow the practice of manual mixing. Majority of farmers paid their attention towards labels on pesticide bottles before use (70 %) while, 30 per cent farmers are not paying any attention on labels for insecticide use.

Regarding the source of information, dose of pesticides, 50 per cent farmers responded that the dose of pesticide was decided by themselves, while 40 per cent gathered information on dose of pesticides from other progressive farmers. However, 5 per cent each of farmers collected information on dose of pesticide either from Agricultural Officers or from pesticide companies.

Applications of pesticides are done at different times of the day. Among the surveyed farmers, 36 per cent of farmers applied pesticides in the morning, 32 per cent in early morning and 16 per cent each at afternoon and evening respectively. Regarding the adoption of Integrated Pest Management, 70 per cent are not adopting while 30 per cent of farmers are following Integrated Pest Management strategies for the effective management of pests of cowpea.

## 4.2 LABORATORY REARING OF SPOTTED POD BORER, *M. VITRATA* IN DIFFERENT DIETS

### 4.2.1 Mass rearing on artificial and natural diets

The results on the shelf life of the three different artificial diets are presented in Table 16. The shelf life was found to be more in diet-2 (120 days) followed by diet-1 (30-45 days) and diet-3 (15-20 days) in normal environmental condition. Due to the high shelf life, diet-2 was selected as the medium for rearing *M. vitrata* in laboratory conditions.

Table 16. Shelf life of the artificial diets prepared for rearing *Maruca vitrata* (F)

| Diets | Shelf life (No. of days) |
|---|---|
| Diet-1 (Diet suggested by Ochieng and Bungu, 1983) | 30-45 |
| Diet-2 (Modification of ingredients of diet-1) | 120 |
| Diet-3 (Modification of ingredients of diet-2) | 15-20 |

### 4.2.2 Comparison between natural and artificial diet

The results of the study on duration of life stages of of *M. vitrata* are given in Table 17 and Plate 4. Mean number of days taken by the first instar larvae was 2.20 days in the population of *M. vitrata* reared in cowpea pod and it was 2 days each when reared in artificial diet and lab-lab bean. Similarly, the average number of days taken by second instar larvae was 3 days in larvae reared on cowpea pod followed by lab-lab bean (2.80 days) and artificial diet (2.20 days). The number of days taken by the third instar larvae ranged from 1.60 -2.80 days in different diets. However, the days taken to complete fourth and fifth instars were 2.64 and 3.60 days respectively when reared in cowpea pods and 2.42 and 3.40 days respectively when reared in lab-lab bean and 2.04 and 2.40 days respectively in artificial diet.

There was significant difference in the total larval period of *M. vitrata* reared in the three different diets. Thus the total larval period of 10.16 was recorded in larvae reared in artificial diet which was significantly lower than the others. The larvae reared in diets *viz.* lab-lab bean and cowpea pod recorded total larval periods of 13.22 and 14.24 days respectively which differed significantly between two. The pre-pupal stage ranged from 1.60 -1.80 days in different diets. Time taken by the pupa to become adult was 8 days in cowpea pod, 7.80 days in artificial diet and 7.60 days in lab-lab bean. The duration of adult stage was 5.32

Table 17. Duration of life stages (days) of *Maruca vitrata* (F) reared on different diets

| Life stages | Mean number of days taken in different diets | | |
|---|---|---|---|
| | Artificial diet | Cowpea pod | Lab-lab bean |
| First instar | 2.00 | 2.20 | 2.00 |
| Second instar | 2.20 | 3.00 | 2.80 |
| Third instar | 1.60 | 2.80 | 2.60 |
| Fourth instar | 2.04 | 2.64 | 2.42 |
| Fifth instar | 2.40 | 3.60 | 3.40 |
| **Total larval period*** | 10.16 | 14.24 | 13.22 |
| Pre-pupa | 1.80 | 1.60 | 1.60 |
| Pupa | 7.80 | 8.00 | 7.60 |
| Adult | 4.38 | 5.32 | 5.64 |
| **Total life cycle**** | 24.14 | 29.16 | 28.06 |

* CD (0.05) of total larval period – 0.113

** CD (0.05) of total life cycle – 0.390

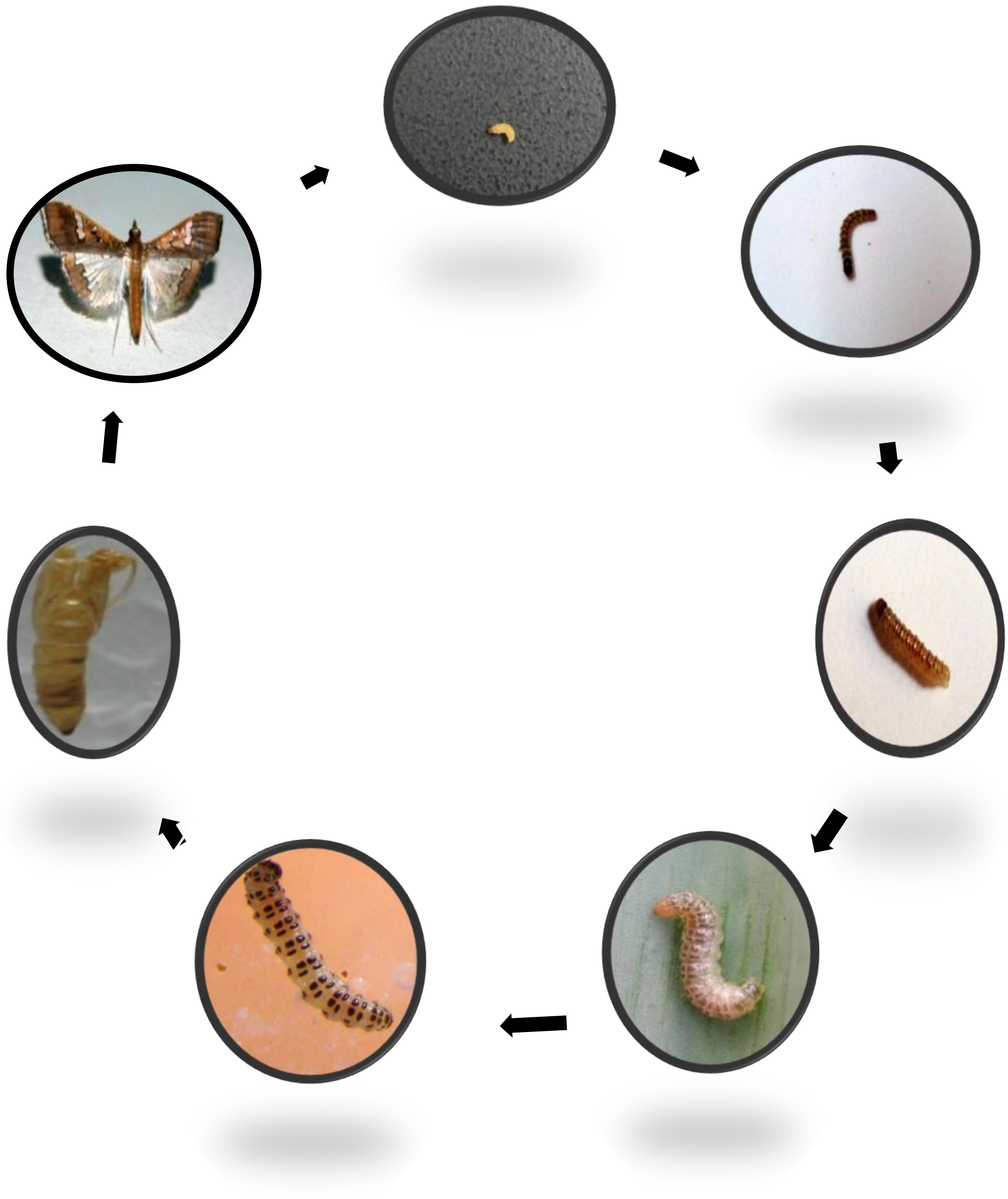


Plate 4. Life stages of *Maruca vitrata* (F)

days when reared on cowpea pod 5.64 days when reared in lab-lab bean and 4.38 days in artificial diet. Significantly lowest total life period was observed in larvae reared in artificial diet (24 – 14 days) followed by lab-lab bean (28.06 days) and cowpea pod (29.16 days).

## 4.3 ASSESSMENT OF INSECTICIDE RESISTANCE IN THE FIELD POPULATION OF *M. VITRATA*

The data on the toxicity of chlorpyriphos to the population of *M. vitrata* collected from the three locations are presented in Table 18. $LC_{50}$ of chlorpyriphos observed after 12, 24 and 48 hours of treatment in the population of *M. vitrata* collected from location I (Venganoor) are 360, 250 and 219 ppm with fiducial limits (95 %) of 279-520, 248-1050 and 200-900 ppm and the slope ± standard error of 0.60 ± 0.085, 0.66 ± 0.145 and 0.605 ± 0.115. Similarly, the $LC_{50}$ of chlorpyriphos are 959, 585 and 501 ppm after 12, 24 and 48 hours of treatment in population of *M. vitrata* sampled from location-II (Instructional farm, Vellayani) with slope ± standard error worked out to be 2.24 ± 0.10, 1.38 ± 0.194, 1.095 ± 0.118. The fiducial limits were 320-1100, 415-994 and 780-1790 ppm. The $LC_{50}$ of chlorpyriphos observed in population collected from location-III (Kalliyoor panchayath) after 12, 24 and 48 hours of treatment are 912, 941 and 642.50 ppm respectively with slope ± standard error are 2.49 ± 0.12, 1.51 ± 0.132 and 0.87 ± 0.148. The fiducial limits worked out to be 503-1014, 761-1120 and 493-1261. The population of *M. vitrata* collected from Location II showed the resistance ratio of 2.66, 2.34 and 2.28 after 12, 24 and 48 hours of treatment respectively. The resistance ratio noticed in population of *M. vitrata* collected from location-III are 2.53, 3.76 and 2.97 after 12, 24 and 48 hours of treatment respectively. The population of *M. vitrata* collected from location-1 showed the resistance ratio of one.

The data on the toxicity of lambda- cyhalothrin to the population of *M. vitrata* collected from three locations are presented in Table 19. $LC_{50}$ of lambda-cyhalothrin in population of *M. vitrata* collected from location I(Venganoor) are

Table 18. Toxicity of chlorpyriphos to the population of *Maruca vitrata* (F) collected from three locations

| Location | HAT | Slope ± SE | $LC_{50}$ (ppm) | 95 % fiducial limits | Resistance ratio |
|---|---|---|---|---|---|
| Location-1 (Venganoor) | 12 HAT | 0.61 ± 0.085 | 360 | 279 - 520 | 1 |
| | 24 HAT | 0.66 ± 0.145 | 250 | 248 - 1050 | 1 |
| | 48 HAT | 0.60 5± 0.115 | 219 | 200 - 900 | 1 |
| Location-2 (College of Agriculture, Vellayani) | 12 HAT | 2.24 ± 0.100 | 959 | 320 - 1100 | 2.66 |
| | 24 HAT | 1.38 ± 0.194 | 585 | 415 - 994 | 2.34 |
| | 48 HAT | 1.095 ± 0.118 | 501 | 780 - 1790 | 2.28 |
| Location-3 (Kalliyoor) | 12 HAT | 2.49 ± 0.120 | 912 | 503 - 1014 | 2.53 |
| | 24 HAT | 1.51 ± 0.132 | 941 | 761 - 1120 | 3.76 |
| | 48 HAT | 0.87 ± 0.148 | 642.5 | 493 - 1261 | 2.97 |

CD (0.05) for $LC_{50}$ - 183.49

HAT- Hours after treatment

Table 19. Toxicity of lambda-cyhalothrin to the populations of *Maruca vitrata* (F) collected from three locations.

| Location | HAT | Slope ± SE | $LC_{50}$ (ppm) | 95 % fiducial limits | Resistance ratio |
|---|---|---|---|---|---|
| Location-1 (Venganoor) | 12 HAT | 0.434 ± 0.08 | 28.50 | 215 - 650 | 1 |
| | 24 HAT | 0.474 ± 0.105 | 19 | 180 - 320 | 1 |
| | 48 HAT | 0.32 ± 0.07 | 9 | 130 - 207 | 1 |
| Location-II (College of Agriculture, Vellayani) | 12 HAT | 0.71 ± 0.32 | 111 | 198 - 623 | 3.89 |
| | 24 HAT | 0.53 ± 0.09 | 86 | 170 - 420 | 4.52 |
| | 48 HAT | 0.365 ± 0.09 | 21.50 | 62.50-160 | 2.38 |
| Location-III (Kalliyoor) | 12 HAT | 0.426 ± 0.246 | 200 | 180 - 352 | 7.01 |
| | 24 HAT | 0.07 ± 0.246 | 75 | 138 - 240 | 3.94 |
| | 48 HAT | 0.4 ± 0.056 | 71.50 | 112 - 225 | 7.94 |

CD (0.05) for $LC_{50}$ - 68.40

HAT- Hours after treatment

28.50, 19 and 9 ppm after 12, 24 and 48 hours of treatment with fiducial limits (95 %) of 215-650, 180-320 and 130-207 respectively. The slope ± standard error worked out to be 0.434 ± 0.08, 0.474 ± 0.105 and 0.32 ± 0.07 in 12, 24 and 48 hours of treatment. The $LC_{50}$ of lambda-cyhalothrin are 111, 86 and 21.50 ppm after 12, 24 and 48 hours of treatment in population of larvae sampled from location-II (Instructional farm, Vellayani) with the slope ± standard error worked out to be 0.71 ± 0.32, 0.53 ± 0.09 and 0.365 ± 0.09 in 12, 24 and 48 hours of treatment respectively. The fiducial limits were 198 - 623, 170 - 420 and 62.50 - 160. However, the $LC_{50}$ of lambda-cyhalothrin observed in the population of *M. vitrata* collected from location-III (Kalliyoor) are 200, 75 and 71.50 ppm respectively with slope ± standard error are 0.426 ± 0.246, 0.07 ± 0.246 and 0.4 ± 0.056. The fiducial limits worked out to be 180 - 352, 138 - 240 and 112 - 225. The resistance ratio of population of *M. vitrata* from location-II showed a resistance ratio of 3.89, 4.52 and 2.38 after 12, 24 and 48 hours of treatment. The resistance ratio noticed in population of *M. vitrata* collected from location-III is 7.01, 3.94 and 7.94 after 12, 24 and 48 hours of treatment respectively. The population collected from location-I showed the resistance ratio of one.

## 4.4 EVALUATION OF EFFICACY OF NEW GENERATION INSECTICIDES AGAINST THE RESISTANT POPULATION OF *M. VITRATA*

### 4.4.1 Mortality of population of *M. vitrata* collected from location-II (Instructional farm, Vellayani) treated with chemicals.

The results of the study on mortality observed at different intervals of the population of *M. vitrata* after treated with new generation insecticides are presented in Table. 20.

The treatments varied significantly on their toxicity to *M. vitrata* after six hours of treatment. However, all the treatments were superior to the standard check *viz.* chlorpyriphos 600 g a.i.$ha^{-1}$. Highest mortality was recorded with emamectin benzoate and indoxacarb + acetamiprid 100 g a.i.$ha^{-1}$after 6 hours of

Table 20. Mean mortality of resistant population of *M. vitrata* collected from location-II (IF, College of Agriculture, Vellayani) treated with new generation insecticides

| Insecticide | Dosage (g a.i.ha$^{-1}$) | Mortality (%) | | | |
|---|---|---|---|---|---|
| | | 6HAT | 12 HAT | 24 HAT | 48 HAT |
| Novaluron | 100 | 9.554 (18.00) | 32.89 (34.99) | 32.89 (34.99) | 53.32 (46.90) |
| Flubendiamide | 100 | 46.61 (43.05) | 67.04 (54.96) | 97.28 (80.50) | 99.97 (89.05) |
| Spinosad | 75 | 53.32 (46.90) | 99.97 (89.05) | 99.97 (89.05) | 99.97 (89.05) |
| Emamectin benzoate | 10 | 97.28 (80.50) | 99.97 (89.05) | 99.97 (89.05) | 99.97 (89.05) |
| Chlorantraniliprole | 30 | 73.76 (59.18) | 79.96 (63.40) | 97.28 (80.50) | 99.97 (89.05) |
| Indoxacarb | 60 | 73.76 (59.18) | 73.76 (59.18) | 97.28 (80.50) | 99.97 (89.05) |
| Indoxacarb+acetamiprid | 100 | 97.28 (80.50) | 99.97 (89.05) | 99.97 (89.05) | 99.97 (89.05) |
| Acephate+imidacloprid | 518 | 53.32 (46.90) | 67.04 (54.96) | 97.28 (80.50) | 99.97 (89.05) |
| Chlorpyriphos | 600 | 0.024 (0.905) | 2.698 (9.455) | 19.98 (26.55) | 39.97 (39.21) |
| CD(0.05) | | (17.130) | (21.072) | (17.443) | (3.807) |

Figures in parentheses are angular transformed values

HAT- Hours after treatment

treatment with the per cent mortality of 97.28 in both cases. The mortality observed in the case of chlorantraniliprole 30 g a.i.ha$^{-1}$, indoxacarb 60 g a.i.ha$^{-1}$, acephate + imidacloprid 518 g a.i.ha$^{-1}$, spinosad 75 g a.i.ha$^{-1}$ and flubendiamide 100 g a.i.ha$^{-1}$ were 73.76, 73.76, 53.32, 53.32 and 46.61 per cent respectively and were on par. However, significantly lower mortality was observed in the population of *M. vitrata* treated with novaluron 100 g a.i.ha$^{-1}$ (9.554 %)

The treatments varied significantly on their toxicity to *M. vitrata* after 12 hours of treatment. However, all the treatments were superior to the standard check *viz.* chlorpyriphos 600 g a.i.ha$^{-1}$. More or less similar results were obtained 12 hours after treatment. Higher mortality was obtained in the population of *M. vitrata* treated with spinosad 75 g a.i.ha$^{-1}$, emamectin benzoate 10 g a.i.ha$^{-1}$ and indoxacarb + acetamiprid 100 g a.i.ha$^{-1}$ (99.97 % each). The mortality observed in population of *M. vitrata* treated with chlorantraniliprole 30 g a.i.ha$^{-1}$ (79.96 %), indoxacarb 60 g a.i.ha$^{-1}$ (73.76 %), acetamiprid + imidacloprid 518 g a.i.ha$^{-1}$ (67.94 %) and flubendiamide 100 g a.i.ha$^{-1}$ (67.04 %) respectively and were on par. The mortality observed was less in larvae treated with novaluron 100 g a.i.ha$^{-1}$ with per cent mortality of 32.89 per cent. The per cent mortality expressed by the population when sprayed with chlorpyriphos 600 g a.i.ha$^{-1}$ was 2.698 per cent.

Similarly, the treatments varied significantly on their toxicity to *M. vitrata* after 24 hours of treatment and all the treatments were superior to the standard check *viz.* chlorpyriphos 600 g a.i.ha$^{-1}$. Significantly higher mortality was recorded in the population treated with spinosad 75 g a.i.ha$^{-1}$ , emamectin benzoate 10 g a.i.ha$^{-1}$ and indoxacarb+acetamiprid 100 g a.i.ha$^{-1}$ (99.97 %). No significant difference was observed in the mortality when treated with chemicals like chlorantraniliprole 30 g a.i. ha$^{-1}$, indoxacarb 60 g a.i.ha$^{-1}$, flubendiamide 100 g a.i.ha$^{-1}$ and acephate+imidacloprid 518 g a.i.ha$^{-1}$ which showed a per cent mortality of 97.28 each and less mortality was recorded in population sprayed with chlorpyriphos 600 g a.i.ha$^{-1}$ (19.98 %) and novaluron 100 g a.i.ha$^{-1}$ (32.89 %).

The per cent mortality 48 hours after treatment were highest in all the treatments except novaluron 100 g a.i.ha$^{-1}$ (53.32 %) and chlorpyriphos 600 g a.i.ha$^{-1}$ (39.97 %).

### 4.4.2 Mortality of population of *M. vitrata* collected from location-III (Kalliyoor panchayath) treated with chemicals.

The results of the study on the mortality of population of *M. vitrata* collected from location-III treated with chemicals are presented in Table 21. All the treatments varied significantly on their toxicity to *M. vitrata* after six hours of treatment. However, the treatments were found to be superior to the standard check *viz.* chlorpyriphos 600 g a.i.ha$^{-1}$. Significantly higher mortality was observed in the population of *M. vitrata* treated with spinosad 75 g a.i.ha$^{-1}$ (80 %), emamectin benzoate 10 g a.i.ha$^{-1}$ (97.28 %) and indoxacarb + acetamiprid 100 g a.i.ha$^{-1}$ (97.28 %) after six hours of treatment as compared to other treatments. The mortality recorded in the population of *M. vitrata* treated with flubendiamide 100 g a.i.ha$^{-1}$ (67.66 %), chlorantraniliprole 30 g a.i.ha$^{-1}$ (67.04 %), acephate + imidacloprid 518 g a.i.ha$^{-1}$ (67.04 %) and indoxacarb 60 g a.i.ha$^{-1}$ (67.04 %) were significantly on par. Significantly lowest mortality was observed in population of *M. vitrata* treated with chlorpyriphos 500 g a.i.ha$^{-1}$ (0.024 %) followed by novaluron 100 g a.i.ha$^{-1}$ (13.33 %).

More or less similar result was obtained in population of *M. vitrata* after 12 hours of treatment. The treatments were found to be superior to the standard check *viz.* Chlorpyriphos 600 g a.i.ha$^{-1}$. Significantly higher mortality was recorded in the population of *M. vitrata* treated with spinosad 75 g a.i.ha$^{-1}$ (94.38 %), emamectin benzoate 10 g a.i.ha$^{-1}$ (99.97 %) and indoxacarb + acetamiprid 100 g a.i.ha$^{-1}$ (100 %). No significant difference was observed in the mortality of population of *M. vitrata* treated with flubendiamide 100 g a.i.ha$^{-1}$ (79.96 %), chlorantraniliprole 30 g a.i.ha$^{-1}$ (79.96 %), acetamiprid + imidacloprid 518 g a.i.ha$^{-1}$ (79.96 %) and indoxacarb 60 g a.i.ha$^{-1}$ (73.76%). However, the lowest

Table 21. Mean mortality of resistant population of *M. vitrata* collected from location-III (Kalliyoor panchayath) treated with new generation insecticides

| Insecticide | Dosage (g a.i.ha$^{-1}$) | Mortality (%) | | | |
|---|---|---|---|---|---|
| | | 6HAT | 12 HAT | 24 HAT | 48 HAT |
| Novaluron | 100 | 9.554 (18.0) | 32.89 (34.99) | 32.89 (34.99) | 53.32 (46.90) |
| Flubendiamide | 100 | 67.66 (55.34) | 79.96 (63.40) | 97.28 (80.50) | 99.97 (89.05) |
| Spinosad | 75 | 90.90 (72.44) | 94.38 (76.28) | 99.97 (89.05) | 99.97 (89.05) |
| Emamectin benzoate | 10 | 97.28 (80.50) | 99.97 (89.05) | 99.97 (89.05) | 99.97 (89.05) |
| Chlorantraniliprole | 30 | 67.04 (54.96) | 79.96 (63.40) | 97.28 (80.50) | 99.97 (89.05) |
| Indoxacarb | 60 | 67.04 (54.96) | 73.76 (59.18) | 97.28 (80.50) | 99.97 (89.05) |
| Indoxacarb+acetamiprid | 100 | 97.28 (80.50) | 99.97 (89.05) | 99.97 (89.05) | 99.97 (89.05) |
| Acephate+imidacloprid | 518 | 67.04 (54.96) | 79.96 (63.40) | 97.28 (80.50) | 99.97 (89.05) |
| Chlorpyriphos | 600 | 0.024 (0.9056) | 2.698 (9.455) | 9.246 (17.70) | 32.89 (34.99) |
| CD(0.05) | | (24.46) | (24.21) | (19.52) | (5.654) |

Figures in parentheses are angular transformed values

HAT- Hours after treatment

mortality was observed in population treated with chlorpyriphos 600 g a.i.ha$^{-1}$ (2.698 %) followed by novaluron 100 g a.i.ha$^{-1}$ (32.89 %).

Highest mortality was recorded in the population of *M. vitrata* treated with spinosad 75 g a.i.ha$^{-1}$, emamectin benzoate 10 g a.i.ha$^{-1}$, indoxacarb + acetamiprid 100 g a.i.ha$^{-1}$ with 99.97 per cent each. No significant difference was observed in treatments chlorantraniliprole 30 g a.i.ha$^{-1}$ (97.28 %), indoxacarb 60 g a.i.ha$^{-1}$ (97.28 %), flubendiamide 100 g a.i.ha$^{-1}$ (97.28 %) and acephate + imidacloprid 518 g a.i.ha$^{-1}$ (97.28 %) after 24 hours of treatment. The lowest mortality was recorded in chlorpyriphos 600 g a.i.ha$^{-1}$ (9.24 %) and novaluron 100 g a.i.ha$^{-1}$ (32.89 %)

The per cent mortality was highest in all treatments except in population treated with novaluron 100 g a.i.ha$^{-1}$ (53.32 %) and chlorpyriphos 600 g a.i.ha$^{-1}$ (32.89 %).

## 4.5 FIELD EVALUATION OF SELECTED NEW GENERATION INSECTICIDES AGAINST THE RESISTANT POPULATION OF *M. VITRATA*

### 4.5.1 Infestation of *M. vitrata* in cowpea flowers

The results of the damage of cowpea flowers by *M. vitrata* in plots treated with selected new generation insecticides are presented in Table 22 and Plate 5. No significant difference was observed in the per cent damage of cowpea flowers treated with insecticides and control plot one day after spraying.

On third day after treatment, the per cent flower damage was significantly lower in the insecticide treated plots than control which recorded a mean flower damage of 33.67 per cent. The per cent flower damage recorded in emamectin benzoate 10 g a.i.ha$^{-1}$ treated plot was the lowest (0.28) and was on par with those recorded in spinosad 75 g a.i.ha$^{-1}$ which recorded a mean per cent damage of 1.06. The plots treated with indoxacarb + acetamiprid 100 g a.i.ha$^{-1}$ recorded a mean damage of 7.04 per cent which was significantly higher than the other treatments.

Table 22. Damage of cowpea flowers by *Maruca vitrata* in cowpea plots treated with selected new generation insecticides

| Treatments | Dosage (g a.i.ha$^{-1}$) | Damage in cowpea flowers (DAS) (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 5 | 7 | 10 | 15 |
| Emamectin benzoate | 10 | 28.15 (32.03) | 0.28 (3.03) | 0 (0) | 0 (0) | 0.81 (5.17) | 8.38 (16.82) |
| Indoxacarb+ Acetamiprid | 100 | 33.28 (35.22) | 7.04 (15.38) | 0 (0) | 0 (0) | 10.0 (18.43) | 15.57 (23.23) |
| Spinosad | 75 | 32.83 (34.94) | 1.06 (5.92) | 0.22 (2.71) | 0 (0) | 13.04 (21.16) | 13.04 (21.16) |
| Untreated control | | 35.24 (36.40) | 33.66 (35.45) | 36.40 (37.13) | 33.59 (35.40) | 36.0 (36.89) | 43.42 (41.2) |
| CD(0.05) | | NS | (8.31) | (5.47) | (3.73) | (11.96) | (9.90) |

Figures in parentheses are angular transformed values

DAS- Days after spraying

Plate 5. Different stages of cowpea flower infested by *Maruca vitrata* (F)

More or less similar result was obtained on seventh day after spraying. No cowpea flowers were infested with *M. vitrata* in plots treated with emamectin benzoate 10 g a.i.ha$^{-1}$, indoxacarb + acetamiprid 100 g a.i.ha$^{-1}$ and spinosad 75 g a.i.ha$^{-1}$ after seventh day of spraying.

However, the infestation started appearing on 10$^{th}$ day after spraying, the lowest per cent infestation was noticed in cowpea plots treated with emamectin benzoate 10 g a.i.ha$^{-1}$ (0.81 %) and it was significantly different from other treatments. The per cent infestation of flowers recorded in the plots treated with indoxacarb + acetamiprid 100 g a.i.ha$^{-1}$ (10 %) and spinosad 75 g a.i.ha$^{-1}$ (13.04 %) were significantly on par.

Similarly 15 days after spraying, lower infestation of cowpea flowers was observed in plots treated with emamectin benzoate 10 g a.i.ha$^{-1}$ (8.38 %), indoxacarb+acetamiprid 100 g a.i.ha$^{-1}$ (15.57 %) and spinosad 75 g a.i.ha$^{-1}$ (13.04 %) which were significantly on par.

### 4.5.2 Infestation of *M. vitrata* in cowpea pods

The results of the study on the infestation of cowpea pods by *M. vitrata* in plots treated with selected insecticides are presented in Table 23 and Plate 6. No significant difference in the per cent infestation of *M. vitrata* was recorded in cowpea pods first and third days after spraying.

On fifth day after treatment, the per cent pod damage was significantly lower in the insecticide treated plots than control which recorded a mean flower damage of 34.05 per cent. The per cent pod damage recorded in indoxacarb + acetamiprid 100 g a.i.ha$^{-1}$ treated plot was the lower (0.52) and was on par with those recorded in spinosad 75 g a.i.ha$^{-1}$ which recorded a mean per cent damage of 1.76 and the plot treated with emamectin benzoate 10 g a.i.ha$^{-1}$ recorded a mean damage of 0.84 per cent.

Table 23. Damage of cowpea pods by *Maruca vitrata* (F) in cowpea plots treated with selected new generation insecticides

| Treatments | Dosage (g a.i.ha$^{-1}$) | Damage in cowpea pods (DAS) (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 3 | 5 | 7 | 10 | 15 |
| Emamectin benzoate | 10 | 20.5 (27.8) | 10.7 (19.27) | 0.84 (5.27) | 0.58 (4.36) | 0 (0) | 5.56 (13.62) |
| Indoxacarb+ Acetamiprid | 100 | 24.27 (29.5) | 19.27 (26.03) | 0.52 (4.14) | 0.74 (4.96) | 11.23 (19.58) | 25.59 (30.37) |
| Spinosad | 75 | 19.09 (25.9) | 11.89 (20.16) | 1.76 (7.62) | 0 (0) | 1.72 (7.53) | 14.94 (22.73) |
| Untreated control | | 23.52 (29.0) | 23.71 (29.12) | 34.05 (35.6) | 36.47 (37.47) | 24.18 (29.4) | 33.4 (35.29) |
| CD(0.05) | | NS | NS | (8.38) | (8.74) | (5.50) | (10.45) |

Figures in parentheses are angular transformed values

DAS- Days after spraying

Plate 6. Damage symptoms of *Maruca vitrata* (F) in cowpea pods

More or less similar trend was observed in plots treated with emamectin benzoate 10 g a.i.ha$^{-1}$ (0.58 %) and indoxacarb + acetamiprid 100 g a.i.ha$^{-1}$ (0.74 %) seven days after spraying. No infestation was recorded in cow pea pods treated with spinosad 75 g a.i.ha$^{-1}$.

The per cent damage in the cowpea pods sprayed with spinosad 75 g a.i.ha$^{-1}$ was 7.53 per cent which was significantly different from pods treated with indoxacarb + acetamiprid 100 g a.i.ha$^{-1}$ (11.23 %) after 10 days of spraying. Whereas no infestation of *M. vitrata* was recorded in cowpea pods sprayed with emamectin benzoate 10 g a.i.ha$^{-1}$.

Significantly lower infestation was recorded in cowpea pods treated with emamectin benzoate 10 g a.i.ha$^{-1}$ (5.56 %) and spinosad 75 g a.i.ha$^{-1}$ (14.94 %) 15 days after spraying. However, the per cent damage in cowpea pods treated with indoxacarb+acetamiprid 100 g a.i.ha$^{-1}$was 25.59.

### 4.5.3 Yield of cowpea in plots treated with different insecticides

Among the treated plots, the highest yield was recorded in the plot treated with emamectin benzoate (16.32 t ha$^{-1}$) and it was significantly higher than other treatments. The yield recorded in cow pea plots treated with spinosad and indoxacarb + acetamiprid varied significantly between them, yield recorded being 14.24 and 11.28 t ha$^{-1}$ respectively. In control plot, yield recorded was only 8.32 t ha$^{-1}$ and is significantly lower than the insecticide treated plots (Table 24).

Table.24. Yield of cowpea pods from field treated with different insecticides

| Treatment | Dosage (g a.i.ha$^{-1}$) | Yield ( t ha$^{-1}$) |
|---|---|---|
| Emamectin benzoate | 10 | 16.32 |
| Indoxacarb+acetamiprid | 100 | 11.28 |
| Spinosad | 75 | 14.24 |
| Control | - | 8.32 |
| CD (0.05) | - | 1.191 |

## 4.6 ESTIMATION OF HARVEST TIME RESIDUES OF INSECTICIDES IN COWPEA PODS

### 4.6.1 Validation of residue method for pesticide residue analysis in cowpea pods

The results of the validation for the estimation of the different insecticides in cowpea pods showed satisfactory recovery for all the compounds fortified. Method validation was accomplished with good linearity and satisfactory recoveries. The mean recovery of all the insecticides under study was within the acceptance range of 70-110 per cent at three levels of fortification. The repeatability of the recovery results as indicated by the relative standard deviations, RSD < 20 per cent, confirmed that the method is sufficiently reliable for pesticide analysis and the results presented in Table 25 to 28.

The mean per cent recovery of emamectin benzoate was 105.73, 78.50 and 83.00 at three different fortification levels *viz.* 0.05, 0.25 and 0.50 ppm respectively with the relative standard deviation in the accepted range of 3.46 to 15.00 per cent. The mean per cent recovery of indoxacarb was 83.13, 103.70 and 93.60 at three different fortification levels *viz.* 0.05, 0.25 and 0.50 ppm respectively with the relative standard deviation in the accepted range of 3.58 to 15.61 per cent. However, in acetamiprid the mean recoveries were 82.00, 97.80 and 82.40 per cent at three different fortification levels *viz.* 0.05, 0.25 and 0.50 ppm respectively with the relative standard deviation in the accepted range of 2.72 to 7.21 per cent. Similarly, the mean per cent recovery of spinosad was 107.30, 93.70 and 99.10 at three different fortification levels *viz.* 0.05, 0.25 and 0.50 ppm respectively with the relative standard deviation in the accepted range of 3.53 to 6.71 per cent.

### 4.6.2 Estimation of harvest time residues of insecticides

The residues of insecticides in cowpea pods harvested 7 and 15$^{th}$ days after spraying is presented in Table 29. The residues of emamectin benzoate,

Table 25. Per cent recovery of emamectin benzoate fortified at different levels using modified QuEChERS method

| Level of fortification (ppm) | Recovery (%) | | | | SD | RSD (%) |
|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | Mean | | |
| 0.05 | 105.60 | 106.80 | 104.80 | 105.73 | 5.30 | 5.01 |
| 0.25 | 81.60 | 77.60 | 76.40 | 78.50 | 2.72 | 3.46 |
| 0.5 | 72.60 | 79.60 | 96.80 | 83.00 | 12.45 | 15.00 |

Table 26. Per cent recovery of indoxacarb fortified at different levels using modified QuEChERS method

| Level of fortification (ppm) | Recovery (%) | | | | SD | RSD (%) |
|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | Mean | | |
| 0.05 | 85.00 | 85.40 | 79.00 | 83.13 | 3.58 | 4.31 |
| 0.25 | 93.60 | 102.40 | 115.20 | 103.70 | 10.86 | 10.40 |
| 0.5 | 98.20 | 106.40 | 76.20 | 93.60 | 15.61 | 1.66 |

SD – Standard deviation

RSD- Relative standard deviation

Table 27. Per cent recovery of acetamiprid fortified at different levels using modified QuEChERS method

| Level of fortification (ppm) | Recovery (%) | | | | SD | RSD (%) |
|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | Mean | | |
| 0.05 | 90.00 | 76.00 | 80.00 | 82.00 | 7.21 | 8.79 |
| 0.25 | 94.80 | 98.80 | 100.00 | 97.80 | 2.72 | 2.78 |
| 0.5 | 86.80 | 80.40 | 80.00 | 82.40 | 3.81 | 4.62 |

Table 28. Per cent recovery of spinosad fortified at different levels using modified QuEChERS method

| Level of Fortification (ppm) | Recovery (%) | | | | SD | RSD (%) |
|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | Mean | | |
| 0.05 | 102.80 | 104.60 | 114.60 | 107.30 | 6.35 | 6.71 |
| 0.25 | 98.80 | 92.60 | 89.90 | 93.70 | 4.56 | 4.86 |
| 0.5 | 102.60 | 99.30 | 95.60 | 99.10 | 3.50 | 3.53 |

SD – Standard deviation

RSD- Relative standard deviation

Table 29. Residues of new generation insecticides in cowpea pods at harvest (mg kg$^{-1}$)

| Days after spraying | Residues of insecticides (mg kg$^{-1}$) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Emamectin benzoate | | | | Indoxacarb + acetamiprid | | | | Spinosad | | | | Control | | | |
| | R1 | R2 | R3 | Mean | R1 | R2 | R3 | Mean | R1 | R2 | R3 | Mean | R1 | R2 | R3 | Mean |
| 7$^{th}$ day | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL |
| 15$^{th}$ day | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL |

LOQ- 0.05 mg kg$^{-1}$

BDL- Below Detectable Limit

indoxacarb + acetamiprid and spinosad were found to be below the quantitative limit of 0.05 mg $kg^{-1}$ on both the days of estimation *viz*. $7^{th}$ and 15 days after treatment.

# *Discussion*

# 5. DISCUSSION

The spotted pod borer, *M. vitrata* is one of the serious pests of legumes in the tropics and sub-tropics because of its wide host range, destructiveness and distribution. Farmers rely mostly on chemical insecticides for their timely control there by mitigating the possible losses which indirectly results in the development of insecticide resistance in the field population. The information gathered from the present study on the extent of insecticide resistance in the population of *M. vitrata* and the efficacy of new generation insecticides to manage this resistant population are discussed under the following heads.

## 5.1 PRELIMINARY SURVEY TO GATHER INFORMATION REGARDING THE PESTICIDE USE AND DEVELOPMENT OF INSECTICIDE RESISTANCE

Spotted pod borer, *M. vitrata* causes economic damage both at the vegetative and reproductive stages of the crop thereby resulting in considerable yield loss in cowpea. Farmers usually adopt frequent application of insecticides to combat the menace. A survey was conducted to collect data from cowpea farmers at Kalliyoor and Venganoor panchayats of Thiruvananthapuram district to correlate the pesticide use pattern, incidence of major pests, effectiveness of plant protection chemicals and awareness of the farmers on insecticide resistance.

The results of the survey revealed that 50 per cent of the farmers applied pesticides by their own decisions without any consulting with technical hands on their label claim. This could be one of the reasons for the wrong selection of insecticides and their erroneous dose either overdoses or underdoses leading to rapid development of resistance in *M. vitrata*. Most striking information gathered is that 70 per cent of farmers surveyed were not adopting integrated strategies to manage the pest. The indiscriminate use of synthetic pesticides may result in reduction of pest biodiversity, outbreak of secondary pests, development of insecticide resistance and pesticide induced resurgence and contamination of food

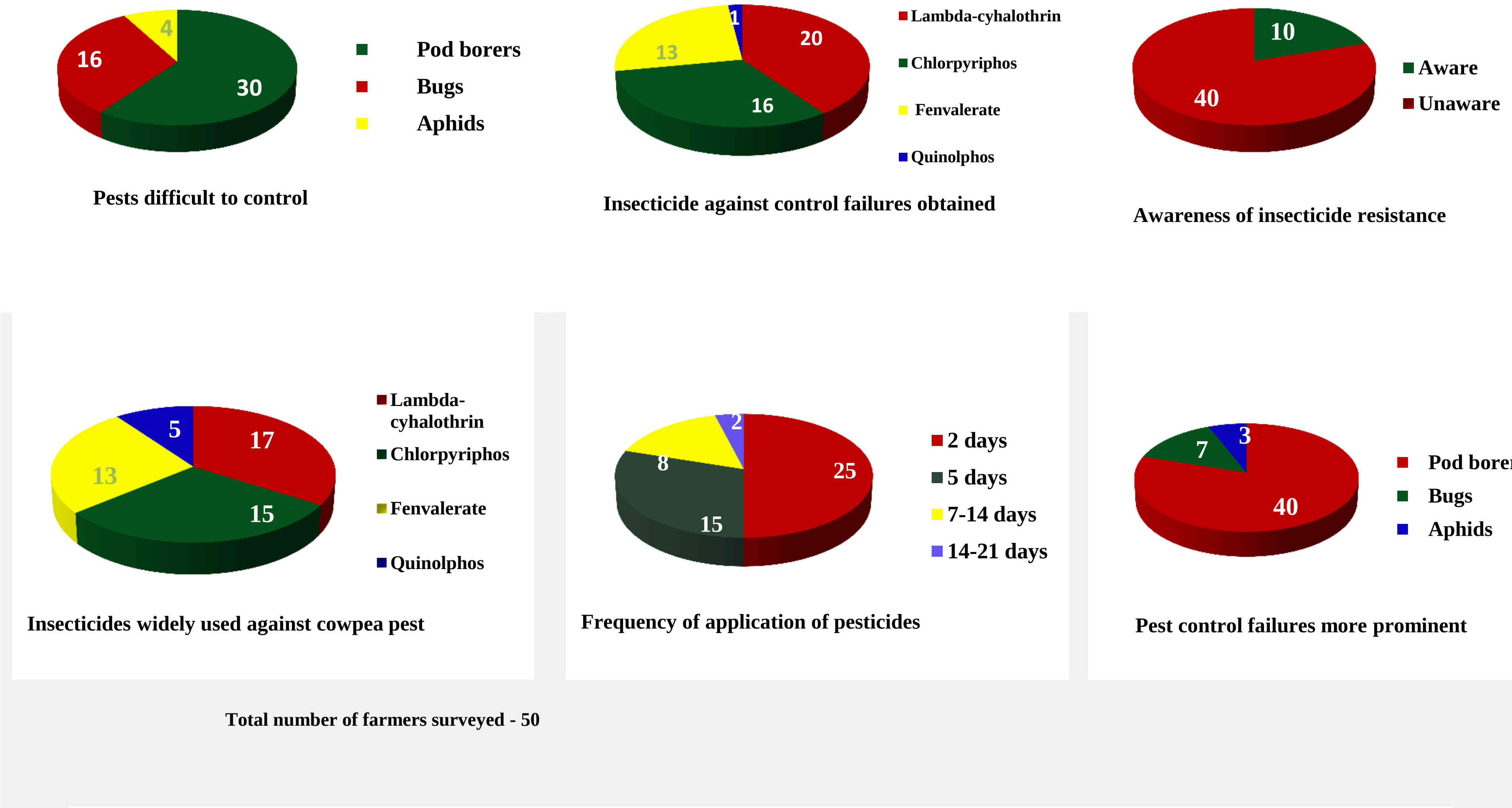



Fig. 1. Response of farmers on details of pests, effectiveness of plant protection chemicals and awareness of insecticide resistance

and the ecosystem (Singh, 2000). The response of farmers on details of pests, effectiveness of plant protection chemicals and awareness of insecticide resistance is presented in Fig.1. Sixty per cent of farmers opined that among the pests infesting cowpea, pod borers are the most difficult to control. However, 31-41 per cent respondents consider that the occurrence of control failure in pod borer of cowpea is due to the continuous application of lambda-cyhalothrin and chlorpyriphos. The frequent occurrence of residues of chlorpyriphos and lambda-cyhalothrin in cowpea sampled from local markets in Kerala [AINP (PR), 2012] is suggestive of their contention. One of the important consequences of the blanket use of insecticides is the development of resistance in the target species. There are reports on development of several fold resistance against synthetic pyrethroids especially lambda-cyhalothrin due to its chemical nature and existence as single isomer (Botwe *et al*., 2012). The development of resistance in insects against insecticides is a bio-chemical mechanism by which they can detoxify the pesticides. If the chemical nature of the pesticide molecule is comprised of a single isomer, the insect system might adjust its biochemical mechanism in such a way that a single enzyme is sufficient to convert the active pesticide molecule to a non-toxic compound.

The results of the survey revealed the development of resistance in the field population of *M. vitrata*. Though instances of insecticide resistance in *M. vitrata* have been reported elsewhere, no study on insecticide resistance in *M. vitrata* was carried out in Kerala. Based on the information collected from the survey, further studies were conducted to manage the problem of resistance development on this devastating pest of cowpea.

## 5.2 LABORATORY REARING OF SPOTTED POD BORER, *M. VITRATA* IN DIFFERENT DIETS

In order to ensure a continuous and adequate supply of larvae for the study, an effort was made to establish a viable rearing technique. Initially, the artificial diet (Diet-1) was prepared based on the procedure described by

Ochieng and Bungu, 1983 (Table 6). Further, two more diets (Diet 2 and Diet 3) were standardised by slightly modifying the procedure and composition (Table 7 and 8).

The effectiveness of artificial diets was assayed in terms of shelf life and palatability. In the present study, shelf life (120 days) and palatability of diet- 2 was higher compared to that of diet 1 and 3. Rearing of larvae of *M. vitrata* in artificial diet was successful and the number of days taken to complete the total life cycle was significantly lesser in the population reared in artificial diet than in natural diet. Though long shelf life and palatability were observed in the artificial diet, subsequent generations could not be maintained in the artificial diet presumably due to the difficulty in mating and egg laying. Similar cases were also observed by Ochieng and Bungu (1983) in rearing *M. vitrata* using an artificial diet based on chickpea. They found a decline in the survival of larval population and failed to obtain adequate numbers of mated pairs of *M. vitrata* after a number of successive generations. Similar cases were also observed by Jackai and Raulston (1988) when they reared *M. vitrata* using soybean based diet. They were also unable to get adequate mating pairs. The present study utilized the procedure given by Ochieng and Bungu (1983), the diet failed to produce the adults with good reproductive potential. This indicated necessity to continue further research on standardization of an effective and viable technique for the mass rearing of *M. vitrata* for continuous supply of larvae for the study of insecticide resistance.

## 5.3 ASSESSMENT OF INSECTICIDE RESISTANCE IN THE FIELD POPULATIONS OF *M. VITRATA*

Resistance is a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species (IRAC, 2014). Insecticide resistance in agricultural systems has been recognised as one of the world's top environmental problems for nearly two decades and it is occurring in over 574 species of insects (APRD, 2012). Insecticide resistance action committee explains that resistance arises through the over-use or misuse of an insecticide/ acaricide against the pest species and results from the selection of resistant forms of the pest and the consequent evolution of populations that are resistant to that insecticide. The data from the survey conducted among cowpea growers served as the benchmark for further studies on the insecticide resistance in *M. vitrata*.

The results presented in Table.18 and 19 revealed that the offspring's from the populations collected from location I (Venganoor) had the lowest $LC_{50}$ value. Low to moderate level of resistance to chlorpyriphos was observed in population collected from location II (College of Agriculture, Vellayani) and location III (Kalliyoor). Resistance ratio ranged from 2.28-2.66 fold in location-II and 2.53-3.76 fold in location-III. Resistance to lambda-cyhalothrin was moderate to high. Resistance ratio in location-II ranged from 2.38 - 4.52 folds, while, in location-III it ranged from 3.94-7.94 fold.

A maiden study conducted at Nigeria on insecticide resistance in *M. vitrata* revealed that the legume pod borer collected from two locations of Nigeria was found to be resistant to cypermethrin, dimethoate and endosulfan. When compared with a susceptible strain, resistance ratios ranged from 17-53 fold for cypermethrin, 27-92 fold for dimethoate and 15-37 fold for cypermethrin, 15-37 fold for endosulfan (Ekesi, 1999). Literature regarding the insecticide resistance in *E. vitella*, *H. armigera*, *S. litura*, and *P. xylostella* showed development of very high levels of insecticide resistance *viz.* 2802.60 (Saini and Ramkuma, 1987), 23 - 8022 (Kranthi *et al.*, 2002), 14 - 229 (Hong Tong *et al*., 2013) and 18 - 1150 (Wang *et al.*, 2013). However, the present study revealed that field population of *M. vitrata* collected from Kalliyoor showed 2.53 – 3.76 fold and 3.94 – 7.94 fold against chlorpyriphos and lambda-cyhalothrin, respectively and indicated the development of resistance. In general, synthetic pyrethroid molecules constituted of single isomer may induce production of detoxifying enzyme and thereby develop resistance rapidly. In the case of organophosphates and carbamates which do not exist in the form of stereo isomer, the insect has to device several mechanisms which need a number of enzyme systems to cause detoxification of the pesticide. Hence, the development of resistance to organophosphates and carbamates are relatively slow and not as easy as in the case of synthetic pyrethroids.

Genetics and indiscriminate application of insecticides are the two factors responsible for the development of insecticide resistance. Development of resistance is mainly a consequence of past and present use of chemicals and comparisons of resistance levels in different locations is an important tool for decision making in insect pest management programmes (Ekesi, 1999). *M. vitrata* has a short life cycle and high reproductive potential as a result they are frequently exposed to multiple applications of several insecticides used to control them resulting in a high selection pressure (Ekesi *et al*., 1997). In location-II (Instructional farm, Vellayani) and III (Kalliyoor), chlorpyriphos and lambda-cyhalothrin were the most commonly used insecticides for the management of cowpea pests. The development of resistance to lambda-cyhalothrin was higher than in chlorpyriphos. Georghiou and Mellon (1983) reported that pest populations that were already resistant to one or more classes of pesticides generally developed resistance more rapidly to new groups of chemicals having same mode of action or same metabolic pathways of detoxification. It has also been reported that genes conferring resistance to chlorpyriphos not only conferred cross-resistance to other organophosphates but also to other carbamates (Ekesi, 1999). Resistance developed by insects against these insecticides especially organophosphates and synthetic pyrethroids has become a serious problem in insect pest control programs in recent years. The major mechanisms of insecticide resistance in insects involve the changes either in insecticide target site or in insecticide detoxifying enzymes.

Though several research works have been conducted on insecticide resistance against pests in different parts of the world, studies on insecticide resistance in *M. vitrata* in particular are scanty except for the work conducted by

Ekesi (1999) in Nigeria. Thus, the present study may be a maiden attempt in assessing the extent of insecticide resistance of *M. vitrata* in India. Further studies have to be taken up to delay the development of resistance through various effective management programmes.

## 5.4 EVALUATION OF EFFICACY OF NEW GENERATION INSECTICIDES AGAINST THE RESISTANT POPULATION OF *M. VITRATA*

The legume pod borer *M. vitrata,* one of the most devastating post flowering pests and is basically a hidden pest, completing its larval development inside the flowers, buds and pods. It is very difficult to manage them as they enter the flowers, buds, pods and the entrance hole is plugged with excreta. Number of insecticides has been tested and few of them were found effective against pod borer of cowpea (Soliman, 2011; Ajeigbe *et al.,* 2012; Dey *et al.,* 2012). However, the research works on the assessment of efficacy of insecticides against the resistant population of *M. vitrata* is meagre.

Laboratory experiments conducted to evaluate the efficacy of new generation insecticides *viz.* Novaluron- 0.015 per cent (inhibitors of chitin biosynthesis), flubendiamide- 0.01 per cent (ryanodine receptor modulators), spinosad- 0.015 per cent (nicotinic acetylcholine receptor allosteric activators), emamectin benzoate- 0.002 per cent (chloride channel activators), indoxacarb 14.5 per cent (voltage-dependent sodium channel blockers) + acetamiprid 7.7 per cent - 0.09 per cent (nicotinic acetylcholine receptor agonists), acephate 50 per cent (acetylcholin esterase inhibitors) + imidacloprid 1.8 per cent -0.20 per cent (nicotinic acetylcholine receptor agonists), chlorantraniliprole-0.03 per cent (ryanodine receptor modulators), indoxacarb- 0.10 per cent (voltage-dependent sodium channel blockers) and chlorpyriphos - 0.05 per cent (as check) against the resistant population of *M. vitrata.* The result showed that the per cent mortality of the second instar larvae treated with insecticides *viz.* emamectin benzoate, indoxacarb + acetamiprid and spinosad were 97.28, 97.28 and 90.90 per cent respectively after 6 hours of treatment. Hence, these insecticides were selected for further field trials against the resistant population of *M. vitrata.*

Unlike the conventional ones, most of the new molecules have excellent toxicological and eco-toxicological profiles and are widely acclaimed as potent compounds for the management of cowpea pests. The Central Insecticide Board & Registration Committee scrutinizes and periodically reviews all pesticides and their usage. In Kerala, in the light of the health hazards reported from Kasaragod consequent to the use endosulfan, Government of Kerala has restricted the sale and use of all red and few yellow labelled pesticides on 7$^{th}$ May 2011 and vide Go (Rt) 99/2011, on 12-1-2011. An expert committee was constituted to suggest suitable alternatives for managing the pest problems in the state. This warranted development of alternative pesticides to substitute the restricted chemicals and quite a substantial amount of work has been done to tackle the problem of *M. vitrata* in recent years. Vijayasree (2013), evaluated the effectiveness of new generation insecticides against *M. vitrata* and found that the anthranilic diamide insecticide, chlorantraniliprole, the oxadiazine insecticide, indoxacarb and the avermectin group insecticide, emamectin benzoate were effective in causing mortality to the tune of 72.22 to 92.96 per cent. In the present study, the avermectin group insecticide, emamectin benzoate (0.002%), the nicotinic acetylcholine receptor allosteric activator, spinosad (0.015%) and an oxadiazine + neonicotinoid mixture (indoxacarb + acetamiprid (0.09%) resulted in 80 to 93.33 per cent mortality to the second instar larvae of the resistant population of *M. vitrata* under the laboratory conditions.

## 5.5 FIELD EVALUATION OF SELECTED NEW GENERATION INSECTICIDES AGAINST THE RESISTANT POPULATION OF *M. VITRATA*

A field experiment was laid out to evaluate the efficacy of the best three treatments selected from the laboratory study *viz.* emamectin benzoate 0.002 per cent, indoxacarb+acetamiprid 0.09 per cent and spinosad 0.015 per cent along with control against the resistant population of *M.vitrata*. The results indicated that all the insecticidal treatments were equally effective in controlling the pest when compared to untreated control. The treatments *viz.* emamectin benzoate 0.002 per cent, indoxacarb + acetamiprid 0.09 per cent and spinosad 0.015 per cent showed a reduction of 81-100, 64-100 and 69.96 - 99.6 per cent in the case of flower infestation (Fig. 2) and 83 -100, 23.38 - 98.40 and 55.26 – 100 per cent respectively in case of pod infestation (Fig. 3). An increase in damage in cowpea flowers and pods due to the infestation of *M.vitrata* was observed 10 and 15$^{th}$ days after spraying (Table 22 and 23). This demands a second spray of chemicals at fortnightly interval to reduce the infestation below economic threshold level. The yield of cowpea was increased to the tune of 96.15, 35.50 and 71.10 per cent over control in the treatments *viz.* emamectin benzoate, indoxacarb+acetamiprid and spinosad respectively (Fig.4). Vijayasree (2013) evaluated the effectiveness of new generation insecticides against field population of *M. vitrata* and found that the anthranilic diamide insecticide, chlorantraniliprole, the oxadiazine insecticide, indoxacarb and the avermectin group insecticide, emamectin benzoate were effective in reducing flower and fruit damage to the tune of 70 to 80.00 per cent. However, in the present study the anthranilic diamide insecticide, chlorantraniliprole was significantly inferior to emamectin benzoate, indoxacarb + acetamiprid mixture and spinosad in the preliminary laboratory screening and hence not selected for the further field evaluation. The present findings on the effectiveness of spinosad in reducing the flower and pod damage by *M. vitrata* is in agreement with the findings of Bhoyar *et al.* (2004), Ladaji (2004) and Sonune *et al.* (2010) who reported spinosad to be effective in containing the infestation of *M. vitrata*. Similarly the effectiveness of indoxacarb in reducing the flower and pod damage of *M. vitrata* was reported by Ladaji, 2004 and Patel *et al.* (2012).

Harvest time residue estimation done on 7$^{th}$ and 15$^{th}$ day after the application of insecticides showed the residues below the quantitative limit of 0.05 mg kg$^{-1}$ revealing its safety for consumption.

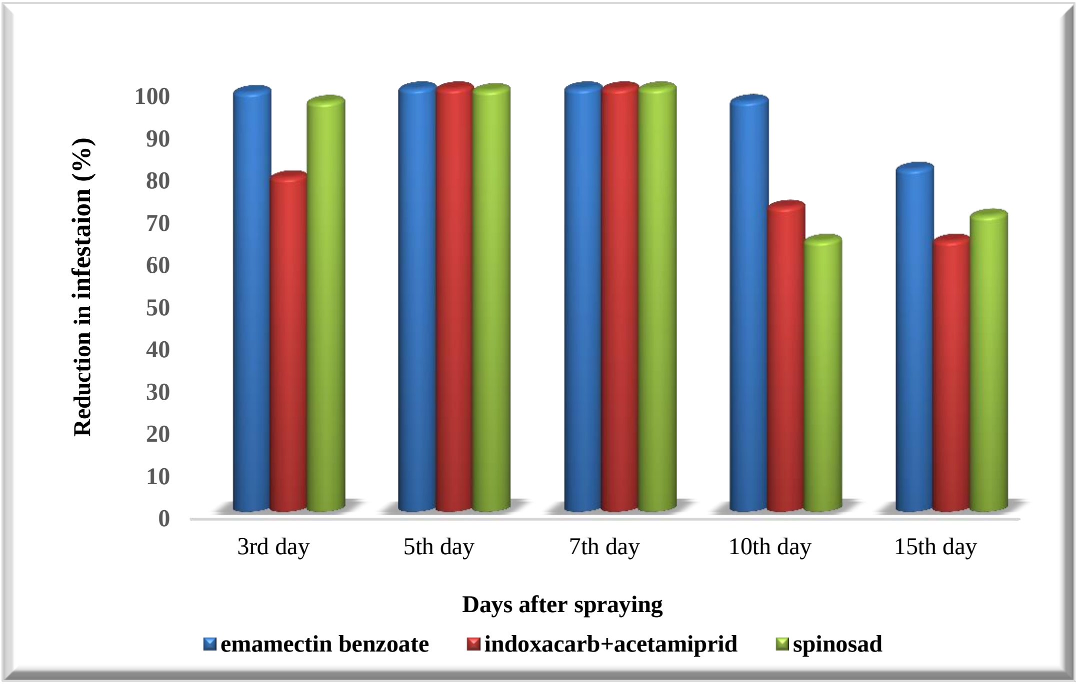



Fig 2. Reduction in infestation of cowpea flowers by *Maruca vitrata* (F)

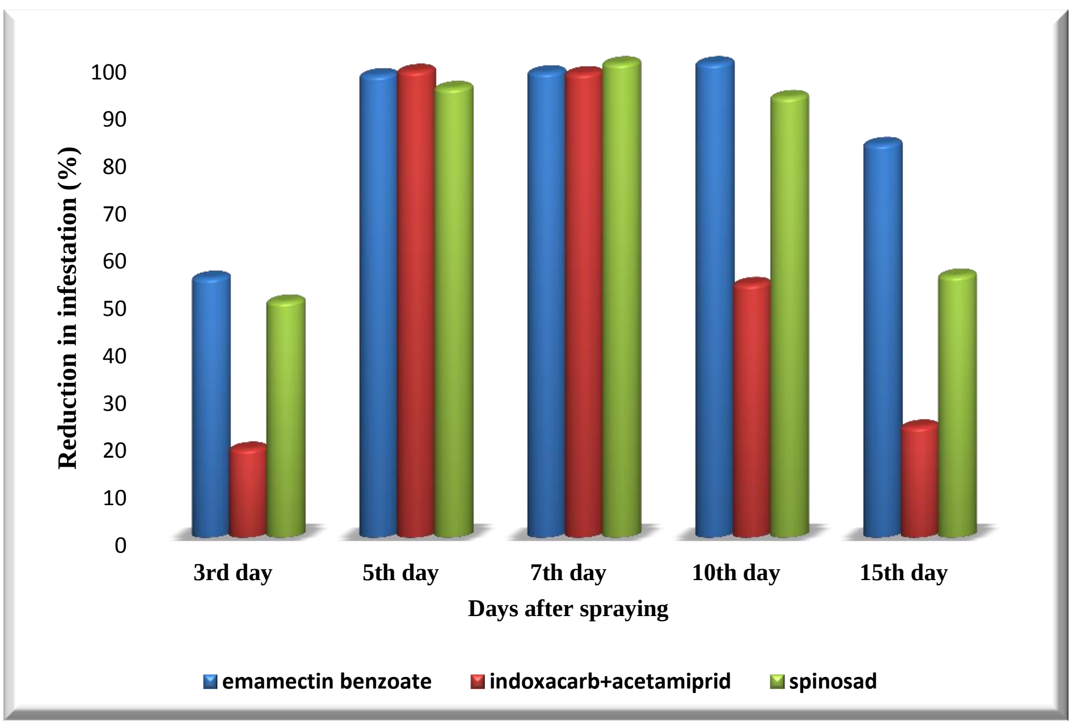



Fig. 3. Reduction in infestation of cowpea pods by *Maruca vitrata* (F)

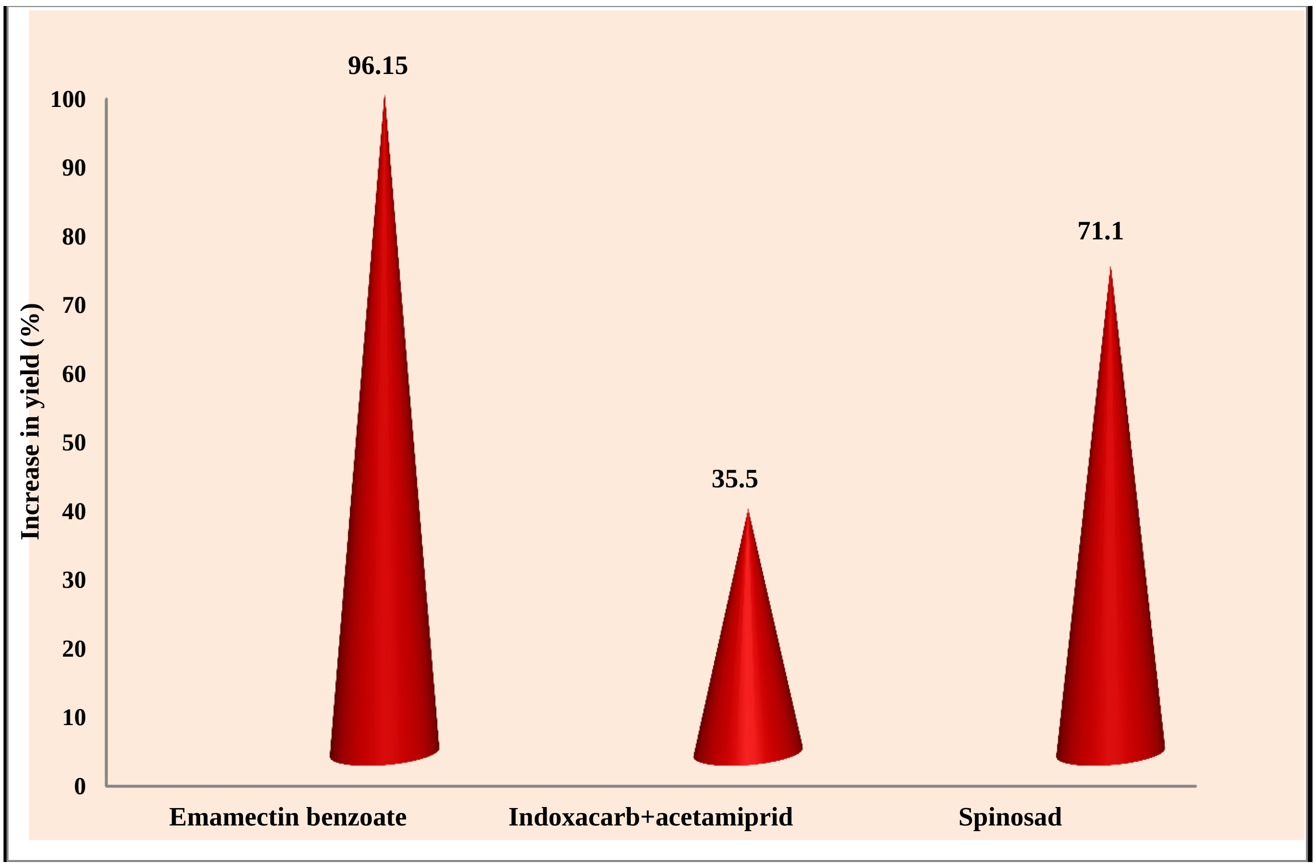



Fig. 4. Increase in yield over control in the experimental plots

The present study could indicate the development of insecticide resistance in the field population of *M.vitrata*. The field study revealed the efficacy of application of emamectin benzoate @ 10 g ai $ha^{-1}$, Indoxacarb + acetamiprid @ 100 g a.i.$ha^{-1}$ and spinosad @ 75 g ai $ha^{-1}$ to manage the resistant population of *M. vitrata* in cowpea. This study forms a maiden attempt in assessing the extent of development of insecticide resistance in the populations *M. vitrata* in Kerala.

Pod damage to cowpea is a matter of concern for the farmers of the State and the existing management strategies are inadequate to meet the requirements. Insecticide resistance in the field population of pod borers may be one of the contributing factors for the control failures. Strategies should be formulated incorporating integrated-pest-management (IPM) techniques and by avoiding repeated use of insecticides from the same chemical group and alternating with compounds from different mode of action (IRAC). Use of insecticides with more than one isomeric form and addition of synergists in the case of synthetic pyrethroids may also to be practised to delay the development of resistance. Along with use of insecticides, non-chemical approaches including the use of bio rational insecticides, resistant varieties, manipulation of planting time, application techniques and use of biological control agents must be followed to curb the development of insecticide resistance.

*Summary*

# 6. SUMMARY

Cowpea is one of the important pulse crops grown over a wide range of environmental conditions throughout the world. Spotted pod borer, *M. vitrata* is the most formidable and potential pests causing substantial damage to the crop. Wide use of pesticides in cowpea against pod borers resulted in the development of insecticide resistance in *M. vitrata.* The present study was undertaken to conduct a preliminary survey among cowpea growers for gathering the information regarding the pesticide use and the development of insecticide resistance, to assess the insecticide resistance in the field populations of *M.vitrata* and to evaluate the efficacy of new generation insecticides *viz*., novoluron 10 EC @ 100 g a.i.ha$^{-1}$, flubendiamide 480 SC @ 100 g a.i.ha$^{-1}$, spinosad 45 SC @ 75 g a.i.ha$^{-1}$, emamectin benzoate 5 SG @ 10 g a.i.ha$^{-1}$, chlorantraniliprole 18.5 SC @ 30 g a.i.ha$^{-1}$, indoxacarb 14.5 SC @ 60 g a.i.ha$^{-1}$, indoxacarb 14.5 per cent + acetamiprid 7.7per cent SC @ 100 g a.i.ha$^{-1}$, acephate 50 per cent + imidacloprid 1.8 per cent SP @ 518 g a.i.ha$^{-1}$ in comparison with one conventional insecticide ( chlorpyriphos 20 EC) against the resistant population of *M. vitrata.* Effective three insecticides were selected for field study. To ensure the safety of cowpea pods for consumption, harvest time residue analysis was done on pods. The results are summarized here under.

- A preliminary survey conducted among cowpea growers in Kalliyoor and Venganoor panchayats of Thiruvananthapuram district revealed control failures in the field population of spotted pod borer due to the continuous application of a single insecticide like chlorpyriphos or lambda cyhalothrin. Survey revealed that 50 per cent of farmers applied pesticides by their own decisions without any consultation with technical hands.
- The survey also revealed the control failures against the pod borers because of continuous application of insecticides like lambda-

cyhalothrin (41 %) and chlorpyriphos (31 %). However, the awareness regarding the insecticide resistance among the farmers is deprived.

- Reduction of life-cycle of *M. vitrata* to 24.14 days was observed in case of larvae reared on artificial diet when compared to cowpea pod (29.16 days) and lab-lab bean (28.06 days) which were significantly different with each other.
- Bioassay was carried out in CRD to assess insecticide resistance in populations of *M. vitrata* collected from three different locations (location I- field with no previous history of pesticide application, location II- field where less control failures and location III- field where more control failures) using two chemicals *viz.* chlorpyriphos and lambda-cyhalothrin at different doses. Results revealed that population collected from location-I was found to be susceptible for both the chemicals with resistance ratio-1, population collected from location-II to be moderately resistant with a resistant ratio of 2.28 and 2.38 and population from location-III to be resistant with resistance ratios of 2.93 and 7.94 for chlorpyriphos and lambda-cyhalothrin respectively.
- Laboratory experiments conducted to evaluate the efficacy of new generation insecticides against the resistant population of *M. vitrata* revealed that emamectin benzoate 0.002 per cent, indoxacarb + acetamiprid 0.09 per cent and spinosad 0.015 per cent found to be superior among all the tested new generation insecticides with per cent mortality of 97.28, 97.28 and 90.90 respectively
- The field experiment conducted with four treatments *viz.* emamectin benzoate 0.002 per cent, indoxacarb + acetamiprid 0.09 per cent and spinosad 0.015 per cent including control against the resistant population of *M. vitrata* resulted in a reduction of 81-100, 64-100 and 69.96 - 99.6 per cent respectively in the case of flower infestation and 83 -100, 23.38 - 98.40 and 55.26 – 100 per cent respectively in case of pod infestation.

- Satisfactory results were obtained while validating the QuEChERS method for the pesticide residue analysis of cowpea with good recovery ranged from 78.50 to 107.30 per cent.
- Harvest time residue estimation done on 7$^{th}$ and 15$^{th}$ days after the application of insecticides showed the residues below the quantitative limit of 0.05 mg $kg^{-1}$ revealing its safety for consumption.

# *References*

# 7. REFERENCES

Abbot, W.S. 1925. A method of computing the effectiveness of an insecticide. *J. Econo. Entomol*. 18: 265-267.

AINP (PR) [All India Network Project on Pesticide Residues]. 2012. *Annual Report 2011-12*. College of Agriculture, Vellayani, 65p.

Ajeigbe, H.A., Adamu, R.S. and Singh, B. 2012. Yield performance of cowpea as influenced by insecticide types and their combinations in the dry savannahs of Nigeria. *Afr. J. Agric. Res*. 7 (44):5930-5938.

Akinfenwa, S. 1975. Biological study of *Maruca testulalis* (Geyer) in the Zaria area of Northern Nigeria. M.Sc. thesis, Ahmadu Bello University, Zaria, Nigeria. 105p

Anabalagan, G. 2001. Studies on insecticide resistance in rice leaf folder complex in Tamil Nadu. Ph.D. (Ag) thesis, Tamil Nadu Agricultural University, Coimbatore. pp. 175.

AOAC (Association of Analytical Chemists) 2012. *Official methods of analysis of AOAC international*. (19th Ed.) Pesticide and Industrial Chemical Residues.

APRD [Arthropod pesticide resistance database]. 2012. [On-line]. Available: http://www.pesticideresistance.com [16 June 2014].

Arulmozhi, K. 1990. Bio-ecology of *Maruca testulalis* (Geyer) and its management. M.Sc. (Ag) thesis, Tamil Nadu Agricultural University, Coimbatore. 117p.

Ashok kumar, C.T. and Shivaraju, C. 2009. Bioefficacy of newer insecticide molecules against tomato fruit borer, *Helicoverpa armigera* (Hubner). *Karnataka J. Agric. Sci*. 22(3): 588-590.

Attique, M.N., Khaliq, A., and Sayyed, A.H. 2006. Could resistance to insecticides in *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) can be overcome by insecticide mixtures? *J. Econ. Entomol.* 130 (2): 122-127.

Barroga, S.F. 1969. Control of lepidopterous pests attacking bush bean, *Phaseolus vulgaris* L. blossoms and pods. *Philippines J. Plant. Int.* 34: 159-162.

Bartek, T., Christoph, T., Zimmer, J.E., and Corinn, S. 2012. Resistance to diamide insecticides in diamondback moth. *P. xylostella* (Lepidoptera: Plutellidae) is associated with a mutation in the membrane- spanning domain of the ryanodine receptor. *Insect Biochem. Mol. Biol.* 42: 873-880.

Betty Varghese, 2003. Evaluation of newer insecticides against major pests of cowpea, *Vigna unguiculata* (L.) walp. and their effect on natural enemies. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 175p.

Bhalani, P.A. and Prasana, G.J. 1987. Relative toxicity of certain newer insecticides to *Maruca testulalis* (Geyer) attacking pigeon pea. *Pesticides.* 21(4): 23-25.

Bhoyar, A.S., Siddha bhatti, P. M., Wadaskar, R. M. and Khan, M. I. 2004. Seasonal incidence and control of pod borer complex in pigeon pea. *Pestology.* 28(9): 99-104.

Booker, R.H. 1965. Pests of cowpea and their control in northern Nigeria. *Bull. Ent. Res.* 55: 663-672.

Botwe, K.P., Vincent. E.Y. and Ebenezer, O.O. 2012. Susceptibility of *Plutella xylostella* (Lepidoptera: Plutellidae) to emamectin benzoate and lambda-cyhalothrin in the greater Accra region of Ghana. *Int. J. Agri. Res.* 1(1): 10-15

Bouvier, J.H., Bues, R., Boivin, T., Boudinhon, L., Beslay, D., and Sauphanor, B. 2001. Deltamethrin resistance in the codling moth

(Lepidoptera: Tortricidae): inheritance and number of genes involved. *Wiley online library*. 87:456-462. Available: http://onlinelibrary.wiley.com/doi/10.1046/j.13652540.2001.00928.x/abstract# [10 October 2001].

Cheema, H.P. 2009. Evaluation of insecticide resistance profile in *Spodoptera litura* (Fab.) population through biological, biochemical and molecular diagnosis. Ph.D. thesis, Punjab Agricultural University, Ludhiana, Punjab. 172p.

Cook, D., Mangano, P., Berlandier, F., Hardie, D., and Cousins. D. 2000. Managing diamondback moth in canola. Agriculture Western Australia, Farm note No. 140/2000

Das, G.P. and Islam, M.A. 1985. *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae), an important pest of country bean in Bangladesh. *Bangladesh J. Agric.* 10: 57- 66.

Dey, P.K., Chakraborty, G., and Somchoudary, A.K. 2012. Evaluation of flubendiamide 480 SC against lepidopteron pod borers of pigeon pea [*Cajanus cajan* (L) Millsp.]. *J. Inter acad*. 16(4): 857-862.

Dharmasena, C.M.D., Subasinghe, S.M.C., Lateef, S.S., Menike, S., Saxena, K.B., and Ariyaratne, H.P. 1992. In: *Pigeon pea - Varietal Adaptation and Production studies in Sri Lanka. Report of work*. *Entomology Research*. Department of Agriculture, Sri Lanka, ICRISAT, Patancheru, Andhra Pradesh, India. pp 104-108.

Dietz, P.A. 1914. Het Katjand VI indertje (het verrmeende Toa-Toh-Motje). Meded. *Deli-Proefstu Medan*. 8: 273-278.

Djamin, A. 1961. The biology of *Maruca testulalis* (Geyer), with a consideration of its control. B.Sc. thesis, University of the Philippines, Quezon City, Philippines. pp. 98

Dunley, J.E. and Welter, S.C. 2000. Correlated Insecticide Cross-Resistance in Azinphosmethyl Resistant Codling Moth (Lepidoptera: Tortricidae) *J. Econ. Entomol.* 93(3): 955-962.

Durairaj, C. and Ganapathy, N. 1998. Bioeffcacy of some newer insecticides and botanicals against pod infesting insects in pigeon pea. *Pestology*. 22:12-14.

Ekesi, S. 1999. Insecticide resistance in field populations of the legume pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Pyralidae) on cowpea, *Vigna unguiculata* (L), Walp in Nigeria. *Int. J. Trop. Agric*. 45 (1): 57-59.

Ekesi, S., Ogunlana, M.O., and Dike, M.C. 1997 (In press). Life-fertility tables for *Maruca testulalis* (Geyer) on cowpea. *Nigerian J. Entomol.* Original not seen.

Elzen, G.W., Leonard, B.R., Graves, J.B., Burris, E., and Micinski, S. 1992. Resistance to pyrethroids, carbamates and organophosphate insecticides in field populations of tobacco budworm (Lepidoptera: Noctuidae) in 1990. *J. Econ. Entomol*. 85: 2064-2972.

Feleke, Y.R.S., Pasquet, F., and Gepts, P. 2006. Development of PCR based chloroplast DNA markers that characterize domesticated cowpea (*Vigna unguiculata* subsp. *unguiculata* var. *unguiculata*) and highlight its crop-weed complex. *Plant Syst. Evol*. 263, 75-87.

Feng, H., Wu, K., Ni, Y., Cheng, D., and Guo. Y. 2005. Return migration of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) during autumn in northern China, *Bull. Entomol. Res.* 95: 361-370.

Fousseni, T., Clementine, L., Dabire-Binsoa, Niango, M., B., Antoine, S., and Barry, R.P. 2013. Feeding preferences of the legume pod borer *Maruca vitrata* (Lepidoptera: Crambidae) larvae and suitability of different flower parts for larval development. *Int. J. Trop. Insect. Sci.* 33(2): 107-113.

Ganapathy, N. 1996. Bio ecology and management of spotted pod borer, *Maruca testulalis* (Geyer) (Pyralidae: Lepidoptera) in pigeon pea. Ph.D. thesis, Tamil Nadu Agricultural University, Coimbatore, India.171p.

Georghiou, G.P. and Mellon, R.B. 1983. Pesticide resistance in time and space. In: Georghiou, G.P. and Saito, T. (eds) *Pest Resistance to Pesticides.* New York: Plenum, pp. 1-46.

Gopali, J.B., Raju, T., Mannur, D.M. and Suhas, Y. 2010. Web-forming lepidopteran, *Maruca vitrata* (Geyer), an emerging and destructive pest in pigeon pea. *Karnataka J. Agric. Sci.* 23: 35-38.

Halder, J. and Srinivasan, S. 2011. Varietal screening and role of morphological factors on distribution and abundance on spotted pod borer, *Maruca vitrata* (Fabricius) on cowpea. *Ann. Pl. Protec. Sci*. 19: 71-74.

Hama, H. 1990. Insecticide resistance of diamondback moth, *Plutella xylostella*. (L.) *Japan Agric. Res. Q.* 24: 22-30.

Hong Tong, Qi, S., Xiaomao, Z., and Lianyang, B. 2013. Field resistance of *Spodoptera litura* (F) (Lepidoptera: Noctuidae) to organophosphates, pyrethroids, carbamates and four newer chemistry insecticides in Hunan, China. *J. Pest. Sci*. 86: 599-609.

IRAC [Insecticide Resistance Action Committee] 2014. IRAC home page [on-line]. Available: http://www.irac-online.org [12 May 2014].

Ishitaq, M., Saleem, M.A., and Razaq, M. 2012. Monitoring of resistance in *Spodoptera exigua* (Lepidoptera: Noctuidae) from four districts of

Southern Punjab, Pakistan to four conventional and six new chemistry insecticides. *Crop Prot.* 33: 13-20.

Jackai, L.E.N. 1981. Relationship between cowpea crop phenology and field infestation by legume pod borer, *Maruca testulalis* (Geyer). *Ann. Ent. Soc. Am.* 74: 402-408.

Jackai, L.E.N. and Raulston, J.R. 1988. Rearing the legume pod borer, *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae) on artificial diet. *Trop. Pest Manag.* 34: 168-172.

Jackai, L.E.N. and Singh, S.R. 1983. Suitability of selected leguminous plants for development of *Maruca testulalis* (Geyer) larvae. *Ent. Exp. Appl.* 34: 174-178.

Karel, A.K. 1985. Yield losses from and control of bean pod borers, *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae) and *Heliothis armigera* (L) (Lepidoptera: Noctuidae). *J. Econ. Entomol*. 78: 1323-1326.

Khakame, S.K., Wang, X., and Wu, Y. 2013. Baseline toxicity of metaflumizone and lack of cross resistance between indoxacarb and metaflumizone in diamondback moth (Lepidoptera: Plutellidae). *J. Econ. Entomol.* 106(3): 1423-1429.

Kishore, N. 2013. Baseline susceptibility, resistance monitoring, detoxifying enzymes, synergists in resistance suppression and selectivity of insecticides to natural enemies of diamondback moth (DBM) *Plutella xylostella* (L.) to chlorantraniliprole 18.5 SC. Ph.D. (Ag) thesis, Tamil Nadu Agricultural University, Coimbatore. pp. 140.

Kranthi, K.R., Jadhav, D.R., Kranthi, S., Wanjari, R.R., Ali, S.S., and Russell, D.A. 2002. Insecticide resistance in five major insect pests of cotton in India. *Crop Prot.* 21: 449-460.

Kranthi, K.R., Jadhav, D.R., Wanjari, R., Russel, D., and Shakir A.S. 2001. Carbamate and organophosphate resistance in cotton pests in India. *Bull. Entomol. Res.* 91: 37-46.

Ladaji, R. 2004. Management of chickpea pod borer, *Helicoverpa armigera* (Hubner) using indigenous materials and newer insecticides. M. Sc. (Ag) thesis, University of Agricultural Sciences, Dharwad, 65p.

Lamb, K.P. 1978. Pests of winged bean and their control in Papua New Guinea. In: Pests of Grain Legumes: Ecology and Control. Singh, S.R., H.F. Van Emden and T.A. Taylor. (eds.), Academic Press, London, UK. pp. 125-136.

Lee, H.S. 1965. Field evaluation of several low toxicity insecticides for controlling the bean pod borer, *Maruca testulalis* (Geyer). *Plant Prot. Bull*. Taiwan, 7: 67-70.

Mau, R.F.L. and Gusukuma-Minuto, L. 2004. Diamondback moth *Plutella xylostella*. (L.), resistance management in Hawaii. University of Hawaii. pp. 23-26.

Melander, A.L. 1914. Can insects become resistant to sprays? *J. Econ. Entomol*. 7: 167–173.

Mohan, M. and Gujar, G.T. 2003. Local variation in susceptibility of the diamondback moth *Plutella xylostella* (L.), to insecticides and role of detoxification enzymes. *Crop. Prot.* 22: 495-504.

Munir Ahmad, Sayyed, A.H., Crickmore, N., and Saleem, M.A. 2007. Genetics and mechanisms of resistance to deltamethrin in a field population of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *Pest Manag. Sci*. 63: 1002-1010.

Munir Ahmad, Sayyed, A.H., Saleema, M.A., and Mushtaq Ahmad. 2008. Evidence for field evolved resistance to newer insecticides in

*Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) from Pakistan. *Crop Prot.* 27: 1367-1372.

Nair, P. 2013. Monitoring and decontamination of pesticide residues in agricultural commodities. M. Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 244p.

Naveen, V., Naik, M.I., Manjunatha, M., Shivanna, B.K., and Sridhar, S. 2009. Biology of legume pod borer, *Maruca testulalis* (Geyer) on cowpea. *Karnataka J. Agric. Sci.* 22: 668-669.

Niranjan Kumar, B.V. and Regupathy, A. 2001. Generating baseline data for insect resistant monitoring in *Spodoptera litura* (Fab.). *Pestic. Res. J.* 12(2): 232-234

Ochieng, R.S. and Bungu, D.O.M. 1983. Studies on the legume pod borer, *Maruca testulalis* (Geyer). IV. A model for mass rearing: Rearing on artificial diet. *Insect Sci. Applic.* 4: 83-88.

Ochieng, R.S., Okeyo-Owuor, J.B. and Dabrowski, Z.T. 1981. Studies on the legume pod borer, *Maruca testulalis* (Geyer): Mass rearing on natural food. *Insect Sci. Applic.* 1: 269-272.

Oei-Dharma, H.P. 1969. Use of pesticides and control of economic pests and diseases in Indonesia. Leiden. *E. J. Brill.* 199p.

Ogunwolu, E.O. 1990. Damage to cowpea by the legume pod borer, *Maruca testulalis* (Geyer), as influenced by infestation density in Nigeria. *Trop. Pest Manag.* 36: 138-140.

Okeyo-Owuor, J.B. and Ochieng, R.S. 1981 Studies on legume pod borer, *Maruca testulalis* (Geyer) its life cycle and behaviour. *Insect Sci. Appl.* 1: 263-268

Onyango, F.O. and Ochieng-Odero, J.P.R. 1993. Laboratory rearing of the legume pod borer, *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae) on a semi-synthetic diet. *Int. J. Trop. Insect Sci.* 14: 719-722.

Passlow, T. 1968. Navy bean pests. *Queens. Agric. J.* 94: 762-763.

Patel, P.S., Patel, I.S., Panickar, B., and Ravindrababu, Y. 2012. Management of spotted pod borer, *Maruca vitrata* Fabricius. in cowpea through newer insecticides. *Trends in Biosci*. 5(2): 149-151.

Patil, S.K., Desmukh, and Patil, J.V. 2008. Efficacy of flubendiamide 480 SC against pod borers in black gram. *Pestology.* 32(9):20-22.

Pradhan, S., Jotwani, M.G., and Sarup, P. 1963. Failure of BHC and DDT to control singhara beetle, *Galleurecella birmanica* Jacoby (Chrysomelidae: Coleoptera). *Indian J. Ent.* 25:177-180

Pu, X., Yang, Y., Wu, S., and Wu, Y. 2010. Characterization of abamectin resistance in a field evolved multi-resistant population of *Plutella xylostella* (L). *Pest Manag. Sci.* 66: 371-378.

Qu Mingjing, Han, Z., Xu, X., and Yue, L. 2003. Triazophos resistance mechanism in the rice stem borer *Chilo suppressalis* Walker. *Pesticide Biochem. Physiol*. 77: 99-105.

Rahaman, M.M. and Rahaman, M.S. 1988. Timing and frequency of insecticide application against *Maruca testulalis* (Geyer) infesting short-duration pigeon pea in Bangladesh. *Leg. Res*. 11:173-179.

Ramasubramanian, G.V. and Sundara Babu, P.C. 1989 Comparative biology of spotted pod borer, *Maruca testulalis* (Geyer) on three host plants. *Leg. Res*. 12: 177-178.

Ramdas Rai, N. 1983. Biology, some aspects of morphology and control of cowpea pod borer, *Maruca testulalis* (Geyer) M.Sc. (Ag) thesis, UAS, Bangalore. 188pp.

Ren, X.X., Han, Z.J., and Wang, Y.C. 2002. Mechanisms of monocrotophos resistance in cotton boll worm, *Helicoverpa armigera* (Hubner). *Arch. Insect Biochem. Physiol*. 51: 103-110.

Saini and Ramkumar. 1987. Development of resistance in *Earias vitella* to fenvalerate and cypermethrin and biology of the resistant & susceptible strains. M.Sc. thesis, Chaudary Charan Singh Haryana Agricultural University, Hissar, Haryana.162pp.

Saleem, M.A., Munir, A., Mushtaq, A., Aslam, M., and Sayyed, A.H. 2008. Resistance to Selected Organochlorin, Organophosphate, Carbamate and Pyrethroid, in *Spodoptera litura* (Lepidoptera: Noctuidae) from Pakistan. *J. Econ. Entomol.* 101(5): 1667-1675.

Sarfraz, A.S., Sayyed, A.H., and Saleem, M.A. 2010. Cross-resistance, mode of inheritance and stability of resistance to emamectin in *Spodoptera litura* (F) (Lepidoptera: Noctuidae). *Pest Manage. Sci*. 66: 839-846.

Sayyed, A.H. and Wright. D.J. 2004. Genetics of spinosad resistance in a multi-resistant field-selected population of *Plutella xylostella* (L). *Pest Manag. Sci.* 60: 827-832.

Sayyed, A.H., Munir, A., and Saleem, M.A. 2008. Cross-Resistance and Genetics of Resistance to Indoxacarb in *Spodoptera litura* (F) (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 101(2): 472-479.

Scott, L.B. 1940. The bean pod borers in the Puerto Rico. *J. Agric. Univ. Puerto Rico.* 24: 35-47.

Sharma, H.C. 1998. Bionomics, host plant resistance and management of legume pod borer, *Maruca vitrata* (Fabricius). *Crop Prot.* 17: 373-386.

Shelton, A.M., Wyman, J.A., Cushing, N.L., Apfelbeck, K., Dennehy, T.J., Mahr, S.E.R., and Eigenbrode, S.D. 1993. Insecticide resistance of diamondback moth (Lepidoptera: Plutellidae) in North America. *J. Econ. Entomol.* 86: 11-19.

Singh, S.P. 2000. Survey of Indian Agriculture. 2000. *The Hindu*, n.d 159-163p.

Singh, S.R. and Allen, D.J. 1980. Pests, diseases, resistance and protection in cowpea. In: Summerfield, R.J. and Bunting, A.H. (eds), *Advances in legume science.* Royal botanical garden, Kew and Ministry of Agriculture, Fisheries and Food, London. pp 419-443.

Smith, J.G. 1978. Pests of soybeans in Brazil. In: Singh, S.R., van Emden, H.F. and Taylor, T.A. (eds), *Pests of Grain Legumes: Ecology* and *Control.* Singh Academic Press, London UK. pp. 167-177

Soliman, K.M. 2011. Changes in concentration of pesticides residues in potatoes during washing and home preparation. *Fd. Chem. Toxic*. 39:887-891.

Somalo, A.P. and Patnaik, H.P. 1986. Efficacy of different insecticides and their frequency of application on the pod borers infesting pigeon pea. *Madras Agric. J.* 73: 352-354.

Sontakke, B.K. and Mishra, P.R. 1991. Comparative efficacy and economics of synthetic pyrethroids in the management of pod borer complex in pigeon pea. *Indian J. Plant. Prot.* 19: 167-170.

Sonune, V.R., Bharodia, R.K., Jethva, D.M., Rathode, R.T., and Deshmukh S.G. 2010. Field efficacy of chemical insecticides against spotted pod borer, *Maruca vitrata* (Fabricius) infesting black gram. *Leg. Res*. 33(4): 287-290.

Srinivasan, G. 2008. Bio efficacy of insecticides against spotted borer, *Maruca vitrata* Fabricius in short duration pigeon pea. *Pesticide Res. J.* 20 (2): 221-223.

Srivastava, B.K. 1964. Pests of pulse crops. In: Entomology in India, 1938-1963 (eds), Pant, N.C. Entomological Society of India, New Delhi. pp. 83-91.

Srivastava, K.M. 1980. Studies of pod borers pests of pigeon pea in Uttar Pradesh, India. *Trop. Pest Manag.*. 26:262-264.

Subasinghe, S.M.C. and Fellowes, R.W.1978. Recent trends in grain legume pest research in Sri Lanka. In: Singh, S.R., Van Emden, H.F. and Taylor, T.A. (eds), *Pests in Grain legumes. Ecology and control*. Singh Academic Press, London, UK. pp. 37-41.

Taylor, T.A. 1967. The bionomics of *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae), a major pest of cowpeas in Nigeria. *J. West Africa Sci. Assoc.* 12:111–129.

Taylor, T.A. 1978. *Maruca testulalis* (Geyer) an important pest of tropical grain legumes. In: Singh, S R., Van Emden, H F. and Taylor, T A. (eds), *Pests of Grain Legumes: Ecology and Control*. Singh Academic Press, London, U.K. pp. 193-200.

Torres-Villa, L.M., Rodriguez-Molina, M.C., Lacasa, P., and Bielza-Lino, P. 2002. Insecticide resistance of *Helicoverpa armigera* (H.) to endosulfan, carbamates and organophosphates: the Spanish case. *Crop Prot*. 21: 1003-1013.

Veeranna, R., Jayaramaiah, M., and Sreeramulu, K.R. 1999. Biology of cowpea pod borer, *Maruca testulalis* (Geyer) (Lepidoptera: Pyralidae). *Leg. Res*. 22: 51-54.

Venkataria, M.V. and Vyas, H.N. 1985. Chemical control of pod borer complex in green gram, *Vigna radiata* L. Wilczek with certain insecticides alone and in combination with Miraculan – a plant grown stimulant. *Leg. Res.* 8:111-113.

Vijayasree, V. 2013. Efficacy and biosafety of new generation insecticides for the management of fruit borers of cowpea, brinjal and okra. Ph.D. (Ag) thesis, Kerala Agricultural University, Thrissur, 220p.

Wang, X., Khakame, S.K., Ye, C., Yang, Y., and Wu, Y. 2013. Characterisation of field evolved resistance to chlorantraniliprole in the diamondback moth, *Plutella xylostella*, from China. *Pest Manag. Sci.* 69(5): 661-665.

Williamson, A.L. 1943. Two foreign bean pod borers discovered in Texas. *J. Econ. Entomol.* 36: 931- 937.

Wolcott, G.N.1933. The lima bean pod borer caterpillar of Puerto Rico. *J. Dep. Agric. Puerto Rico.* 17: 241 - 255.

Yu, S.J. and Nguyen. S.N. 1992. Detection and biochemical characterization of insecticide resistance in the diamondback moth. *Pestic. Biochem. Physiol.* 44: 74-81.

Yu, S.J. and Nguyen. S.N. 1996. Insecticide susceptibility and detoxification enzymes activities in permethrin- selected diamondback moths. *Pestic. Biochem. Physiol.* 56(1): 69-72.

Zanella, R., Primel, E.G., Gonclaves, F.F., and Martins, A.F. 2000. Development of validation of high performance chromatography on the determination of cloma zone residues in surface water. *J. Chromatography*. 9: 257-262.

Zhao, J.Z., Collins, H.L., Li, Y.X., Mau, R.F.L., Thompson, G.D., Hertlein, M., Andaloro, J.T., Boykin, R., and Shelton, A.M. 2006. Monitoring of diamondback moth (Lepidoptera: Plutellidae) Resistance to spinosad, indoxacarb and emamectin benzoate. J. Econ. Entomol. 99(1): 176-181.

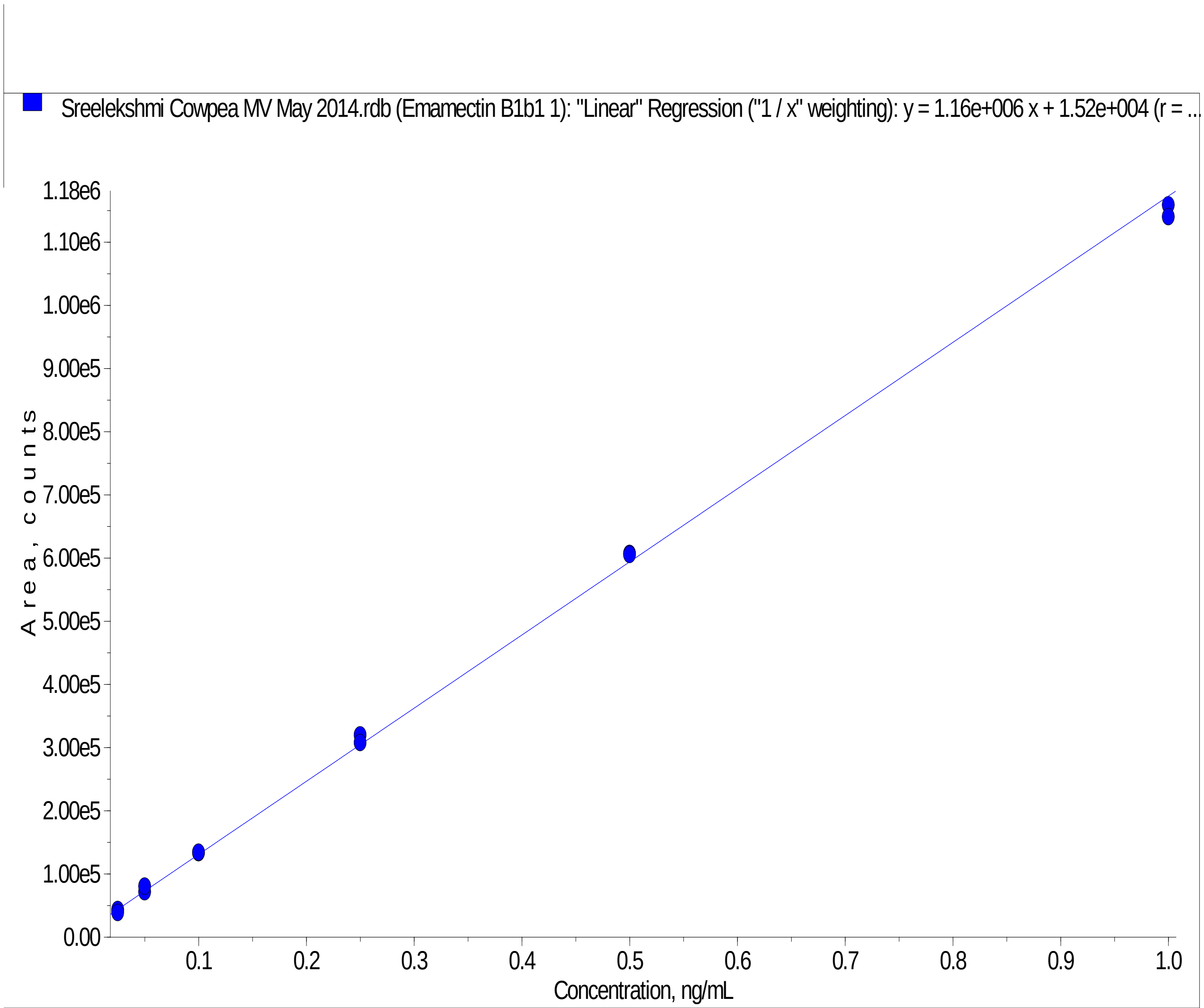
# APPENDIX-I

## PROFORMA FOR SURVEY ON PESTICIDE USE PATTERN IN COWPEA AGAINST SPOTTED POD BORER, *MARUCA VITRATA* (F)

| Sl. no. | Particulars | Response of farmers |
|---|---|---|
| 1 | Location | |
| 2 | Name and address of Farmer | |
| 3 | Age | |
| 4 | Source of technical information regarding crop protection | |
| | a) Agriculture officers | |
| | b) Company representatives | |
| | c) Other progressive farmers | |
| | d) Own decisions | |
| | e) Media | |
| 5 | Source of plant protection chemicals | |
| 6 | Source of information on dose of pesticides | |
| | a) Agricultural officers | |
| | b) Pesticide shops | |
| | c) Other progressive farmers | |
| | d) Own decisions | |
| 7 | Is there any practice of manual mixing of pesticides and spraying? | Yes/No |
| 8 | Is there any prophylactic application of PP chemicals | Yes/No |
| 9 | Whether following integrated pest management strategies | Yes/No |
| 10 | Whether following the directions in the pesticide label during handling and application of pesticides? | Yes/No |
| 11 | Most frequently used insecticides | |

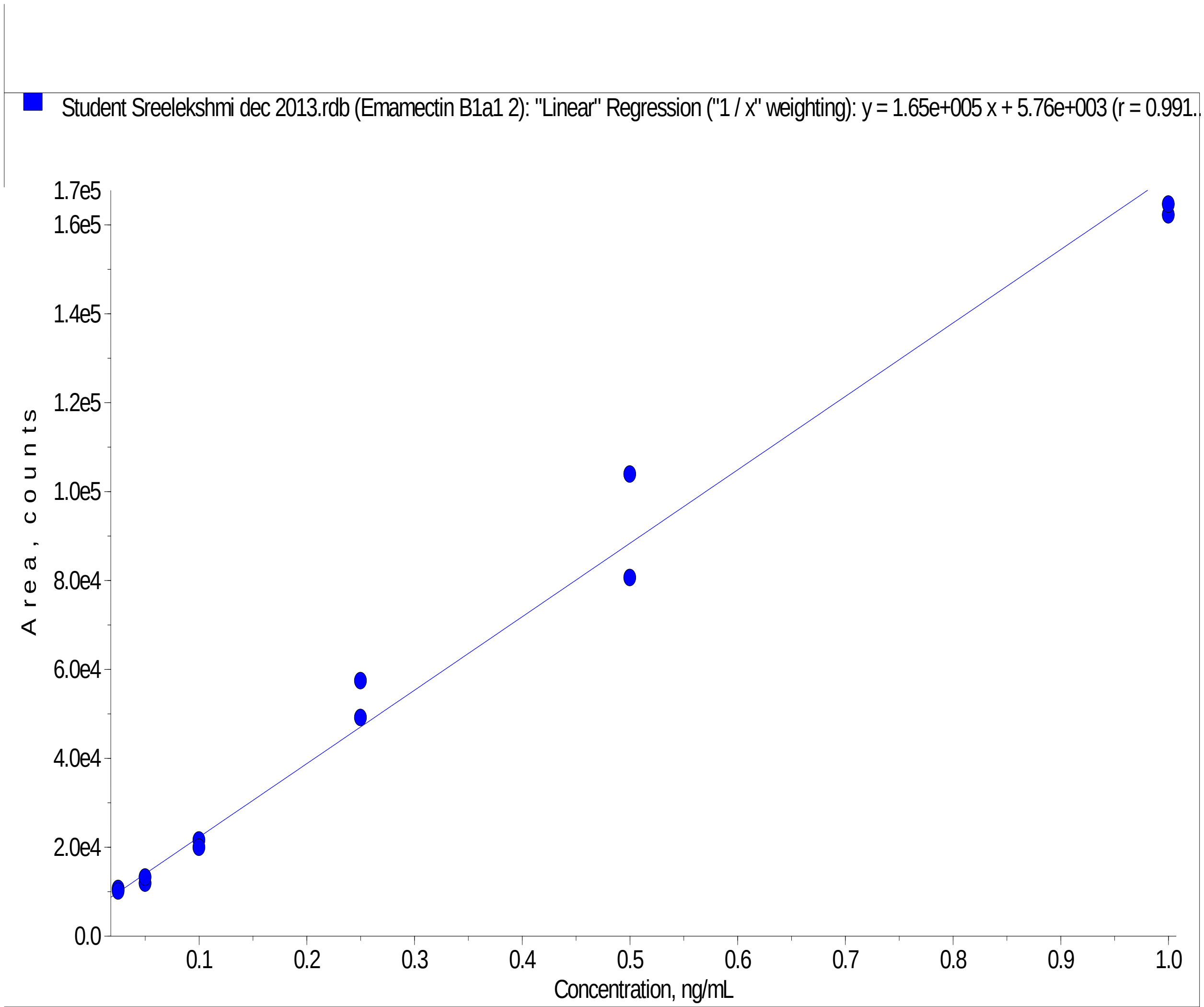
| | | |
|---|---|---|
| 12 | Which insecticide against control failures obtained | |
| 13 | Time of application of pesticides | |
| | a) Early morning | |
| | b) Morning | |
| | c) Afternoon | |
| | d) evening | |
| 14 | Any control failures noticed after the application of any pesticides | Yes/No |
| 15 | Name of pest which is very difficult to control | |
| 16 | Do you aware of insecticide resistance | Yes/No |
| 17 | Source of information on insecticide resistance | |
| | a) Training classes | |
| | b) Media | |
| | c) Other farmers | |

Appendix II a



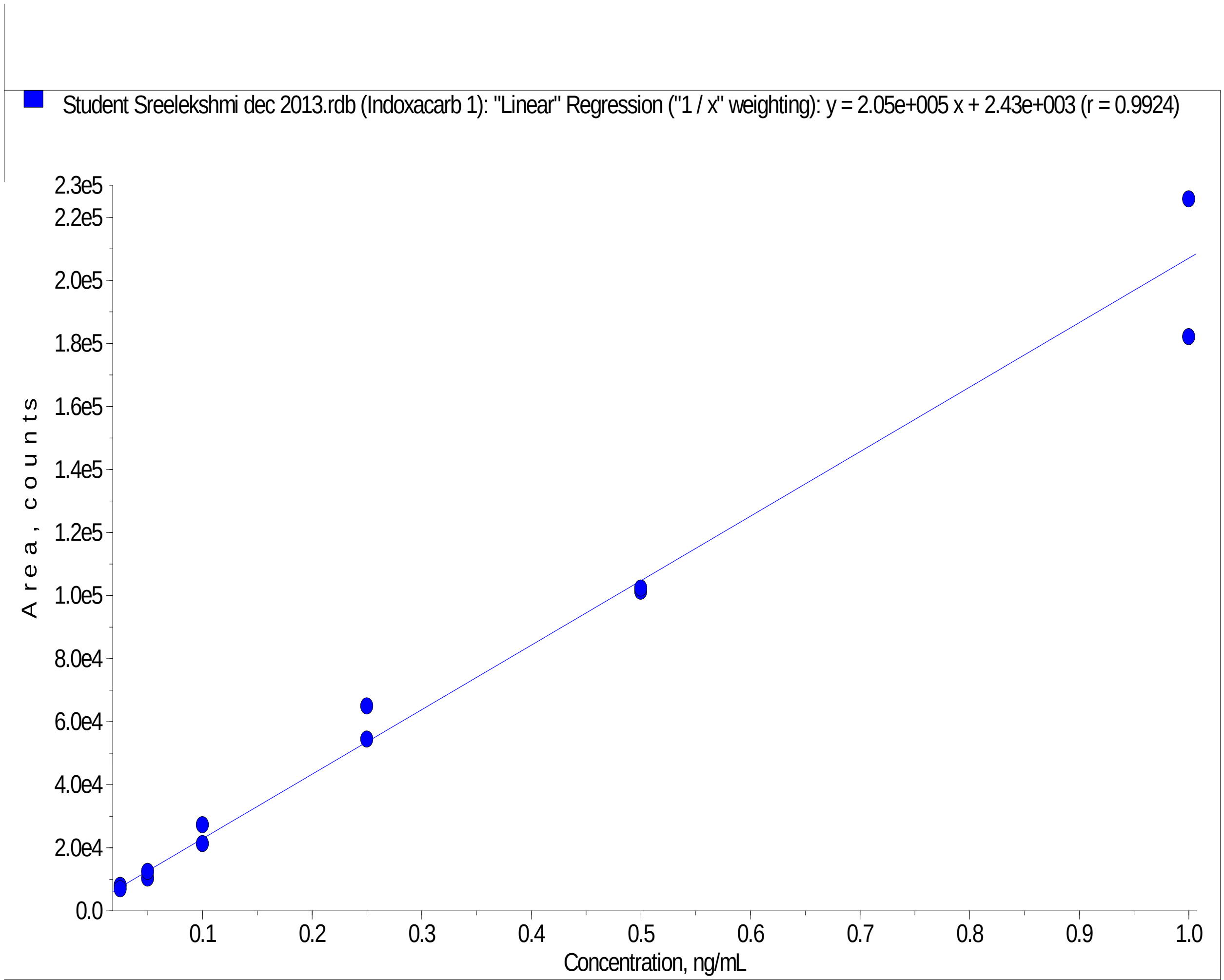

Calibration curve of Emamectin B1b1

Appendix II b



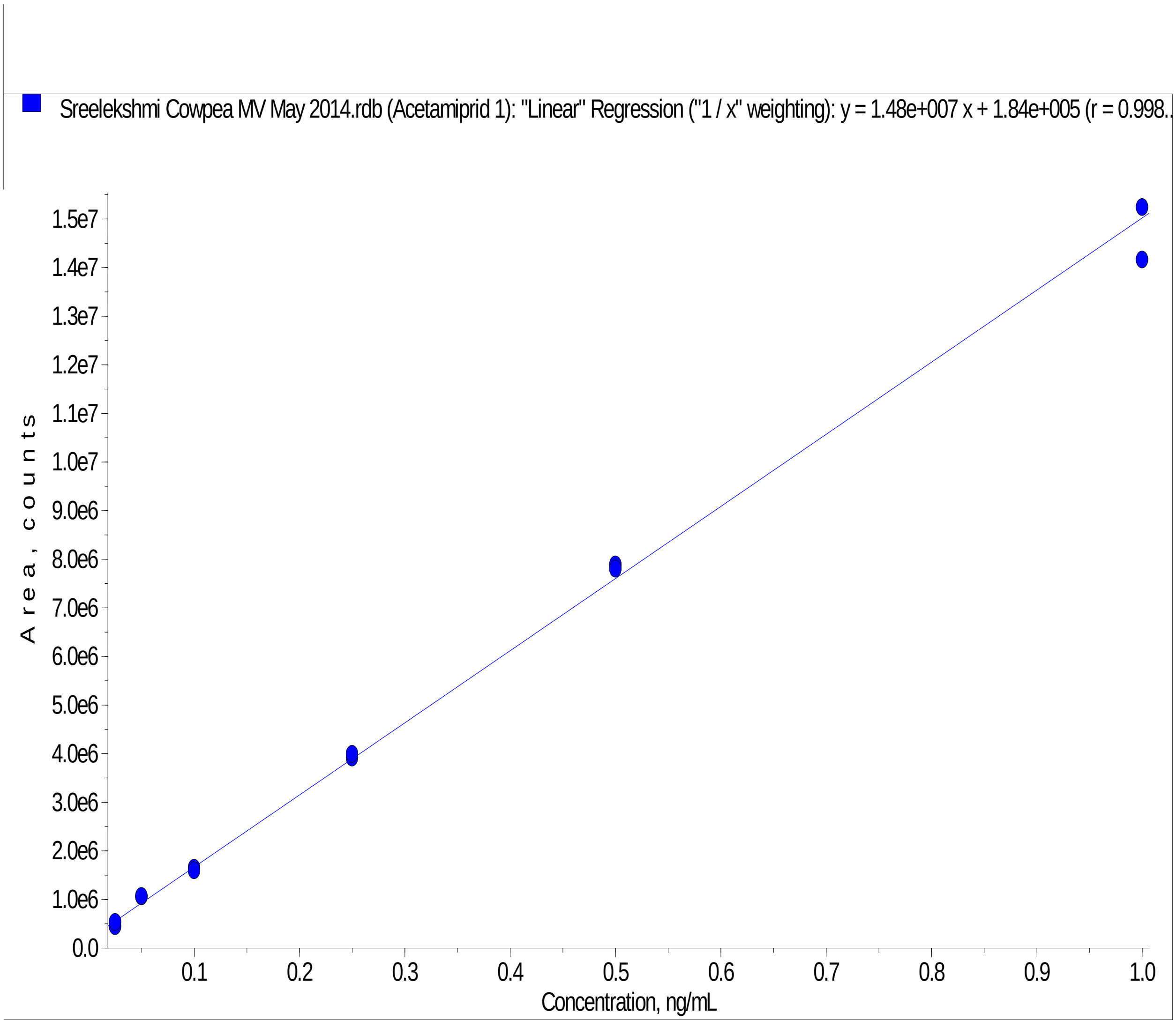

Calibration curve of Emamectin B1A1

# Appendix III



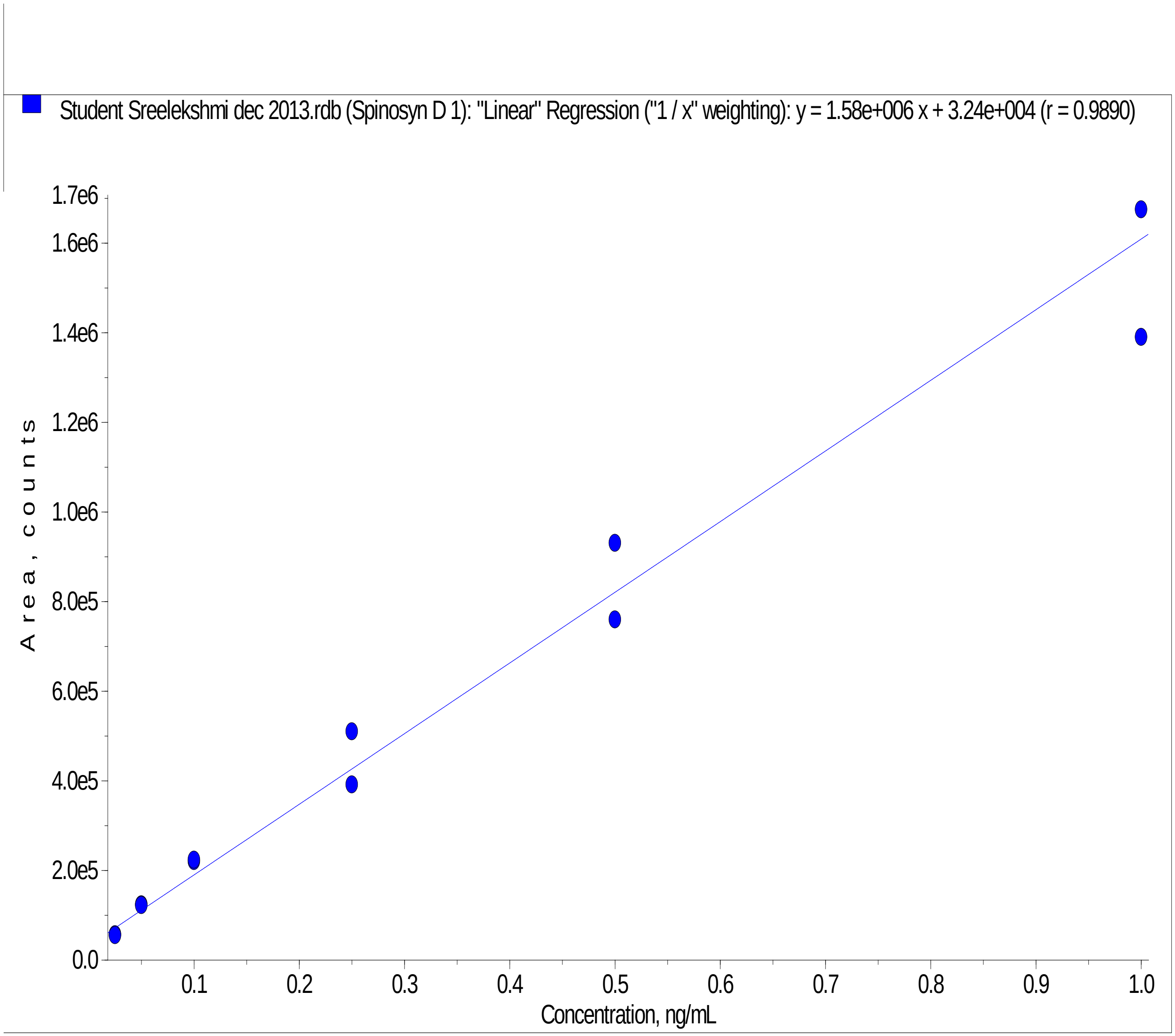

Calibration curve of Indoxacarb

Appendix IV



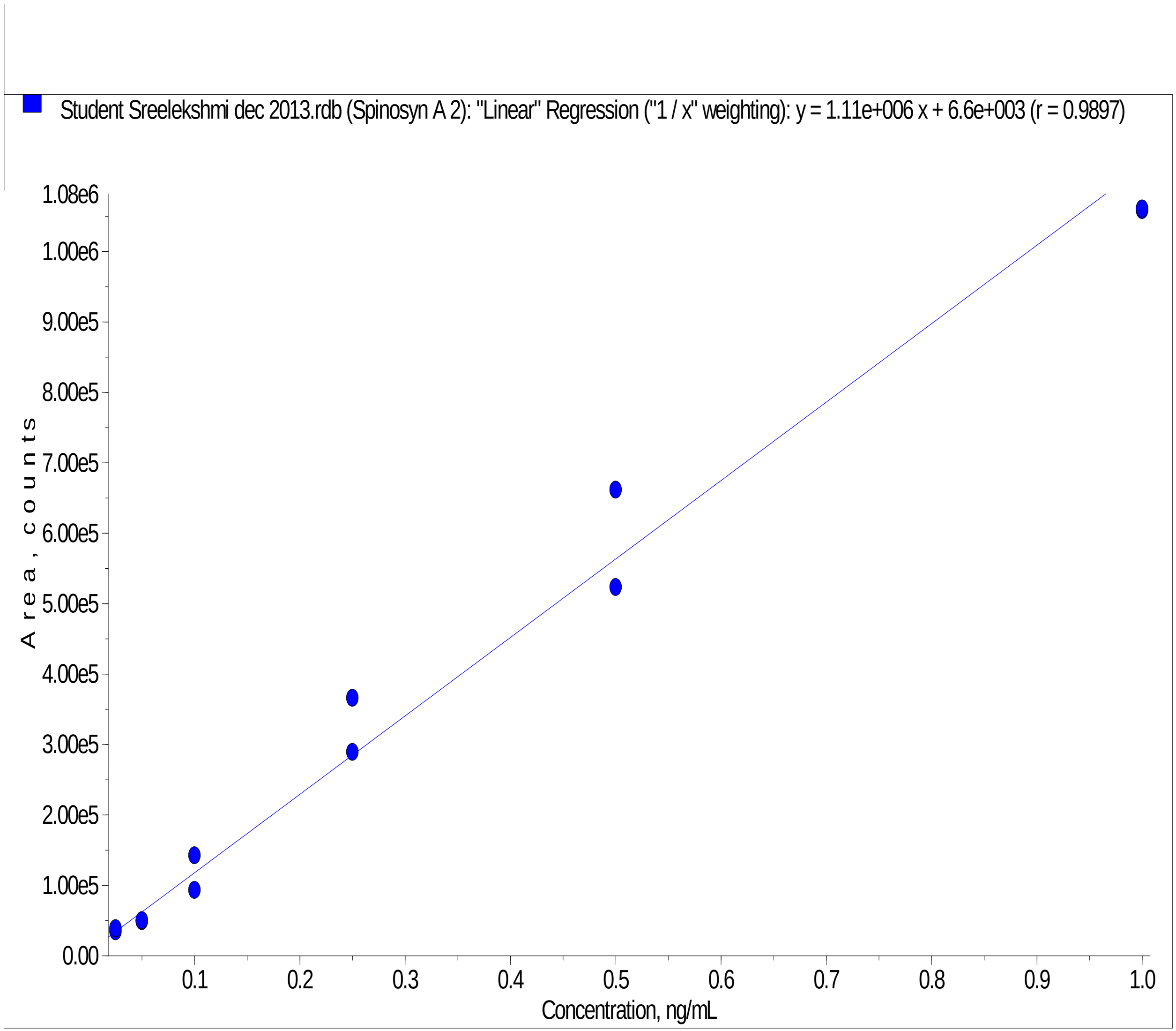

Calibration curve of Acetamiprid

Appendix V a



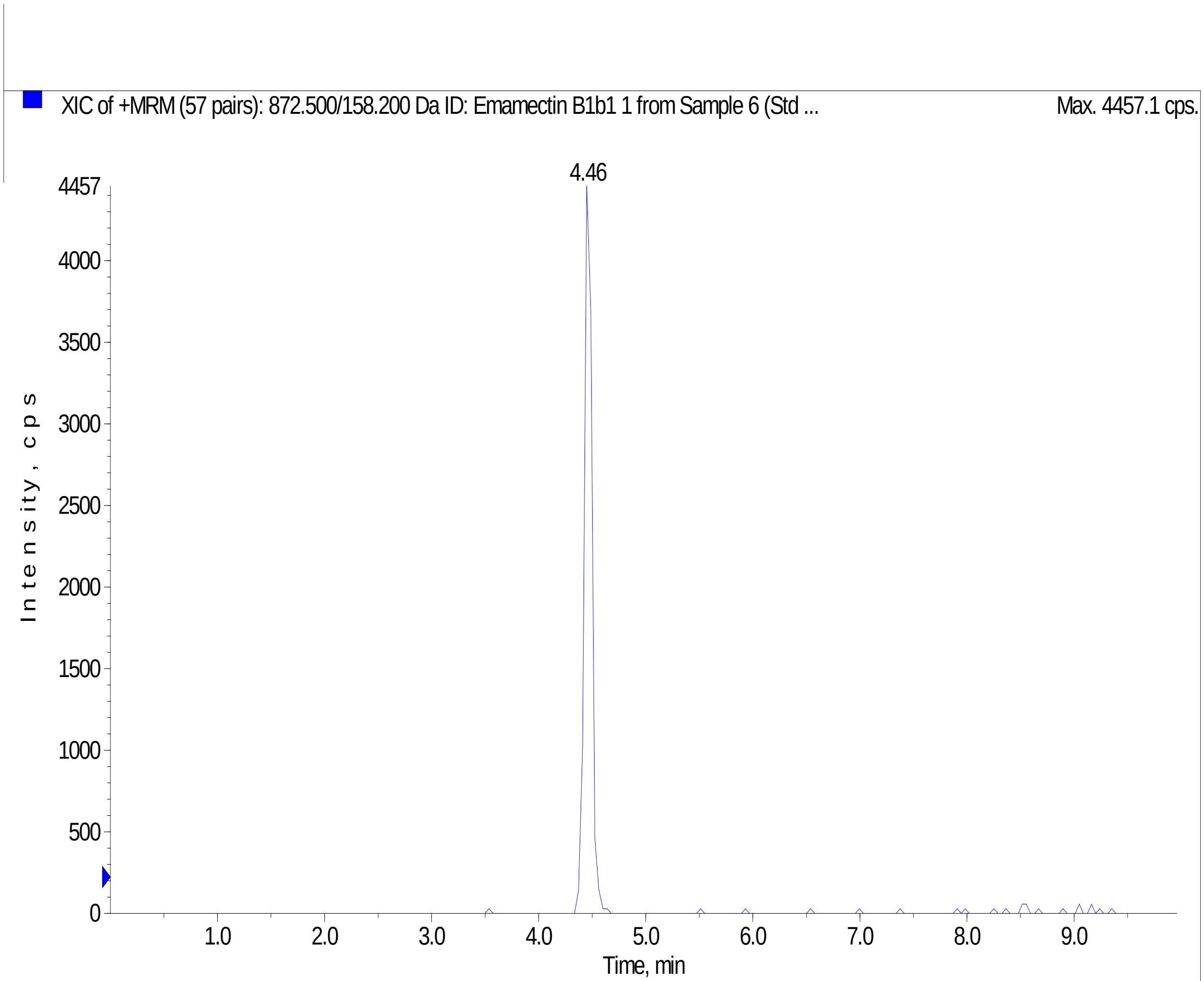

Calibration curve of Spinosyn D

Appendix V b



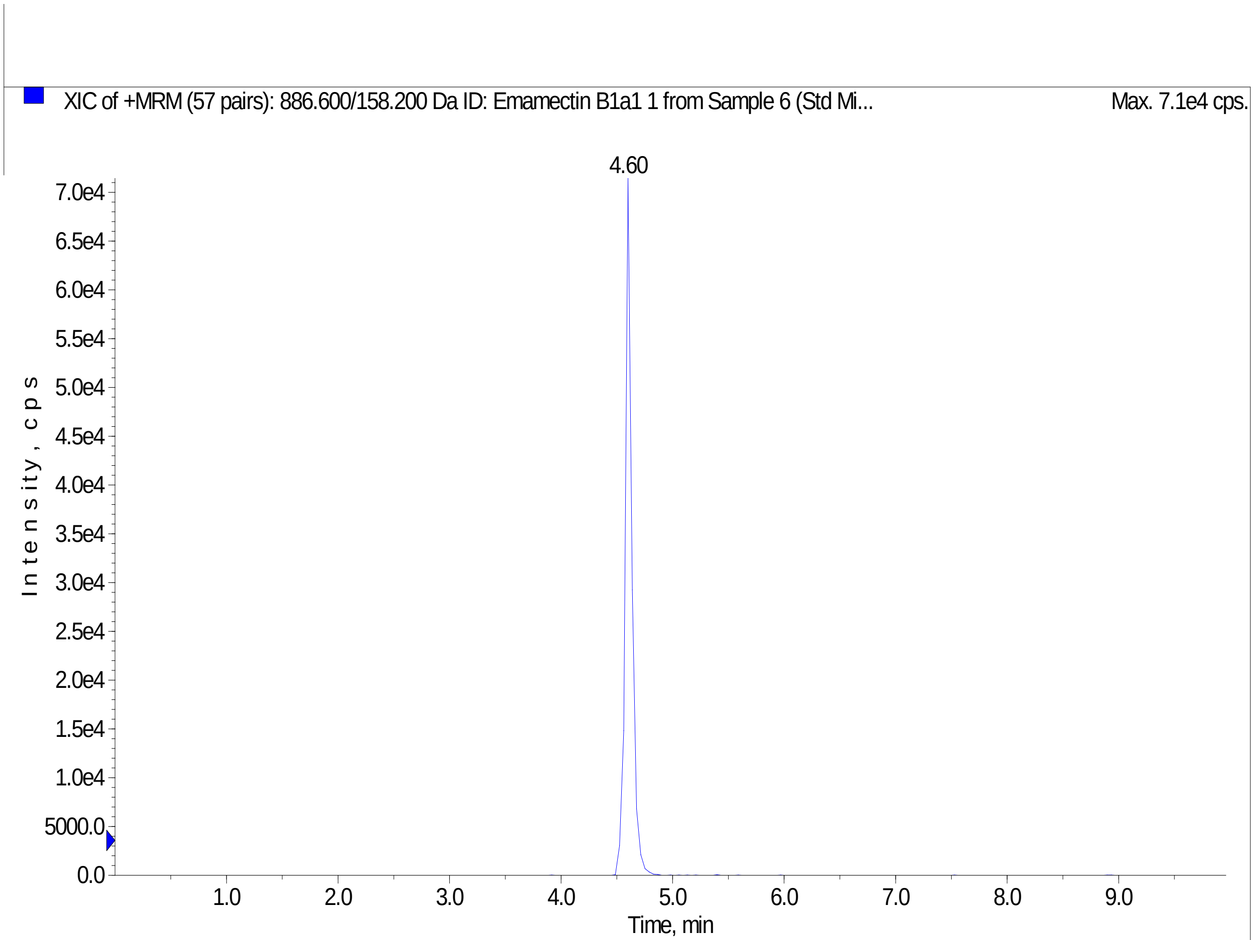

Calibration curve of Spinosyn A

Appendix VI a



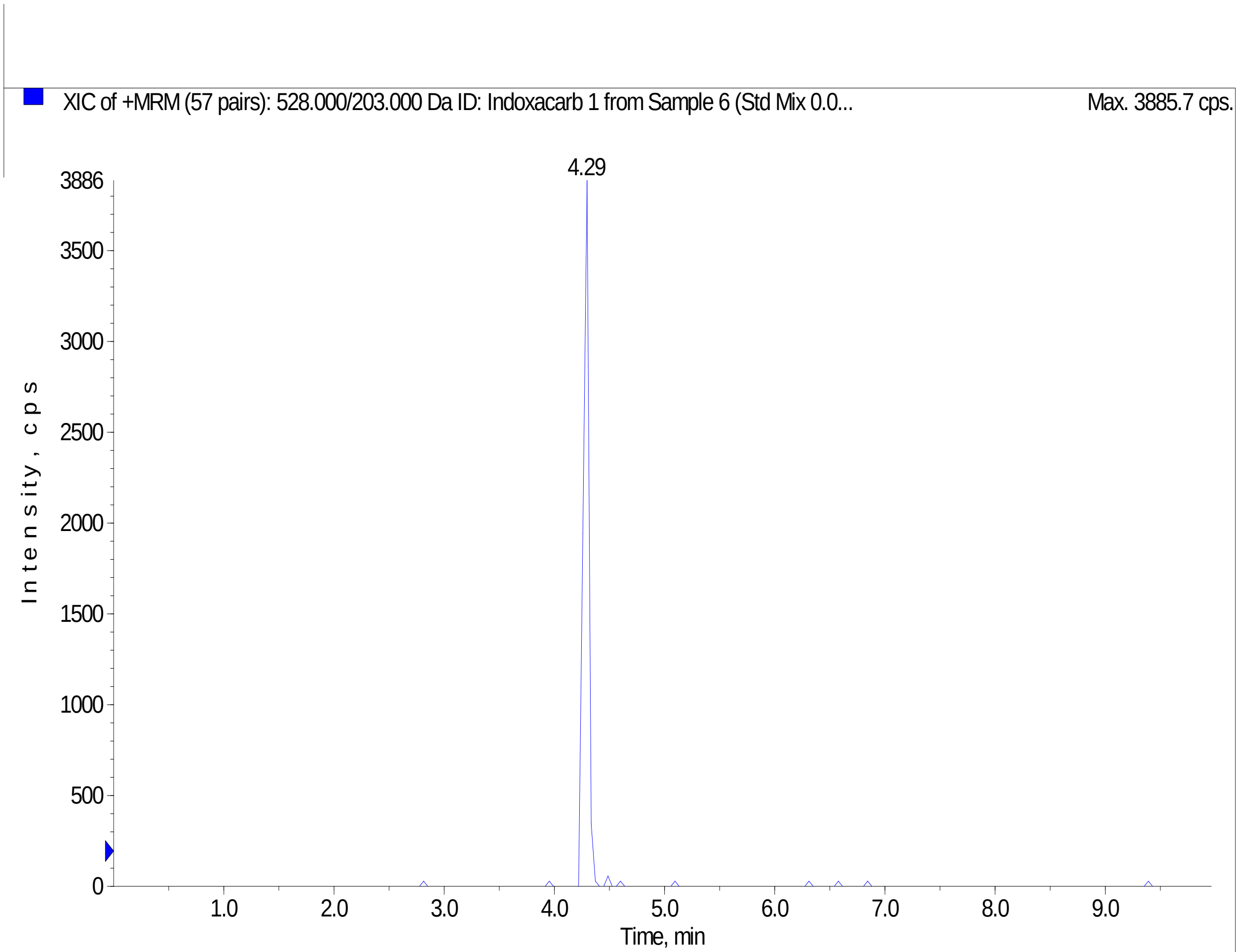

LC-MS/MS chromatogram of Emamectin benzoate B1b1

Appendix VI b



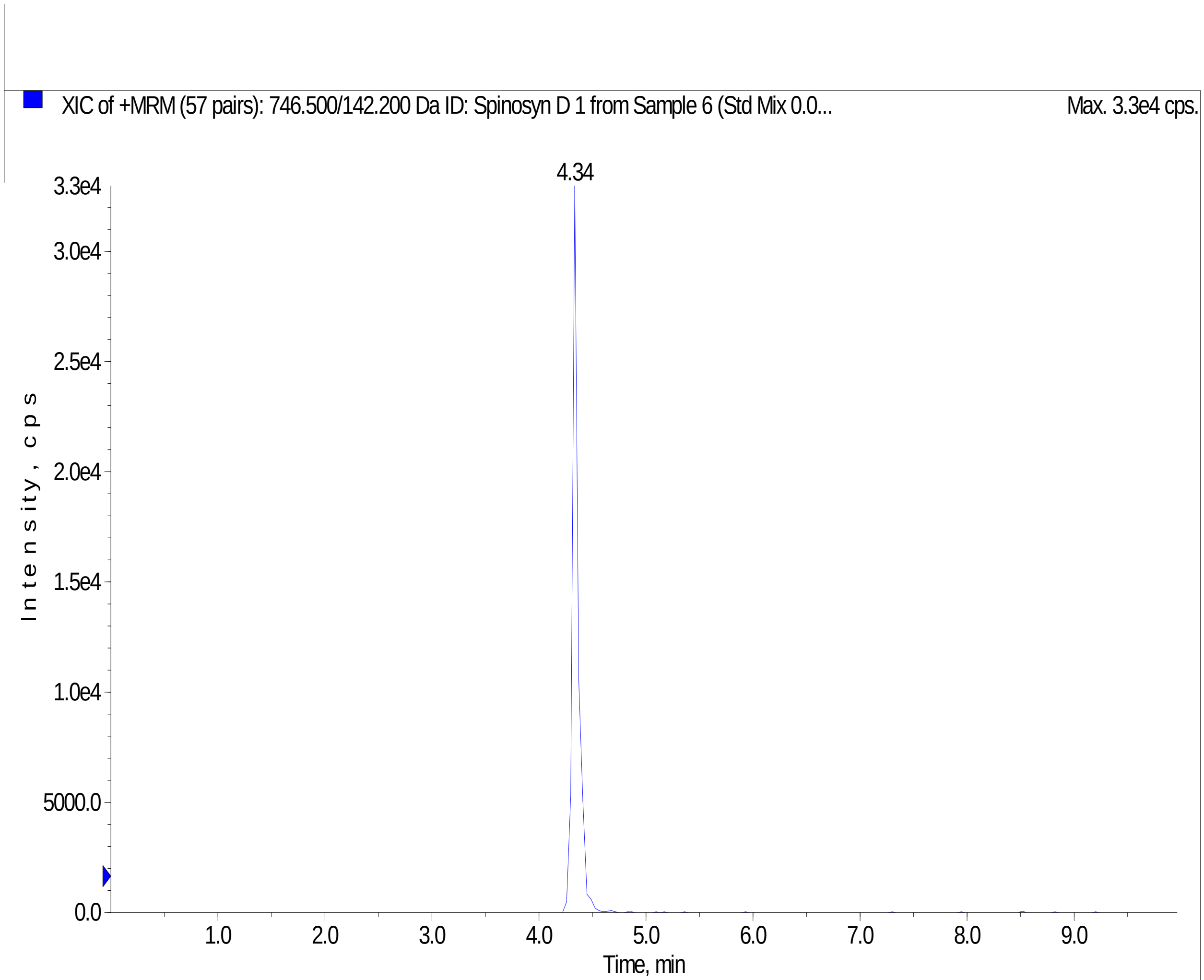

LC-MS/MS chromatogram of Emamectin benzoate B1A1

# Appendix VII



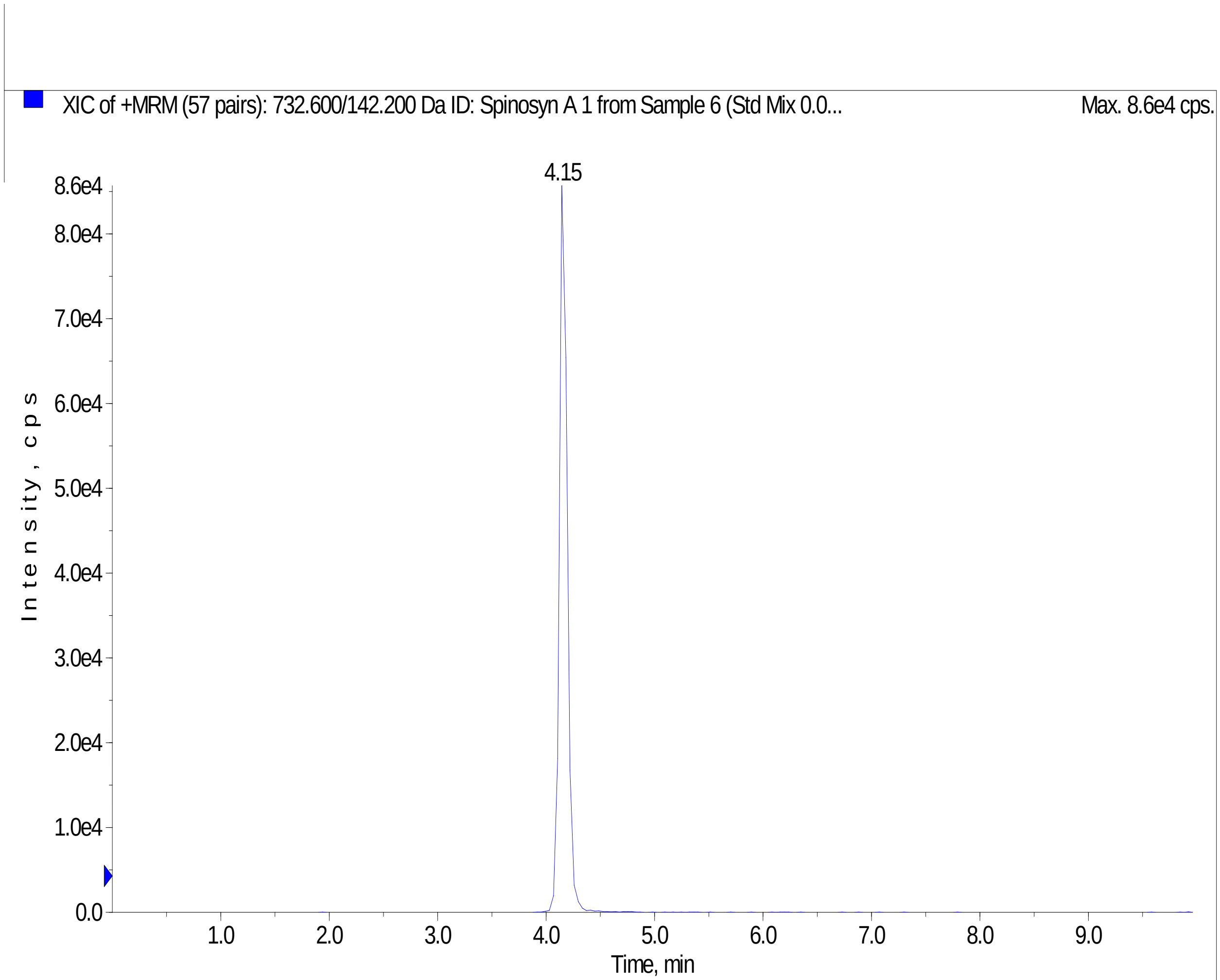

LC-MS/MS chromatogram of Indoxacarb

Appendix VIII a



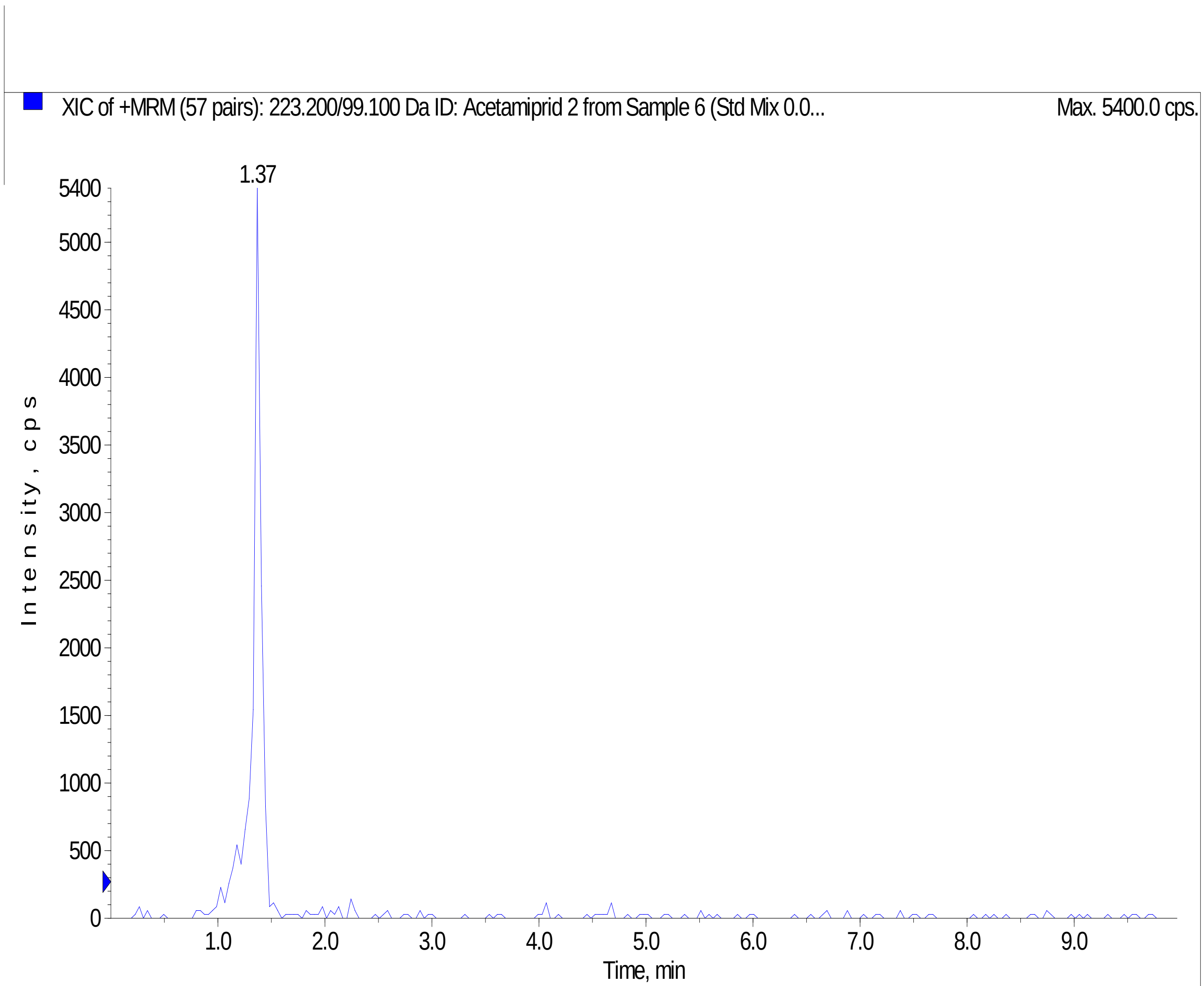

LC-MS/MS chromatogram of Spinosyn D

Appendix VIII b




LC-MS/MS chromatogram of Spinosyn A

Appendix IX




LC-MS/MS chromatogram of Acetamiprid

# INSECTICIDE RESISTANCE IN SPOTTED POD BORER, *Maruca vitrata* (Fabricius) ON VEGETABLE COWPEA AND ITS MANAGEMENT

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**(2012-11-189)**

**ABSTRACT**
**of the thesis submitted in partial fulfillment of**
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**MASTER OF SCIENCE IN AGRICULTURE**

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**Kerala Agricultural University**

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# ABSTRACT

A study on "Insecticide resistance in spotted pod borer *Maruca vitrata* (Fabricius) on vegetable cowpea and its management" was undertaken at College of Agriculture, Vellayani and in the farmer's field at Kalliyoor and Venganoor panchayaths during January, 2013 to May, 2014. The main objectives were to assess the insecticide resistance in field population of spotted pod borer, *M. vitrata,* evaluate the efficacy of new generation insecticides against the resistant population and determine the harvest time residues in cowpea pods.

A preliminary survey conducted among cowpea growers in Kalliyoor and Venganoor panchayaths of Thiruvananthapuram district revealed control failures in the field population of spotted pod borer due to the continuous application of a single insecticide like chlorpyriphos or lambda cyhalothrin. The survey data served as the benchmark for further studies on the insecticide resistance in *M. vitrata*.

Bioassay was carried out in CRD to assess insecticide resistance in populations of *M.vitrata* collected from three different locations (location I- field with no previous history of pesticide application, location II- field where less control failures and location III- field where more control failures) using two chemicals *viz.* chlorpyriphos and lambda-cyhalothrin at different doses. Results revealed that population collected from location-I was found to be susceptible for both the chemicals with resistance ratio-1, population collected from location-II to be moderately resistant with a resistant ratio of 2.28 and 2.38 and population from location-III to be resistant with resistance ratios of 2.93 and 7.94 for chlorpyriphos and lambda-cyhalothrin respectively.

Laboratory experiments conducted to evaluate the efficacy of new generation insecticides *viz.* novaluron - 0.015 per cent, flubendiamide - 0.01 per cent, spinosad - 0.015 per cent, emamectin benzoate - 0.002 per cent, indoxacarb 14.5 per cent + acetamiprid 7.7 per cent SC- 0.09 per cent, acephate 50 per cent

+ imidacloprid 1.8 per cent SP - 0.20 per cent, chlorantraniliprole - 0.03 per cent, indoxacarb - 0.10 per cent and chlorpyriphos - 0.05 per cent (as check) against the resistant population of *M.vitrata* revealed that the per cent mortality of the second instar larvae treated with insecticides *viz.* emamectin benzoate, indoxacarb+acetamiprid and spinosad were 97.28, 97.28 and 90.90 per cent respectively after 6 hours of treatment. Hence, these insecticides were selected for further field trials against the resistant population of *M .vitrata.*

The field experiment was conducted in RBD with four treatments *viz.* emamectin benzoate 0.002 per cent, indoxacarb+acetamiprid 0.09 per cent and spinosad 0.015 per cent including control against the resistant population of *M.vitrata*. Application of emamectin benzoate, indoxacarb + acetamiprid and spinosad resulted in a reduction of 81-100, 64-100 and 69.96 - 99.6 per cent respectively in the case of flower infestation and 83 -100, 23.38 - 98.40 and 55.26 – 100 per cent respectively in case of pod infestation. Harvest time residue estimation done on 7$^{th}$ day after the application of insecticides showed the residues below the quantitative limit of 0.05 mg kg$^{-1}$ revealing its safety for consumption.

The study could indicate the development of insecticide resistance in the field population of *M.vitrata*. On the basis of the present study it could be concluded that spraying of emamectin benzoate @ 10 g ai ha$^{-1}$ or indoxacarb + acetamiprid @100 g ai ha$^{-1}$ or spinosad @ 75 g ai ha$^{-1}$ could effectively manage the resistant population of *M. vitrata* in cowpea. This study forms a maiden attempt in assessing the extent of insecticide resistance development in the populations of *M. vitrata* in Kerala. Further studies have to be taken up to develop and popularize an Insecticide Resistance Management (IRM) strategy against this devastating pest of cowpea.