COMPARATIVE STUDIES ON GENETIC, BIOCHEMICAL AND INSECTICIDES TOXICITY IN *Spodoptera litura* (Fab) FROM DIFFERENT HOST CROPS

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COMPARATIVE STUDIES ON GENETIC, BIOCHEMICAL AND INSECTICIDES TOXICITY IN *Spodoptera litura* (Fab) FROM DIFFERENT HOST CROPS

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CERTIFICATE

This is to certify that the thesis entitled **"COMPARATIVE STUDIES ON GENETIC, BIOCHEMICAL AND INSECTICIDES TOXICITY IN *Spodoptera litura* (Fab) FROM DIFFERENT HOST CROPS"** submitted by **Mr. TUKARAM. A. H** for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **AGRICULTURAL ENTOMOLOGY** to the University of Agricultural Sciences, Raichur, is a record of research work carried out by him during the period of his study in this University, under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.

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AFFECTIONATELY DEDICATED

TO

MY BELOVED PARENTS
Sri. ALLAPPA AND Smt.RATNAVVA

AND

SISTERS

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With regardful memories……

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Raichur

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LIST OF ABBREVIATIONS

Abbreviations		Expansion
%	:	per cent
µl	:	micro litre
ppm	:	parts per million
Fig.	:	figure
hrs	:	hours
mM	:	Micro molar
RH	:	relative Humidity
^{0}C	:	degree Celsius
LC_{50}	:	median lethal concentration
LL	:	lower limit
UP	:	upper limit
mU/mL	:	mili-Units per mili-liter
SC	:	Soluble concentrate
EC	:	Emulsifiable concentrate
s	:	second

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INTRODUCTION

I. INTRODUCTION

The tobacco caterpillar, *Spodoptera litura* (Fabricius) is distributed worldwide and it is a member of the economically important polyphagous pest (>120 host plants) and causes serious crop losses (Singh and Jalali, 1997). In India, it is a major pest that attacks a wide variety of economically important crops such as tobacco, cotton, groundnut, castor, chilli, potato, soybean, cauliflower, cabbage, tomato, beans, sunflower and onion. The *S. litura* larva initially scrape the leaf tissue gregariously in clusters and skeletonise the leaves and is next only to american bollworm *Helicoverpa armigera* (Hub.) in economic importance both at national and global level. Recently, the pest has been appearing in severe form, especially during rainy and post rainy seasons causing heavy yield losses throughout India. It was observed that the incidence of *S. litura* in groundnut ecosystem may cause 26 to 100 per cent yield loss (Dhir *et al*., 1992).

The *S. litura* has remarkable features such as polyphagous, high reproductive potential, overlapping generations, ability of adults to migrate over large distances and frequent control failures due to development of resistance most of the routinely used to available insecticides. The last four decades witnessed a massive transition in the development of insecticide resistance in *S. litura*. However, the first case of insecticide resistance in *S. litura* was documented by Srivatsava and Joshi (1965) who observed frequent control failures of *S. litura* with BHC in areas around Ajmer and Jaipur.

Intensive application of organchlorines, organophosphates and carbomate insecticides were led to the development of resistance in *S. litura*. Similarly, to combat the unprecedented *S. litura* pressure, synthetic pyrethroids were introduced in the country during 1980's primarily for the control of *S. litura* and pyrethroids were effective to an extent that *S. litura* was relegated to the status of a minor pest of cotton. Field strains of *S. litura* which showed resistance to almost all classes of insecticides have been reported from different parts of the world (Armes *et al.,* 1997).

The chemical control of *S. litura* has been extensively reported in relation to various crops in India. Until 1968, this pest was held in check by methyl-parathion, but then insect developed resistance to this compound. Since then, numerous other organophosphate, carbamate and synthetic pyrethroid developed resistance and cross resistance in many cases

(Issa *et al.*, 1984; Abo-El-Ghar *et al*., 1986). In Egypt Sawicki (1986) showed the synthetic pyrethroid can stop the new resistance appearance on cotton when applied one time in a year.

The development of molecular markers is directly related to advances in the field of molecular biology. The recent characterization of genomes, completely or partially, and knowledge of the molecular basis of genetic variation have been very important sources for the development of markers and establishment of evolutionary models at the inter and intra-specific levels (Li, 1997). In the past decades numerous molecular markers have been characterized. Most of these are derived from sequence differences within mitochondrial DNA (mtDNA) and microsatellite loci. These molecular markers have been applied in studies of a great variety of organisms.

Though mtDNA sequence data have proved valuable in determining phylogenetic relationships, the choice of gene is also of great significance (Simon *et al*. 1994; Lunt *et al*. 1996). The size and structure of cytochrome oxidase subunit 1 (COI) gene has been well conserved in the animal groups analysed so far, a feature which makes it especially suitable for evolutionary studies (Lunt *et al*. 1996). Therefore, it is imperative that data collected from molecular markers be employed for defining the phylogenetic relationships among different taxa in the family.

Digestive enzymes are of fundamental importance for understanding the digestive processes, feeding habits and host selection (Snodgrass, 1935). Most digestion occurs in the midgut, where a variety of enzymes are available in abundance (Engelmann, 1969; Persaud and Davey, 1971; Hori *et al*., 1981). In certain insects, digestion commences in the foregut by virtue of salivary gland secretions or enzyme regurgitation from the midgut. Rare instances of extra-intestinal digestion have also been reported in some insects (Chapman, 1972).

A wide range of digestive enzymes has been recorded in the alimentary canal of insects (House, 1965; Applebaum, 1985; Chapman, 1985a,b; Terra and Ferreira, 1996). The nature of the enzymes secreted is related to the nature of the meal that an insect can assimilate (Hubert *et al*., 1999; Agusti and Cohen, 2000; Zeng and Cohen, 2000a, 2000b; Torres and Boyd, 2009). Whereas herbivorous insects secrete more carbohydrases (Day and Powning, 1949), carnivorous insects secrete mainly proteases (Gooding and Rolseth, 1976).

Digestive enzymes specific for zoophagous animals include proteases (e.g., trypsin, chymotrypsin, cathepsin), hyaluronidases and phospholipases (Cohen, 1998b

and 2000). Digestive enzymes specific for phytophagous animals include amylases and pectinases (Cohen, 1996)

The evolution of resistance to insecticides is a threat to modern agriculture, to tackle this problem bioassay of insecticides through leaf dip method, genetic relatedness and enzyme activity may help in better understanding of resistance mechanism. Keeping this view the following objectives were planned as indicated below

1. To study the insecticide resistance in *Spodoptera litura* collected from different host crops to selected insecticides.
2. To study the genetic relatedness among the *Spodoptera litura* population collected from different host crops using cytochrome oxidase subunit-I DNA-Marker.
3. To study the digestive enzyme activity among the *Spodoptera litura* population collected from different host crops.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

It is envisaged in the present investigation to study the insecticide resistance, genetic relatedness and digestive enzyme activity of *Spodoptera litura* (Fab.). The literature pertaining to the objectives enlisted is presented here.

2.1 To study the insecticide resistance in *Spodoptera litura* collected from different host crops to selected insecticides

Armes *et al.* (1997) collected twenty two strains of *S. litura* from eight locations of India to study the resistance level to commonly used insecticide. Results indicated that the development of resistance in *S. litura* to cypermethrin and fenvelarate in field condition ranged from 0.22 to 197 and 8 to 121 fold, respectively.

Adamczyk *et al.* (1999) reported the superiority of spinosad over thiodiocarb on fifth instar larvae of fall army worm, *Spodoptera frugiperda* (Smith) under laboratory condition where spinosad recorded low LC_{50} values indicating its superiority while thiodiocarb recorded high LC_{50} value. Similarly, susceptibility response of five commonly used insecticides *viz.*, endosulfan, chlorpyriphos, profenophos, fenvalarate and deltamethrin were conducted on tobacco caterpillar, *S. litura* (Fab.) through topical bioassay method.

Niranjan Kumar and Reghupathy (2000) studied the susceptibility response of five commonly used insecticides *viz;* endosulfan, chlorpyriphos, Profenophos, fenvalarate and deltamethrin on *S. litura* through topical bioassay method. Chlorpyrifos recorded LC_{50} value (0.035 ppm) which was followed by profenophos (0.037 ppm), deltamethrin (0.064 ppm), fenvalarate (0.067 ppm) and endosulfan (0.762 ppm). Overall *S. litura* showed highest susceptibility to chorpyrifos compared to endosulfon.

Kapoor *et al.* (2002) studied relative toxicity of different insecticides against third instar larvae of *H. armigera* using leaf disc residue technique and reported that the order of toxicity was deltamethrin > fenvalerate > chlorpyriphos > quinalphos > carbaryl > alphametrin > endosulfan. They compared with recommended dosages and suggested that about 2 to 112 fold decrease in susceptibility of the pest population to different insecticides was noticed. The decrease in susceptibility of *H. armigera* to synthetic pyrethroids was maximum. The result clearly indicated that *H. armigera* has developed resistance to synthetic pyrethroids compared to organophosphate molecules.

Kathuria *et al*. (2005) reported that cypermethrin was highly effective on *S. litura* which recorded lowest toxicity value (LC_{50}) of 178.6 ppb compared to conventional insecticide *Viz*, endosulfan, quinalphos and monocrotophos which recorded 484.1, 535.3 and 643.4 ppb LC_{50} values, respectively.

Ahmad *et al.* (2005) conducted bioassay of new insecticides *Viz;* emamectin benzoate, spinosad, lufenuron, indoxacarb and abamectin on tobacco caterpillar, *S. litura* through topical application. Among the new chemistries, emamectin benzoate recorded higher toxicity followed by lufenuron in a time oriented mortality and abamectin recorded low toxicity.

Thilangam (2006) conducted the bioassay of flubendiamide on major lepidopteran pest namely rice leaf folder *Cnaphalocoris medinalis* (Guea), spotted boll worm, *Earias vitella* (F) and fruit borer *Helicoverpa armigera* (Hub.). Among the lepidopteran pests *C. medinalis* was found highly susceptible to flubendiamide which recorded lowest LD_{50} value (0.0024 µg/gm) followed by *E. vitella* (0.0043 µg/gm) and *H. armigera* (0.001131 µg/gm). The calculated discriminating dose of flubendiamide was 0.70 ppm to *H. armigera.*

The LC_{50} value of flubendiamide was 4.75 ppm and was relatively more toxic to the third instar larvae of *S. litura* than the other insecticides tested. On the basis of LC_{50} values, the next best insecticides were emamectin benzoate (5.09 ppm), indoxacarb (7.86 ppm) and fipronil (136.48 ppm). Methoxyfenozide showed lowest acute toxic effect with a highest LC50 value (738.41 ppm). The order of relative toxicity of different insecticides after 24 hr of exposure to *S. litura* was flubendiamide (155.35 ppm) > emamectin benzoate (144.93 ppm) > indoxacarb (93.93 ppm) > fipronil (5.41 ppm) > methoxyfenozide (1.00 ppm) in laboratory conditions (Ghosh *et al*., 2007).

Kodandaram and Dhingra (2007) conducted the bioassay on five pyrethroids against third instar larvae of *S. litura* collected from two locations *viz;* Kapurthala and Delhi through topical application and leaf dip method. *S. litura* population collected from Kapurthala showed 20.74, 16.74, 15.46, 12.90 and 9.59 fold resistance to deltamethrin, fenvalrate, alpha cypermethrin, cypermethrin and beta cyfluthrin. While Delhi population showed 16.74, 11.50, 13.22, 21.18 and 4.83 fold resistance to deltamethrin, fenvalarate, alpha cypermethrin, and cypermethrin and beta cyfluthrin respectively.

Dhawan *et al.* (2007) studied the bioassay of novel insecticides on *S. litura* larvae using topical bioassay technique and reported that LC_{50} values of emamectin benzoate, novaluron, pyridalyl, flubendiamide, chlorantraniliprole and thiodiocarb were 0.0001, 0.0020, 0.0037, 0.0040, 0.0390 and 0.0410 per cent respectively. On the basis of LC_{50} values the order of toxicity of different insecticides was emamectin benzoate > novaluron > pyridalyl > flubendiamide > chlorantraniliprole > chlorpyriphos and thiodiocarb with respective toxicity of 390.0, 19.50, 10.54, 9.75, 8.86, 1.00 and 0.95 ppm respectively.

Toxicity of emamectin benzoate against *S. litura* by three different assay techniques *viz.*, leaf-dip, potter's tower and thin-film were done under laboratory condition. The study concluded that median lethal concentrations of emamectin benzoate 1.9 EC against five, seven and nine days old larvae were 0.00005, 0.00017 and 0.0007 per cent respectively (Ajanta *et al*., 2008).

Joshua *et al*. (2008) studied the susceptibility levels of rynaxypyr and cypermethrin against lepidopteran insect pest using adult vial test. The cypermethrin LD_{50} values of two pyrethroid susceptible colonies were 0.20 and 0.21 µg/g larval weight where as in rynaxypyr treated diet assay showed bollworm colonies had LC_{50} values ranging from 0.038 to 0.089 µg/ml of diet.

Adeel *et al*. (2011) studied the susceptibility of *S. litura* to four insecticides *Viz*, emamectine benzoate, spinosad, imidacloprid and profenofos through diet incorporation method. The calculated LC_{50} value at first generation was low in emamectine benzoate (1.59 ppm) which was followed by spinosad (7.77 ppm), profenofos (258.75 ppm) and imidacloprid (686.5 ppm) while there was drastic reduction in LC_{50} values when the same molecules were exposed to eleventh generations and the decreases in resistance was to the extent of 11.36, 11.11, 16.67 and 9.61 fold for imidacloprid, spinosad, emamectine benzoate and profenofos, respectively.

Qamar *et al*. (2012) evaluated that effectiveness of different insecticides against field populations of second instar larvae of *S. exigua* under laboratory conditions. Bioassays were performed through leaf dip method to arrive the dose and time mortality response for emamectine benzoate, lufenuron, chlorpyrifos and cypermethrin. Significant variation was revealed in lethal concentration and lethal time values. The order of effectiveness in terms of LT_{50} values was; cypermethrin > chlorpyriphos > emamectin benzoate > lufenuron.

Hong Tong *et al*. (2013) studied the the resistance in the populations of five districts of Hunan Province in China to various insecticides from 2010 to 2012 using a standard leaf dip bioassay method for organophosphates and pyrethroids. Resistance ratios were compared with a susceptible Lab-BJ strain. The result shown that the range of resistance ratio was 14 to 229 fold for organophosphate and 12 to 227 fold for pyrithroids, respectively. Similarly, relative low levels of resistance to emamectin, indoxacarb, and chlorfenapyr were observed in all the five populations. In contrast, the resistance to carbamates (thiodicarb or methomyl) was significantly higher than that of organophosphates, pyrethroids and new molecules.

Umair *et al*. (2013) Studied the insecticide resistance in *H. armigera* to conventional group of neuro-toxic insecticides such as endosulfan, profenofos, carbosulfan, deltamethrin, emamectin benzoate, abamectin, spinosad, lufenuron and methoxyfenozide. Two bioassay techniques i.e. residual method through leaf dip and topical method through micro-applicator were used for comparison. Low to moderate levels of resistance were recorded against these conventional and new chemistry insecticides at different locations. The results indicated the development of multiple resistances in the field populations of *H. armigera*. Resistance ratios in topical method were found higher as compared to the residual method.

2.2 To study the genetic relatedness among the *Spodoptera litura* population collected from different host crops using cytochrome oxidase subunit-I DNA-Marker

Mitochondrial genes well suited for tracing the history of population as well as for estimation of migration and gene flow. The mitochondrion is a haploid and maternally inherited in most insects (Avise, 1994)

Mun *et al*. (1999) studied the genetic variation among Asian populations of plant hoppers, *Nilaparvata lugens* (Stal) and *Sogetella furcifera* (Horvath) through mitochondrial DNA sequence. The study indicated that 71 individuals of plant hoppers collected from 11 locations of seven countries showed presence of different haplotypes. However, three haplotypes of *N. lugens* was found in all the localities and samples collected from Indonesian pinisular did not record any genetic variation within or between the population which proves the hypothesis that regular migration was uniform when sequenced through 850 bp region of mtDNA CO-I.

Genetic variability in *S. litua* population collected frem different locations of Tamilnadu. A total of 40 random primers were screened to reveal the existence of

polymorphism between the populations of *S. litura*. Among the 40 primers eight primers showed scorable banding pattern while three showed distinct banding pattern, overall the *S. litura* population collected from the Chenggalpattu and Chennai were closely related while population collected from Coimbatore were distinct (Janarthanan *et al.*, 2002).

Kavar *et al*. (2006) studied the variation of Southern green stink bug, *Nezara viridula* (Linnaeus) collected from 11 geographically separated location (Solvenia, France, Greece, Italy, Madeira, Japan, Guadeloupe, Galapagos, California, Brazil and Botswana) by sequencing 16s and 28s rDNA, cytochrome b and cytochrome c oxidase subunit I gene fragments and random amplified polymorphic DNA (RAPD) analysis. Sequencing revealed 11 distinct haplotypes clustering into lineages A, B and *C. lineages* C was characteristic for a single analysed specimen from Botswana, lineage B was detected from the Japan while lineage A was represented from Europe and America.

Subramanian and Mohankumar (2006) reported that the bollworm *H. armigera* collected from six host crops namely tomato, chilli, cotton, bhendi, pigeon pea and black gram, a total of 10 microsatellite simple sequence repeat (SSR) markers were used and out of ten SSR nine showed polymorphism of 75 to 100 per cent. The *H. armigera* population collected from tomato and bhendi were found to be closely related with a coefficient of 0.741, while the population from cotton and blackgram was found to differ widely with a coefficient value of 0.348. The dendrogram revealed the existence three principles clusters and a single sub clusters the population occurring on cotton stood out in a single cluster (A) while the population occurring on redgram and chilli grouped together in cluster B, and the population occurring on black gram, bhendi and tomato grouped together in cluster C.

Bajpai and Tewari (2010) studied the phylogenetic relationship among flesh flies of the genus Sarcophaga (Sarcophagidae: Diptera) through mitochondrial DNA sequence. The mitochondrial genes *Viz*, cytochrome oxidase-I and WAD dehydrogenase sub units (WD5), the five species of Sarcophaga analysed showed little genetic difference.

Saldamando and Arango (2010) studied the host plant association and genetic differentiation of corn and rice strains of false army worm *S. frugiperda* in Colombia. The 253 collected false army worm (FAW) larvae, a total of 143 individuals were genotyped as corn strain, and 49 individuals were genotyped as rice strain. Two types of hybrids were found the hybrids could be the product of bidirectional crosses between the strains, producing F1 generations, or backcrosses of F1 individuals to parentals, suggesting that interstrain

mating occurs easily in Tolima. Results show genetically differentiated FAW populations between the four crops (corn, cotton, sorghum and rice) with significant PhiPT values for both markers: PhiPT = 0.309 for the COI region and PhiPT = 0,168 for the FR marker.

Veeramani *et al*., (2012) studied the genetic relatedness of six South Indian agriculturally important moth species based on 28S rRNA-D2 region sequence. The average percentage of nucleotide composition among lepidopteran agricultural insect pests was A-21.85, T-20.95, C-27.68 and G-29.52%. The molecular phylogenetic tree derived out of multiple sequence alignment revealed three major clusters for the six different species of lepidopteran moths belonging to the family Noctuidae. One cluster consisted of *H. armigera* and *S. litura*. The second cluster contained with all hairy caterpillars including the red hairy caterpillar, *Amasacta albistriga* (Walker) hairy caterpillar, *Euproctis fraternal* (Moore) and woolly bear moth, *Pericallia ricini* (Fab.) while the third cluster had the spotted bollworm, *Earias vitella* (Fab.). At the superfamily level, all the six species were shown to be closely related to each other with similarity index range values of 0.85 to 0.93.

2.3 To study the digestive enzyme activity among the *Spodoptera litura* population collected from different host crops

The chemical structure and catalytic action of these enzymes are quite similar in all animals (Penzlin, 2003). However, the storage of digestive enzymes, the activation of enzymes, the site where the enzymes are released varies considerably between vertebrates and insects (Nation, 2002). The hormonal and neural regulation of digestive enzyme release in vertebrates is well understood, but not in insects (Penzlin, 1991).

Insect α-amylases (α-1,4-glucan-4-glucanohydrolases) are a group of glycoside hydrolases that are widely distributed in animal tissues, which catalyse the hydrolysis of the α-(1,4) glycosidic linkage found in starch, glycogen and other polysaccharides. These enzymes are well adapted to the slightly acidic to neutral conditions found in the more anterior regions of the midgut in many insects. The central calcium ion stabilises the 3-dimensional structure of α-amylase and protects it against digestion by proteases (Stein and Fischer, 1958).

Baker (1982) revealed that the hydrolysis of two general proteinase substrates and seven substrates specific for trypsin and chymotrypsin, aminopeptidases, and carboxy peptidases were demonstrated in adults of *Sitophilusoryzae* (L.), *Sitophilus*. *Zeamais*

(Motchulsky), and *Sitophilus granarius* (L.). Endopeptidase activity was very low in all species, whereas amino peptidases and carboxy peptidases were relatively active. Hydrolysis of α-*N*-benzoyl-DL-arginine-*p*-nitroanilide (BApNA) by larvae and adults of *S. granarius* was due to a single enzyme, inhibited by *N*-α-tosyl-L-lysinechloromethyl ketone (TLCK).

Mahammad (1982) studied the digestive enzymes of *Chrotogomus trachypterus* Blanch. *Acheta domestica* (L.) and *Periplanata americana* (L.). Proteinase could not be detected in *C. trachypterus* and other two insect *Viz*., *A. domestica* and *P. americana* trypsin and pepsin were recorded both from fore and hind gut. Quantitative estimation of trypsin showed a higher activity of the enzyme in mid-gut (26.1 ±3.09) than in the fore-gut (16.6 ±3.19) of *A. domestica* while in *P. americana* there was no significant difference in the activity of the trypsin in the fore-gut (22.75 ±3.30). However, activity of the enzyme in each gut counter part of the insects, did not show any significant difference from one another.

In false army worm, *S. frugiperda* trypsin and α-amylase are secreted into the ectoperitrophic space and diffuse into the endoperitrophic space, whereas aminopeptidase and disaccharidases are secreted into the ectoperitrophic space and remain attached to the membrane complex of the gut epithelium. Aminopeptidase is both found to the microvillar membranes and present as a soluble enzyme trapped in the cell glycocalyx of the ventriculus (Klinkowstrom *et al*., 1994).

The total lipase activity of *S. frugiperda* is low, but the lumen fraction of the total is relatively high (Lwalaba *et al*., 2009) and it is the first report of high lipase activitiy in the gut lumen of Lepidoptera larvae. Based on studies with radiolabelled trioleine, dietary triacylglycerol in *Manduca sexta (L.)* larvae is completely hydrolyzed to 22 free fatty acids in the lumen before absorption into the cells (Tsuchida and Wells, 1988). Finally they suggest, that because lipase and amylase have similar molecular weights (50-60 kD) it would be expected that soluble lipase also passes into the endoperitrophic space at the anterior end of the ventriculus. Amylase is secreted at the anterior end of the ventriculus and passes through the pores of the peritrophic membrane into the endoperitrophic space (Ferreira *et al*., 1994 a and b).

Yu-Cheng Zhu and James., 1999, studied the protein digestion in the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) complex of serine proteinases present in the midgut. In this study partially characterized trypsin-like enzyme activity

against N-a-benzoyl-l-arginine *p*-nitroanilide (BA*p*NA) in midgut preparations and cloned and sequenced three cDNAs for trypsinogen like proteins. BA*p*NAase activity in *R. dominica* midgut was significantly reduced by serine proteinase inhibitors and specific inhibitors of trypsin, whereas BA*p*NAase activity was not sensitive to specific inhibitors of chymotrypsin or aspartic proteinases. However, trans epoxysuccinyl-l-leucylamido-(4-guanidino) butane (E-64) inhibited BA*p*NAase activity by about 30 per cent. BA*p*NAase was most active in a broad pH range from about pH 7 to 9.5. The gut of *R. dominica* is a tubular tract approximately 2.5 mm in length. BA*p*NAase activity was primarily located in the midgut region with about 1.5-fold more BA*p*NAase activity in the anterior region compared to that in the posterior region.

Mohammadi *et al,* (2010) studied the protease activity of cotton bollworm, *H. armigera* and beet army worm, *S. exigua*. Insects were reared on special artificial diets in controlled conditions. Different developmental stages including, larval stages, 5-6 inter-moult period, pre-pupa, male and female pupa and adult's protease activity measured using azocaseionolytic assay method. Specific protease activity in 4^{th} larval stages of both studied insects was more than other developmental stages. Protease activity in male and female pupa and adults of *S. exigua* was not significantly different, but in *H. armigera* male pupa and female adults have significantly more protease activity than other related sexes.

Tierno *et al*. (2011) revealed that digestive enzymes of two stoneflies species, *Hemimelaena flaviventris* (Pictet.) and *Isoperla morenica* (Tierno) were studied for the first time. The enzymatic activities of digestive amylase, lipase, protease, trypsin and chymotrypsin were determined for each species at the same developmental stage. The results showed that *H. Flaviventris* has a greater digestive enzymatic pool and higher relative and absolute protease, lipase and trypsin activities than *I. morenica.* The latter has a relative higher amylase activity. As higher amylase activity is typical of phytophagous species and higher protease activity typical of carnivorous species; these results revealed that *H. flaviventris* is a more efficient zoophagous species than *I. morenica*.

Eslam *et al*. (2013) studied the response of metal salts like potassium phosphate, magnesium nitrate and NaCl. Buffers like EDTA, Tris, SDS and ethanol on midgut lipase activity of greater wax moth, *Galleria mellonella* (Fab.) which is an ideal insect to study the physiology. The study indicated that lipase activity was reduced by 75 per cent in ethanol while, in magnesium nitrate, SDS, NaCl and potassium phosphate the activity of lipase was increased.

Arash and Mahamadi *et al*. (2012) studied the biochemical purification and characterization of a digestive lipase from larval midgut of *Naranga aenescens* Moore (Lepidoptera: Noctuidae) in the larval instars, lipase showed the highest activity in the third instar larvae comparing with other two instars. After final purification, the enzyme was purified 12.28-fold with a recovery of 8.21 per cent and a specific activity of 5.6 μmol/min/mg protein as well as a molecular weight approximately 51 kDa. Results of the biochemical characterization showed the purified lipase had the highest activity at pH 10 and temperature 35-40 °C as well as stability for 24 hours in the optimal pH and 6 hours in the mentioned temperatures. Different cations like Ca2+, Mg2+, Na+ and K+ increased the enzyme activity and some of the synthetic inhibitors had a significant reduction on the enzyme activity.

Mahbobe *et al*. (2012) studied the insect digestive enzyme of rosaceous branch borer, *Osphranteria coerulescens*, (Redtenbacher.) were assayed for protease activity in the presence of protease inhibitors in order to determine the dominant protease types. The maximum total proteolytic activity in the midgut extract using azocasein as substrate was observed at pH 8, suggesting the presence of serine proteases. Also, the highest activity using azocasein as substrate was revealed at temperature 55°C. The presence of serine proteases was confirmed remarkable inhibition due to specific inhibitor.

Gholamzadeh *et al*. (2013*)* studied the biochemical properties of proteases from the digestive system of the fig tree skeletonizer moth, *Choreutis nemorana* (Hub.) were determined. Gut extracts of *C. nemorana* larvae were analysed using different specific peptide substrates and proteinase inhibitors. The optimal pH and temperature for proteolytic activities using azocasein as substrate were obtained as pH 11 and 45 °C, respectively. In the case of *N*-benzoyl-l-arg-*p*-nitroanilide as substrate, the enzyme showed the maximum tryptic activity at pH 11. The kinetic parameters of trypsin-like proteases indicated that the *Km* and *V*max values of trypsin in the gut of *C. nemorana* were 0.157 ± 0.006mM and 0.188 ± 0.005 μmol/min/mg protein.

Oluwakemi *et al*. (2014) studied the male and female cockroaches, *Periplaneta americana* (L.) were assayed for the presence of digestive enzymes in the fore-, mid- and hindgut regions. Activities of α-amylase, β-amylase, γ-amylase, proteinase and lipase were detected in the three gut regions except for the absence of γ-amylase and lipase in female hindgut. The presence of these enzymes partly explains the polyphagous feeding habit of *P*.

americana, enabling the insect species to digest a wide variety of food substances. In some instances, significant differences in enzyme activities were observed between sexes and among gut regions. Generally, enzyme activity was highest in the midgut followed by the fore- and hindgut in descending order. Despite this trend, a considerable level of proteinase and male lipase was observed in the hindgut suggesting that it might be necessary to give extra attention to hindgut activities in future studies.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

The details of materials and methods adopted in the course of investigations are presented here under. All the experiments were carried out at the Department of Agricultural Entomology, College of Agriculture and Main Agricultural Research Station, Raichur during 2013-14.

3.1 To study the insecticide resistance in *Spodoptera litura* collected from different host crops to selected insecticides

Nine commercially available insecticides were used for bioassay on population collected *S. litura* collected from different host crops.

3.1.1 Maintenance of pure culture of *S. litura* on various crops

Spodoptera litura larvae collected from different host crops were reared on respective host crops which were maintained at laboratory of Department of Agricultural Entomology and Main Agricultural Research Station, Raichur during the study period 2013-14. Pupae were kept separately for moth emergence in a plastic jar of size 15 cm diameter where in the pupae were placed on a moist sand. To facilitate egg laying fresh host plant leaves were kept within the plastic jar and 10 per cent honey solution with yeast was provided as a adult food and each plastic jar was covered with muslin cloth. Eggs collected from each host crops were sterilized with one per cent sodium hypochlorite solution (4 % available chlorine added in 250 ml concentration sodium hypochlorite and made volume to 1000 ml of distilled water) to avoid any entomopathogenic contamination. After sterilization, eggs of *S. litura* collected from respective host crops were kept for incubation in a BOD incubator.

Neonate larvae of *S. litura* from different host crops were reared separately in a bread box 18 X 11 cm size and the fresh leaves of different host crops were provided and in each bread box it was lined with moist foam to maintain the turgidity of the leaves. Later instar larvae such as second and third were reared separately on different host crops in a plastic basin of 30 cm size diameter and the basin were covered with muslin cloth. The different host crops leaves were changed daily and the muslin cloth was moistened by sprinkling water with water atomizer regularly.

3.1.2 Bioassay of various insecticides on *S. litura*

Total nine insecticides representing various group of insecticides were selected and commercial formulation of insecticide namely, indoxacarb, flubendiamide, thiodicarb, cypermethrin, novaluron, spinosad, emamectin benzoate, quinalphos and chlorpyrifos were used for bioassay on third instar larvae of *S. ltura.*

Primary stock solutions of all insecticides were calculated and bracketing was done to arrive the different concentration of different insecticides on third instar larvae of *S. litura.* For each insecticide bioassay was done as detailed below (Plate 1).

3.1.2.1 Bioassay of indoxacarb

Different concentrations of 4.50, 3.50, 2.50, 1.50, 0.50 ppm of indoxacarb was prepared and compared with untreated control. The each concentration thirty, third instar larvae of uniform age were used for leaf dip bioassay technique. Leaves of respective host crops were collected from the field, washed and dipped into the insecticide solutions, for ten seconds, with the gentle agitation and shade dried on the tissue papers, such leaves were placed in the bread box of 18 X 11 cm size and then ten larvae per box were released and was covered with muslin cloth. Mortality was recorded at 72 hrs after exposure.

3.1.2.2 Bioassay of flubendiamide

Different concentrations of 6.00, 5.00, 4.00, 3.00 and 2.00 ppm of flubendiamide was prepared and compared with untreated control. The each concentration thirty, third instar larvae of uniform age were used for leaf dip bioassay technique. Leaves of respective host crops were collected from the field, washed and dipped into the insecticide solutions, for ten seconds, with the gentle agitation and shade dried on the tissue papers, such leaves were placed in the bread box of 18 X 11 cm size and then ten larvae per box were released and was covered with muslin cloth. Mortality was recorded at 72 hrs after exposure.

3.1.2.3 Bioassay of quinalphos

Different concentrations of 55.00, 45.00, 35.00, 25.00 and 15.00 ppm of quinalphos was prepared and compared with untreated control. The each concentration thirty, third instar larvae of uniform age were used for leaf dip bioassay technique. Leaves of respective host crops were collected from the field, washed and dipped into the insecticide solutions, for ten seconds, with the gentle agitation and shade dried on the tissue papers, such leaves were placed in the bread box of 18 X 11 cm size and then ten larvae per box were released and was covered with muslin cloth. Mortality was recorded at 72 hrs after exposure.

Preparation of concentration

Experimental setup for bioassay studies

Rearing of *S. litura* larvae

Plate 1. Experimental set up for bioassay of *S. litura* collected from major host crops

3.1.2.4 Bioassay of novaluron

Different concentrations of 10.00, 8.00, 6.00, 4.00 and 2.00 ppm of novaluron was prepared and compared with untreated control. The each concentration thirty, third instar larvae of uniform age were used for leaf dip bioassay technique. Leaves of respective host crops were collected from the field, washed and dipped into the insecticide solutions, for ten seconds, with the gentle agitation and shade dried on the tissue papers, such leaves were placed in the bread box of 18 X 11 cm size and then ten larvae per box were released and was covered with muslin cloth. Mortality was recorded at 92 hrs after exposure.

3.1.2.5 Bioassay of spinosad

Different concentrations of 15.00, 12.00, 9.00, 6.00 and 3.00 ppm of spinosad was prepared and compared with untreated control. The each concentration thirty, third instar larvae of uniform age were used for leaf dip bioassay technique. Leaves of respective host crops were collected from the field, washed and dipped into the insecticide solutions, for ten seconds, with the gentle agitation and shade dried on the tissue papers, such leaves were placed in the bread box of 18 X 11 cm size and then ten larvae per box were released and was covered with muslin cloth. Mortality was recorded at 72 hrs after exposure.

3.1.2.6 Bioassay of emamectin benzoate

Different concentrations of 1.50, 1.20, 0.90, 0.60 and 0.30 ppm of emamectin benzoate was prepared and compared with untreated control. The each concentration thirty, third instar larvae of uniform age were used for leaf dip bioassay technique. Leaves of respective host crops were collected from the field, washed and dipped into the insecticide solutions, for ten seconds, with the gentle agitation and shade dried on the tissue papers, such leaves were placed in the bread box of 18 X 11 cm size and then ten larvae per box were released and was covered with muslin cloth. Mortality was recorded at 72 hrs after exposure.

3.1.2.7 Bioassay of thiodicarb

Different concentrations of 50.00, 40.00, 30.00, 20.00 and 10.00 ppm of thiodicarb was prepared and compared with untreated control. The each concentration thirty, third instar larvae of uniform age were used for leaf dip bioassay technique. Leaves of respective host crops were collected from the field, washed and dipped into the insecticide solutions,

for ten seconds, with the gentle agitation and shade dried on the tissue papers, such leaves were placed in the bread box of 18 X 11 cm size and then ten larvae per box were released and was covered with muslin cloth. Mortality was recorded at 72 hrs after exposure.

3.1.2.8 Bioassay of cypermethrin

Different concentrations of 60.00, 50.00, 40.00, 30.00 and 20.00 ppm of cypermethrin was prepared and compared with untreated control. The each concentration thirty, third instar larvae of uniform age were used for leaf dip bioassay technique. Leaves of respective host crops were collected from the field, washed and dipped into the insecticide solutions, for ten seconds, with the gentle agitation and shade dried on the tissue papers, such leaves were placed in the bread box of 18 X 11 cm size and then ten larvae per box were released and was covered with muslin cloth. Mortality was recorded at 72 hrs after exposure.

3.1.2.9 Bioassay of chlorpyrifos

Different concentrations of 500, 400, 300, 200, 100 ppm of chlorpyrifos was prepared and compared with untreated control. The each concentration thirty, third instar larvae of uniform age were used for leaf dip bioassay technique. Leaves of respective host crops were collected from the field, washed and dipped into the insecticide solutions, for ten seconds, with the gentle agitation and shade dried on the tissue papers, such leaves were placed in the bread box of 18 X 11 cm size and then ten larvae per box were released and was covered with muslin cloth. Mortality was recorded at 72 hrs after exposure.

3.2 To study the genetic relatedness among the *Spodoptera litura* population collected from different host crops using cytochrome oxidase subunit-I DNA-Marker

S. litura population were collected from eight different hosts *Viz*., cotton, chilli, castor, groundnut, cabbage, soybean, sunflower and onion at peak infestation levels during 2013-14 season and were reared on respective host crops in the laboratory till they reach final instar stage. Thus collected larvae were used for diversity analysis studies.

3.2.1 Genomic DNA isolation

Twenty field collected healthy well grown 5th instar larvae were randomly collected in 70 % alcohol from each host crops. Samples were brought to laboratory and kept at -20^0 c until the isolation of DNA was done. At the time of isolation of total DNA, each larva was dissected and gut contents were completely removed to avoid any contamination of DNA by the food material it eats. Resulting skin and legs were used to prepare total DNA as procedure described by Doyle and Doyle, 1998 with suitable modification (Plate 3).

3.2.2 Solutions

CTAB extraction buffer

0.1M Tris-HCL (pH 8.0)

1.4mM Nacl

0.02 M EDTA

4% CTAB

0.2 % β –mercaptoethanol

3.2.3 Equipment

Pestle and porter

Refrigerated centrifuge (Beckman GPR, Beckman G2-21)

Centrifuge tubes 50 ml

Micro Centrifuge tubes 1.5 ml

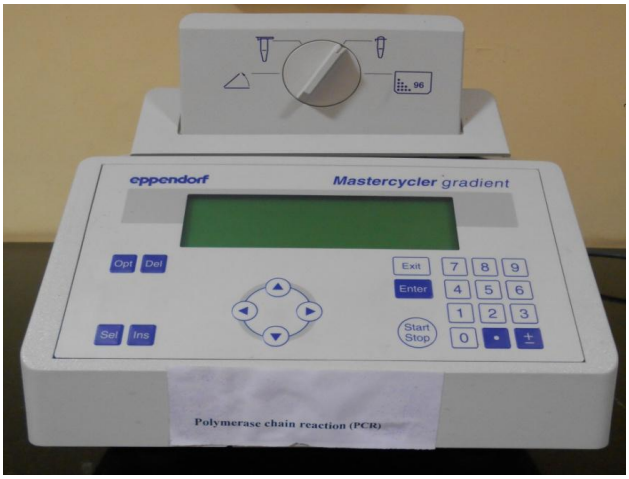
Thermostat 37 ^{0}C

Water bath 60 ^{0}C and -20^0 C freezer

3.2.4 Genomic DNA extraction protocol

The larvae stored in alcohol were removed and kept on tissue paper for one minute. The larva was dipped in liquid nitrogen and immediately in to 1.5 ml micro centrifuge tubes. The larvae were grinded individually using micro pestle in micro centrifuge tubes containing 100 µl of preheated CTAB extraction buffer and incubated at 65^0 C for 30-45 min with occasional mixing. After incubation, the tubes were cooled to room temperature and equal volume of chloroform: isoamyl mixture (24: 1) was add and mixed by inversion for 15 min. the suspension was centrifuge at 8000 rpm for 30 min at 40 ^{0}C. The supernatant was

transferred to fresh tube and equal (0.7 ml) volume of ice-cold isopropanol was added and mixed gently by inversion and kept at -20 ^{0}C overnight for DNA precipitation. The clear aqueous phase was transferred to a new micro centrifuge tube and the DNA pellet was separated from aqueous phase by brief centrifugation and the pellet was air dried. Then the DNA was dissolved in 100-150 µl of $T_{10}E_1$ (10 mM Tris 1mM EDTA) buffer (Plate 2).



Polymerase chain reaction (PCR)

Insect tissue homogenizer

Spectrophotometer

Plate2. Equipments used for genetic relatedness and digestive enzyme of *S. litura* collected from major host crops

3.2.4 Purification of genomic DNA

The genomic DNA isolated was purified according to the protocol described by Maniatis *et al.* (1982) to eliminate contaminating RNA, RNase (10 µl / 100 µl) was added to DNA and incubated at 37^0 C for 30 min by adding equal volume of chloroform: isoamyl alcohol (100 µl) and mixed thoroughly by repeated inversions. The mixture was centrifuged at 8000 rpm for 10 min at 40^0 C, and the aqueous phase was transferred to another micro centrifuge to which two volumes of absolute alcohol was added and incubated at -20^0 C overnight. DNA was washed pellet by brief centrifugation and the supernant was discarded. The pellet was washed with 70 % alcohol and centrifuge at 8000 rpm for 5 min at 4^0 C, the alcohol was discarded and the DNA pellet was air dried completely. Depending upon the size of the pellet, DNA was dissolved in 25-50 µl of Tris-EDTA and stored at 4^0 C. In order to make the better representation of each location equal amount of DNA from0 each of 5 larvae for each location was pooled and the resulting 14 bulked DNA samples were used for PCR reaction. The concentration of DNA was measured using Nano Drop® ND-1000 spectrophotometer (Nanidrop Technologies, Wilmington, DE), and the equality was checked by 0.8 % agarose gel electrophoresis before being used as a template in PCR.

3.2.5 Quantification of genomic DNA

The genomic DNA isolated was quantified by ethidium bromide fluorescent quantification method described by Maniatis *et al*., 1982. The band intensity of isolated DNA in agarose dissolved in 40 ml of 1X TAE buffer containing ethidium bromide @ 0.5 µg per ml was compared with the band intensity of reference λ- DNA Hind III digest.

The quality and concentration of DNA was assessed by a spectrophotometer and also by the gel electrophoresis using 0.8 per cent agarose with unknow concentration of uncut λ- DNA

$$\text{DNA (µg / µl)} = \frac{\text{OD260 x 50 (dilution factor) x 50 µl /ml}}{1000}$$

OD260/OD280 ratio was used to assed the purity of DNA. A ratio of 1.6 of less indicated that may be protein and / or other UV absorbance in the samples whereas ratio higher than the 2.0 indicated that sample may be contaminated with chloroform of phenol.

3.2.5 PCR amplification

The mitochondrial cytochrome oxidase sub unit I gene (850 bp) was amplified from eight host strains using the primer pair (5′-TTGATTTTTTGGTCATCCAGAAGT-3´) and (5′-TCCAATGCACTA ATCTGCCATATTA-3’) as described by (Simon *et al*., 2006).

Reaction of 30 µl total volume contained 50 mM KCl, 10 mM Tris-Hcl. pH 8.3.1.5mm$MgCl_2$, 200 mol dNTP, 0.2 mol of ech primer, 1U of Taq DNA polymerase (M/S Bangalore Genei Pvt. Ltd., Bangalore), and 50 to 100 ng genomic DNA. Touchdown thermal cycling programs encompassing 5^0 C span of annealing temperatures at 50-55^0 C were also used for the amplification in a thermal cycler (Bio-Rad, Hercules, CA). after an initial denaturation at 94^0 C for 4 min, cycling parameters were 10 cycles of 94^0 C for 20 s, highest anneling temperature (decreased 0.5^0 C per cycle) for 30 s, and 72^0 C for 30 s, and 30 cycles of 94^0 C for 20 s, 50^0 for 30 s, and 72^0 C for 30 s. this was followed by an extension step at 72^0 C for 5 min (Plate 4).

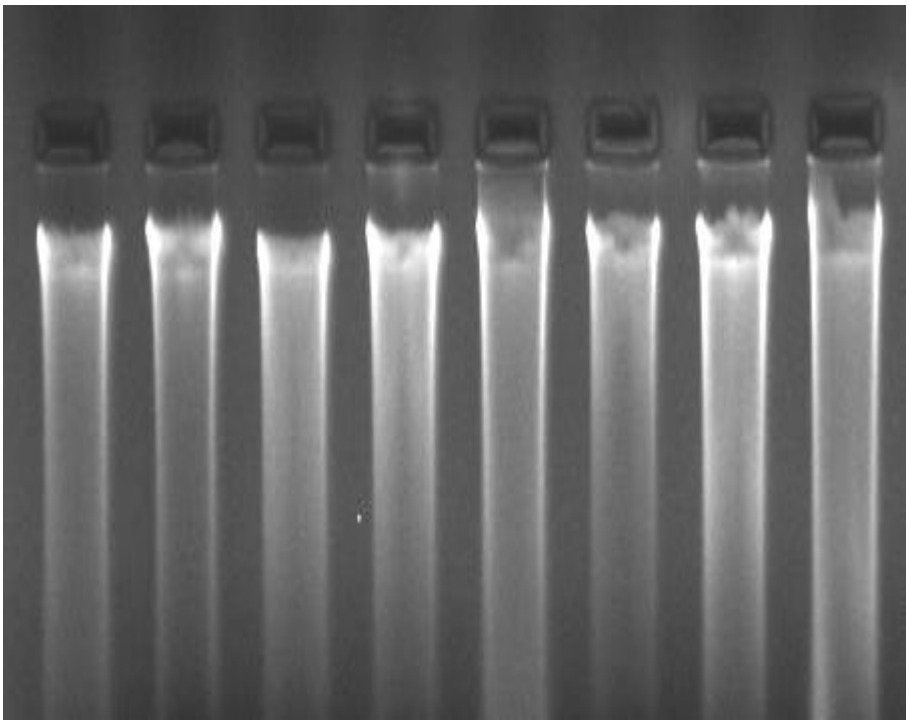


Plate 3. Genomic DNA extracted from *S. litura* collected from major host crops

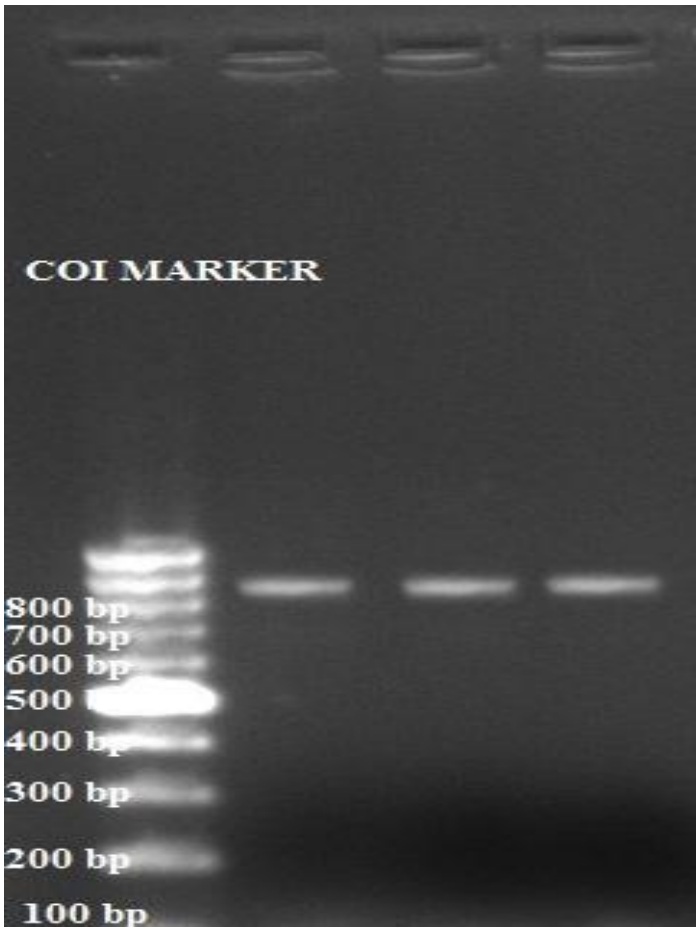


Plate 4. PCR amplification of Cytochrome oxidase-I DNA marker at 850 bp

3.2.6 Cloning, sequencing and phylogenetic analysis

Electrophoresis of PCR product was performed in 1.5 per cent agarose gel containing ethidium bromide solution (10 mg / ml) along with 100 bp DNA ladder and amplicon product were measured using gel documentation unit. Following PCR amplification, the resulting amplicon of about 850 bp of mitochondrial COI gene were purified using the spin column PCR product purification kit according to manufactures specifications. The purified PCR product were then inserted into the pGEM-T Esay vector system (Promega) and transferred into competent *E. coli* strain DH5α by following manufactures instructions. Plasmid DNA was isolate and the presence of the insert was confirmed by restriction digestion of plasmid DNA with E *co*RI restriction enzyme (Fermentas life sciences canada), clones of inserted were bi- directionally sequenced with the amplification primers commercially (Xcelris Labs Ltd, Ahmadabad, India). All the sequences were confirmed with NCBI BLAST database of the identification of the isolates

based on previously published database sequences online multiple sequence alignment tools (www. Genomic. Jp/tools/clustal w) were used to construct the phylogenetic tree using UPGMA-NJ method.

3.2.7 Data analysis

The evolutionary history using the Neighbour-Joining method (Saitou and Nei, 1987) and optimal tree with the sum of branch length = 0.08093345 is shown. The percentage of replicate tree in which the association taxa clustered together in the bootstrap test (1000 replicates are shown) next to branches (Felsenstein, 1985). The tree is drawn to scale with branch length in the same unit as those of the evolutionary distance used to infer the phylogenetic tree. The evolutionary distance were computed using the Poisson correction method (Zuckerkandl and Pauling, 1965) and are in the units of the number of amino acid substitution per site. The analysis involved 16 amino acid sequences. All ambiguous position were removed for each sequence pair. These were a total of 210 position in the final dataset. Evolutionary analyses were conducted in MEGA 6 (Tamura *et al*., 2013).

3.3. To study the digestive enzyme activity among the *Spodoptera litura* population collected from different host crops

Spodoptera larvae were collected from different ecosystems like Groundnut, Castor, Sunflower, Soybean, Onion, Chilli and Cabbage at approximately same developmental stage. Samples were immediately placed in liquid nitrogen and stored at -80º C until analysis.

3.3.1 Samples preparation

Spodoptera larvae were homogenized on ice in ice-cold buffer respective enzymes with an electric homoginiser and homogenates were spun at 10,000 rpm for 10 min at 4ºC and supertant was used for protein estimation and for enzyme assays.

3.3.2 Protein estimation

3.3.3. Reagents

1. Alkaline copper reagent

2. Solution A- Sodium carbonate (2 %) in 0.1 N NAOH

3. Solution B – Sodium potassium tartrate (1 %)

4. Solution C – Copper sulfate (0.5 %)

5**.** Mixed solutions A, B and C in 100 : 1 : 1 proportion just bifore use

3.3.4 Protein satndard Preparation

Dissolved 50mg of bovine serum albumin in distilled water and volume was made up to 50ml in a volumetric flask. 10ml of this solution was made upto 100ml with distilled water in a volumetric flask which contains 100mg of protein per ml.

3.3.5 Folin- Ciocalted Reagent (FCR), 1N

S. litura larvae were homogenized and homogenate was centrifuged at 10,000 rpm for 10 minutes and supernatant was collected and 10 µl of homogenate sample was added to 490 µl of distilled water and incubated with 2.5 ml alkaline copper sulfate solution for 10 minutes at room temperature. Then 250 µl of Folin's reagent (1:1 diluted with water) was added and the color was read after 30 min at 670 nm. Bovine serum albumin (BSA) was used as the standard.

3.3.6 Trypsin activity

Trypsin activity was spectrophotometrically assayed following the method of Faulk *et al*. (2007) homogenised sample of *S. litura* (10 µL) was used for the assay, with the substrate N-benzoyl-DL-arginine-4-nitroanilide (BAPNA) 1.5 µL was added (43.7 mg of substrate was dissolved in 5 ml DMF solution), 50 µL of Tris–HCl (50 mM) and 20 µL of $CaCl_2$ (20 mM, pH 8.2) were incubated for one hour at 37 ºC in a water bath and absorbance was measured at 410 nm.

3.3.7 Chymotrypsin activity

Chymotrypsin activity was assayed following the method of Faulk *et al*. (2007), homogenized sample of *S. litura* (10 µL) was used for the assay, with the substrate Benzoyl-L-tyrosine ethyl ester (BTEE) (0.566 mM) dissolved in double distilled water and 1.698 µL taken for assay and added 28 µL of Tris–HCl, 7.5 µL of NaCl (25 mM), mixed and maintained the pH 7.8 and incubated for one hour at 37 ºC in a water bath and absorbance was recorded at 256 nm and 37 °C.

3.3.8 Lipase activity

Lipase activity was measured following the method of Faulk *et al*. (2007), homogenized sample of *S. litura* (10 µL) was used for the assay, with the substrate 4-

nitrophenyloctanoate as substrate were dissolved in Tris HCl and added 20 µL (0.35 mM) and maintained pH 7.4 buffer 0.5 mM, and with these 40 µL sodium taurocholic acid (TA) (6 mM), 10 µL NaCl (1 M) and incubated for one hour at 37º C and absorbance was read at 400 nm.

3.3.9 Alpha- Amylase activity:

The assay method described by Fuwa (1994) was followed with slight modification to 0.2 ml 1% (w/v) soluble starch solution was added 0.1ml of enzyme solution with mixing and 0.7 ml of distilled water. The mixture was incubated at room temperature for 30 minute in water bath. The reaction was terminated by the addition of 1 ml Dinitrosalicylic acid (DNSA) and mixed thoroughly; 0.1 ml of iodine mixture was added to the mixture. The optical densities of both blank and experimental sample were read at 670 nm in the spectrophotometer (Plate 2).

EXPERIMENTAL

RESULTS

IV. EXPERIMENTAL RESULTS

The present investigations on bioassay, genetic relatedness and digestive enzyme of *Spodoptera litura* (Fab) was carried out at Department of Agricultural Entomology, College of Agriculture Raichur and Main Agricultural Research Station, Raichur during 2013-14 are presented bellow.

4.1 To study the insecticide resistance in *S. litura* collected from different host crops to selected insecticides

4.1.1 Bioassay of Indoxacarb 14.5 % SC on *S. litura* collected from major host crops.

Maximum LC_{50} value was observed in population collected from the cabbage host crop (2.50 ppm), followed by chilli (2.47 ppm). Where in population collected from ground nut was (2.33 ppm), in soybean (2.19 ppm). Similarly the population collected from the castor the LC_{50} values was (2.08 ppm), followed by onion (1.98 ppm). Out of seven host crops *S. litura* population collected from sunflower showed lowest LC_{50} value was (1.89 ppm) (Table 1).

Table.1 Bioassay of indoxacarb 14.5 % SC on *Spodoptera litura* collected from major host crops.

Crops	LC_{50} (ppm)	Fiducial limit (95%)		χ^2 P= 0.05	Regression equation (slope)
		LL (ppm)	UL (ppm)		
Castor	2.08	0.86	5.62	0.54	0.79 + 4.74X
Groundnut	2.33	0.91	13.35	0.44	0.71 + 4.73X
Chilli	2.47	1.01	22.80	0.35	0.70 + 4.72X
Sunflower	1.89	0.85	3.77	0.82	0.88 + 4.75X
Soybean	2.19	0.88	7.77	0.40	0.75 + 4.75X
Cabbage	2.50	0.92	64.80	0.29	0.66 + 4.73X
Onion	1.98	0.87	4.37	0.59	0.84 + 4.74X

4.1.2 Bioassay of Flubendiamide 39.35 % SC on *S. litura* collected from major host crops.

The maximum LC_{50} value in the population of *S. litura* collected from chilli (3.29 ppm) followed by soybean and cabbage which recorded 2.94 and 2.90 ppm respectively. The LC_{50} value in the population collected from onion was 2.86 ppm, in ground nut was 2.82 ppm and the correspondence lower and upper values was 1.99 to 3.41 respectively at 95 per cent. The LC_{50} value of in sunflower was 2.81 ppm and a correspondence lower and upper value was 2.0 to 3.37 and. The lowest LC_{50} value of 2.66 ppm was with lower and upper values was 1.78 and 3.25 was noticed in castor (Table 2).

Table.2 Bioassay of flubendiamide 39.35 % SC on *Spodoptera litura* collected from major host crops.

Crops	LC_{50} (ppm)	Fiducial limit (95%)		χ^2 P= 0.05	Regression equation (slope)
		LL (ppm)	UL (ppm)		
Castor	2.66	1.78	3.25	2.92	2.53 + 3.92X
Groundnut	2.82	1.99	3.41	1.87	2.59 + 3.82X
Chilli	3.29	2.58	3.94	2.19	2.73 + 3.58X
Sunflower	2.81	2.05	3.37	2.91	3.75 + 2.76X
Soybean	2.94	2.10	3.57	3.30	2.51 + 3.82X
Cabbage	2.90	2.06	3.51	2.61	2.53 + 3.82X
Onion	2.86	2.03	3.46	2.13	2.56 + 3.82X

4.1.3 Bioassay of Quinalphos 25 % EC on *S. litura* collected from major host crops.

Maximum LC_{50} value of 35.10 ppm was found in larval population of *S. litura* collected from chilli which was followed by cabbage (34.64 ppm). Population collected from onion the LC_{50} value recorded of 31.86 ppm. Where in sunflower LC_{50} value was 30.40 ppm. *S. litura* collected from castor The LC_{50} value of 29.77 ppm (20.60 to 39.99). Where in soybean LC_{50} value was (29.19 ppm) and correspondence fiducial limits of lower and upper values ranged from 20.40 to 38.47 respectively at 95 per cent with calculated chi squire value

of heterogeneity was 0.79. The least LC_{50} value (28.28 ppm) was found in population collected from ground nut (Table 3).

Table.3 Bioassay of quinalphos 25 % EC on *Spodoptera litura* collected from major host crops.

Crops	LC_{50} (ppm)	Fiducial limit (95%)		χ^2 P= 0.05	Regression equation (slope)
		LL (ppm)	UL (ppm)		
Castor	29.77	20.60	39.99	0.91	1.79 + 2.35X
Groundnut	28.28	20.60	35.82	1.91	2.09 + 1.96X
Chilli	35.10	20.64	91.78	0.29	1.22 + 3.10X
Sunflower	30.40	20.83	41.83	1.21	1.72 + 2.44X
Soybean	29.19	20.40	38.47	0.79	1.86 + 2.27X
Cabbage	34.64	20.50	90.71	0.50	0.91 + 3.58X
Onion	31.86	21.02	47.87	1.10	1.54 + 2.07X

4.1.4 Bioassay of Novaluron 10 % EC on *S. litura* collected from major host crops.

Novaluron at 96 hrs after treatment showed maximum LC_{50} value in chilli it was 9.94 ppm, followed by cabbage (9.18 ppm) with correspondence fiducial limits of lower and upper values ranged from 5.49 to 8.38 respectively at 95 per cent. The calculated chi squire value of heterogeneity was 0.53. Where in Ground nut LC_{50} value was 7.86 ppm (5.06 to 43.33). In onion LC_{50} value was 7.39 ppm and correspondence fiducial limits of lower and upper values ranged from 4.89 to 23.83 respectively at 95 per cent with the calculated chi squire value of heterogeneity was 3.18, followed by castor (6.68 ppm). In soybean LC_{50} value was (6.42 ppm). Among all seven host crop population the lowest LC_{50} value was observed in sunflower 6.40 ppm fiducial limits of lower and upper values ranged from 4.49 to 11.26 (Table 4).

Table.4 Bioassay of novaluron 10 % EC on *Spodoptera litura* collected from major host crops.

Crops	LC_{50} (ppm)	Fiducial limit (95%)		χ^2 P= 0.05	Regression equation (slope)
		LL (ppm)	UL (ppm)		
Castor	6.68	4.60	13.28	3.18	1.32 + 3.49X
Groundnut	7.86	5.06	43.33	1.13	1.07 + 4.03X
Chilli	9.94	5.73	50.37	0.44	0.86 + 4.13X
Sunflower	6.40	4.49	11.26	4.18	1.40 + 3.86X
Soybean	6.42	4.51	11.30	4.19	1.41 + 3.90X
Cabbage	9.18	5.49	8.38	0.53	0.91 + 4.11X
Onion	7.39	4.89	23.83	1.66	1.15 + 3.99X

4.1.5 Bioassay of Spinosad 45 % SC on *S. litura* collected from major host crops.

The maximum LC_{50} value was observed population collected from chilli 10.50 ppm, followed by population collected from cabbage which recorded (9.60 ppm) and onion (9.27 ppm). The LC_{50} value in ground nut was 8.98 ppm with correspondence lower and upper values was 6.39 to 14.03. In castor LC_{50} value was (8.71 ppm) fiducial limits of lower and upper values ranged from 6.24 to 13.06. The larvae collected from soybean recorded (8.06 ppm). The lowest LC_{50} value was observed in sunflower (7.51 ppm) with fiducial limit of lower and upper values was 5.52 to 9.87 at 95 per cent and the calculated chi squire value for heterogeneity was 4.83 (Table 5).

Table.5 Bioassay of spinosad 45 % SC on *Spodoptera litura* collected from major host crops.

Crops	LC_{50} (ppm)	Fiducial limit (95%)		χ^2 P= 0.05	Regression equation (slope)
		LL (ppm)	UL (ppm)		
Castor	8.71	6.24	13.06	3.76	1.56 + 3.52X
Groundnut	8.98	6.39	14.03	3.72	1.51 + 3.55X
Chilli	10.50	7.11	25.14	2.34	1.23 + 3.73X
Sunflower	7.51	5.52	9.87	4.83	1.88 + 3.34X
Soybean	8.06	5.71	11.45	2.08	1.61 + 3.53X
Cabbage	9.60	6.73	16.84	4.18	1.40 + 3.62X
Onion	9.27	6.55	15.27	3.86	1.45 + 3.58X

4.1.6 Bioassay of Emamectin benzoate 5 % SG on *S. litura* collected from major host crops.

The calculated LC_{50} value of *S. litura* collected from different host crop ranged from 0.55 to 0.75 at 72 hrs after treatment. Highest LC_{50} value was noticed in cabbage (0.75 ppm), followed by chilli (0.71 ppm). The LC_{50} value in ground nut was (0.66 ppm), followed by onion (0.61 ppm) and correspondence lower and upper values was 0.45 to 0.77 respectively at 95 per cent. In soybean the LC_{50} value observed was (0.60 ppm) and castor was (0.57 ppm). The lowest LC_{50} value was observed in sunflower was (0.55 ppm) with correspondence lower and upper values was 0.38 to 0.59 at 95 per cent. The calculated chi squire value for heterogeneity was 3.48 (Table 6).

Table.6 Bioassay of emamectin benzoate 5 % SG on *Spodoptera litura* collected from major host crops.

Crops	LC_{50} (ppm)	Fiducial limit (95%)		χ^2 P= 0.05	Regression equation (slope)
		LL (ppm)	UL (ppm)		
Castor	0.57	0.39	0.72	3.22	2.03 + 5.49X
Groundnut	0.66	0.49	0.82	5.85	2.26 + 5.45X
Chilli	0.71	0.56	0.86	4.48	2.55 + 5.37X
Sunflower	0.55	0.38	0.59	3.48	2.02 + 5.51X
Soybean	0.60	0.43	0.75	3.28	2.12 + 5.46X
Cabbage	0.75	0.62	0.90	3.69	2.96 + 5.35X
Onion	0.61	0.45	0.77	3.62	2.16 + 5.45X

4.1.7 Bioassay of Thiodicarb 75 % WP on *S. litura* collected from major host crops.

The LC_{50} value of *S. litura* collected from different host crop when expose to thiodicarb at 72 hours after treatment was maximum in chilli 38.04 ppm (22.22 to 54.10), followed by population collected from cabbage (36.92 ppm) and in onion it was (32.23 ppm) (14.62 to 50.75) respectively at 95 per cent. The LC_{50} value in ground nut was 31.98 ppm and correspondence lower and upper values was 13.24 to 52.07 respectively at 95 per cent and. In castor and soybean the LC_{50} value observed was 31.68 and 28.23 ppm. The lowest LC_{50} value was observed in sunflower (26.39 ppm) with correspondence fiducial limits lower and upper values was 9.22 to 45.25 at 95 per cent (Table 7).

Table.7 Bioassay of thiodicarb 75 % WP on *Spodoptera litura* collected from major host crops.

Crops	LC_{50} (ppm)	Fiducial limit (95%)		χ^2 P= 0.05	Regression equation (slope)
		LL (ppm)	UL (ppm)		
Castor	31.68	18.33	44.59	0.95	1.82 + 2.26X
Groundnut	31.98	13.24	52.07	3.69	1.16 + 3.24X
Chilli	38.04	22.22	54.10	0.81	1.61 + 2.44X
Sunflower	26.39	9.22	45.25	2.51	1.12 + 3.40X
Soybean	28.23	10.62	47.34	1.96	1.14 + 3.33X
Cabbage	36.92	19.59	54.82	1.86	1.41 + 2.77X
Onion	32.23	14.62	50.75	3.19	1.27 + 3.08X

4.1.8 Bioassay of Cypermethrin 10 % EC on *S. litura* collected from major host crops.

Cypermethrin at 72 hours after treatment the highest LC_{50} value was observed in onion (31.35 ppm), followed by castor (30.26 ppm) and. The LC_{50} value in ground nut and soybean was 28.08 and 26.91 ppm. Where as in cabbage the LC_{50} value observed was 26.29 ppm and the fiducial limits of lower and upper values was 21.66 to 29.97 at 95 per cent. The calculated chi squire value for heterogeneity was 3.61. In sunflower the LC_{50} value was 25.43 ppm and the lower and upper value was 19.97 to 29.52 at 95 per cent. Lowest LC_{50} value of 24.17 ppm was observed in the population collected from chilli. (Table 8).

Table.8 Bioassay of cypermethrin 10 % EC on *Spodoptera litura* collected from major host crops

Crops	LC_{50} (ppm)	Fiducial limit (95%)		χ^2 P= 0.05	Regression equation (slope)
		LL (ppm)	UL (ppm)		
Castor	30.26	26.99	33.26	2.13	6.06 + 4.00X
Groundnut	28.08	24.16	31.42	2.46	4.99 + 2.23X
Chilli	24.17	14.74	30.03	4.99	2.49 + 1.54X
Sunflower	25.43	19.97	29.52	6.15	3.79 + 0.33X
Soybean	26.91	22.54	30.46	3.16	4.54 + 1.50X
Cabbage	26.29	21.66	29.97	3.61	4.33 + 1.15X
Onion	31.35	28.31	34.20	3.03	6.80 + 5.17X

4.1.9 Bioassay of Chlorpyrifos 20 % EC on *S. litura* collected from major host crops.

The highest LC_{50} value was noticed in chilli (245.48 ppm), followed by cabbage (236.41 ppm). The LC_{50} value in Onion was 225.80 ppm and the correspondence lower and upper values was 188.90 to 338.97 respectively at 95 per cent. The calculated chi squire value for heterogeneity was 5.38. The LC_{50} value in Sunflower was 215.32 ppm and correspondence lower and upper values was 160.51 to 268.74 respectively at 95 per cent and the calculated chi squire value was 3.47. In soybean and ground nut the LC_{50} value observed was 176.25 ppm and 167.73 ppm with fiducial limits of lower and upper values was 114 to 227.57 and 100.69 to 220.90 at 95 per cent. The calculated chi squire value for heterogeneity was 0.72. The lowest LC_{50} value was observed in castor (158.6 ppm) the correspondence lower and upper values was 88.78 to 213.89 at 95 per cent. The calculated chi squire value for heterogeneity was 0.68 (Table 9).

Table.9 Bioassay of chlorpyrifos 20 % EC on *Spodoptera litura* collected from major host crops.

Crops	LC_{50} (ppm)	Fiducial limit (95%)		χ^2 P= 0.05	Regression equation (slope)
		LL (ppm)	UL (ppm)		
Castor	158.66	88.78	213.89	0.68	1.69 + 1.27X
Groundnut	167.73	100.69	220.90	0.72	1.81 + 0.95X
Chilli	245.48	183.02	316.97	5.17	1.98 + 0.25X
Sunflower	215.32	160.51	268.74	3.47	2.25 + 0.16X
Soybean	176.25	114.26	227.57	0.83	1.94 + 0.16X
Cabbage	236.41	177.95	299.43	5.75	2.10 + 0.00X
Onion	225.80	188.90	338.97	5.38	1.86 + 0.50X

4.1.10 Estimation of median lethal concentration of various insecticides on *S. litura* collected from the different host crops.

The calculated LC_{50} value for indoxacarb ranged from 1.89 to 2.50 ppm when the *S.litura* population collected from the seven host crops were exposed to indoxacarb while in flubendiamide it range from 2.66 to 3.29 ppm *S. litura* larvae collected from different host crops when expose to quinalphos and novaluron the LC_{50} values ranged from 28.28 to 35.10 and 6.40 to 9.94 ppm, respectively. In spinosad LC_{50} values ranged from 7.51 to 10.50 ppm. Among the insecticides the lowest LC_{50} values were noticed in emamectin benzoate which ranged from 0.55 to 0.75 ppm and highest LC_{50} value was noticed when larvae were exposed to chlorpyrifos which ranged from 167.73 to 245.48 ppm. The LC_{50} values were almost similar when *S. litura* larvae were exposed to thiodicarb and cypermethrin 26.39 to 38.04 and 24.17 to 31.35 ppm, respectively (Table 10).

2.2 To study the genetic relatedness among the *S. litura* population collected from different host crops using cytochrome oxidase subunit-I DNA-Marker

Strains of *S. litura* collected from eight different hosts were established as two distinct groups (Table 13). In PCA (Principle component analysis), the first two components

accounted for 100 per cent of the similarity. The first group (A) comprised seven host strains while the distinct second group (B) consisted of only one host strain. On the basis of simple matching coefficient, all the selected host strains were grouped into three clusters. Cluster 1 had maximum of four host strains followed by cluster 2 with three host strains and cluster 2 single host strains. The highest genetic variation (0.026) was found between the host strains of cotton and cabbage followed by chilli and groundnut strain and sunflower and chilli strains (0.020). The genetic variation was nil between the host strains *viz*., groundnut and sunflower, groundnut and castor, sunflower and castor, soybean and castor, chilli and castor host strains.

The A group was subdivided into A1 and A2 at a coefficient value of 0.004, where A2 comprised of three host strains. The A1 group comprising four different host strains included two distinct groups (at a coefficient value of 0.003) A1.1 and A1.2; A1.1 group consisting of chilli, castor and soybean host strains. While A1.2 comprised of single strain from cabbage. The A2 group comprising three different host strains included two distinct groups (at a coefficient value of 0.003) A2.1 and A2.2 and A2.2: A2.1 group consisting of onion strain. While A2.2 comprised of two host strains from groundnut and sunflower. Group B comprised of single host strains from cotton. (Table 11).

Table 10. Estimation of median lethal concentration of various insecticides on *Spodoptera litura* collected from the different host

crops	Insecticides LC_{50} (ppm)								
	Indoxacarb	**Flubendamide**	**Quinalphos**	**Novaluron**	**Spinosad**	**Emamectin benzoate**	**Thiodicarb**	**Cypermethrin**	**Chlorpyrifos**
Castor	2.47	3.29	29.77	9.18	8.71	0.71	36.92	30.26	245.48
Groundnut	2.50	2.82	28.28	7.86	9.60	0.66	31.98	28.08	236.41
Chilli	2.08	2.66	35.10	6.40	10.50	0.55	38.04	24.17	158.66
Sunflower	2.19	2.86	30.40	9.94	8.06	0.61	26.39	25.43	215.32
Soybean	1.89	2.94	29.19	6.42	7.51	0.60	28.23	26.91	176.25
Cabbage	2.33	2.81	31.86	6.68	8.98	0.57	31.68	26.29	167.73
Onion	1.98	2.90	34.64	7.39	9.27	0.75	32.23	31.35	225.80

Table 11. Co-efficient values of host strains of *Spodoptera litura*.

	Sl 1	Sl 2	Sl 3	Sl 4	Sl 5	Sl 6	Sl 7	Sl 8
Sl 1								
Sl 2	0.000							
Sl 3	0.020	0.020						
Sl 4	0.006	0.006	0.020					
Sl 5	0.022	0.022	0.020	0.006				
Sl 6	0.006	0.006	0.026	0.006	0.006			
Sl 7	0.007	0.007	0.020	0.007	0.007	0.013		
Sl 8	0.000	0.000	0.020	0.000	0.000	0.006	0.007	

*Sl – *Spodoptera litura*

Sl 1: Groundnut; Sl 2: Sunflower; Sl 3: Cotton; Sl 4: Soybean; Sl 5:Chilli; Sl 6: Cabbage; Sl 7: Onion; Sl 8: Castor.

Table 12. Digestive enzyme activity of *Spodoptera litura* collected from different host Crops

Sl. No	Crops	Digestive enzymes (mU/mL)			
		Trypsin	**Chymotrypsin**	**Lipase**	**Alpha-Amylase**
1	Castor	6.091 ± 4.25	19.069 ± 0.10	27.459 ± 1.10	17.71 ± 4.21
2	Sunflower	31.695 ± 6.25	19.905 ± 0.20	22.882 ± 2.20	129.89 ± 3.27
3	Groundnut	10.372 ± 3.10	7.222 ± 0.11	30.306 ± 1.26	59.00 ± 1.20
4	Soybean	49.304 ± 5.20	19.735 ± 0.52	26.866 ± 1.00	59.00 ± 2.34
5	Cabbage	2.572 ± 3.75	7.738 ± 0.62	21.763 ± 0.10	39.69 ± 6.20
6	Chilli	5.375 ± 3.18	21.067 ± 3.10	13.221 ± 2.31	157.77 ± 5.32
7	Onion	8.272 ± 2.94	19.199 ± 0.32	18.713 ± 1.25	30.18 ± 1.20

4.3 To study the digestive enzyme activity among the *S. litura* population collected from different host crops

4.3.1 Digestive enzyme activity of *S. litura* collected from different host Crops

The insects obtain their nutritional requirements by utilizing food from environment and proper digestion of ingested food. Digestion following ingestion of food from various origins is a process by which food molecules (macromolecules such as carbohydrates, lipids and proteins) are broken down into smaller molecules to be absorbed by cells in the gut tissue. This process is perfectly controlled by digestive enzymes that depend on their site of activity in the insect gut. Present investigation on digestive enzyme activity in *Spodoptera litura* populations obtained from different host crops are envisaged here under.

4.3.2 Trypsin activity

Trypsin activity in the gut of larvae was measured by BAPNA as a substrate. The result obtained after the determination of the different digestive enzymes of *S. litura* collected from different host crops were reared till they reach third instar and enzymatic activity of trypsin was expressed per ml of extract of *S. litura* population collected from different host crops. The higher trypsin activity was observed in soybean (49.304 ± 5.20),followed by sunflower (31.695 ± 6.25), groundnut (10.372 ± 3.10), onion (8.272 ± 2.94), castor (6.091 ± 4.25), chilli (5.375 ± 3.18) and lowest activity was found in cabbage (2.572 ± 3.75) this result shows that activity of the trypsin varies from different host crops in the insect gut system. (Table 12)

4.3.3 Chymotrypsin ativity

Quantitative estimation of the chymotrypsin enzyme activity of *S. litura* was higher in the larvae collected from chilli (21.067 ± 3.10) followed by Sunflower (19.905 ± 0.20), soybean (19.735 ± 0.52), onion (19.199 ± 0.32), castor (19.069 ± 0.10), cabbage (7.738 ± 0.62) and among all seven host crops the lowest activity was observed in population collected from ground nut (7.222 ± 0.11) crops (Table 12).

4.3.4 Lipase activity

The lipase activity of *S. litura* collected from different host crops, the higher lipase activity was found in ground nut (30.306 ± 1.26) followed by castor (27.459 ± 1.10) soybean (26.866 ± 1.0), sunflower (22.882 ± 2.20), cabbage (21.763 ± 0.10) and onion (18.713 ± 1.25)

Out of seven host crops the lowest activity lipase activity was noticed in the larvae collected from chilli (12.221 ± 2.31) crop (Table 12).

4.3.5 Alpha-amylase

The higher activity of alpha- amylase in *S. litura* was observed the larvae collected from the chilli (157.37 ± 5.32) followed by sunflower (129.89 ± 3.27), soybean (59.37 ± 2.34), groundnut (59.00± 1.20), cabbage (39.69 ± 6.20), onion (30.18 ± 1.20), and lowest activity was found in castor (17.17 ± 4.21) crop (Table 12).

DISCUSSION

V. DISCUSSION

The results of the investigations on bioassay, genetic relatedness and enzyme activity of *Spodoptera litura* (Fab) was carried out at Department of Agricultural Entomology, College of Agriculture and Main Agricultural Research Station, Raichur during 2013-14. The results obtained are discussed in this chapter.

5.1 To study the insecticide resistance in *Spodoptera litura* collected from different host crops to selected insecticides

The data indicated that the LC_{50} value was significantly influenced by the host plants on which insect larvae were reared. Differential resistance was compared on the basis of resistance parameter i.e. LC_{50} values. In case of indoxacarb, lowest LC_{50} value of 1.89 ppm was observed when *S. litura* larvae collected on sunflower as compared to larvae reared on onion (1.98 ppm), castor (2.08 ppm), soybean (2.19 ppm), ground nut (2.33 ppm) and chilli (2.47 ppm) and highest LC_{50} value was in cabbage (2.50 ppm). It indicated that larvae reared on sunflower were more susceptible to indoxacarb. These results are contradict with the Munir ahmad, *et al*.,2006, LC_{50} value of second instar larvae of *S. litura* at 48 hrs after treatment was 42.60 µg/mL. The variation in LC_{50} values might be due to the fact that they used second instar larvae and the method followed for bioassay was potters tower. The variation in LC_{50} value occurs may be due to nutrition of host crops. The present study is in conformity with Goel and Sachan (2004) who reported that susceptibility of mustard aphid, *Lipaphis erysimi* (Kaltenbach) to deltamethrin, monocrotophos and endosulfan differenced due to variation in the nutritional status of the host on which insect was reared (Fig 1).

In case of flubendiamide, larvae collected on chilli recorded highest LC_{50} value (3.29 ppm) as compared to soybean (2.94 ppm), cabbage (2.90 ppm), onion (2.86 ppm), ground nut (2.82 ppm), sunflower (2.81 ppm) and in castor (2.66 ppm). It indicated that larva collected on soybean showed tolerance to flubendiamide. The changes in responce may be due to biochemical composition of the crop plants. These results are in conformity with the Sufian, *et al.,* 2013, where in second instar *S. litura* larvae exposed to flubendiamide recorded 2.77 ppm of LC_{50} value (Fig 1).

Whereas, in case of quinalphos, larvae collected on chilli recorded highest LC_{50} value 35.10 ppm as compared to cabbage (34.64 ppm), onion (31.86 ppm), sunflower (30.40

ppm), castor (29.77 ppm) and soybean (29.19 ppm), and lowest LC_{50} value was observed in ground nut (28.28 ppm) the variation in LC_{50} may be attributed to the host plants (Fig 1).

The population collected on chilli exposed to novaluron has recorded highest LC_{50} value (9.94 ppm) as compared to cabbage (9.18 ppm), ground nut (7.86 ppm), onion (7.39 ppm), castor (6.68 ppm), soybean (6.42 ppm), and sunflower (6.40 ppm). It indicated that larvae collected on chilli it was more tolerance as compared to other host crops. These results contradicts with that of Umair *et al.* (2013) where in the LC_{50} value of the *Helicoverpa armigera* (Hub) larvae to lufenuron in cotton (6.6589 ppm), in wheat (2.275 ppm), in maize (0.8562 ppm) and in berseem (6.81 ppm). The variation in LC_{50} values might be due to the facts that change biochemical composition of different host crops (Fig 1).

Where as in case of spinosad, larvae collected from onion recorded highest LC_{50} values (10.50 ppm) as compared to cabbage (9.60 ppm), onion (9.27 ppm), ground nut (8.89 ppm), castor (8.71 ppm), soybean (8.06 ppm), and lowest LC_{50} value was observed in sunflower (7.51 ppm). Indicated that larvae reared on chilli were found to be tolerance to spinosad (Fig 1).

Larvae reared on cabbage had highest LC_{50} value (0.75 ppm) to emamectin benzoate as compared with other selected host crops like chilli (0.71 ppm), ground nut (0.66 ppm), onion (0.61 ppm), soybean (0.60 ppm), castor (0.57 ppm) and lowest LC_{50} value was observed in sunflower (0.55 ppm). It indicated that larvae reared on cabbage showed much tolerance to emamectine benzoate as compared to other host crops. Present finding are in agrrement with Ravishanker, *et al*., 2010, where *S. litura* larvae were reared on different host plants and to semi-synthetic diet when treated with different doses of SlNPV. The LC_{50} value against the pest which was reared on different host plants were as follows: cabbage 0.42, cotton 0.61, potato 0.75, ground nut 0.93 and rose 1.28 POB/mm^2. The variation in LC_{50} values might be due to the facts that change in biochemical composition of host crops, nutrientional composition of host crops and insect virus (SlNPV). And Emamectin benzoate was the most effective insecticide among all other new chemistry insecticides against *Spodoptera litura* (Ahmad *et al.,* 2008) (Fig 1).

When *S. litura* population collected on the chilli exhibited the highest LC_{50} values (38.04 ppm) as compared to other selected host crops *Viz.,* castor (36.92 ppm) onion (32.33 ppm) ground nut (31.98 ppm) cabbage (31.68 ppm) and sunflower (28.23 ppm) and out of these host crops lowest LC_{50} value was observed in soybean (26.39 ppm). Therefore the

biochemical composition of the crop plant has a role in determining the susceptibility of larvae against the insecticides. Such observations have also been made by Massarat (2007) who reared the larvae of *S. litura* on different host plants *Viz.;* cabbage, castor groundnut and cauliflower and treated them with an entomopathogenic fungi, *Beauveria bassiana*. Larvae reared on cabbage and castor were more susceptible to the infection of *B. bassiana* in terms of per cent mortality as compared to cauliflower and groundnut having lower LT_{50} value when fed on cabbage and castor (Fig 1).

In case of cypermethrin, out of seven host crops population of *S. litura* tested against cypermethrin insecticide by leaf dip method, larvae reared on onion showed the highest LC_{50} value (31.35 ppm) as compared to other host crops like castor (30.26 ppm), ground nut (28.08 ppm), soybean (26.91 ppm), cabbage (26.29 ppm), sunflower (25.43 ppm) and lowest LC_{50} value was observed in case of chilli (24.17ppm). It indicated that larvae reared on the onion were more tolerance to cypermethrin. These results contradicts with that of Ajin, (2007), who collected larvae of *S. litura* from vegetable field and maintained under laboratory condition at 25-30^{o} C with artificial diet and larvae were tested against cypermethrin, showed highly toxicity the lethal dose in term of LC_{50} showed different value that significant increasing from first to fifth instars larvae at 2.08, 2.92, 9.93, 11.64 and 18.38 ppm, respectively. The correlation between concentration and mortality showed as 0.95-0.97 in second to fifth instar indicated that the effect of cypermethrin were highly correlated with mortality. This variation in LC_{50} value might be due to change in method of rearing and stage of larvae used for bioassay was leaf disc (Fig 1).

The bioassay of chlorpyrifos against *S. litura* collected from different host crops, the larvae collected on chilli exhibited highest LC_{50} value (245.25 ppm) as compared to cabbage (236.41 ppm), onion (225.80 ppm), sunflower (215.32 ppm), soybean (176.25 ppm) and ground nut (167.73 ppm) and lowest LC_{50} value was observed in case of castor it was (158.66 ppm). It indicated that larvae reared on chilli showed much tolerance as compared to other host crops. These results contradict with that of Sheikh (2012), where in the LC_{50} for fourth instar larvae of *Spodoptera litura* at 42 hrs after treatment was 17.50 ppm. The variation in LC_{50} values might be due to the fact that they used fourth instar larvae and the method followed for bioassay was leaf disc (Fig 1).

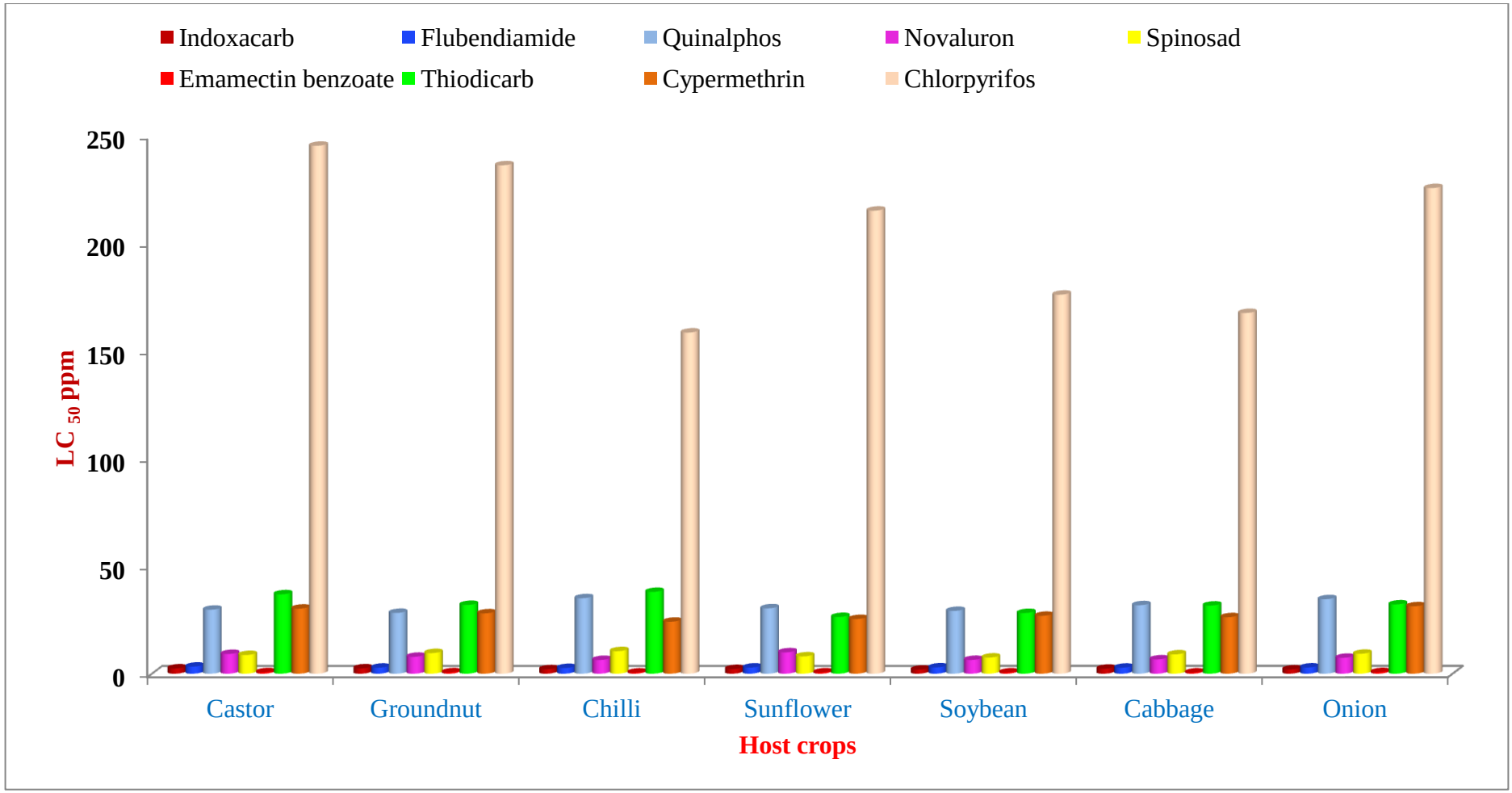


Fig.1. Comparative LC $_{50}$ values of different insecticides on *S. litura* collected from different host crops

5.2 To study the genetic relatedness among the *Spodoptera litura* population collected from different host crops using cytochrome oxidase subunit-I DNA-Marker

The present study revealed very low level of genetic variation among *S. litura* host strains. The coefficient values varied 0.000 to 0.026 across the selected host strains. Significant genetic similarity was found among host strains in close proximity, as well as among those from distant parts of the range (Fig 2).

On a larger scale, genetic differences among populations appeared to result from low dispersal rates between host strains. The genetic variation detected in present study, among host strains, was on lower side (0.000 to 0.026). The number of introductions, the size of each introduction, and subsequent drift and selection pressures that occur after introduction all can affect the amount of haplotypes and genetic variation in invasive populations (Nei *et al*., 1975). Low mitochondrial DNA variation has been repeatedly reported in taxa that have undergone severe bottlenecks or founder effects (Grapputo *et al*., 2005:Saw *et al*., 2006). Therefore, the *S. litura* populations from different host strains that we studied are likely to have each undergone bottlenecks, as suggested by the low nucleotide and amino acid diversity observed.

Extremely low genetic variation was observed in the Chinese population of *Pectnophora gossypiella* (Sunders) in the two mitochondrial regions among all populations examined. The low level of population genetic variation of *P. gossypiella* is attributed to invasion bottlenecks, which may have been subsequently strengthened by its non migratory biology and the mosaic pattern of agricultural activities (Yudi Liu *et al*., 2010).

The pine processionary month *Thaumetopoea wilkinsoni* (Tams) showed a reduction in genetic variability at both mitochondrial COI and COII regions at the expanding boundary of the range, founded by few individuals expanding from nearby localities (Simnato *et al*., 2007). The low haplotype and nucleotide diversities among Australian diamondback moth *Plutella xylostella* L. mtDNA suggested a relatively recent bottleneck in population size (Saw *et al*., 2006).

Genetic relatedness of six South Indian agriculturally important moth species based on 28S rRNA-D2 region sequence. The average percentage of nucleotide composition among lepidopteran agricultural insect pests was A-21.85, T-20.95, C-27.68 and G-29.52%. The molecular phylogenetic tree derived out of multiple sequence alignment revealed three major

clusters for the 6 different species of lepidopteran moths belonging to the family Noctuidae. One cluster consisted of *H. armigera* and *S. litura*. The second cluster contained with all hairy caterpillars including the red hairy caterpillar, hairy caterpillar, and woolly bear moth, while the third cluster had the spotted bollworm, at the superfamily level, all the 6 species were shown to be closely related to each other with a range of similarity index values 0.85 to 0.93. (Veeramani, *et al*. 2013)

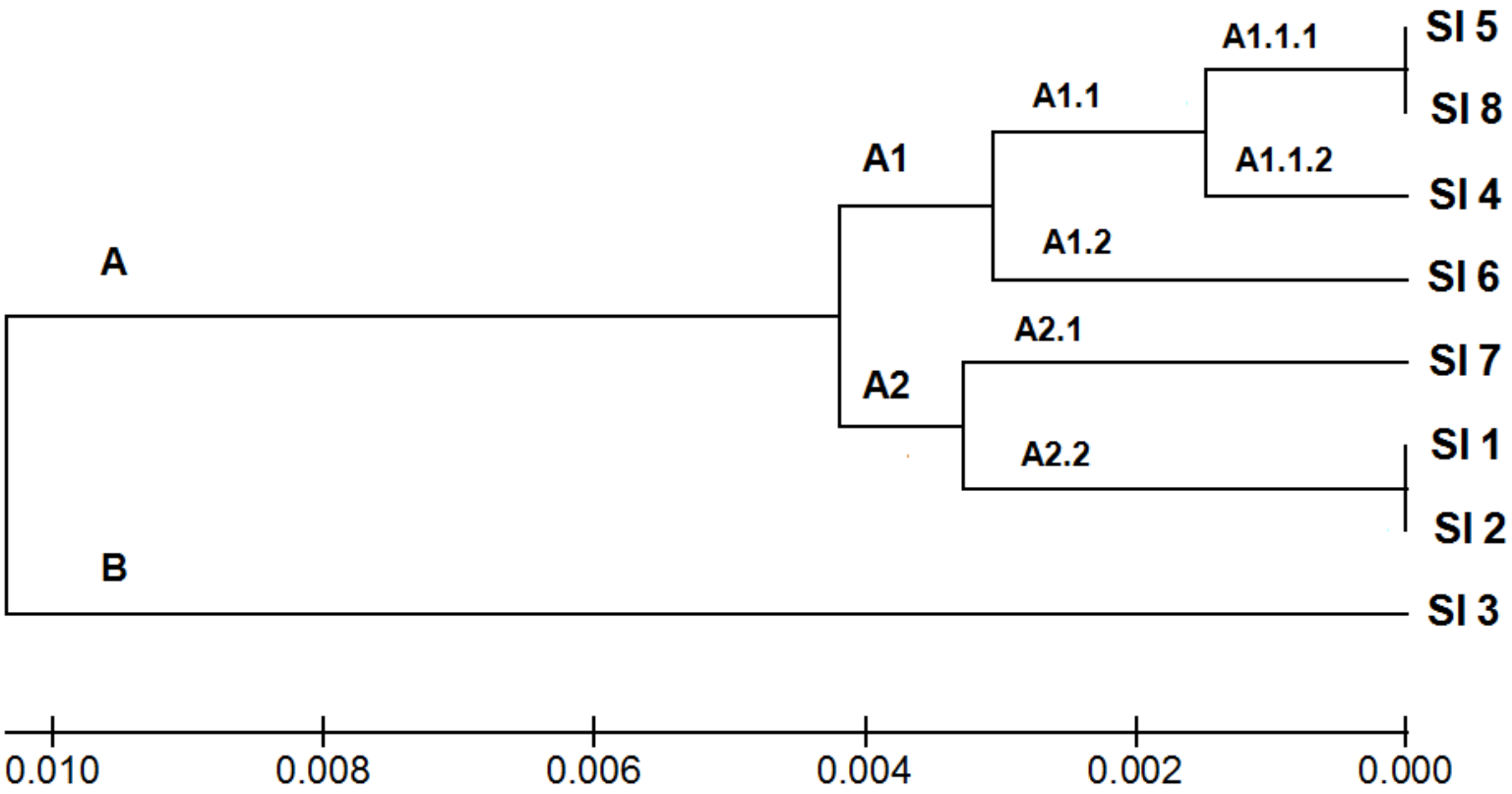


Fig.2. Dendrogram constructed based on coefficients with UPGMA-SAHN clustering method among eight host strains of *S.litura* using multiple sequences alignment tools. Sl 1: Groundnut; Sl 2: Sunflower; Sl 3: Cotton; Sl 4: Soybean; Sl 5:Chilli; Sl 6: Cabbage; Sl 7: Onion; Sl 8: Castor.

5.3 To study the digestive enzyme activity among the *Spodoptera litura* population collected from different host crops

5.3.1 Digestive enzyme activity in *Spodoptera litura*

Digestion is a phase of Insect Physiology on which surprisingly little research has been done, considering the economic importance of the food of insects and the fact that our most important control measures involve the action of digestive juices on poisons taken into the digestive tract. Understanding the functioning of the digestive machinery helps to explain the utilisation of nutrients at the digestive level (Glass *et al*., 1989; Hidalgo *et al*., 1999; Kolkovski, 2001). In addition, the pattern of digestive enzymes can reflect the feeding habits of an animal (Hofer and Kock, 1989). Plant defense against insect herbivores are mediated, in part, by enzymes that impair digestive processes in the insect gut. Little is known about the evolutionary origins of these enzymes, their distribution in the plant kingdom, or the mechanisms by which they act in the protease-rich environment of the animal digestive tract (Chen *et al*., 2007).

The proteinases are a major group of hydrolytic enzymes in insects and are involved in digestive processes, proenzyme activation, liberation of physiologically active peptides, complement activation, and inflammation processes amongst others. The proteinases are classified according to their mechanism of catalysis: (1) serine proteinases; (2) cysteine proteinases; (3) aspartic proteinases, and (4) metalloproteinases (Bode and Huber, 1992). For an efficient management of pest control through proteinase inhibitor transgenes, it is imperative to know the type of enzymes present in the gut of insects and pests. The two major proteinase classes in the digestive systems of phytophagous insects are the serine and cysteine proteinases (Haq *et al*., 2004).

Proteinase inhibitors are proteins widely distributed in plants. Among these, the serine proteinase inhibitors, of which trypsin inhibitors are the most studied, are thought to participate in the array of defensive substances that plants synthesize for protection against pest and pathogens. Due to their biological basis, in several species, changes in digestive enzyme activities demonstrated during: (1) ontogenetic development (Lovett and Felder, 1990; Biesiot and McDowell Capuzzo, 1990; Fang and Lee, 1992; Lemos *et al*., 1999); (2) ovarian maturation (Ferna´ndez *et al*., 1997); (3) circadian cycles (Herna´ndez-Corte´s *et al*., 1999).

5.3.2 Trypsin activity

Present finding showed that digestive enzyme activity of third instars larvae of *S. litura* collected from major host crops. Higher trypsin activity was observed in larvae collected from soybean field it (49.304 ± 5.20 mU/mL), when compared to control. Followed by sunflower (31.695 ± 6.25 mU/mL), groundnut (10.372 ± 3.10 mU/mL), onion (8.272 ± 2.94 mU/mL), castor (6.091 ± 425 mU/mL), chilli (5.375 ± 3.18 mU/mL) and cabbage (2.572 ± 3.75 mU/mL) (Fig 3). This result contradicts with that of Tierno *et al.* (2011). Where in the total digestive enzymatic activity was detected in two species of *H. flaviventris* and *I. Morenica.* The trypsin activity in *I. morenica* was (92.4 ± 15.9 mU/mL) and in *H. Flaviventris* was (236.2 ± 46.7 mU/mL).

The quantitative estimation of trypsin activity was revealed that a higher activity of the enzyme in mid-gut (26.1 ± 3.09 mU/mL) than in fore-gut (16.6 ±3.19 mU/mL) of *A. domestica* while in *P. americana* there was no significant difference in the activity of trypsin in the fore-gut (22.75 ± 3.30 mU/mL) or mid-gut (22.50 ± 5.36 mU/mL) (Muhammad, 1982).

The variation of enzyme activity might be due to change in host crops, insects and gut system of insects (Gholamzadeh, *et al*., 2013). Enzymes play important roles in insect growth, development and reproduction, enzyme activation, toxin activation/detoxification, and inflammation processes (Terra *et al*. 1996) (Fig 3).

5.3.3 Chymotrypsin activity

In case of chymotrypsin the higher activity was noticed larvae collected from the chilli field it was (21.067 ± 3.10 mU/mL), when compared to control, followed by sunflower (19.905 ± 0.20 mU/mL), soybean (19.735 ± 0.52 mU/mL), and onion (19.199 ± 0.32 mU/mL), castor (19.069 ± 0.10 mU/mL), cabbage (7.738 ± 0.62 mU/mL) and lower activity was noticed in ground nut (7.222 ± 0.11 mU/mL) (Fig 3). These results contradict with that of Tierno *et al.* (2011). The total digestive enzymatic activity was detected in two species of Plecoptera *Hemimelaena flaviventris* and *Isoperla morenica* chymotrypsin activity in *I. morenica* was (51.6 ± 5.3 mU/mL)) and in *H. Flaviventris* was *(*78.6 ± 10.6mU/mL) (Fig 3).

5.3.4 Lipase activity

Higher lipase activity was noticed in case of ground nut population (30.306 ± 1.26 mU/mg/protein), castor (27.459 ± 1.10 mU/mL), soybean (26.866 ± 1.00 mU/mL), sunflower (22.882 ± 2.20 mU/mL), cabbage (21.763 ± 0.10 mU/mL**)**, onion (18.713 ± 1.25 mU/mL) and chilli (13.221 ± 2.31 mU/mL**)** among all the larval population collected from different host crops chilli population showed lowest lipase activity (Fig 3). These results contradict

with that of Tierno *et al.* (2011). The total digestive enzymatic activity was detected in two species of Plecoptera *Hemimelaena flaviventris* and *Isoperla morenica* lipase activity in *I. morenica* was (171.1 ± 13.2 mU/mL)) and in *H. flaviventris* was (325.3 ± 18.5 mU/mL).

Measurement of lipase activity (Crude extract) in all three larval instars (first, second and third) of *Naranga aenescens* (Moore) demonstrated that the first instar larvae had the lowest lipase activity and the third instar larvae had the highest one so that the lipase activity in the third instar larvae was 4.33-fold and 2-fold more that of the first and the second instars, respectively. (Arash and Mahmoud, 2012) (Fig 3).

5.3.5 Alpha- amylase activity

Higher activity of alpha- amylase was noticed in case of chilli (157.37 ± 5.32 mU/mL) followed by sunflower (129.89 ± 3.27 mU/mL), soybean (59.37 ± 2.34 mU/mL), groundnut (59.37 ± 1.20 mU/mL), cabbage (39.69 ± 6.20 mU/mL), onion (30.18 ± 1.20 mU/mL), and castor (17.17 ± 4.21 mU/mL) (Fig 3). The present findings on alpha- amylase is also in line with (Cohen, 1996; Hidalgo *et al*., 1999; Furné *et al*., 2005; De Almeida *et al*., 2006; Ferro Correa *et al*., 2007, Studies carried out in insects and in other animals have demonstrated that greater amylase activity is typical of phytophagous animals. However the results were comparable with Fuse *et al*. (1995). Mendiola-Olaya *et al*. (2000), showed that alpha-amylase activity reached its highest value in the second larval instar of *P. truncates*, also enzyme activity in second larval stage was more than 3rd instar and only a little activity was detected in pupal stage, thay suggest that no digestive processes are taking place during pupal stage (Fig 3).

Presence of adaptive mechanism(s) under the stress of protease inhibitors are expected since polyphagous insects must respond to a large array of different plant protease-inhibitors. This is true for other Spodoptera species, but some other insects show less effective adaptation (Jongsma and Bolter, 1997). This may be due to certain physiological or biochemical factors or some factors concerning the plants themselves. So, a single inhibitor cannot be used against all herbivorous insects, and an inhibitor may lose its potency due to changes in the relative proportion of digestive enzyme(s) in the mid gut. Thus, in case of plant transformation with gene coding with more than one protein inhibitor, good results can be achieved in plant defense against herbivorous insects.

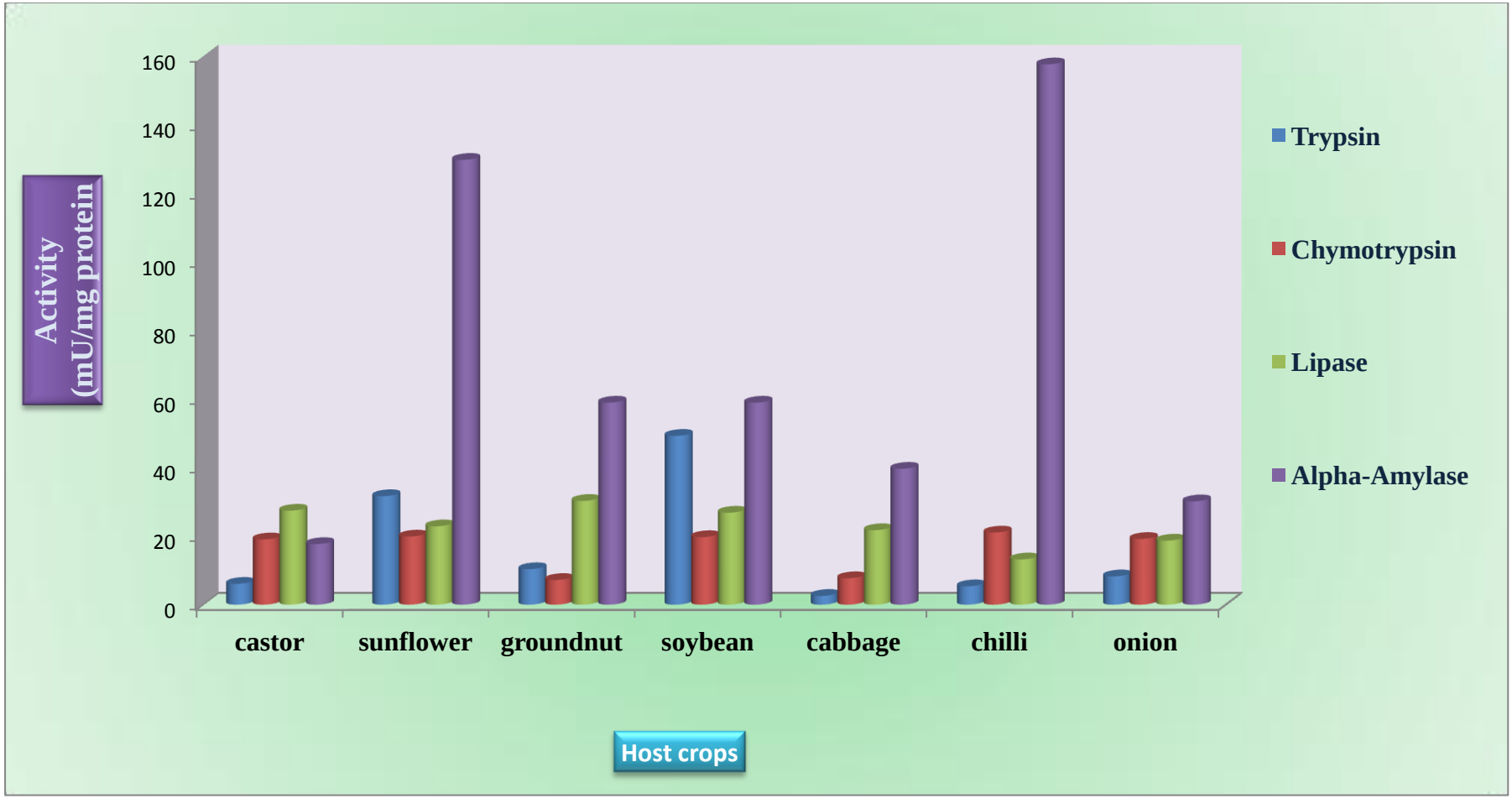


Fig.3.Digestive enzyme activity of *Spodoptera litura* collected from different host Crops

SUMMARY AND CONCLUSIONS

VI. SUMMARY AND CONCLUSIONS

The present investigation on bioassay, genetic relatedness and estimation of digestive enzyme activity in the *Spodoptera litura* was carried out at the Department of Agricultural Entomology, College of Agriculture and Main Agricultural Research Station, Raichur, Karnataka during 2013-14. The outcome of these investigations are summarised here under.

The LC_{50} values of indoxacarb from larvae collected from the different host crops like Castor, Groundnut, Chilli, Sunflower, Soybean, Cabbage and Onion at 72 hours after treatment was 2.08, 2.33, 2.47, 1.86, 2.19, 2.50 and 1.98 ppm respectively. Similarly, the bioassay of flubendiamide LC_{50} values were find out at 72 hours after treatment was 2.66, 2.82, 3.29, 2.81, 2.94, 2.90 and 2.86 ppm. And LC_{50} values Quinalphos were noticed 72 hours after treatment was 29.77, 28.28, 35.10, 30.40, 29.19, 34.64 and 31.86 ppm. Where in Novaluron LC_{50} values were observed at 96 hours after treatment was 6.68, 7.86, 9.94, 6.40, 6.42, 9.18 and 7.39 ppm. And in case of Spinosad LC_{50} values 72 hours after treatment 8.71, 8.98, 10.50, 7.51, 8.06, 9.60 and 9.27 ppm respectively. Similarly in case of Emamectin benzoate LC_{50} values was 0.75, 0.66, 0.71, 0.55, 0.60, 0.75 and 0.61 ppm respectively. Where as in case of Thiodicarb LC_{50} values was recorded 31.68, 31.98, 38.04, 26.39, 28.23, 36.92 and 32.23 ppm. In cypermethrin LC50 value was 30.26, 28.08, 24.17, 25.43, 26.91, 26.29 and 31.35 ppm. Among the various selected insecticides highest LC_{50} values was observed in case of Chlorpyriphos at 48 hours after treatment was 168.66, 167.73, 245.48, 215.32, 176.25, 236.41 and 225.80 ppm respectively.

Study revealed very low level of genetic variation among *S. liture* host strains. The coefficient values varied 0.000 to 0.026 across the selected host strains. Significant genetic similarity was found among host strains in close proximity, as well as among those from distant parts of the range.

The digestive enzyme activity of third instar larvae *S. litura* collected from the different host crops like Castor, Groundnut, Chilli, Sunflower, Soybean, Cabbage and Onion. The highest trypsin activities were observed the larvae collected from the soybean populations 49.304 ± 5.20 mU/mL. Where in the chilli field population found to be highest chymotrypsin activity was observed 21.067 ± 3.10 mU/mL. Similarly ground nut population showed highest lipase activity was noticed 30.306 ± 1.26 mU/mL. And highest Alpha-

amylase activity were found to be the third instar larvae collected from the chilli field population 157.77 ± 5.32mU/mL.

Future line of work.....

- Comparative biology of *S. litura* on different host crops.
- Biochemical analysis of different host crops in response to *S. litura*.

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VII. REFERENCES

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COMPARATIVE STUDIES ON GENETIC, BIOCHEMICAL AND INSECTICIDES TOXICITY IN *Spodoptera litura* (Fab) FROM DIFFERENT HOST CROPS

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ABSTARCT

The present investigation on comparative studies on genetic, biochemical and insecticides toxicity in *Spodoptera litura* (Fab) was carried out in the college of Agriculture and Main Agricultural Research Station, Raichur, during 2013-14. *Spodoptera litura* collected from different host crops *Viz;* Castor, Groundnut, Chilli, Sunflower, Soybean, Cabbage and onion showed differential resistance to different insecticides. Among the different host crops the toxicity of indoxacarb 14.5 SC was minimum (1.89 ppm) When *S. litura* larvae collected from sunflower host crop while highest LC_{50} value was noticed in larvae collected from cabbage host crop. A minimum LC_{50} value of 0.55 ppm was noticed when *S. litura* larvae collected from sunflower host crop when exposed to emamectin benzoate 5 SG while maximum LC_{50} value of 0.75 ppm was observed from cabbage host crop. Similarly when *S. litura* larvae collected from different host crops and exposed to different concentration of Flubendiamide 39.35 SC showed that lowest LC_{50} value of 2.66 ppm from castor host crop while the highest LC_{50} value of 3.29 ppm was noticed from chilli host crop.

The genetic relatedness among the *S. litura* population collected from different host crops using cytochrome oxidase subunit-I DNA-Marker showed very low level of genetic variation. Strains of *S. litura* collected from eight different hosts were established as two distinct groups. The first group (A) comprised seven host strains while the distinct second group (B) consisted of only one host strain. On the basis of simple matching coefficient, all the selected host strains were grouped into three clusters. Cluster 1 had maximum of four host strains followed by cluster 2 with three host strains and cluster 2 single host strains. The digestive enzyme activity of third instars larvae of S. *litura* collected from the different host crops showed that the highest trypsin activity was observed in Soybean (49.304 ± 5.20 mU/mL) while larvae collected from Chilli host crop had highest chymotrypsin (21.067 ± 3.10 mU/mL) and alpha- amylase (157.77 ± 5.32mU/mL) activity and larvae collected from Ground nut had highest lipase activity (30.306 ± 1.26 mU/mL).