DAMAGE POTENTIAL, GROWTH AND DEVELOPMENT OF *Spodoptera litura* (FAB.) ON DUAL TOXIN TRANSGENIC *Bt* COTTON

BY

Y. N.VENKATESH

B.Sc. (Ag.)

THESIS SUBMITTED TO THE ACHARYA N. G. RANGA AGRICULTURAL UNIVERSITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE IN AGRICULTURE

(ENTOMOLOGY)

CHAIRPERSON: Dr. N.V.V.S. DURGA PRASAD

DEPARTMENT OF ENTOMOLOGY

AGRICULTURAL COLLEGE, BAPATLA

ACHARYA N.G. RANGA AGRICULTURAL UNIVERSITY

2015

DECLARATION

I**, Mr. Y. N. VENKATESH,** hereby declare that the thesis entitled **“DAMAGE POTENTIAL, GROWTH AND DEVELOPMENT OF *Spodoptera litura* (Fab.) ON DUAL TOXIN TRANGENIC *Bt* COTTON”** submitted to the **Acharya N.G. Ranga Agricultural University** for the degree of **Master of Science in Agriculture** is the result of original research work done by me. I also declare that no material contained in the thesis has been published earlier in any manner.

Place: Bapatla	**(VENKATESH. Y. N.)**
Date:	**I.D. No. BAM 13-35**

CERTIFICATE

Mr. Y. N. VENKATESH has satisfactorily prosecuted the course of research and that thesis entitled **"DAMAGE POTENTIAL, GROWTH AND DEVELOPMENT OF *Spodoptera litura* (Fab.) ON DUAL TOXIN TRANSGENIC *Bt* COTTON**" submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that neither the thesis nor its part there of has been previously submitted by him for a degree of any university.

(N.V.V.S. DURGA PRASAD)
Major Advisor
Senior Scientist
(Entomology)
RARS, Lam, Guntur

Place: Bapatla

Date :

CERTIFICATE

This is to certify that the thesis entitled **"DAMAGE POTENTIAL, GROWTH AND DEVELOPMENT OF *Spodoptera litura* (Fab.) ON DUAL TOXIN TRANSGENIC *Bt* COTTON"** submitted in partial fulfillment of the requirements for the degree of '**Master of Science in Agriculture'** of the Acharya N. G. Ranga Agricultural University, Hyderabad is a record of the bonafide original research work carried out by **Mr. Y. N. VENKATESH** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

Thesis approved by the Student Advisory Committee

Chairperson **Dr. N.V.V.S. Durga Prasad**
Senior Scientist (Entomology)
RARS, Lam, Guntur

Member **Dr. D. V. Sai Ram Kumar**
Associate Professor
Department of Entomology
Agricultural College, Bapatla

Member **Dr. V. Prasanna Kumari**
Assistant Professor
Department of Plant Pathology
Agricultural College, Bapatla

Date of final viva-voce:

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DAMAGE POTENTIAL, GROWTH AND DEVELOPMENT OF *Spodoptera litura* (Fab.) ON DUAL TOXIN TRANSGENIC *Bt* COTTON

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	per cent
@	:	at the rate of
^{0}C	:	degree Celsius
/	:	Per
>	:	greater than
&	:	And
AD	:	after death
Avg.	:	Average
BG	:	Bollgard
Bt	:	*Bacillus thuringiensis*
C.D. (P=0.05%)	:	critical difference at 5 per cent
cm	:	Centimeter
cm^2	:	square centimeter
Cry	:	Crystalline
DAR	:	days after release
DAS	:	days after sowing
et al.	:	co-workers
Fig.	:	Figure
HAF	:	hours after feeding
HAT	:	hours after treatment
h	:	Hours
i.e.	:	that is
kg	:	Kilogram
Ltd.	:	Limited
m	:	Meter
m^2	:	square meter
Max.	:	Maximum
MBG	:	Mallika bollgard
mg	:	Milligram
mm	:	Millimeter
Min.	:	Minimum

M/S	:	Manufacturers
NBG	:	non bollgard
NS	:	non-significant
RBD	:	Randomized Block Design
RH	:	relative humidity
SEm±	:	Standard Error of mean
Tab	:	Table
viz.,	:	Namely
µg	:	Microgram

ABSTRACT

Name of the Author	**:**	**Y. N. VENKATESH**
Title of the thesis	**:**	**DAMAGE POTENTIAL, GROWTH AND DEVELOPMENT OF *Spodoptera litura* (Fab.) ON DUAL TOXIN TRANGENIC *Bt* COTTON**
Submitted for the award of	**:**	**Master of Science in Agriculture**
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Major Advisor	**:**	**Dr. N.V.V.S. DURGA PRASAD**
University	**:**	**ACHARYA N.G. RANGA AGRICULTURAL**
Year of submission	**:**	**2015**

A field experiment was conducted at Regional Agricultural Research Station, Lam, Guntur, A.P. during *kharif*, 2014-15 to carry out the experiment on **"DAMAGE POTENTIAL, GROWTH AND DEVELOPMENT OF *Spodoptera litura* (Fab.) ON DUAL TOXIN TRANGENIC *Bt* COTTON".** In the investigation assessing the damage potential of *S. litura* on BG II *Bt* cotton, influence of cotton hybrids containing Cry1Ac (BG I) and Cry 1Ac + Cry 2Ab (BG II) of Jaadoo, Mallika and their corresponding non *Bt* versions on larva of *S. litura*, were studied.

There was a significant difference of leaf, square and boll damage in BG II cotton hybrid at different days of release of *S. litura.* The leaf, square and boll damage was less at 50 and 80 DAS crop compared to 110 and 140 DAS crop this may due to expression of Cry 2Ab toxin in leaves which was effective against *S .litura* damage. At 110 and 140 DAS crop leaf, square and boll damage was more this was due to decrease in protein expression of Cry 2Ab.

Leaf area consumption was compared with 50 DAS larvae released crop with 110 and 140 DAS larvae released crop in which the percentage leaf area consumption has gradually increased at 110 DAS larvae released crop and it was double the percentage of leaf area consumption at 140 DAS larvae released crop, this shows that *S. litura* larvae causing the more damage to later stages of the crop this my due to decrease in Cry 2Ab toxin in the plant.

Yield has gradually decreased with increase in larval numbers/plant and yield was more in 50 and 80 DAS larvae released crop compared to 110 and 140 DAS larvae released crop. The per cent yield reduction over control was increasing with increase in duration of crop period. When compared the yield of 50 DAS larvae released crop with 110 and 140 DAS larvae released crop the percent yield reduction has increased this shows that the damage potential was more at later stages of the crop and cause economic losses to farmers.

The mortality of 1^{st} and 2^{nd} instar larvae of *S. litura* was high with reduced mortality rates in 3^{rd} and there was no mortality in 4^{th} and 5^{th} instar larvae on BG II hybrids, where as the mortality was very low and more or less similar in BG I and non *Bt* cotton hybrids. The BG II cotton hybrids were found high larval mortality. This might be due to high level expression of dual toxin at vegetative stages of the crop. The survival of early larval instars of *S. litura* was very low than the later instars on the leaves and squares of both the BG II cotton hybrids and the 1^{st} instar larvae showed highest mortality compared to 2^{nd} instar larvae. The survival rate of 3^{rd} instar larvae was higher and recorded only 30 - 35 per cent mortality, whereas the influence of BG II hybrids on grown up 4^{th} and 5^{th} instar larvae was very minimal and equal to BG I and non *Bt* hybrids.

The mean weight of larva fed on BG II cotton leaves was found to be very low. The weight reduction of late instars of *S. litura* was lower than the early instars on the leaves of both the BG II versions, where as BG I hybrids shows marginal influence and non *Bt* versions did not effect the larval or pupal weight and weight gains were more or less similar.

The mean leaf area consumed by 1^{st} instar larvae of *S. litura* on BG II cotton hybrids were found to be significantly very low due to higher mortality rates and the same trend was also observed in 2^{nd} instar larvae. The mean leaf area consumption by 3^{rd}, 4^{th} and 5^{th} instar larvae of *S. litura* was high the larvae were not effected by BG II hybrids. In BG I and non *Bt* hybrids the leaf area consumption was very high for all the instars.

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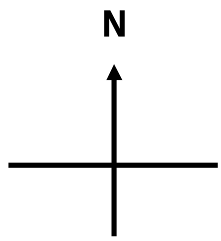
I extend my gratitude to the ***ICAR, New Delhi for providing financial support (JRF Scholarship)*** *during my course of study.*

Place:

Date: ***(Y. N. Venkatesh)***



R1	R2	R3
T1	T8	T2
T2	T7	T5
T3	T6	T1
T4	T5	T7
T5	T4	T6
T6	T3	T8
T7	T2	T4
T8	T1	T3

60 cm

105 cm

Treatments:

T_1- Complete protection

T_2- Releasing 3 larvae/plant

T_3- Releasing 6 larvae/plant

T_4- Releasing 9 larvae/plant

T_5- Releasing 12 larvae/plant

T_6- Releasing 15 larvae/plant

T_7- Releasing 18 larvae/plant

T_8- Releasing 21 larvae/plant

Fig 3.1. Layout of the experimental field

Table 4.1. Leaf area consumption of *S. litura* larvae on BG II *Bt* cotton

Treatments	Leaf Area Consumption (cm^2) (Mean of Three Replications)															
No. of Larvae released/plant	50 DAS			Total	80 DAS			Total	110 DAS			Total	140 DAS			Total
	3DAR	7DAR	11DAR		3DAR	7DAR	11DAR		3DAR	7DAR	11DAR		3DAR	7DAR	11DAR	
Control	0.00 (1.00)^{e}	0.00 (1.00)^{f}	0.00 (1.00)^{g}	0.00	0.00 (1.00)^{e}	0.00 (1.00)^{f}	0.00 (1.00)^{f}	0.00	0.00 (1.00)^{g}	0.00 (1.00)^{e}	0.00 (1.00)^{g}	0.00	0.00 (1.00)^{g}	0.00 (1.00)^{f}	0.00 (1.00)^{f}	0.00
3	7.05 (2.80)^{d}	9.40 (3.15)^{e}	9.52 (3.24)^{f}	25.97	8.47 (3.06)^{d}	10.37 (3.31)^{e}	10.27 (3.28)^{e}	29.11	9.83 (3.24)^{f}	12.47 (3.66)^{d}	10.90 (3.43)^{f}	33.20	14.37 (3.88)^{f}	16.53 (4.14)^{e}	15.50 (4.03)^{e}	46.40
6	14.03 (3.85)^{c}	16.33 (4.16)^{de}	17.64 (4.31)^{ef}	48.00	14.67 (3.87)^{d}	19.94 (4.45)^{de}	18.42 (4.34)^{de}	53.03	15.45 (4.05)^{ef}	23.93 (4.96)^{cd}	17.60 (4.28)^{ef}	56.99	22.13 (4.77)^{ef}	23.63 (4.86)^{e}	22.37 (4.81)^{de}	68.13
9	18.25 (4.32)^{c}	25.57 (5.12)^{cd}	25.30 (5.11)^{de}	69.12	25.37 (5.07)^{c}	29.03 (5.45)^{cd}	25.47 (5.08)^{cd}	79.87	23.20 (4.90)^{de}	31.87 (5.72)^{bc}	24.20 (4.95)^{de}	79.27	30.90 (5.64)^{de}	38.57 (6.28)^{d}	33.40 (5.79)^{cd}	102.87
12	30.07 (5.53)^{b}	35.30 (6.01)^{bc}	30.73 (5.60)^{cd}	96.10	32.43 (5.77)^{bc}	40.60 (6.42)^{bc}	35.73 (6.03)^{bc}	108.76	34.03 (5.89)^{cd}	45.87 (6.84)^{ab}	32.27 (5.74)^{cd}	112.17	39.70 (6.35)^{cd}	45.90 (6.84)^{cd}	41.83 (6.45)^{bcd}	127.43
15	34.40 (5.91)^{b}	40.80 (6.41)^{b}	40.47 (6.35)^{bc}	115.67	40.67 (6.43)^{ab}	47.00 (6.89)^{ab}	45.40 (6.75)^{ab}	133.07	39.03 (6.29)^{bc}	53.93 (7.40)^{a}	41.07 (6.43)^{bc}	134.03	50.57 (7.16)^{bc}	55.27 (7.49)^{bc}	53.43 (7.33)^{abc}	159.27
18	48.87 (7.05)^{a}	50.63 (7.12)^{ab}	45.30 (6.73)^{ab}	144.80	45.39 (6.77)^{ab}	55.63 (7.50)^{ab}	55.50 (7.47)^{ab}	156.53	50.50 (7.12)^{ab}	57.07 (7.61)^{a}	53.13 (7.32)^{ab}	160.70	59.07 (7.66)^{ab}	65.03 (8.11)^{ab}	63.23 (7.94)^{ab}	187.33
21	55.77 (7.53)^{a}	60.07 (7.76)^{a}	55.80 (7.50)^{a}	171.63	55.13 (7.45)^{a}	65.53 (8.12)^{a}	60.80 (7.80)^{a}	181.47	58.67 (7.69)^{a}	67.17 (8.06)^{a}	63.93 (8.04)^{a}	189.77	70.40 (8.44)^{a}	73.77 (8.63)^{a}	74.97 (8.70)^{a}	219.13
F-test	Sig	Sig	Sig		Sig	Sig	Sig		Sig	Sig	Sig		Sig	Sig	Sig	
SEm±	0.28	0.40	0.37		0.35	0.45	0.52		0.39	0.45	0.39		0.38	0.33	0.56	
CD (P=0.05)	0.85	1.23	1.13		1.05	1.37	1.52		1.19	1.37	1.19		1.16	1.00	1.70	

Figures in parentheses are square root transformed values

Numbers followed by same superscript are not statistically different (P=0.05)

Table 4.2. Damage on squares by larvae of *S. litura* on BG II *Bt* cotton

Treatments	**Number of squares damaged/plant (Mean of Three Replications)**															
No. of Larvae released/plant	**50 DAS**			**Total**	**80 DAS**			**Total**	**110 DAS**			**Total**	**140 DAS**			**Total**
	3DAR	**7DAR**	**11DAR**		**3DAR**	**7DAR**	**11DAR**		**3DAR**	**7DAR**	**11DAR**		**3DAR**	**7DAR**	**11DAR**	
Control	0.00 (1.00)^{d}	0.00 (1.00)^{d}	0.00 (1.00)^{d}	0.00	0.00 (1.00)^{d}	0.00 (1.00)^{e}	0.00 (1.00)^{e}	0.00	0.00 (1.00)^{d}	0.00 (1.00)^{d}	0.00 (1.00)^{c}	0.00	0.00 (1.00)^{e}	0.00 (1.00)^{e}	0.00 (1.00)^{e}	0.00
3	1.00 (1.38)^{cd}	1.33 (1.52)^{c}	1.00 (1.41)^{cd}	3.33	1.00 (1.38)^{cd}	1.33 (1.52)^{d}	1.00 (1.38)^{de}	3.33	1.33 (1.52)^{c}	1.67 (1.57)^{c}	1.33 (1.52)^{b}	4.33	1.67 (1.62)^{d}	1.33 (1.52)^{d}	1.33 (1.47)^{de}	4.33
6	1.00 (1.38)^{cd}	1.67 (1.62)^{bc}	1.00 (1.41)^{cd}	3.67	1.67 (1.63)^{bc}	1.67 (1.62)^{cd}	1.67 (1.60)^{cd}	5.00	1.67 (1.57)^{c}	2.00 (1.71)^{bc}	1.67 (1.57)^{b}	5.33	2.67 (1.91)^{cd}	2.67 (1.91)^{cd}	2.33 (1.82)^{cd}	7.67
9	1.33 (1.52)^{bc}	2.00 (1.65)^{bc}	1.33 (1.48)^{c}	4.67	2.33 (1.82)^{abc}	2.67 (1.91)^{bc}	2.00 (1.71)^{bcd}	7.00	3.00 (1.98)^{bc}	3.67 (2.15)^{ab}	3.00 (1.98)^{ab}	9.67	3.67 (2.14)^{bc}	4.00 (2.22)^{bc}	3.00 (1.98)^{bc}	10.67
12	2.33 (1.82)^{abc}	2.67 (1.91)^{abc}	1.67 (1.60)^{bc}	6.67	2.67 (1.91)^{ab}	3.00 (2.00)^{abc}	2.33 (1.79)^{abcd}	8.00	3.33 (2.07)^{b}	4.00 (2.22)^{ab}	4.00 (2.22)^{a}	11.33	4.67 (2.36)^{ab}	5.00 (2.44)^{ab}	4.00 (2.22)^{abc}	13.67
15	2.67 (1.90)^{ab}	3.33 (2.07)^{ab}	2.33 (1.80)^{abc}	8.33	3.00 (1.99)^{ab}	3.67 (2.13)^{ab}	3.00 (1.98)^{abc}	9.67	4.00 (2.21)^{ab}	4.67 (2.36)^{a}	4.33 (2.30)^{a}	13.00	5.00 (2.42)^{ab}	6.00 (2.64)^{ab}	5.00 (2.44)^{ab}	16.00
18	2.67 (1.91)^{ab}	4.00 (2.22)^{a}	3.00 (1.98)^{ab}	9.67	3.67 (2.15)^{a}	4.33 (2.29)^{ab}	4.00 (2.20)^{ab}	12.00	5.00 (2.40)^{ab}	5.67 (2.58)^{a}	4.67 (2.36)^{a}	15.33	6.00 (2.64)^{a}	6.67 (2.76)^{a}	6.00 (2.64)^{a}	18.67
21	3.67 (2.14)^{a}	4.33 (2.30)^{a}	4.00 (2.22)^{a}	12.00	4.00 (2.20)^{a}	4.67 (2.36)^{a}	4.33 (2.30)^{a}	13.00	5.67 (2.57)^{a}	6.00 (2.60)^{a}	5.33 (2.49)^{a}	17.00	6.33 (2.68)^{a}	7.00 (2.79)^{a}	6.33 (2.69)^{a}	19.67
F-test	Sig	Sig	Sig		Sig	Sig	Sig		Sig	Sig	Sig		Sig	Sig	Sig	
SEm±	0.15	0.15	0.15		0.15	0.13	0.18		0.16	0.14	0.17		0.14	0.15	0.16	
CD (P=0.05)	0.47	0.47	0.45		0.45	0.39	0.55		0.48	0.42	0.50		0.43	0.45	0.47	

Figures in parentheses are square root transformed values

Numbers followed by same superscript are not statistically different (P=0.05)

Table 4.3. Damage on bolls by larvae of *S. litura* on BG II *Bt* cotton

Treatments	Number of bolls damaged/plant (Mean of Three Replications)															
No. of Larvae released/plant	50 DAS			Total	80 DAS			Total	110 DAS			Total	140 DAS			Total
	3DAR	7DAR	11DAR		3DAR	7DAR	11DAR		3DAR	7DAR	11DAR		3DAR	7DAR	11DAR	
Control	0.00	0.00	0.00	0.00	0.00 (1.00)^{d}	0.00 (1.00)^{e}	0.00 (1.00)^{d}	0.00	0.00 (1.00)^{e}	0.00 (1.00)^{e}	0.00 (1.00)^{d}	0.00	0.00 (1.00)^{d}	0.00 (1.00)^{e}	0.00 (1.00)^{d}	0.00
3	0.00	0.00	0.00	0.00	0.67 (1.27)^{cd}	1.00 (1.41)^{d}	0.33 (1.13)^{cd}	2.00	0.33 (1.13)^{de}	0.67 (1.27)^{de}	0.33 (1.13)^{cd}	1.33	0.33 (1.13)^{d}	0.33 (1.13)^{e}	0.33 (1.13)^{cd}	1.00
6	0.00	0.00	0.00	0.00	1.00 (1.38)^{bc}	1.33 (1.52)^{cd}	0.67 (1.27)^{bcd}	3.00	1.00 (1.41)^{cd}	1.00 (1.38)^{cde}	0.67 (1.27)^{bcd}	2.67	1.33 (1.52)^{cd}	1.67 (1.62)^{d}	1.00 (1.38)^{bcd}	4.00
9	0.00	0.00	0.00	0.00	1.00 (1.41)^{bc}	1.67 (1.62)^{bcd}	1.00 (1.38)^{abcd}	3.67	1.33 (1.52)^{cd}	1.67 (1.60)^{bcd}	1.67 (1.62)^{abc}	4.67	1.67 (1.55)^{cd}	2.33 (1.82)^{cd}	1.33 (1.52)^{abcd}	5.33
12	0.00	0.00	0.00	0.00	1.33 (1.52)^{bc}	2.00 (1.73)^{bcd}	1.33 (1.52)^{abc}	4.67	2.00 (1.71)^{bc}	2.33 (1.80)^{bc}	2.00 (1.71)^{ab}	6.33	2.00 (1.71)^{bc}	2.67 (1.91)^{bcd}	1.67 (1.57)^{abc}	6.33
15	0.00	0.00	0.00	0.00	2.00 (1.71)^{ab}	2.33 (1.82)^{bc}	1.67 (1.62)^{ab}	6.00	2.67 (1.91)^{ab}	3.00 (1.95)^{ab}	2.33 (1.80)^{a}	8.00	3.33 (2.06)^{abc}	3.67 (2.14)^{abc}	2.00 (1.71)^{ab}	9.00
18	0.00	0.00	0.00	0.00	2.67 (1.91)^{a}	2.67 (1.88)^{ab}	2.00 (1.71)^{ab}	7.33	3.00 (1.97)^{ab}	3.33 (2.07)^{ab}	2.67 (1.86)^{a}	9.00	3.67 (2.12)^{ab}	4.00 (2.22)^{ab}	2.67 (1.88)^{ab}	10.33
21	0.00	0.00	0.00	0.00	3.00 (2.00)^{a}	3.67 (2.15)^{a}	2.33 (1.80)^{a}	9.00	4.00 (2.22)^{a}	4.33 (2.29)^{a}	3.00 (1.98)^{a}	11.33	4.33 (2.29)^{a}	4.67 (2.36)^{a}	3.00 (1.98)^{a}	12.00
F-test					Sig	Sig	Sig		Sig	Sig	Sig		Sig	Sig	Sig	
SEm±	0	0	0		0.12	0.11	0.15		0.13	0.16	0.17		0.19	0.13	0.18	
CD (P=0.05)	0	0	0		0.37	0.32	0.46		0.38	0.48	0.52		0.56	0.39	0.55	

Figures in parentheses are square root transformed values

Numbers followed by same superscript are not statistically different (P=0.05)

Table 4.4. Seed cotton yield and percent yield reduction over control

Treatment	Seed cotton yield (g/plant)				Per cent reduction over control			
	Released larvae at 50 DAS	Released larvae at 80 DAS	Released larvae at 110 DAS	Released larvae at 140 DAS	50 DAS	80 DAS	110 DAS	140 DAS
Control	293.33	276.67	275.00	270.33	0.00	0.00	0.00	0.00
3	276.00	249.67	245.33	245.33	5.91	9.76	10.79	9.25
6	271.67	235.00	233.33	212.67	7.38	15.06	15.15	21.33
9	270.00	221.67	202.67	194.33	7.95	19.88	26.30	28.11
12	263.33	211.67	187.67	170.33	10.23	23.49	31.76	36.99
15	253.33	202.67	168.00	145.00	13.64	26.75	38.91	46.36
18	246.67	178.33	154.33	126.33	15.91	35.54	43.88	53.27
21	230.00	166.67	135.00	119.33	21.59	39.76	50.91	55.86
SEm±	18.87	17.37	15.21	15.33				
CD (P=0.05)	57.25	52.68	46.14	47.12				
CV %	12.43	13.81	13.16	14.51				

Table 4.5. Comparison of per cent leaf damage at 50, 110 and 140 DAS

Treatments No. of Larvae released/plant	Leaf damage (cm^2)			% Leaf damage increase over 50 DAS larvae released crop	
	50 DAS	**110 DAS**	**140 DAS**	**110 DAS**	**140 DAS**
Control	0.00	0.00	0.00	0.00	0.00
3	25.97	33.20	46.40	21.77	44.02
6	48.00	56.99	68.13	15.77	29.55
9	69.12	79.27	102.87	12.80	32.81
12	96.10	112.17	127.43	14.32	24.59
15	115.67	134.03	159.27	13.70	27.38
18	144.80	160.70	187.33	9.89	22.70
21	171.63	189.77	219.13	9.56	21.68

Table 4.6. Comparison of per cent seed cotton yield at 50, 110 and 140 DAS

Treatment	Yield (g/plant)			% Reduction of yield over 50 DAS	
No. of Larvae released/plant	**50 DAS**	**110 DAS**	**140 DAS**	**110 DAS**	**140 DAS**
Control	293.33	275.00	270.33	6.25	7.84
3	276.00	245.33	245.33	11.11	11.11
6	271.67	233.33	212.67	14.11	21.72
9	270.00	202.67	194.33	24.94	28.03
12	263.33	187.67	170.33	28.73	35.32
15	253.33	168.00	145.00	33.68	42.76
18	246.67	154.33	126.33	37.43	48.79
21	230.00	135	119.33	41.30	48.12

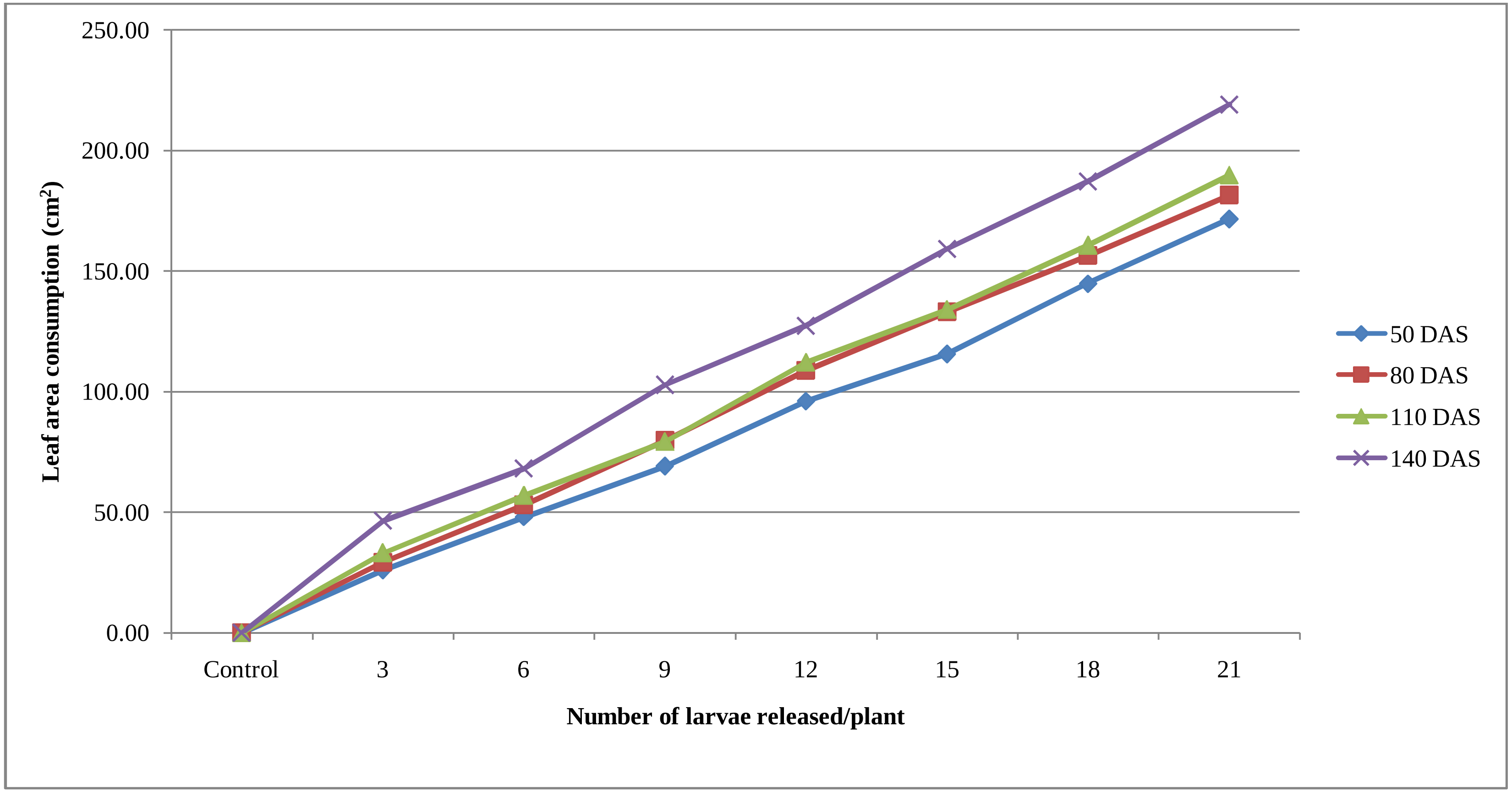


Fig4.1: Leaf area consumption of *S. litura* larvae on BG II *Bt* cotton

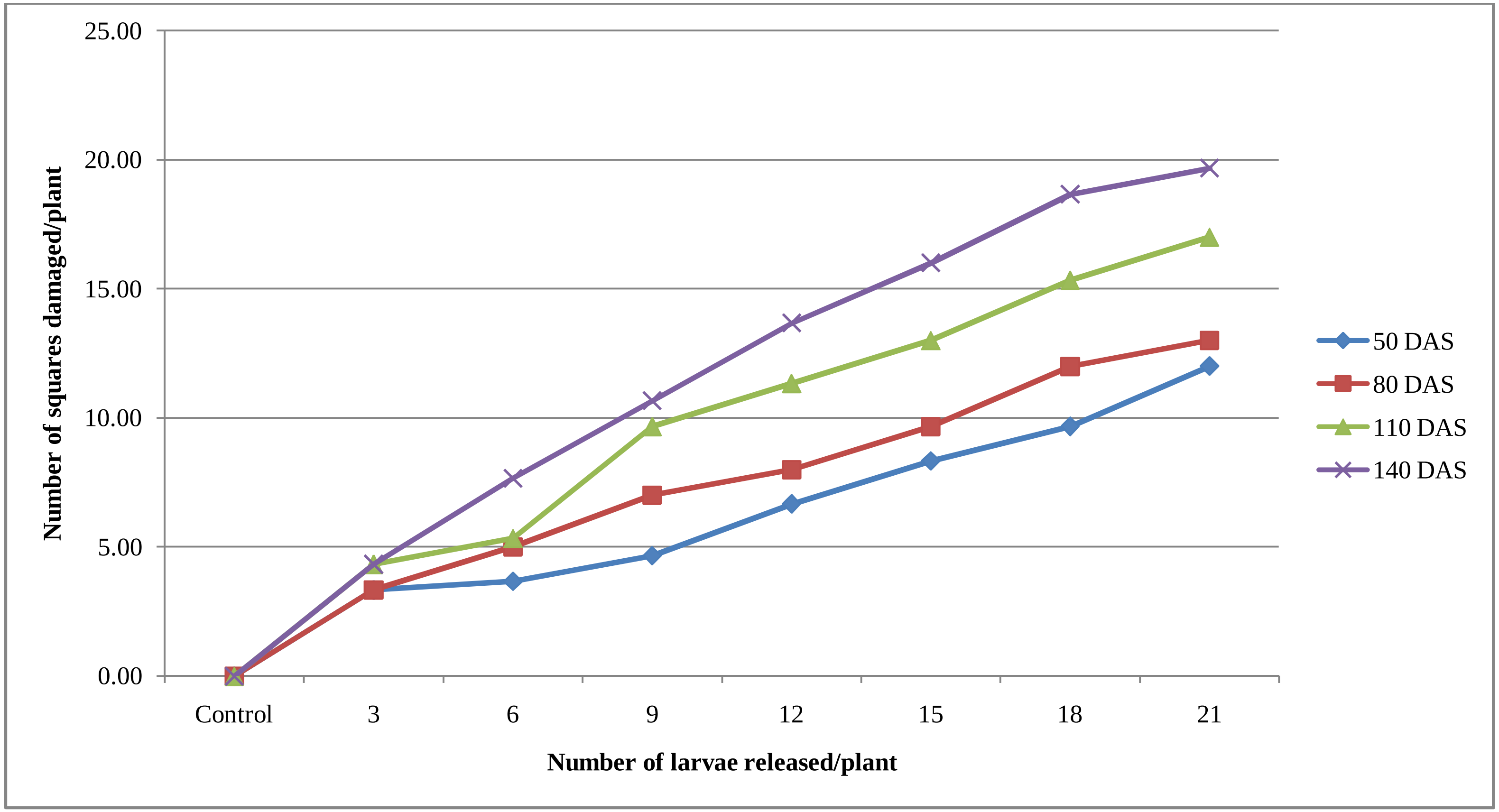


Fig 4.2: Square damage by larvae of *S. litura* on BG II *Bt* cotton

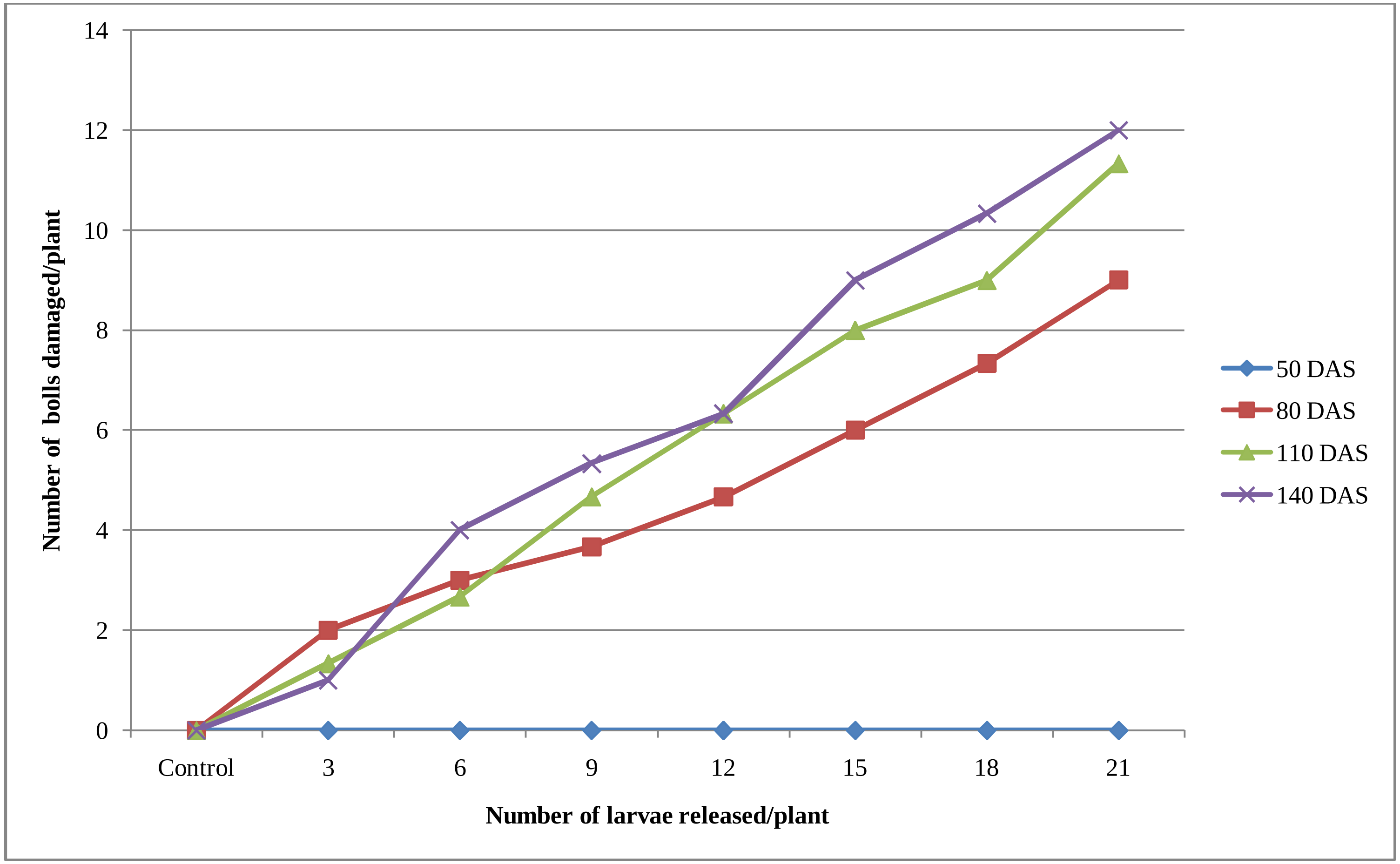


Fig 4.3: Boll damage by larvae of *S. litura* on BG II *Bt* cotton

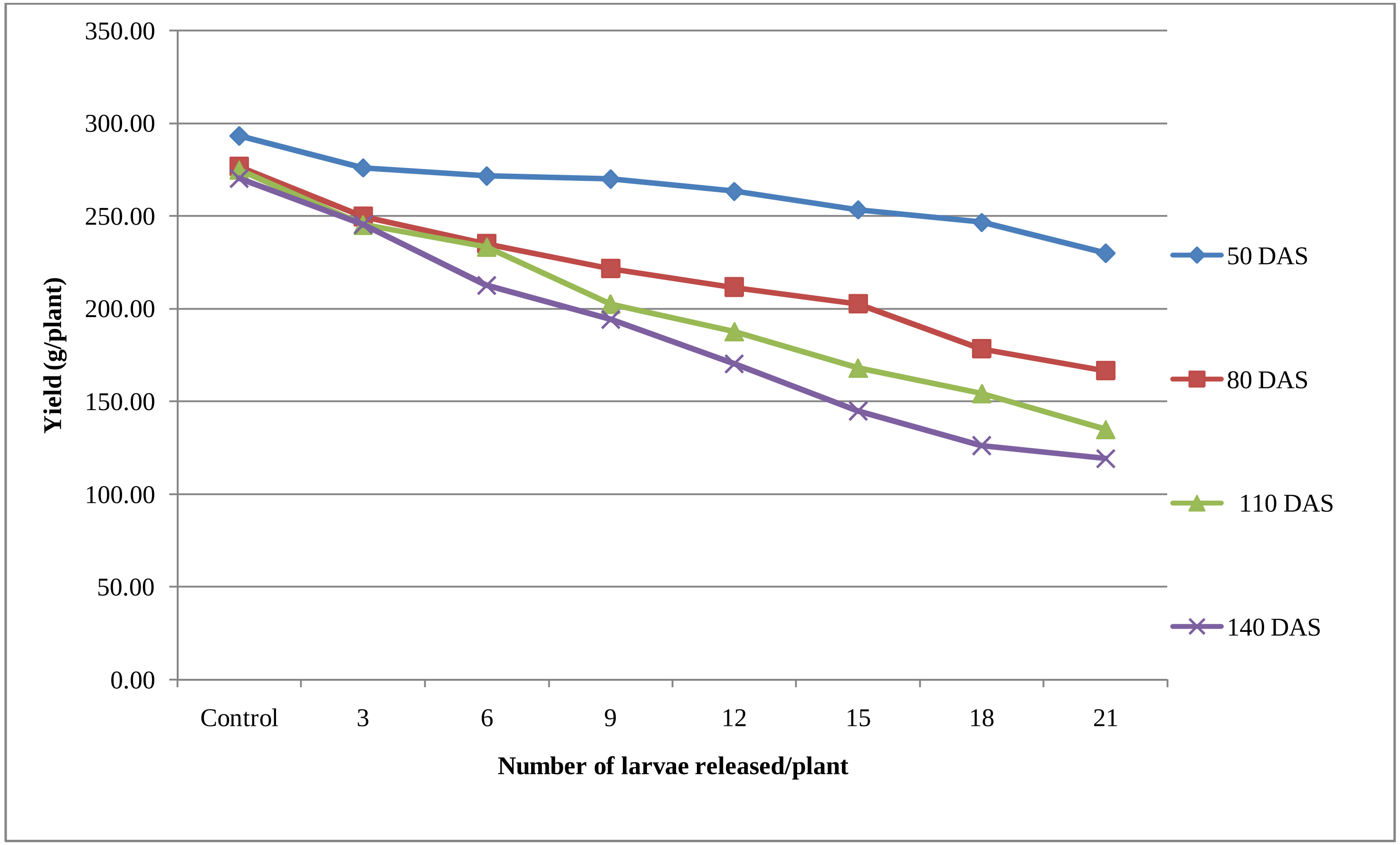


Fig 4.4: Seed cotton yield at different levels of larvae (*S. litura*) released at various days after sowing

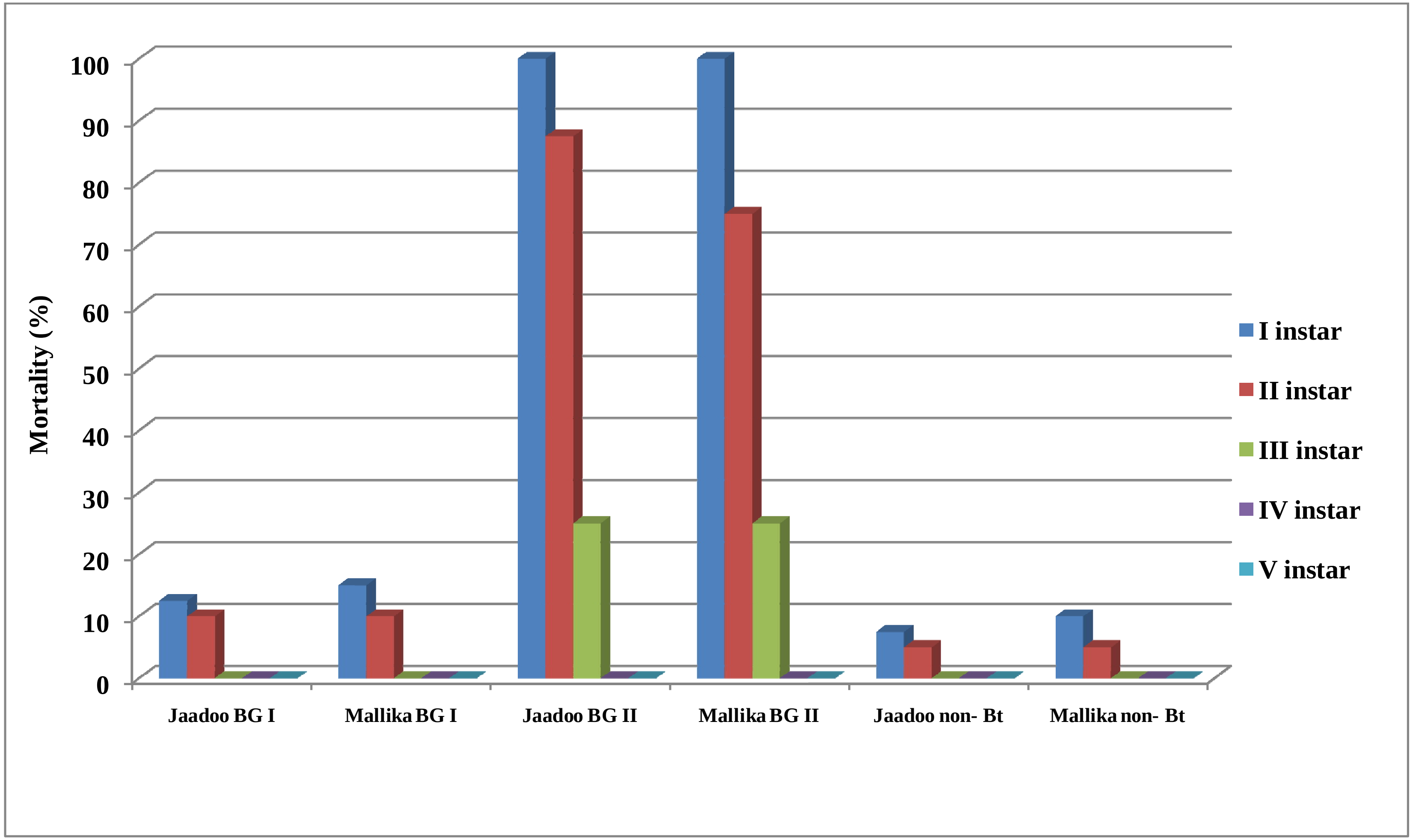


Fig 4.5. Percent mortality of larval instars of *S. litura* on leaves of test hybrids

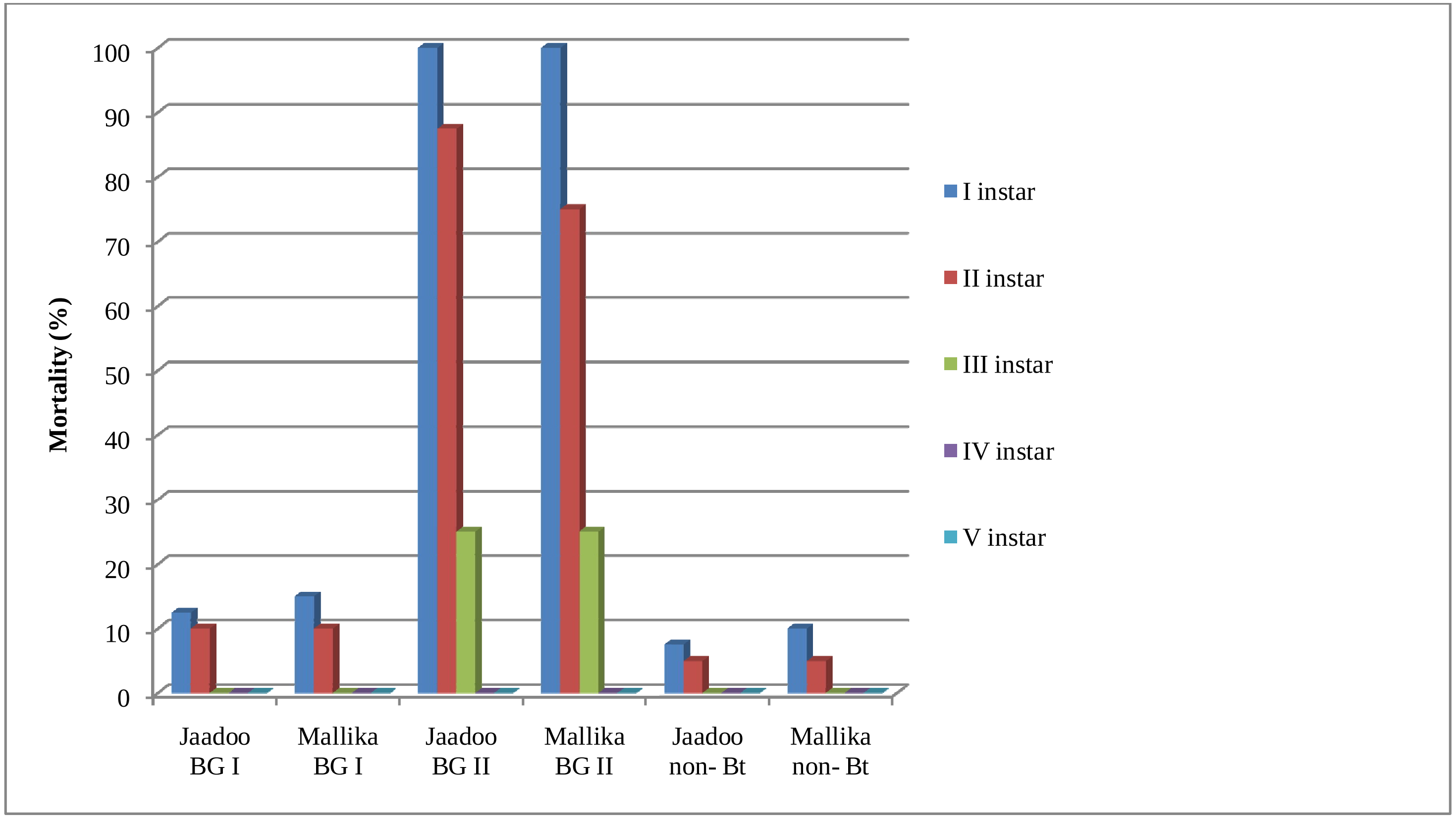


Fig 4.6. Percent mortality of larval instars of *S. litura* on squares of test hybrids

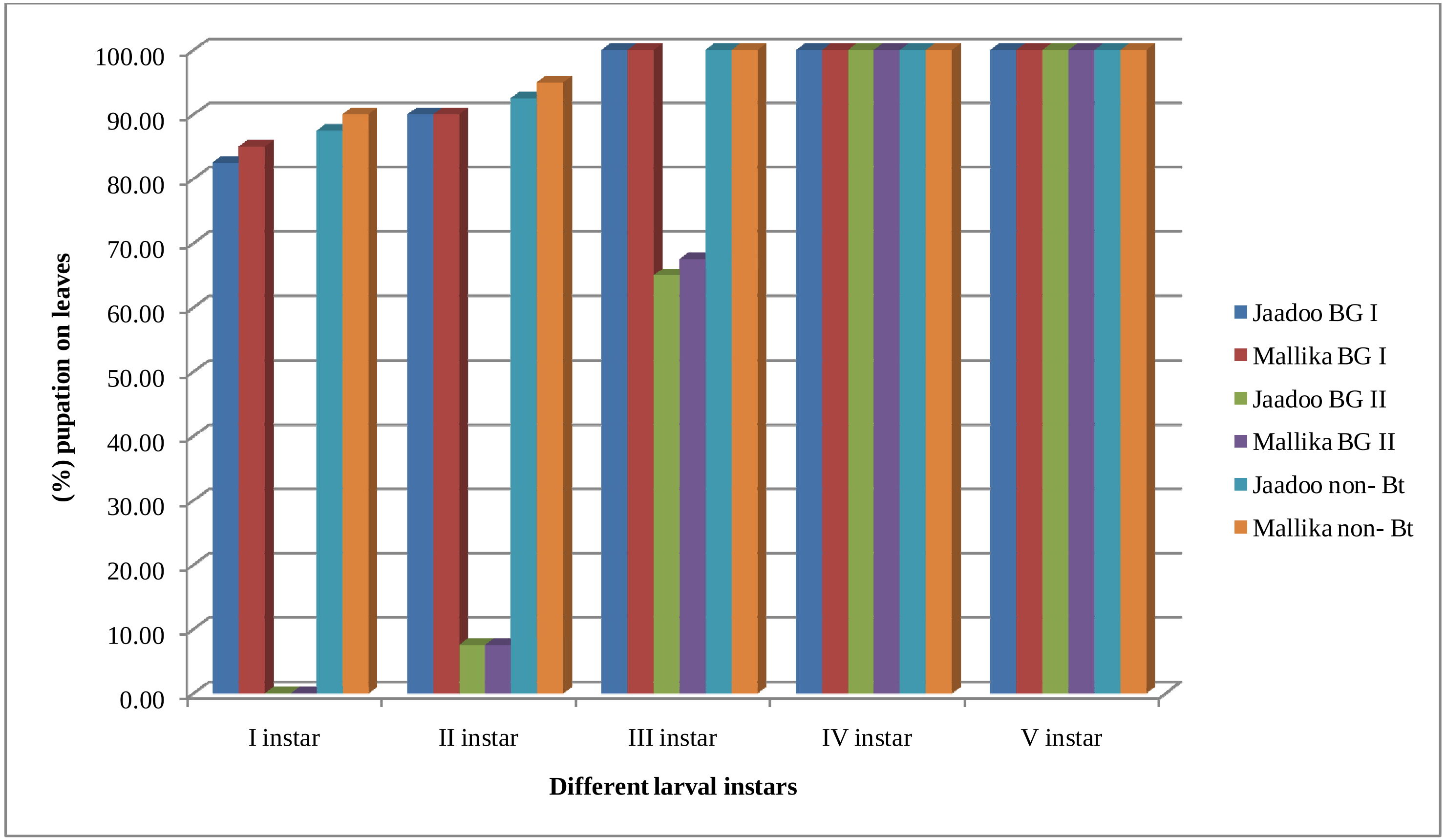


Fig 4.9. Percent pupation of surviving larvae of *S. litura* on leaves of test hybrids

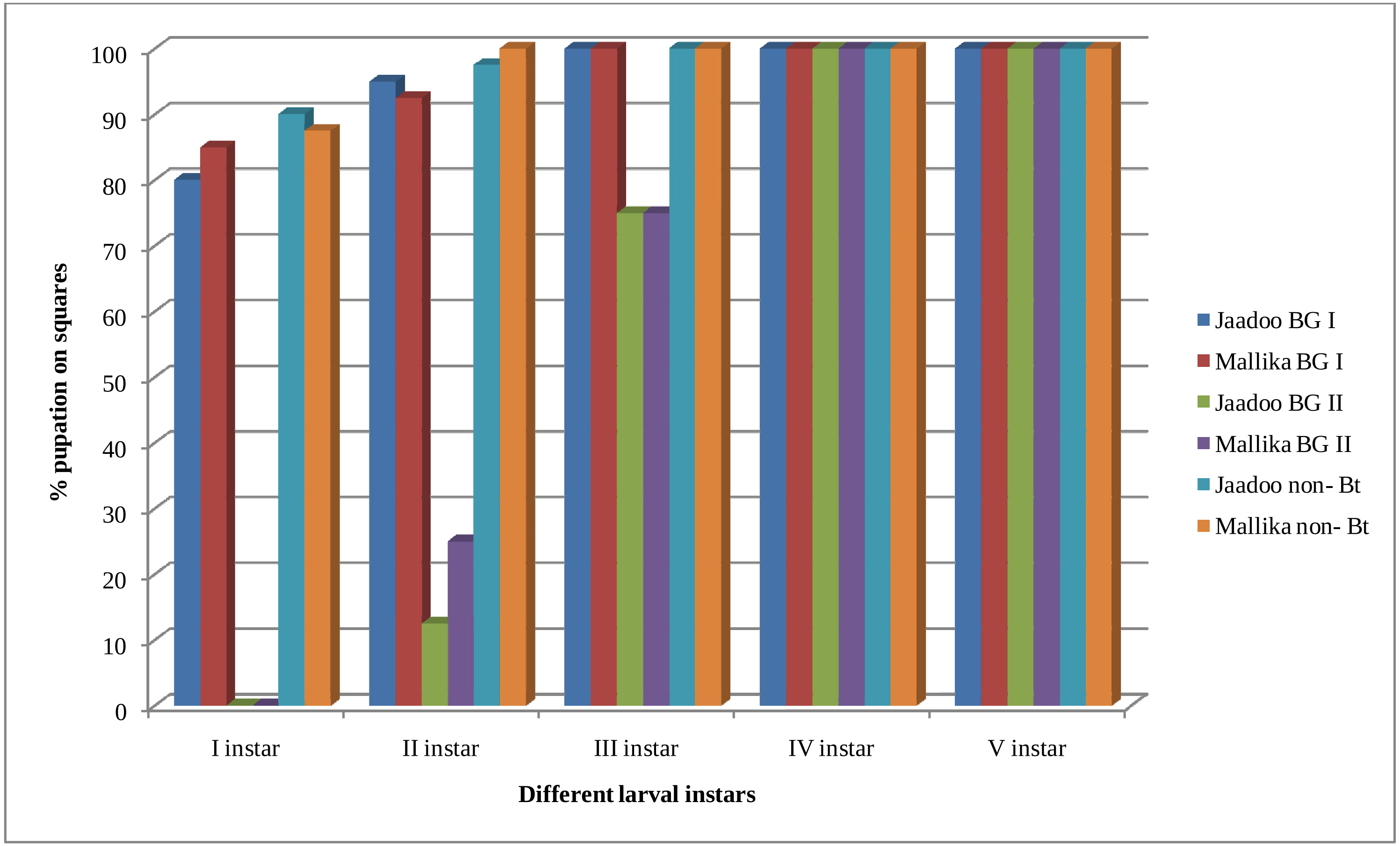


Fig 4.10. Percent pupation of surviving larvae of *S. litura* on squares of test hybrids

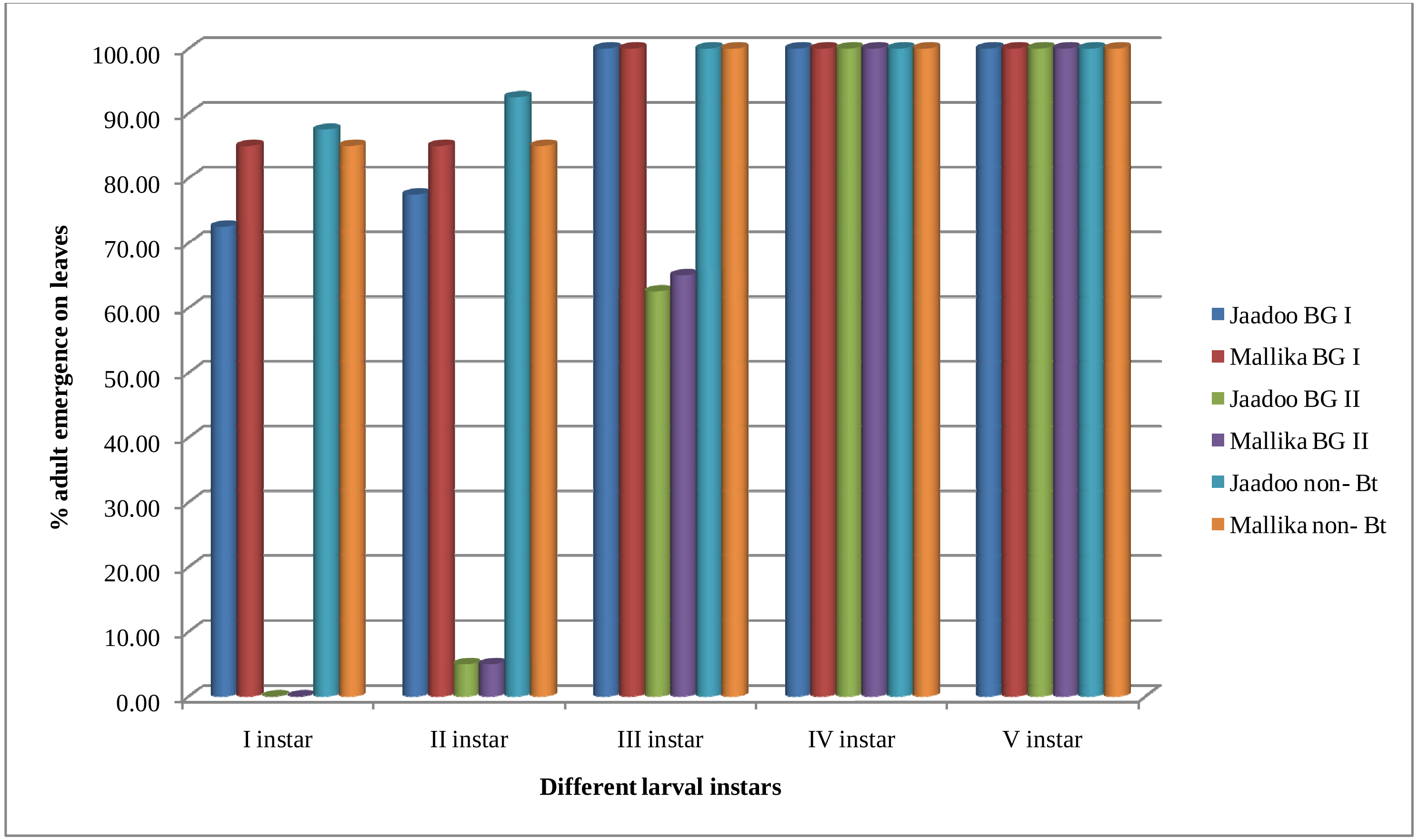


Fig 4.11: Percent adult emergence of *S. litura* on leaves of test hybrids

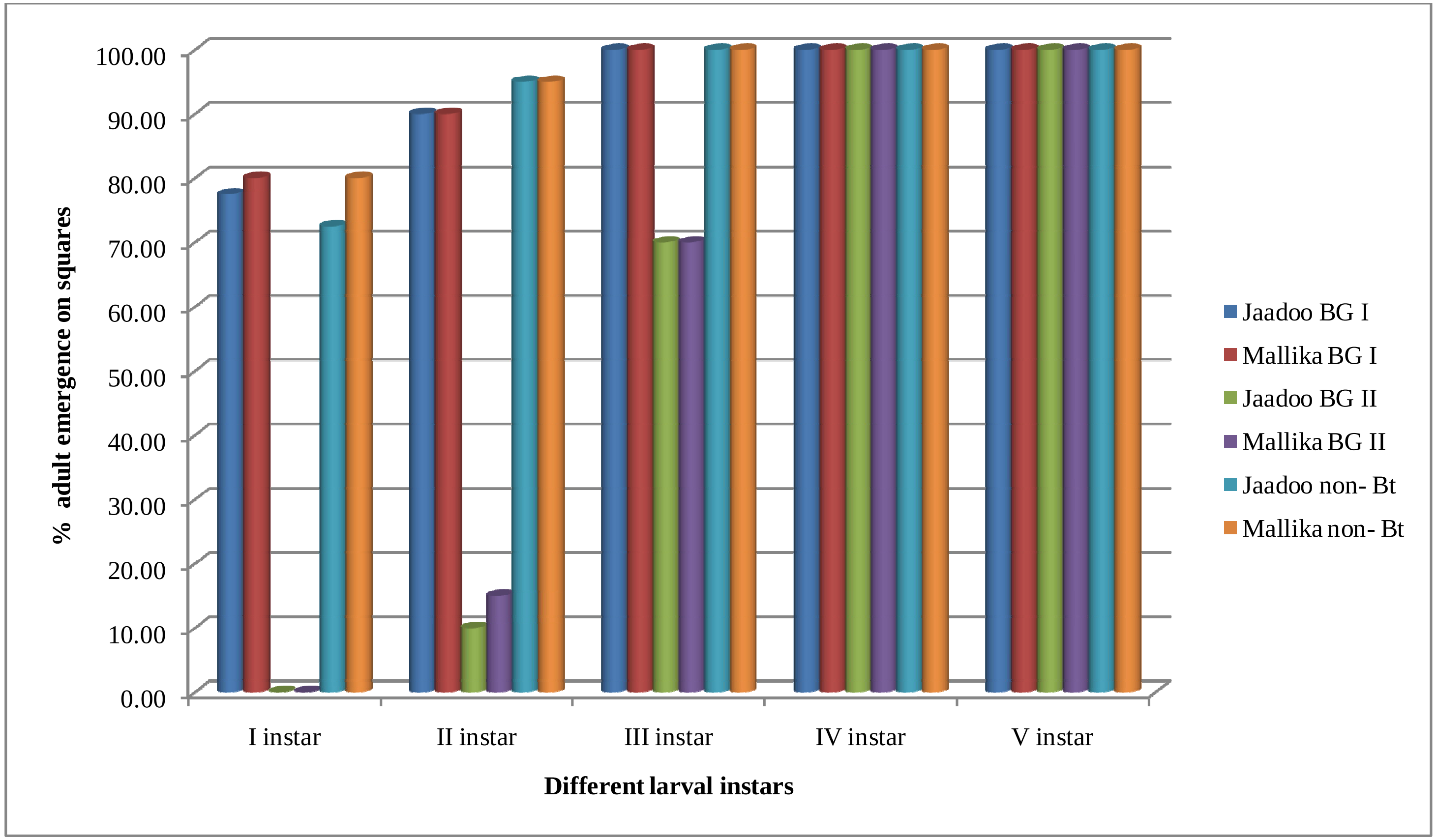


Fig 4.12. Percent adult emergence of *S. litura* on squares of test hybrids

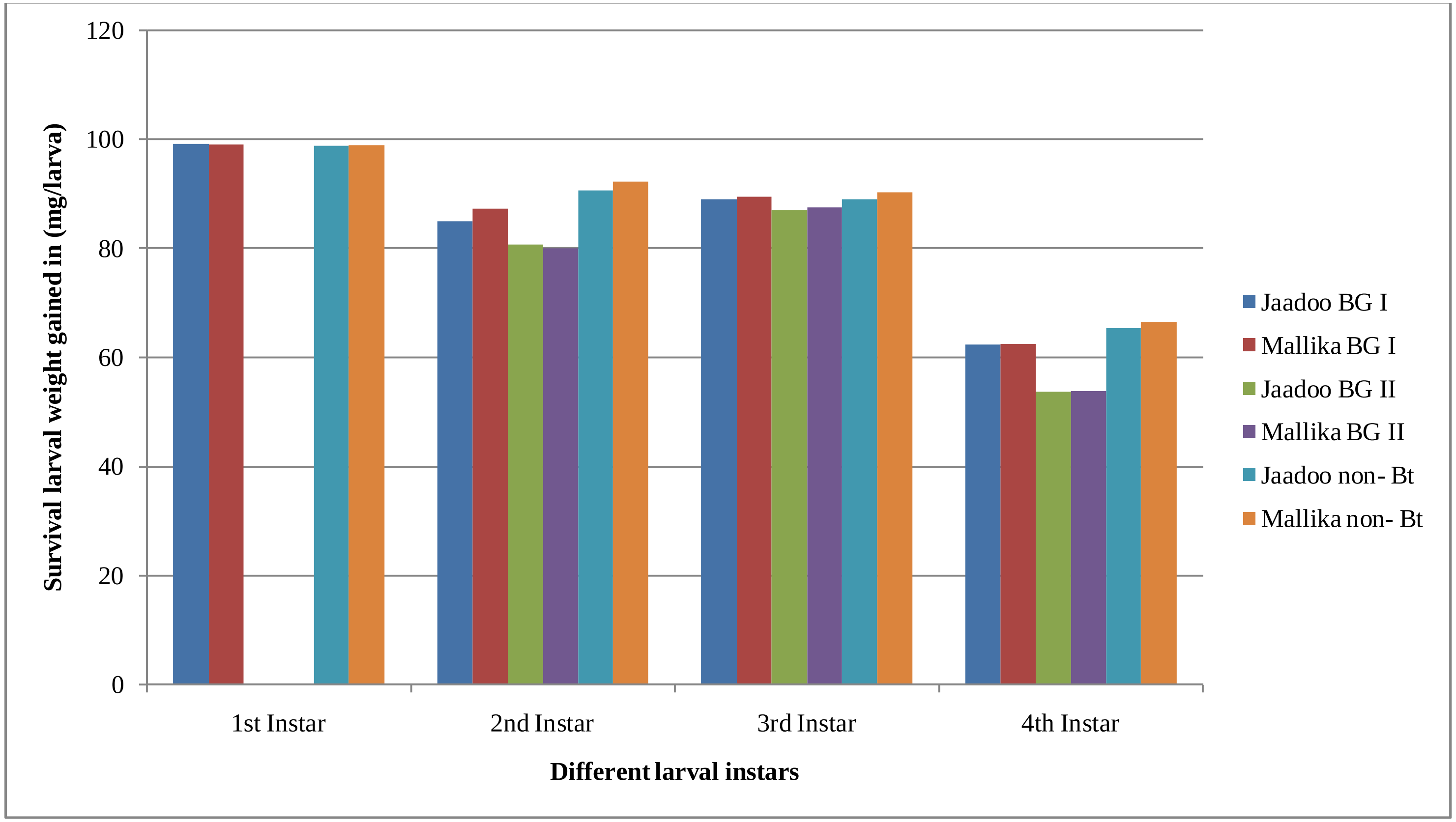


Fig 4.7. Weight gain of different larval instars of *S. litura* on leaves of test hybrids

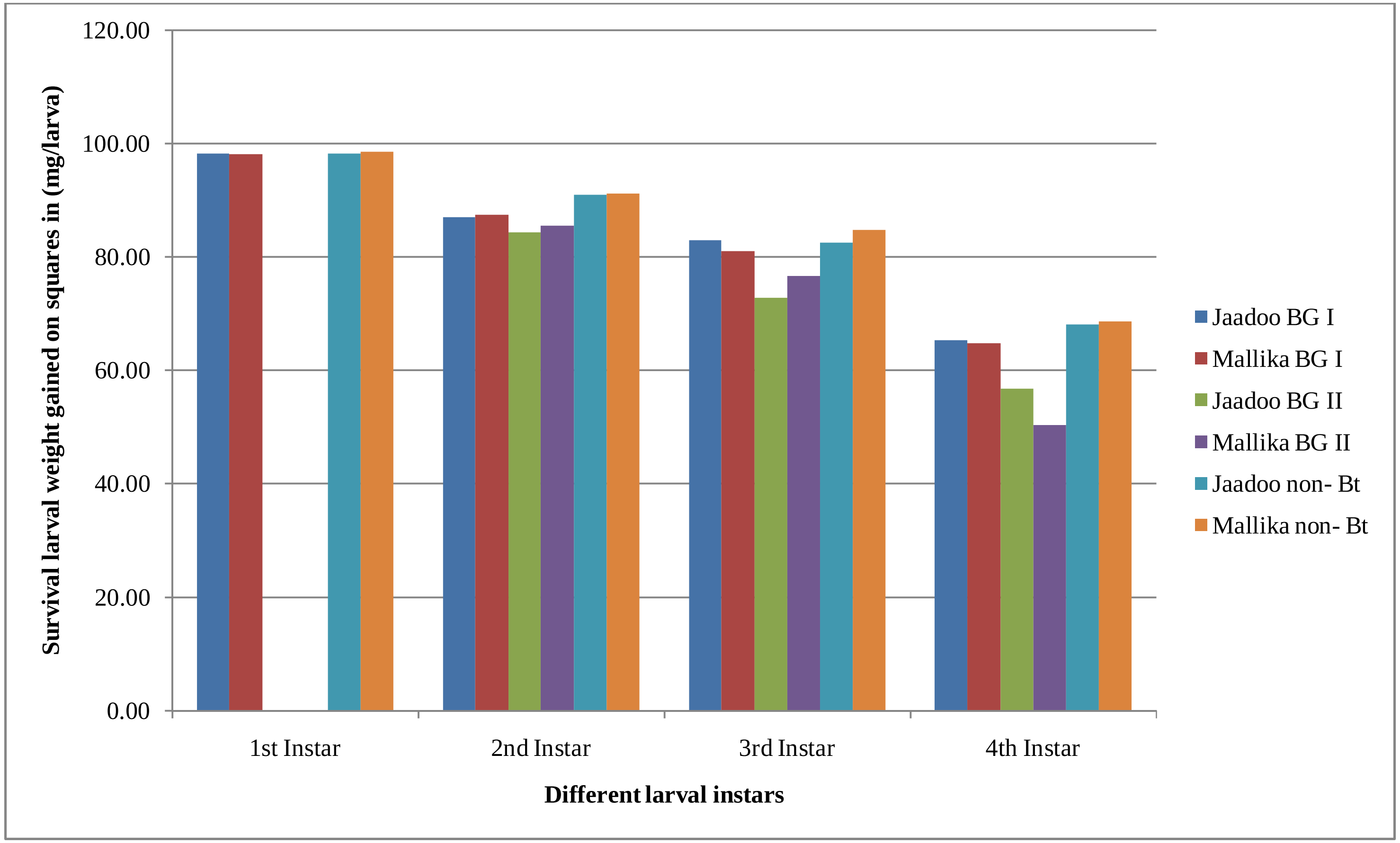


Fig 4.8. Weight gain of different larval instars of *S. litura* on squares of test hybrids

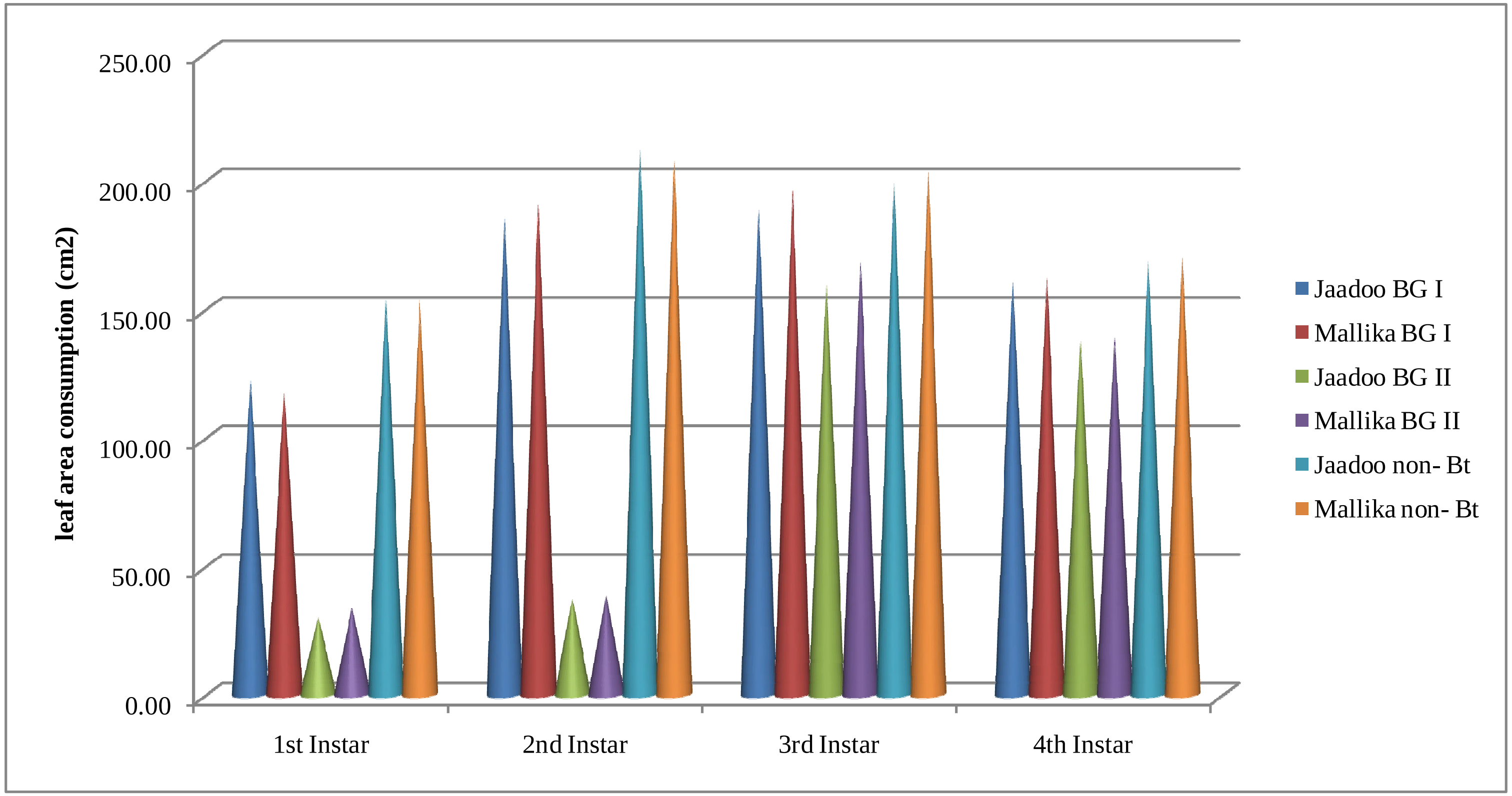


Fig 4.13. Leaf area consumption by larval instars of *S. litura* on test hybrids

Chapter I

INTRODUCTION

Cotton, *Gossypium* spp. (L.) the "King of fibers" is an important commercial crop grown in more than 70 countries under diverse agro-climatic conditions. Cotton plays a predominant role in Indian economy as it provides 80 per cent of raw material to the textile industry and livelihood for more than 100 Million people through its production, processing and marketing (Rakesh and Kathane, 1989).

India is an important grower of cotton on a global scale. India ranks first in cultivated area (117.27 lakh ha) and second in production (398.00 lakh bales) and productivity (577 kg ha^{-1}) in the world. It is being cultivated in 22.69 lakh ha with production of 78.00 lakh bales and productivity of 584 kg ha^{-1} in Andhra Pradesh (Cotton Advisory Board, 2013- 14).

India is planning to produce 40 million bales of lint by 2050 AD to meet the anticipated domestic and export requirements. To fulfill this projected requirement, the cotton production has to be increased by 15 per cent over existing levels which has to come mainly from increased productivity. Reasons for the low productivity of cotton in India are cultivation under rainfed conditions, predominance of pests and diseases, inadequate and unscientific method of cultivation.

It has been reported that 162 insect pests attack cotton in India (Lingappa, 2001). But key production constraints are sucking pests and bollworms are which cause losses to the tune of 30-80% (Patil, 1998).

At this juncture, advances made in the genetic engineering led to the development of first biotechnological tool in the world, the *Bacillus thuringiensis* (*Bt*) *Cry1Ac* gene (crystal protein delta endotoxin) incorporated cotton (Bollgard) which was commercially cultivated since 2002 in India. The first successful example of the biotechnology in crop protection is Bollgard. The Bollgard imparts insect resistance by virtue of which the cotton plant is able to resist attack of lepidopteron insect pests. This in-built protection in Bollgard cotton is provided by the presence of the *Bt* gene in the seed. Bollgard cultivars express the Cry1Ac protein and are active against larval stages of selected lepidopteron insects (Perlak *et al*., 1990). The technology has been anticipated to address bollworm infestation and significant reduction in the overall use of insecticides (Fernandez and McBride, 2000). *Bt* cotton technology has a capability of reducing insect pest infestations by 60-90 per cent under field conditions (Kranthi *et al*., 2004).

Among the bollworms, tobacco caterpillar (*Spodoptera litura)* is one of the key pests on cotton. The three R's (Resistance, Resurgence and Residue) are the most potential problems associated with indiscriminate use of insecticides in cotton agro-ecosystem (Armes *et al*., 1992). Moreover, the modern production technologies which rely heavily on the use of chemicals have significantly lowered the cost benefit ratio in cotton.

Tobacco caterpillar *S. litura* has been noted as emerging pest in BG-I genotypes in India (Jeyakumar *et al*., 2007) with the advent of *Bt* genotypes. Transgenic *Bt* cotton with *Cry1Ac* proved not to be effective against *S. litura*. (Arshad and Suhail, 2011; Selvi *et al*., 2012; Lalitha *et al*., 2012). It has been found that *S. litura* has a greater potential to survive in the presence of *Bt* toxins when compared to other bollworms. Thus, the transgenic cotton expressing more than one *Bt* toxin targeting different insect pest complex has been considered as an ideal one.

Dual gene (stacked) genotypes known as Bollgard II (BG-II) event, where *Cry 2Ab* is incorporated along with *Cry1Ac* have assumed significance and provide season long control of key bollworms. The stacked *Bt* cotton technology

(Bollgard II) has two genes working at the same time, which delays the development of resistance to *Bt* toxin. This tendency was necessitated because, insects can develop resistance to one gene faster than to two genes working in the same genotype (Crickmore *et al.*, 1998; Sivasupramaniam *et al.*, 2003). Thus, there is a necessity to generate the information on bio-efficacy of these events to potential pest *S. litura* which can be addressed in the present investigation.

Keeping in view of this, the present study was proposed with the following objectives

Objectives:

1. Assessing the damage potential of *S. litura* on Bollgard II (BG II) *Bt* cotton.

2. Studies on the influence of Bollgard II *Bt* cotton (Cry 1Ac + Cry 2Ab) plants against *S. litura*.

Chapter II

REVIEW OF LITERATURE

Cotton is one of the important commercial fiber crop infested by wide range of insect pests at various stages of crop growth compared to any other crop. The tobacco caterpillar, *S. litura* is one among the nine key pests on cotton, causing considerable yield losses. However, the rapid development of resistance is another problem associated with *Spodoptera* management due to indiscriminate use of insecticides leading to ecocidal effects. Development of an eco friendly and potent method to reduce the incidence of *Spodoptera* is highly imperative at this juncture. The literature on the cultivation of new generation *Bt* cotton genotypes as a newly emerged tactic to reduce the amount of pesticide usage in cotton against this pest is scanty. However, an attempt has been made to consolidate the available literature pertaining to the performance of new generation *Bt* cotton genotypes against *S. litura*, expression of Cry protein in new generation *Bt* cotton genotypes at different crop growth stages any damage potential in comparison with non transgenic conventional cotton in this chapter.

2.1 ASSESSING THE DAMAGE POTENTIAL OF *S. litura* ON BG II *Bt* COTTON

Katayama and Sano (1989) studied the injurious effect of *S. litura* on red bean (*Vigna angularis*) plants. As the defoliation rate increased, the number of pods and the weight of grains decreased, especially at the end of the defoliation cycle. The average leaf area of *V. angularis* consumed by *S. litura* was 203.9 cm^2, of which 82% was consumed by final-instar larvae. Based on relationships between the defoliation area and the yield index of *V. angularis,* the number of final-instar larvae which caused 5% loss of yield (tolerable injury level) was estimated to be approximately two per plant.

El-Sherif and Mesbah (1992) conducted an experiment at different growth stages of cotton plant. The losses up to 50% were reported at vegetative growth (24-28%), budding (28-49%), flowering (35-37%), square (27-40%) and boll stages (14-18%) decreased the cotton yield. It was suggested that the economic injury levels of *S. littoralis* on cotton in Giza could be approximated to 40, 31, 44, 60 and 142 egg masses or 0.38, 0.3, 0.42, 0.58 and 1.35 larvae/plant at each of the growth stages, respectively.

Dhir *et al.* (1992) reported that groundnut plant at 30 DAS and flowering stages single larva of *S. litura* per plant consumed about 54.7% leaf area and reduced pod yield by 25.8%, at flowering, it consumed 49.1% leaf area and reduced the yield by 19%, at pegging, it consumed about 38.8% leaf area and resulted in a yield loss of 5.7%.

Luttrell and Mink (1999) reported the damage caused by different larval instars confined for 48 h in cotton bags containing fruiting structures. Third instars damaged 0.63 squares, 0.72 small bolls, and 0.40 large bolls, fourth instars damaged 0.71 squares, 0.76 small bolls, and 0.63 large bolls; and fifth instars damaged 0.83 squares, 0.81 small bolls, and 0.66 large bolls. Damage to squares by all instars resulted in a significant reduction in yield.

Qin HouGuo *et al*. (2000) studied the damage caused by *S. litura* in cotton through artificial inoculation. It can consume 3.61 cotton leaves, 0.97 square, 0.37 flower and 0.27 boll. The damage increased in exponential function with the rise of larval numbers. Based on the control cost and cotton price, the economic threshold was determined to be 2.6 larvae per plant when the population of fourth generation larvae in the third instar is at a peak.

Kang *et al.* (2004) showed that there was no significant difference between transgenic *Bt* cotton Kemian 1 and non transgenic *Bt* cotton Yumian 1 in the rate of damage to leaves, flowers and buds by *S. litura*. There was no difference in the development duration and survival rate of *S. litura* but high population growing trend index was reported in Kemian 1 than Yumian 1. Hence, they reported that Kemian 1 was susceptible to *S. litura*.

Lee *et al*. (2006) conducted an experiment to study the damage aspects of soyabean by common cutworm, *S. litura*, at different larval densities and different growth stages of soyabean. Based on the relationships between the densities of *S. litura* larvae and the yield index of soyabean, the number of larvae (2^{nd} - 3^{rd} instar) which caused 5% loss of yield (tolerable injury level). The average soyabean leaf areas consumed by the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, and 5^{th} larvae of *S. litura* for 24 h at 28^{0}C was 0.3, 0.7, 2.6, 4.0, 20.1 and 55.8 cm^2, respectively.

Abhilash and Patil (2008) reported that per cent pod damage which ranged from zero in completely protected plant to 25.24 where six larvae per plant were released. The plants which received 1, 2, 3 and 6 larvae per plant differed significantly with each other. The correlation between the number of larvae per plant and the per cent pod damage was positive and significant.

Zahid *et al*. (2008) studied the damage potential and economic threshold levels (ETL) of *Maruca vitrata* larvae on mungbean which were worked out at on an average single larva per meter row reduced the yield to an extent of 154 kg ha^{-1} and 106 kg^{-ha} in a two year study. Economic injury level (EIL) for *M. vitrata* were determined as 1.0 and 1.2 larvae per meter row in two year study. On the basis of means of two years the EIL and ETL values of *M. vitrata* were 1.08 & 0.81 larvae per meter row, respectively.

Zahid *et al.* (2008) conducted an experiment to determine the damage potential and ETL for *Helicoverpa armigera* larvae on chickpea crop. On an average, single larva per meter row reduced the yield to the extent of 155 kg $^{-ha}$ and 157 kg^{-ha} in a two year study. EILs for *H. armigera* were determined as 12.0 and 0.95 per meter row and ETLs was at 09.0 and 0 .73 larvae per m row length of the crop.

Bheemanna *et al.* (2010) studied the damage potential and EIL for *S. litura* larvae on *Bt* cotton (NCS 145 BG) at different days after sowing during 2008-09 and 2009-10. EIL was 2.64, 3.47 and 4.27 larvae per plant at 90, 120 and 135 days after sowing, respectively during 2008-09. Similarly during 2009-10, EIL was 1.68, 2.44, 2.54 and 3.59 larvae per plant at 90, 105, 120 and 135 DAS, respectively.

Chavan *et al.* (2010) studied the losses caused by bollworms using desi cotton variety PA- 255. The mean infestation at the time of harvesting was 6.67 per cent in protected and 11.88 % in unprotected plot. Overall infestation in locules was observed to be 4.31% in protected and 7.32 % in unprotected plots. The avoidable losses due to major insect pests (sucking + bollworms) observed to be 2.94 q/ha or 28.13 %.

Dhawan *et al.* (2010) conducted an experiment to study losses caused due to insect pests in four *Bt* cotton hybrids-RCH 134, RCH 317, MRC 6301 and MRC 6304 in Punjab. Among all the hybrids RCH 134 recorded higher avoidable losses to an extent of 4.1, 6.5 and 8.8 %, when chemical protection is given against sucking pest, tobacoo caterpillar and both sucking pests and tobacco caterpillar, respectively.

Santos *et al.* (2010) reported that the larva of *S. eridania* damaged 1.7 flower buds, but did not damage bolls, while larva of *S. cosmioides* damaged 5.2 flower buds and 3.0 cotton bolls. *S. eridania* and *S. cosmioides* can be considered as species with potential to cause economic damage to cotton plants.

Sahebari and Radjab (2010) studied the (EIL) of chickpea pod borer *Heliothis viriplaca* by using two varieties including Jam and Beevanij. Number of larvae per row with four metre length was counted and the amount of crop losses caused by one larva was estimated to be 0.01463 and 0.00403 kg for Jam and 0.1283 and 0.00267 kg for Beevanij in high precipitation and normal precipitation years respectively. Estimated EIL rates were 0.35 for Jam and 0.4 for Beevanij varieties in high rainfall condition, 1.25 for Jam and 1.9 for Beevanij in normal rainfall years.

Badiger *et al.* (2011) studied the stacked *Bt* hybrids and reported that, Steplon BG-II and Kashinath hybrids were better compared to other hybrids. All the stacked *Bt* hybrids recorded significantly lower pink bollworm and tobacco caterpillar larval population, green boll and locule damage, tobacco caterpillar infested leaves per plant and damaged area per infested leaf compared to single gene *Bt* hybrids.

Shashikant *et al*. (2011) studied the second generation genotypes MRC-7201 and MRC-6322 with *Cry1Ac* + *Cry2Ab* genes which have shown high level of resistance to all the three species of bollworms. The incidence of bollworms did not cross ETL in BG-II hybrids. MRC-7201 recorded 0.13 larva of *H. armigera* per plant and 4.98 per cent fruiting body damage. MRC-6322 BG-II was on par with MRC-7201. RCH-368 *Bt* was found better with 0.1 larvae per plant of *E. vittella* and 0.54 *H. armigera* larvae per plant and 5.73 per cent fruiting body damage among genotypes with Cry1Ac.

Badiger *et al*. (2012) revealed that mean population of *S. litura* on BG II cotton hybrid registered low population of *S. litura* larvae (0.22 larva plant^{-1}) as compared to BG I (0.55 larva plant^{-1}) though the differences were non significant. BG II exhibited only 0.76 damaged leaves per infested plant against 3.11 damaged leaves /infested plant in BG I. The damaged area per infested leaf was 85.40 per cent lower in BG II as compared to BG I hybrid.

Vashisth *et al.* (2012) conducted an experiment to determine the damage potential of *S. litura* which reveled that damage was the maximum in cabbage (5.30 g), followed by cucumber (5.08 g), tomato (4.45g) but was minimum in sweet pepper (2.89 g).

2.2. STUDIES ON THE INFLUENCE OF BOLLGARD II *Bt* COTTON (CRY 1AC + CRY 2AB) PLANTS AGAINST *S. litura*

Halcomb *et al.* (1996) studied the survival and growth of bollworm and tobacco budworm on transgenic and non transgenic cotton and found that these pests were susceptible to *Bt* cotton.

Leonard *et al.* (1997) reported that *Bt* technology serves primarily as an insurance policy to reduce the probability of severe economic losses by tobacco bud worms (*Spodoptera* spp.)

Cry1C protein derived from *B. thuringiensis* subsp. *aijawai* expressed in tobacco conferred significant insecticidal activity towards the Egyptian cotton leaf worm *S. littoralis*. Expression of the Cry1C toxin peaked at early stages of plant development and it decreased as the plants aged (Mazier *et al.*, 1997).

Adamczyk *et al.* (1998) reported that there was no significant difference in fall armyworm survival on the normal and *Bt* cotton varieties. It was also reported that larval weights were significantly higher at 6 and 12 days after exposure on DP 5415 (normal) leaves than NuCOTN (*Bt*) leaves and the time to pupation and adult eclosion were significantly shorter on DP 5415 leaves than on NuCOTN 33B leaves.

Chakrabarti *et al*. (1998) reported that the toxins of Cry1Ac and Cry1F that expressed together in transgenic crop plants gave effective control of *H. armigera*.

Zeng *et al.* (1998) evaluated the toxicity of *Bacillus thuringiensis* against *S. exigua* and the results showed that the development, rate of pupation, emergence and sex ratio were normal. Factors such as developmental period of the test larvae, length of the incubation period after infection and temperature would affect LC_{50} values.

Greenplate (1999) reported that terminal fully expanded leaves of *Bt* cotton express more delta endotoxin compared with other fruiting structures like flowers.

The effects of plant nitrate levels, neonate diet, instar and leaf development stage on expression of resistance to *S. exigua* were evaluated in three varieties of *Bt* cotton *i.e.*, DP90B, DP35B and DP33B in a field trials at Artesia. First instar larvae fed DP90B from high nitrogen plots had six per cent mortality, compared to 38% mortality of larvae fed on less nitrogen plots of DP90B. Seven per cent mortality was observed in larvae fed on DP90B cotton after three to six hours on artificial diet compared to 38% mortality of *S. exigua* fed on DP90B throughout the test. Leaf development stage, did not effect mortality (Pierce *et al.*, 1999)

Allen *et al.* (2000) studied the effectiveness of stacked *Bt* cotton against foliage and fruiting caterpillars and showed that Bollguard II cultivars exhibited far lower number of beet armyworm, tobacco budworm, cabbage looper and soybean looper than *Bt* and non *Bt* varieties.

Ashfaq *et al.* (2000) studied the effects of *Bt* cotton on larval instars of *H. zea* and reported that the larval weight was reduced with an increase in feeding time on *Bt* cotton.

Development, survival and fecundity of *S. exigua* were determined on *Bt* and non *Bt* cotton cultivars, where in growth, survival and fecundity were negatively affected by the *Bt* toxin contained in transgenic NUCOTN 35B. Larval weight and size as well as pupal weight were reduced by the toxin. Developmental times were similar on *Bt* and non *Bt* cotton. Larval survival decreased from 72% in insects not exposed to the *Bt* toxin to 26% in those fed on cotton leaves with the toxin (Camberos *et al*., 2000).

Fitt *et al.* (2000) studied the efficacy of Cry2Ab cottons against minor lepidopteran pests, cotton tipworm, cotton leaf perforator and cotton looper and reported that small numbers of larvae that survived up to five days weighed not more 0.5 mg in than on Cry2Ab cotton compared to 1.25 to 2 mg on INGARD plants (*Bt* cotton) and 3.75 mg for those on non *Bt* cotton.

Greenplate *et al.* (2000) reported that stacked *Bt* cotton had two genes (*Cry1Ac+Cry2Ab*) which could increase activity against lepidopterans because of increased potentiation by two toxins.

Jackson *et al.* (2000) conducted field and green house studies to evaluate the performance of bollworm on Bollgard II cotton genotypes and stated that Bollgard II lines sustained significantly lower terminal, square and boll damage compared to the commercial Bollgard variety (DP50B) and a conventional cotton variety (DP50) and also found that Bollgard II lines significantly reduced penetration of fruiting structures for the field and laboratory selected strains of bollworm compared to *Bt* variety.

Johnson *et al.* (2000) reported that the commercial transgenic variety (DP50B) provided an acceptable level of control against *H. zea* and *H. virescens* than standard variety (DP50). It was also found that use of varieties with the Bollgard II gene suppressed the beet armyworm (*S. exigua*).

Development, survival and fecundity of *S. exigua* were determined on *Bt* and non *Bt* cotton cultivars, where in growth, survival and fecundity were negatively affected by the *Bt* toxin contained in transgenic NUCOTN 35B. Larval weight and size as well as pupal weight were reduced by the toxin. Developmental times were similar on *Bt* and non *Bt* cotton. Larval survival decreased from 72% in insects not exposed to the *Bt* toxin to 26% in those fed on cotton leaves with the toxin (Camberos *et al*., 2000).

Field comparison studies of genetically modified cotton containing one and two strains of *B. thuringiensis* subsp. *kurstaki* (*B.t.k*) proved that bollworm and soybean looper population was significantly lower in Bollgard II than in Bollgard I (Ridge *et al*., 2000).

Adamczyk *et al.* (2001) conducted field efficacy studies and found that populations of *S. exigua* and soybean looper were significantly lower in Bollgard II compared with Bollgard I. They also demonstrated that single and dual toxin genotypes remained superior compared with conventional cotton against the tobacco budworm (*H. virescens*).

Gore *et al.* (2001) conducted the fresh tissue bioassays to investigate bollworm survival on Bollgard, Bollgard II and conventional cotton (Deltapine 5415), in which bollworm survival did not correlate with protein expression levels, however survival was lower on all structures of Bollgard II than Bollgard and conventional cotton.

Henneberry *et al.* (2001) reported high susceptibility of *H. virescens* to *Bt* leaves or flower buds with 100% and 96% mortality within four days, respectively compared to 95% on *Bt* leaves and 47% mortality on flower buds of *Trichoplusia ni* and 57% on *Bt* leaves and 37% mortality on flower buds of *S. exigua*.

Marchosky *et al.* (2001) studied the efficacy of stacked *Bt* and *Bt* cotton hybrids against pink bollworm *Pectinophora gossypiella* (Saunders) and reported that dual stacking with *Cry1Ac* + *Cry2Ab* genes have 10 folds advantage over *Cry1Ac* genotypes in United States of America.

Rodriguez *et al.* (2001) evaluated the insecticidal activity of Colombian native strains (Cry1Aa, Cry1Ab, Cry1B, Cry1C and Cry1D) of *B. thuringiensis* against *S. frugiperda* and concluded that the strains were effective and faster.

Stewart *et al.* (2001) conducted field and laboratory assays to compare the relative efficacy of *Bt* and stacked *Bt* cotton. The results of the experiment revealed that the dual toxin *Bt* cultivar was more toxic to *S. frugiperda* and *S. exigua* than single toxin cultivar.

Janakiraman and Gupta (2002) indicated that Cry1Ac toxin had no impact on the growth and development of *S. litura* even at the highest dose. There was no significant difference between transgenic *Bt* cottons and non transgenic cottons in respect of damage to leaves, flowers and buds due to *S. litura*. There was no difference in the developmental period and survival rate of *S. litura* on *Bt* and non *Bt* cotton hybrids.

Sparks and Norman (2002) studied the survival of beet armyworm larvae on leaves of stacked *Bt* cotton and the results of the experiment showed 88.30 % mortality with one day old larvae. Further larval survival, larval weight, pupal weight, pupation rate, adult emergence rate and life span were decreased on *Bt* cultivars compared to non *Bt* cultivars.

Chitkowski *et al*. (2003) studied the efficacy of Bollgard II which express two *B. thuringiensis* proteins Cry1Ac + Cry2Ab against *S. frugiperda* (Hubner) and *S. exigua* (Hubner) and found that the mortality was significantly greater on Bollgard II than on Bollgard I and conventional cotton.

Sivasupramaniam *et al.* (2003) compared the effects of feeding of beet armyworm on vegetative and floral parts of stacked *Bt* cotton and found that none of the larvae survived beyond second larval stage or seven days after treatment.

Adamczyk and Gore (2004) investigated the efficacy of transgenic *Bt* cotton genotypes containing *Cry1Ac*, *Cry1F* and *Cry1Ac* stacked with *Cry1F* against beet armyworm, *S. exigua* (Hubner) and the fall armyworm *S. frugiperda* and revealed that the cotton containing *Cry1F* was more toxic compared to cotton containing only *Cry1Ac*.

Radhika *et al*. (2004) conducted field experiment to study the relative incidence of pest complex on *Bt* and non *Bt* cotton hybrids under protected and unprotected conditions. The results of the experiment revealed that there is no much variation in the incidence of *S. litura* between *Bt* and non *Bt* cotton hybrids.

Raja *et al.* (2004) reported that the lepidopteran pests were noticed on *Bt* cotton leaves of MECH 162 from fourth week to nineteenth week after sowing and the relative percentage of incidence of *H. armigera* was very less (2.55 larvae / 5 plants), whereas high incidence of *Earias vittella* (Fab.) and *S. litura* was recorded (2.99 and 4.15 larvae / 5 plants, respectively).

Yueshu *et al.* (2004) observed that there was no significant difference between transgenic *Bt* cotton Kemian 1 and non transgenic *Bt* cotton Yumian 1 in respect of damage to leaves, flowers and buds by *S. litura* and also found that there was no difference in duration and survival rates of larvae.

Hagerty *et al.* (2005) reported that the toxin of *B. thuringiensis* subsp. *kurstaki* expressed in Bollgard I or Bollgard II cotton have no adverse impact on predaceous arthropods and the dual toxins of DP50BII (Bollgard II) were highly effective on all major lepidopteran pests, even under disrupted in predator population.

Jech and Henneberry (2005) evaluated the effect of *Bt* and non *Bt* cottons on pink bollworm, cabbage looper, beet armyworm and tobacco budworm. The results of the experiment revealed that pink bollworm and tobacco budworm were highly susceptible compared to beet armyworm and cabbage looper to all *Bt* cottons after 72 hours of feeding period.

Kranthi *et al.* (2005) tested the quantitative levels of Cry1Ac and the seasonal decline in expression of Cry proteins among eight commercial Bollgard hybrids and reported that *Cry1Ac* expression decreased consistently as the plant aged.

Polania *et al.* (2005) studied the influence of Cry1Ac toxin of *Bt* cotton on the development of the fall armyworm, *S. frugiperda* (Hubner) and found that there was a significant decrease in pupal weight.

Donglin *et al.* (2006) studied the effect of *S. litura* on stacked *Bt* cotton (Cry1Ac+Cry2Ab), non *Bt* cotton and found that the mortality of second instar larvae reared on transgenic cotton was significantly greater than non *Bt* cotton. Further the growth stage of neonate larvae was longer, whereas fourth and fifth instar larvae were shorter with lower pupal weight on *Bt* cotton.

Guifen *et al.* (2006) studied the development of *S. litura* on *Bt* cotton cultivars (GK-12 and NuCOTN 33B, producing the fused Cry1Ab/Ac toxin and Cry1Ac toxin, respectively) and found that the survival rate of *S. litura* was decreased. Further the time required to reach the second instar was prolonged and the bodymass was lowered when reared on NuCOTN 33B compared to those on non *Bt* cotton.

Li *et al*. (2006) conducted a field experiment to evaluate the effects of *Bt* cotton expressing Cry1Ac and Cry2Ab and non *Bt* cotton on *T. ni* and found that the percentage of feeding damage on *Bt* cotton was only 15.7%, while 84.5% damage was recorded in non *Bt* cotton.

Subramanian *et al.* (2006) studied food utilization by *S. litura* on various cotton cultivars and found that feeding and growth parameters were positively correlated to carbohydrates, proteins and water content of the host plants, whereas food utilization parameters showed negative correlation with lipid and phenolic content of the host plants.

Vennila *et al.* (2006) studied the growth and survival of *H. armigera* and *S. litura* on transgenic *Bt* cotton and reported low larval mortality and higher survival index for *S. litura* on *Bt* cotton. Further, the larval development period between *Bt* and non *Bt* cultivars was non significant.

Jeyakumar *et al.* (2007) reported that the difference between the incidence of *S. litura* in *Bt* cotton (0.25 to 0.28 larva/ plant) and non *Bt* cotton (0.10 to 0.28 larva/ plant) remained statistically non significant.

Spafford *et al.* (2007) studied on whole leaf bioassay of Bollgard II cotton varieties indicated, reduced larval survival on the Bollgard II varieties compared to a non-transgenic. Neonates that fed on young leaves of Bollgard II varieties showed no significant variation to the non-transgenic control in relative mortality or weight gain. Their study also indicated differential expression of the *B. thuringiensis* Berliner toxin in Bollgard II cotton leaves that makes it in adequate to control *S. litura* in the field.

Santos (2007) conducted a field assay to compare the relative efficacy of *Bt* and stacked *Bt* cotton. The results of the experiment revealed that dual toxin *Bt* cultivar DP50 expressed greater concentration of the Cry2Ab and Cry1Ac toxic proteins and was more toxic to *S. frugiperda*.

Seshamahalakshmi (2007) reported that the mortality of *H. armigera* was higher on leaves compared to squares for all the larval instars. The larvae that survived after exposure to *Bt* plant parts weighed less and developed into smaller pupae than the larvae fed on non *Bt* plant parts.

The efficacy of transgenic cotton lines containing vegetative insecticidal protein Vip3A (d- endotoxin), Cry1Ab and both Vip3A and Cry1Ab were evaluated by Adamczyk and Mahaffey (2008) and the results showed that the cotton line containing Vip3A was found more efficacious against *S. exigua* and *S. frugiperda* compared to the Cry1Ab cotton line.

Adamczyk *et al.* (2008) conducted a field experiment to measure the efficacy and levels of δ-endotoxin in two cotton varieties (‘DP 451B/RR’ and ‘NUCOTN 33B’) against bollworm and fall armyworm and the results showed that the larvae fed on DP 451B/RR completed development faster and exhibited better survivorship than those larvae fed on NUCOTN 33B. The lower levels of δ-endotoxin were detected in plant parts from DP 451B/RR compared with NUCOTN 33B.

In a field study, Adamczyk *et al.* (2008) examined the efficacy of Bollgard, Bollgard II and Widestrike traits against beet armyworm, *S. exigua* and fall armyworm *S. frugiperda*. The results suggested that both dual gene traits were more efficacious against these armyworm species than Bollgard.

Basavaraja *et al.* (2008) evaluated the impact of transgenic *Bt* cotton hybrids (Ankur 2534 *Bt,* Ankur 651 *Bt,* RCH 134 *Bt*, RCH 317 *Bt*, and MRC 6304 *Bt)* and their corresponding non *Bt* hybrids against *S. litura*. The results showed that there was no significant difference between *Bt* and non *Bt* hybrids on various biological parameters (larval period, larval weight, larval survival, pupal period, pupal weight and adult emergence) of *S. litura.*

Bheemanna *et al.* (2008) evaluated the second generation transgenic *Bt* cotton, Bollgard II against bollworms of cotton and reported that the incidence of *S. litura* larvae was nil in BG II where as in BG I and non *Bt* cotton recorded 4.34 and 5.41 larvae per meter row. Similarly, per cent defoliation due to *S. litura* was also nil in Bollgard II, while it varied from 20 to 35 per cent in Bollgard I and non *Bt* cotton.

Buntin (2008) conducted a field experiment to compare the efficacy of two transgenic cotton hybrids expressing the Cry1Ab (MON 810 event) and Cry1F (TC 1507 event) against *S. frugiperda* and concluded that TC 1507 provided greater protection from whorl injury than MON 810 under severe fall armyworm infestation.

In a field study conducted in Saint Louis, second instar larvae of *S. exigua* and *S. frugiperda* showed higher mortality on Bollgard II *Bt* cotton compared to Bollgard I *Bt* cotton. The mortality of second instars of *S. exigua* and *S. frugiperda* on Bollgard II *Bt* cotton ranged from 69 to 93%, whereas in Bollgard I *Bt* cotton the mortality ranged from 20 to 69% (Sivasupramaniam *et al.,* 2008).

Siebert *et al*. (2008) evaluated the efficacy of Maize (TC 1507 event) and cotton (DAS-24236-5 event) varieties expressing Cry1F insecticidal crystal protein of *Bacillus thuringiensis* (*Bt*) and reported that Cry1F protein provided high level of control against fall armyworm *S. frugiperda*.

Soujanya (2009) investigated the effect of *B.t.* toxins (Cry1Ac + Cry2Ab) on the development of *S. litura* and revealed that the mortality of early larval instars was higher than the later instars on both leaves and squares of stacked *Bt* cotton hybrids, while no mortality was reported in *Bt* cotton hybrids.

Naik (2009) evaluated the mortality rates of *S. litura* on BG II cotton hybrids and indicated that the mortality of early instars was higher than the later instars on both leaves and squares of RCH 2 BG II hybrid.

Tindall *et al.* (2009) evaluated the efficacy of cotton plants expressing Cry1Ac and Cry1F insecticidal crystal proteins of *B. thuringiensis* Berliner against fall armyworm, *S. frugiperda*, *S. exigua* and *Pseudoplusia includens* and reported high reduced levels of damage (leaf defoliation) and significantly higher mortality (90 to 100%) of *S. frugiperda* and *Pseudoplusia includens* compared with that of *S. exigua*. The *S. frugiperda* was relatively less sensitive to *Cry1Ac* + *Cry1F* cotton plants.

Greenberg *et al.* (2010) examined the transgenic cottons containing Bollgard, Bollgard II and Widestrike traits along with non *Bt* cotton to evaluate the efficacy against 1st and 2nd instar larvae of beet armyworm, *S. exigua* at 70 DAS and 80 DAS and the results showed that the mortality was higher on Bollgard II than Widestrike and Bollgard traits. The mortality was 85 % and 60 % in 1st and 2nd instar larvae respectively on Bollgard II after two days of release.

Govindan *et al.* (2010) studies on *Bt* cotton and non *Bt* cotton hybrids showed that, RCH 2 *Bt* top fully opened young leaves showed highest per cent mortality followed by squares, middle leaves and young green bolls followed by top fully opened young leaves, squares, middle leaves and young green bolls of RCH 515 *Bt* which recorded 46.67, 38.34, 37.67 and 30.00 per cent mortality, respectively after 168 HAT when compared to squares, green bolls, top fully opened leaves and middle leaves of RCH 2 non *Bt* cotton which recorded 4.83, 4.17, 3.33 and 0.00 per cent mortality respectively after 168 HAT.

Pradeep (2011) reported that mortality of 1st and 2nd instar larvae of *S. litura* was high with reduced mortality rates in 3rd and 4th instar larvae on BG II hybrids, where as the mortality was very low and more or less similar in BG I and non *Bt* cotton hybrids. The mean weight of larva fed on BG II cotton leaves was found to be very low. The weight reduction of late instars of *S. litura* was lower than the early instars on the leaves of both the BG II hybrids of the crop, whereas BG I and non *Bt* hybrids did not effect the larval or pupal weight and weight gains were more or less similar.

Hallad *et al.* (2011) conducted the quantitative bioassays at 80 and 110 DAS by leaf disc feeding method for *H. armigera* and *S. litura* using five different *Bt* cotton event genotypes for characterization of resistance to early and late instars. Mortality of *S. litura* 2nd instar larvae was 80.3 and 71.3 % at 80 and 110 DAS in Tulasi 4BG-II and the mortality of 3rd and 4th instar was 72.6 % and 64.2 % which recorded in Tulasi 4BG-II. Whereas Tulasi 4BG-I recorded least mortality for 2nd instar which was 7.9 and 5.8 per cent at 80 and 110 DAS.

According to Soujanya *et al.* (2011) first instar larvae survived beyond 72 h, when reared continuously on the leaves of dual toxin expressing *Bt* cotton cultivars and none of the larvae survived beyond five days. In second, third, fourth and fifth instars when fed on leaves and squares of dual toxin *Bt* cotton cultivars, survival was noticed, but final weight of each instar was reduced and they developed into smaller pupae. The growth and survival indices for III instar was significantly low when the larvae were fed with dual toxin *Bt* cotton cultivars as compared to single toxin *Bt* cultivars and non *Bt* cotton.

Naik *et al.* (2013) in a laboratory study on the growth and development of *S. litura,* revealed that the larval mortality was more when fed on leaves and squares of RCH 2 BG-II. The susceptibility level of *S. litura* to RCH 2 BG-II varied instar wise. Late instars had very little effect on the survival and development of the insect. The development of *S. litura* was drastically affected as indicated by lowest growth and survival indices.

Peche *et al.* (2013) conducted the bioassay of new BG-II cotton hybrids against *S. litura* which revealed that all BG-II cotton hybrids were superior with higher per cent mortality of *S. litura* as compared to *Bt* cotton hybrid. RCH-2 BG-II was most promising BG-II cotton hybrid, recording highest per cent mortality of *S. litura* on leaves (60.42%), squares (59.05%) and young green bolls (45.56%). This was followed by Ankur-3228 BG-II, Ankur-3224 BG-II and IAHH-2 BG-II. The per cent mortality of *S. litura* was highest at 75 DAS in all the BG-II cotton hybrids. In general per cent mortality was higher on leaves.

Saini and Dhawan (2013) studied toxin expression of Cry 1Ac and Cry 2Ab in plant parts of five Bollgard II (BG II) genotypes *viz.* MRC 7031, MRC 7017, RCH 134, Ankur Jassi and Tulsi 4. Expression of both toxins was maximum at 60 days after sowing (DAS) in leaves and squares. But in bolls, it was maximum at 120 DAS. Maximum mortality of *H. armigera* and *S. litura* was observed when larvae were fed with RCH 134 BG II. Mortality of *S. litura* was maximum when it was fed with squares. The mortality of different instars of *S. litura* on RCH 134 BG I genotype was very less, which indicated that BG I genotype has no effect on *S. litura*.

Hallad *et al.* (2014) studied five different transgenic events of *Bt* cotton and non *Bt* for their efficacy against different cry proteins expressed at different days of interval. Mortality of *S. litura* was highest in Tulasi 4 BG-II (83.7%) followed by Nathbaba (81.6%) and least mortality was in Tulasi 4 BG-I (12.1%) at 50 DAS. The mortality was decreased to 57.3, 52.1, 47.9 per cent (Tulasi 4 BG-II, Nathbaba & MH 5174) respectively and Tulasi 4 BG-I recorded least mortality (3.2%) at 135 DAS respectively.

Chapter III

MATERIAL AND METHODS

The present investigation on **“DAMAGE POTENTIAL, GROWTH AND DEVELOPMENT OF *Spodoptera litura* (Fab.) ON DUAL TOXIN TRANGENIC *Bt* COTTON”** was conducted at RegionalAgricultural Research Station, Lam, Guntur, A.P. during *kharif*, 2014-15. The materials used and methods employed in conducting these experiments are elucidated in this chapter.

3.1 COTTON CULTIVARS

The two cotton hybrids *viz*., Mallika BG I and Jaadoo BG I containing Cry1Ac, the two stacked *Bt* cotton hybrids *viz*., Mallika BG II and Jaadoo BG II containing Cry 1Ac + Cry 2Ab and their corresponding non *Bt* versions *i.e.*, Mallika non *Bt* and Jaadoo non *Bt* were chosen for the research work. Seed material was obtained from M/S Kaveri seeds Pvt. Ltd and Nuzuveedu seeds Pvt. Ltd, Guntur.

3.2 CULTIVATION ASPECTS

3.2.1 Land Preparation

Experimental area was prepared by deep summer ploughing thoroughly with tractor drawn implements which was leveled and planked with the on set of pre-monsoon rains before sowing. Marking was done with bullock drawn implements during *kharif*, 2014.

3.2.2 Sowing

After receipt of sufficient rains, under saturated condition of soil, sowing was taken up with a spacing of 105 × 60 cm between rows and plants, respectively. Hand dibbling of seeds was done to ensure proper plant population and uniform geometry during second week of August in *kharif*, 2014-2015. Gap filling was done once within ten days after sowing and thinning was done to retain one healthy seedling per hill after 10^{th} day of gap filling to maintain uniform population.

3.2.3 Fertilization

The recommended fertilizer dose of 120 kg N, 60 kg P and 60 kg $ha^{-1}$was applied in 3 split doses. The entire dose of phosphorus was applied at the time of sowing as basal dose, whereas nitrogen and potash were applied in three equal splits at 30, 60 and 90 DAS as pocket application. Pocketing was done by placing nitrogen and potash fertilizers at 7-10 cm deep in soil at 7-10 cm distance from plant.

3.2.4 Irrigation

The crop was grown under rainfed conditions hence, did not give any irrigation during the entire season.

3.2.5 Inter cultivation and Weeding

During the crop season *i.e., kharif*, 2014-15 four hand weeding were given to keep the crop free from weeds. Pendimethalin 50 EC was applied @ 2.5 l ha^{-1} as pre-emergence herbicide one day after sowing. Intercultivation with bullock drawn implements were carried twice during the season to control the weeds.

3.3 ASSESSING THE DAMAGE POTENTIAL OF *S. litura* ON BG II *Bt* COTTON.

Assessing the damage of *S. litura* was done based on the economic losses in yield resulted due to insect pest. An experiment in randomized block design (RBD) was laid out during 2014-15 with eight treatments and three replications using the popular *Bt* cotton hybrid Mallika BG II

3.3.1 Treatments :

T_1	-	Complete protection
T_2	-	Releasing 3 larvae per plant
T_3	-	Releasing 6 larvae per plant
T_4	-	Releasing 9 larvae per plant
T_5	-	Releasing 12 larvae per plant
T_6	-	Releasing 15 larvae per plant
T_7	-	Releasing 18 larvae per plant
T_8	-	Releasing 21 larvae per plant

3.3.2 Procedure

Egg masses of *S. litura* were collected from field and they were reared in laboratory on cotton leaves up to pupation. After emergence of moths from pupae they were kept for oviposition, eggs laid by the moths were reared in the laboratory and 3rd instar larvae were released on caged plants at 50, 80, 110 and 140 days after sowing. Care was taken to ensure for presence of larvae.

3.3.3 Methodology

For each treatment, three replications were maintained with a single plant under caged condition, as it was covered with fine nylon mesh to prevent infestation from outside. The cages were designed in such a way that they did not interrupt ventilation and aeration to the growing plants inside and they did not allow free passage of larvae. The bottom edges of the cages were inserted into the soil on all the sides so as to check the escape or entry of larvae. Nylon net cages were erected on bamboo sticks fixed in four corners.

Third instar larvae of *S. litura* obtained from laboratory culture were released on cotton plants for four times *i.e.*, at 50, 80, 110 and 140 days after sowing in respective treatments. Different blocks were maintained for releasing the larvae at different intervals after sowing to assess the damage potential.

The leaf area damage (Graphic method), number of squares and bolls damaged in each caged plant were recorded at 3, 7, 11 days after release of larvae. The yield at harvest was collected from two pickings and total weight of seed cotton yield from all the blocks was recorded.

3.4 STUDIES ON THE INFLUENCE OF BOLLGARD II *Bt* COTTON (Cry 1Ac + Cry 2Ab) PLANTS AGAINST *S. litura*

A bulk plot of 19.44 m^2 each of BG I (Cry1Ac), stacked *Bt* (Cry1Ac + Cry2Ab) (BG II) cotton hybrids of Mallika, Jaadoo and their corresponding non *Bt* hybrids were raised by following recommended agronomic practices with insecticidal protection to control sucking pests during the crop season. The experiment was conducted in CRD design in the lab with 6 treatments and 4 replications for each replication with 10 larvae.

Treatments :

T1 - Jaadoo BG I

T2 - Mallika BG I

T3 - Jaadoo BG II

T4 - Mallika BG II

T5 - Jaadoo non-*Bt*

T6 - Mallika non-*Bt*

3.4.1 Procedure

Comparative growth and development studies on *S. litura* larval instars were conducted for all the larval instars *i.e.,* from first to last instar by allowing them to feed on leaves and squares of different transgenic *Bt* cotton hybrids and non *Bt* cotton hybrids.

Plant parts namely tender leaves (top) and squares of 70-80 days old plants were plucked from transgenic and corresponding non transgenic *Bt* hybrids at experimental field. They were carried to the laboratory in polythene covers, washed with distilled water and blot dried. The cleaned leaves and squares were placed individually in small boxes. The field collected egg masses of *S. litura* were used to initiate the mass culturing under laboratory conditions. The egg masses were kept in the egg cage. After emergence, first instar larvae were transferred to the castor leaves which were changed and the fecal pellets removed from the container for every 24 hrs. The grown up larvae were allowed to pupate in soil. Moths were collected on emergence and released in oviposition cage for egg laying. The required larvae for the different treatments were taken from the culture. Prior to larval release, the larvae were weighed with electronic balance. First instar larvae were weighed in mass, while remaining instars were weighed individually. After bioassay, larvae were observed daily and changed to respective fresh food material up to pupation.

3.4.2 Survival and Development of *S. litura*

Bioassay was conducted with BG I (Cry1Ac) and stacked *Bt* (Cry1Ac + 2Ab) (BG II) cotton hybrids of Mallika, Jaadoo and their corresponding non *Bt* versions against *S. litura* to study the per cent mortality, larval survival, pupal weight and leaf area consumed at 3, 7 and 11days interval of all larval instars (For first instar larvae, leaf area consumed was taken on a grading score based on leaf area scrapping, Score I - 1-10 %, Score II - 11-20 %, Score III-21-30 %, Score IV- 31-40%, Score V- 41-50 %, ScoreVI- 51-60 %, Score VII- 61- 70 %, Score VIII- 71- 80 %, Score IX- 81-90 %, Score X – 91-100%).

Investigation was conducted in the laboratory on the leaves and squares of 70 to 80 days old crop with the five larval instars *i.e.* from first to five instars by feeding the leaves of *Bt* and stacked *Bt* cottons and their corresponding non *Bt* versions as control. Laboratory reared 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} instar larvae were released separately on leaves and squares of 70 to 80 days old crop with four replications. Ten larvae of each instar were released on the leaves and squares of cotton with the help of camel hair brush in each of the replications.The mortality of all the five instars of *S. litura* (1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th}) on tender leaves and squares, weight of surviving larvae and pupae (with an electronic balance) and leaf area consumed (Graphic method) were taken at 3, 7 and 11 days after release for each stage of the larvae in the lab. Adult emergence, was observed on all the three versions of Mallika and Jaadoo hybrids.

3.5 STATISTICAL PROCEDURES

Assessing the damage potential

The data obtained from field experiment were subjected to Square root transformation and put to statistical analysis in Randomized Block Design (RBD)

Survival and Development of *S. litura*

The larval survival, pupal weight and leaf area consumed was subjected to square root transformation and the variance was calculated. The data obtained on per cent mortality in bioassay was subjected to arcsin transformation and statistically analyzed by Completely Randomized Design (CRD). Means in simple CRD analysis were separated by Duncan’s multiple range test (Duncan, 1951).

Table 4.7.Mortality of larval instars of *S. litura* on leaves of test hybrids

Treatments	% mortality															
	I instar			Total mortality	II instar			Total mortality	III instar			Total mortality	IV instar		Total mortality	V instar
	3DAR	7DAR	11DAR		3DAR	7DAR	11DAR		3DAR	7DAR	11DAR		3DAR	7DAR		
Jaadoo BG I	7.50 (13.82)^{b}	7.50 (13.82)^{b}	2.50 (4.60)	17.50	7.50 (13.82)^{b}	2.50 (4.60)^{b}	0.00 (0.00)^{c}	10.00	0.00 (0.00)^{c}	0.00 (0.00)^{b}	0.00 (0.00)	0.00	0.00 (0.00)	0.00 (0.00)	0.00	0.00
Mallika BG I	2.50 (4.60)^{b}	7.50 (13.82)^{b}	5.00 (9.21)	15.00	7.50 (13.82)^{b}	2.50 (4.60)^{b}	0.00 (0.00)^{c}	10.00	0.00 (0.00)^{c}	0.00 (0.00)^{b}	0.00 (0.00)	0.00	0.00 (0.00)	0.00 (0.00)	0.00	0.00
Jaadoo BG II	47.50 (43.55)^{a}	52.50 (46.44)^{a}	0.00 (0.00)	100.00	25.00 (29.88)^{a}	47.50 (43.55)^{a}	20.00 (26.56)^{a}	92.50	22.50 (28.28)^{a}	12.50 (20.46)^{a}	0.00 (0.00)	35.00	0.00 (0.00)	0.00 (0.00)	0.00	0.00
Mallika BG II	52.50 (46.44)^{a}	47.50 (43.55)^{a}	0.00 (0.00)	100.00	40.00 (39.23)^{a}	40.00 (39.23)^{a}	12.5 (20.46)^{b}	92.50	17.50 (24.67)^{b}	15.00 (22.50)^{a}	0.00 (0.00)	32.50	0.00 (0.00)	0.00 (0.00)	0.00	0.00
Jaadoo non- *Bt*	2.50 (4.60)^{b}	5.00 (9.21)^{b}	2.50 (4.60)	10.00	5.00 (9.21)^{b}	0.00 (0.00)^{b}	0.00 (0.00)^{c}	5.00	0.00 (0.00)^{c}	0.00 (0.00)^{b}	0.00 (0.00)	0.00	0.00 (0.00)	0.00 (0.00)	0.00	0.00
Mallika non- *Bt*	5.00 (9.21)^{b}	5.00 (9.21)^{b}	2.50 (4.60)	12.50	5.00 (9.21)^{b}	2.50 (4.60)^{b}	0.00 (0.00)^{c}	7.50	0.00 (0.00)^{c}	0.00 (0.00)^{b}	0.00 (0.00)	0.00	0.00 (0.00)	0.00 (0.00)	0.00	0.00
F-Test	Sig	Sig	NS		Sig	Sig	Sig		Sig	Sig	NS		NS	NS		NS
SEM ±	4.00	4.14	3.92		4.13	3.31	0.83		0.6	1.27	-		-	-		-
CD (P=0.05)	11.90	12.33	11.64		12.29	9.84	2.47		1.79	3.77	-		-	-		-

Figures in parentheses are angular transformed values

Numbers followed by same superscript are not statistically different (P=0.05)

Table4.8. Mortality of larval instars of *S. litura* on squares of test hybrids

Treatments	% mortality															
	I instar			Total mortality	II instar			Total mortality	III instar			Total mortality	IV instar		Total mortality	V instar
	3DAR	7DAR	11DAR		3DAR	7DAR	11DAR		3DAR	7DAR	11DAR		3DAR	7DAR		
Jaadoo BG I	5.00 (9.21)^{b}	5.00 (9.21)	2.50 (4.60)	12.50	7.50 (13.82)^{bc}	2.50 (4.60)^{b}	0.00 (0.00)^{c}	10.00	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)	0.00	0.00 (0.00)	0.00 (0.00)	0.00	0.00
Mallika BG I	5.00 (9.21)^{b}	5.00 (9.21)	5.00 (9.21)	15.00	7.50 (13.82)^{bc}	2.50 (4.60)^{b}	0.00 (0.00)^{c}	10.00	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)	0.00	0.00 (0.00)	0.00 (0.00)	0.00	0.00
Jaadoo BG II	92.50 (76.17)^{a}	7.50 (11.25)	0.00 (0.00)	100.00	22.50 (28.22)^{a}	55.00 (47.88)^{a}	10.00 (18.43)^{b}	87.50	12.50 (20.61)^{a}	12.50 (20.46)^{a}	0.00 (0.00)	25.00	0.00 (0.00)	0.00 (0.00)	0.00	0.00
Mallika BG II	92.50 (76.17)^{a}	7.50 (11.25)	0.00 (0.00)	100.00	17.50 (24.53)^{ab}	40.00 (39.23)^{a}	17.50 (24.53)^{a}	75.00	12.50 (20.46)^{a}	12.50 (20.46)^{a}	0.00 (0.00)	25.00	0.00 (0.00)	0.00 (0.00)	0.00	0.00
Jaadoo non- *Bt*	2.50 (4.60)^{b}	2.50 (4.60)	2.50 (4.60)	7.50	2.50 (4.60)^{c}	2.50 (4.60)^{b}	0.00 (0.00)^{c}	5.00	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)	0.00	0.00 (0.00)	0.00 (0.00)	0.00	0.00
Mallika non-*Bt*	5.00 (9.21)^{b}	2.50 (4.60)	2.50 (4.60)	10.00	2.50 (4.60)^{c}	2.50 (4.60)^{b}	0.00 (0.00)^{c}	5.00	0.00 (0.00)^{b}	0.00 (0.00)^{b}	0.00 (0.00)	0.00	0.00 (0.00)	0.00 (0.00)	0.00	0.00
F-Test	Sig	NS	NS		Sig	Sig	Sig		Sig	Sig	NS		NS	NS		NS
SEm±	4.98	5.61	3.92		3.91	3.94	0.83		0.98	1.17			-	-		-
CD (P=0.05)	14.79	16.68	11.64		11.63	11.72	2.47		2.90	3.49			-	-		-

Figures in parentheses are angular transformed values

Numbers followed by same superscript are not statistically different (P=0.05)

Table4.9. Effect of test hybrids leaves on *S. litura* larval weight

Treatments	Mean weight (mg/larva) of I instar after			Weight gain (%)	Mean weight (mg/larva) of II instar after			Weight gain (%)	Mean weight (mg/larva) of III instar after		Weight gain (%)	Mean weight (mg/larva) of IV instar after	Weight gain (%)
	3 DAR	7 DAR	11 DAR		3 DAR	7 DAR	11 DAR		3 DAR	7 DAR		3 DAR	
Jaadoo BG I	6.85 (2.80)^{a}	14.79 (3.97)^{b}	252.65 (15.87)^{a}	99.21	50.03 (7.14)^{b}	99.56 (9.99)^{bc}	226.61 (15.03)^{bc}	85.08	175.38 (13.25)^{bc}	528.87 (23.02)^{b}	88.96	466.23 (21.59)^{ab}	61.37
Mallika BG I	4.76 (2.39)^{b}	14.42 (3.93)^{b}	216.85 (14.75)^{ab}	99.08	50.40 (7.16)^{b}	125.39 (11.23)^{ab}	267.27 (16.35)^{b}	87.35	178.80 (13.39)^{bc}	559.25 (23.66)^{ab}	89.56	480.00 (21.93)^{a}	62.48
Jaadoo BG II	0.79 (1.33)^{c}	5.23 (2.49)^{c}	0.00 (1.00)^{d}	**	41.08 (6.48)^{c}	88.09 (9.33)^{c}	176.38 (13.21)^{c}	80.84	150.17 (12.23)^{c}	452.12 (21.27)^{c}	87.08	389.23 (19.70)^{b}	53.73
Mallika BG II	1.10 (1.45)^{c}	4.75 (2.38)^{c}	0.00 (1.00)^{d}	**	38.95 (6.32)^{c}	80.31 (9.00)^{c}	169.07 (13.04)^{c}	80.01	146.56 (12.11)^{c}	470.12 (21.70)^{c}	87.58	389.78 (19.69)^{b}	53.79
Jaadoo non- *Bt*	4.29 (2.29)^{b}	19.56 (4.53)^{a}	178.65 (13.36)^{c}	98.88	55.63 (7.52)^{ab}	104.82 (10.28)^{abc}	361.04 (18.99)^{a}	90.64	201.65 (14.23)^{b}	529.50 (23.03)^{b}	88.97	519.93 (22.82)^{a}	65.36
Mallika non- *Bt*	5.32 (2.51)^{b}	14.99 (3.99)^{b}	199.86 (14.17)^{bc}	99.00	58.45 (7.71)^{a}	129.96 (11.43)^{a}	437.53 (20.90)^{a}	92.27	283.75 (16.79)^{a}	602.22 (24.54)^{a}	90.30	537.10 (23.17)^{a}	66.47
F- Test	Sig	Sig	Sig		Sig	Sig	Sig		Sig	Sig		Sig	
SEm ±	0.09	0.12	0.43		0.14	0.45	0.71		0.61	0.42		0.65	
CD (P = 0.05)	0.27	0.35	1.28		0.42	1.34	2.11		1.80	1.25		1.93	
Initial weight	2				33.8				58.4			180.1	

** No survival Larvae

Figures in parentheses are square root transformed values

Numbers followed by same superscript are not statistically different (P=0.05)

Table4.10. Effect of test hybrid squares on *S. litura* larval weight

Treatments	Mean weight (mg/larva) of I instar after			Weight gain (%)	Mean weight (mg/larva) of II instar after			Weight gain (%)	Mean weight (mg/larva) of III instar after		Weight gain (%)	Mean weight of (mg/larva) of IV instar after	Weight gain (%)
	3 DAR	7 DAR	11 DAR		3 DAR	7 DAR	11 DAR		3 DAR	7 DAR		3 DAR	
Jaadoo BG I	28.85 (5.43)^{a}	75.09 (8.72)^{ab}	174.90 (13.25)^{ab}	98.28	54.20 (7.42)^{a}	116.30 (10.30)^{b}	275.59 (16.63)^{b}	87.01	297.00 (17.25)^{ab}	502.88 (22.44)^{b}	83.02	486.93 (22.09)^{a}	65.44
Mallika BG I	35.75 (6.05)^{a}	72.89 (8.60)^{b}	162.90 (12.71)^{b}	98.16	57.70 (7.65)^{a}	117.25 (10.87)^{ab}	285.50 (16.92)^{b}	87.46	350.40 (18.74)^{a}	451.33 (21.22)^{c}	81.08	480.00 (21.93)^{a}	64.94
Jaadoo BG II	3.60 (2.11)^{b}	0.00 (1.00)^{c}	0.00 (1.00)^{c}	**	42.68 (6.60)^{b}	75.18 (8.71)^{c}	230.80 (15.19)^{b}	84.49	164.42 (12.57)^{c}	314.55 (17.76)^{e}	72.85	389.23 (19.70)^{b}	56.76
Mallika BG II	3.17 (2.03)^{b}	0.00 (1.00)^{c}	0.00 (1.00)^{c}	**	42.93 (6.62)^{b}	76.28 (8.79)^{c}	248.19 (15.76)^{b}	85.58	128.81 (11.37)^{c}	366.43 (19.16)^{d}	76.69	339.78 (18.43)^{b}	50.47
Jaadoo non- *Bt*	31.15 (5.66)^{a}	81.43 (9.05)^{ab}	174.96 (13.25)^{ab}	98.29	61.13 (7.88)^{a}	126.76 (11.30)^{ab}	398.62 (19.91)^{a}	91.02	268.95 (16.34)^{ab}	488.58 (22.12)^{bc}	82.52	527.43 (22.98)^{a}	68.09
Mallika non- *Bt*	31.53 (5.69)^{a}	89.06 (9.45)^{a}	210.54 (14.53)^{a}	98.58	60.68 (7.85)^{a}	129.90 (11.44)^{a}	408.91 (20.09)^{a}	91.24	239.80 (15.51)^{b}	565.73 (23.80)^{a}	84.90	537.10 (23.17)^{a}	68.67
F- Test	Sig	Sig	Sig		Sig	Sig	Sig		Sig	Sig		Sig	
SEm ±	0.23	0.28	0.44		0.19	0.20	0.79		0.81	0.41		0.51	
CD (P = 0.05)	0.67	0.84	1.30		0.55	0.60	2.36		2.40	1.21		1.51	
Initial weight	3				35.8				85.4			168.3	

** No survival Larvae

Figures in parentheses are square root transformed values

Numbers followed by same superscript are not statistically different (P=0.05)

Table4.12. Effect of test hybrids on pupal weight of *S. litura*

Treatments	Pupal weight (mg/pupa)									
	Leaves					Squares				
	I instar	**II instar**	**III instar**	**IV instar**	**V instar**	**I instar**	**II instar**	**III instar**	**IV instar**	**V instar**
Jaadoo BG I	219.76 (14.78)^{bc}	126.28 (11.25)^{a}	206.53 (14.40)^{a}	281.75 (16.81)^{a}	256.58 (16.02)^{a}	171.35 (13.12)^{b}	118.54 (10.93)^{ab}	160.83 (12.70)^{a}	244.78 (15.65)^{b}	268.25 (16.40)^{a}
Mallika BG I	183.00 (13.54)^{c}	138.86 (11.81)^{a}	210.55 (14.54)^{a}	267.57 (16.37)^{a}	242.75 (15.60)^{ab}	176.39 (13.30)^{b}	114.77 (10.75)^{ab}	152.13 (12.37)^{a}	281.58 (16.80)^{a}	260.23 (16.15)^{a}
Jaadoo BG II	0.00 (1.00)^{d}	92.90 (9.68)^{b}	167.27 (12.93)^{b}	223.37 (14.97)^{b}	210.90 (14.55)^{c}	0.00 (1.00)^{c}	63.93 (7.93)^{c}	117.83 (10.89)^{b}	208.53 (14.46)^{c}	243.58 (15.63)^{ab}
Mallika BG II	0.00 (1.00)^{d}	98.31 (9.89)^{b}	166.75 (12.95)^{b}	213.80 (14.65)^{b}	220.43 (14.87)^{bc}	0.00 (1.00)^{c}	102.16 (10.15)^{b}	117.66 (10.88)^{b}	202.95 (14.27)^{c}	223.1 (15.07)^{b}
Jaadoo non- *Bt*	269.96 (16.42)^{a}	149.94 (12.28)^{a}	224.13 (14.99)^{a}	278.20 (16.70)^{a}	261.05 (16.18)^{a}	183.55 (13.56)^{ab}	130.69 (11.44)^{ab}	166.73 (12.94)^{a}	249.3 (15.80)^{ab}	260.6 (16.17)^{a}
Mallika non- *Bt*	233.6 (15.30)^{ab}	140.44 (11.88)^{a}	220.90 (14.89)^{a}	271.75 (16.51)^{a}	268.25 (16.39)^{a}	198.35 (14.11)^{a}	134.72 (11.61)^{a}	165.38 (12.89)^{a}	265.88 (16.32)^{ab}	268.75 (16.40)^{a}
F-test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
SEm±	0.48	0.38	0.43	0.26	0.3	0.24	0.44	0.45	0.34	0.28
CD (P=0.05)	1.43	1.12	1.27	0.77	0.9	0.72	1.32	1.35	1.02	0.82

Figures in parentheses are square root transformed values

Numbers followed by same superscript are not statistically different (P=0.05)

Table4.11. Per cent pupation of surviving larvae of *S. litura* on test hybrids

Treatments	Pupation (%)									
	Leaves					Squares				
	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar
Jaadoo BG I	82.50 (65.46)^{b}	90.00 (71.56)^{a}	100.00 (90.00)^{a}	100.00 (90.00)	100.00 (90.00)	80.00 (63.80)^{b}	95.00 (80.78)^{ab}	100.00 (90.00)^{a}	100 (90.00)	100 (90.00)
Mallika BG I	85.00 (67.5)^{ab}	90.00 (71.56)^{a}	100.00 (90.00)^{a}	100.00 (90.00)	100.00 (90.00)	85.00 (67.50)^{ab}	92.50 (76.17)^{b}	100.00 (90.00)^{a}	100 (90.00)	100 (90.00)
Jaadoo BG II	0.00 (0.00)^{c}	7.50 (13.82)^{b}	65.00 (53.77)^{b}	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)^{c}	12.50 (20.46)^{c}	75.00 (60.11)^{b}	100 (90.00)	100 (90.00)
Mallika BG II	0.00 (0.00)^{c}	7.50 (13.82)^{b}	67.50 (55.28)^{b}	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)^{c}	25.00 (29.88)^{c}	75.00 (60.63)^{b}	100 (90.00)	100 (90.00)
Jaadoo non- *Bt*	87.50 (69.53)^{ab}	92.50 (76.17)^{a}	100.00 (90.00)^{a}	100.00 (90.00)	100.00 (90.00)	90.00 (71.56)^{a}	97.50 (85.39)^{ab}	100 (90.00)^{a}	100 (90.00)	100 (90.00)
Mallika non- *Bt*	90.00 (71.56)^{a}	95.00 (80.78)^{a}	100.00 (90.00)^{a}	100.00 (90.00)	100.00 (90.00)	87.50 (69.53)^{a}	100 (90.00)^{a}	100 (90.00)^{a}	100 (90.00)	100 (90.00)
F-test	Sig	Sig	Sig			Sig	Sig	Sig	NS	NS
SEm±	1.51	3.92	0.94	-	-	1.77	3.62	1.98	-	-
CD (P=0.05)	4.50	11.64	2.40	-	-	5.26	10.75	5.90	-	-

Figures in parentheses are angular transformed values

Numbers followed by same superscript are not statistically different (P=0.05)

Table4.13. Per cent adult emergence of *S. litura* on test hybrids

Treatments	Adult emergence (%)									
	Leaves					Squares				
	I instar	II instar	III instar	IV instar	V instar	I instar	II instar	III instar	IV instar	V instar
Jaadoo BG I	72.50 (58.45)^{b}	77.50 (61.77)^{b}	100.00 (90.00)^{a}	100.00 (90.00)	100.00 (90.00)	77.50 (61.77)^{a}	90.00 (71.56)^{a}	100.00 (90.00)^{a}	100.00 (90.00)	100.00 (90.00)
Mallika BG I	85.00 (67.50)^{a}	85.00 (67.50)^{b}	100.00 (90.00)^{a}	100.00 (90.00)	100.00 (90.00)	80.00 (63.80)^{a}	90.00 (71.56)^{a}	100.00 (90.00)^{a}	100.00 (90.00)	100.00 (90.00)
Jaadoo BG II	0.00 (0.00)^{c}	5.00 (9.21)^{c}	62.50 (52.33)^{b}	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)^{b}	10.50 (18.43)^{b}	70.00 (56.94)^{b}	100.00 (90.00)	100.00 (90.00)
Mallika BG II	0.00 (0.00)^{c}	5.00 (9.21)^{c}	65.00 (53.99)^{b}	100.00 (90.00)	100.00 (90.00)	0.00 (0.00)^{b}	15.00 (22.50)^{b}	70.00 (56.94)^{b}	100.00 (90.00)	100.00 (90.00)
Jaadoo non- *Bt*	87.50 (69.53)^{a}	92.50 (76.17)^{a}	100.00 (90.00)^{a}	100.00 (90.00)	100.00 (90.00)	72.50 (58.45)^{a}	95.00 (80.78)^{a}	100.00 (90.00)^{a}	100.00 (90.00)	100.00 (90.00)
Mallika non- *Bt*	85.00 (67.50)^{a}	85.00 (67.50)^{b}	100.00 (90.00)^{a}	100.00 (90.00)	100.00 (90.00)	80.00 (63.80)^{a}	95.00 (80.78)^{a}	100.00 (90.00)^{a}	100.00 (90.00)	100.00 (90.00)
F-test	Sig	Sig	Sig	NS	NS	Sig	Sig	Sig	NS	NS
SEm±	1.73	3.91	1.99	-	-	1.99	3.82	1.49	-	-
CD (P=0.05)	5.13	11.60	5.9	-	-	5.92	9.56	3.78	-	-

Figures in parentheses are angular transformed values

Numbers followed by same superscript are not statistically different (P=0.05)

Table 4.14. Leaf area consumption by larval instars of *S. litura* on test hybrids

Treatments	Leaf area consumption (cm^2) (Mean of four replication)															
	I instar			Total	II instar			Total	III instar			Total	IV instar		Total	V instar
	24 h of feeding*	7DAR	11DAR		3DAR	7DAR	11DAR		3DAR	7DAR	11DAR		3DAR	7DAR		
Jaadoo BG I	III	43.60 (6.67)^{b}	77.98 (8.88)^{b}	121.58	40.23 (6.41)^{b}	55.85 (7.51)^{c}	88.78 (9.47)^{b}	184.85	49.15 (7.07)^{b}	67.90 (8.29)^{b}	71.43 (8.50)^{a}	188.48	81.25 (9.06)^{a}	81.90 (9.10)^{a}	163.15	78.73 (8.92)^{a}
Mallika BG I	III	40.13 (6.40)^{b}	77.38 (8.84)^{b}	117.50	39.98 (6.39)^{b}	61.85 (7.92)^{bc}	89.38 (9.50)^{b}	191.20	53.70 (7.39)^{ab}	73.55 (8.63)^{ab}	69.33 (8.38)^{a}	196.58	83.23 (9.17)^{a}	81.48 (9.08)^{a}	164.71	79.15 (8.95)^{a}
Jaadoo BG II	I	11.90 (3.57)^{c}	**	11.90	12.23 (3.63)^{c}	12.78 (3.70)^{d}	11.83 (3.57)^{c}	36.83	37.03 (6.15)^{c}	49.38 (7.09)^{c}	51.48 (7.23)^{b}	137.86	66.88 (8.22)^{b}	70.55 (8.45)^{b}	137.43	70.03 (8.42)^{b}
Mallika BG II	I	14.50 (3.92)^{c}	**	14.50	13.24 (3.77)^{c}	12.70 (3.69)^{d}	12.90 (3.72)^{c}	38.84	40.93 (6.47)^{c}	51.09 (7.21)^{c}	51.55 (7.24)^{b}	143.57	69.53 (8.39)^{b}	69.10 (8.37)^{b}	138.63	67.53 (8.27)^{b}
Jaadoo non- *Bt*	IV	58.33 (7.70)^{a}	95.38 (9.81)^{a}	153.70	45.15 (6.79)^{a}	67.78 (8.28)^{ab}	99.10 (10.00)^{a}	212.03	53.30 (7.36)^{ab}	77.75 (8.87)^{a}	67.68 (8.27)^{a}	198.73	87.50 (9.40)^{a}	80.88 (9.04)^{a}	168.38	81.90 (9.10)^{a}
Mallika non- *Bt*	IV	61.03 (7.87)^{a}	92.35 (9.66)^{a}	153.38	42.70 (6.60)^{ab}	70.38 (8.44)^{a}	94.83 (9.78)^{a}	207.90	55.35 (7.50)^{a}	75.93 (8.76)^{a}	72.43 (8.56)^{a}	203.70	87.15 (9.38)^{a}	82.88 (9.15)^{a}	170.03	79.65 (8.97)^{a}
F-test		Sig	Sig		Sig	Sig	Sig		Sig	Sig	Sig		Sig	Sig		Sig
SEm±		0.15	0.14		0.09	0.17	0.09		0.12	0.17	0.13		0.12	0.09		0.12
CD (P=0.05)		0.43	0.40		0.26	0.51	0.28		0.35	0.51	0.39		0.36	0.27		0.35

* Larval scrapping area was taken on grading score

** 100 per cent mortality

Figures in parentheses are square root transformed values , Numbers followed by same superscript are not statistically different (P=0.05)

Plate 1. General view of the experimental field

Plate 4 : Caged cotton plant used to release of 3rd instar larvae of *S. litura*

Hatching of *S. litura* eggs in egg mass

Plate 2: Field collected egg mass of *S. litura* for rearing purpose

Plate 3 : Rearing of *S. litura* on non *Bt* leaves

Plate 5: Damaged leaf and squares of *S. litura* larvae inside the cage

Score III – 21 – 30 %

Score I – 1 – 10 %

Score IV – 31 – 40%

Plate 6 : Leaf damage (scrapping) area after 24 h of feeding of first instar larvae of *S. litura* on test hybrids of BG I, BG II and non-*Bt* was taken on the grading score

Pupa from BG I

Pupa from BG II

Pupa from non-*Bt*

Plate7: Pupae from the larvae fed on BG I, BG II and non-*Bt* leaves of test hybrids

Chapter IV

RESULTS AND DISCUSSION

The present investigation was undertaken to study the "**Damage Potential, Growth and Development of *Spodoptera litura* (Fab.) on Dual Toxin Transgenic *Bt* Cotton**" at Regional Agricultural Research Station (RARS), Lam, Guntur, Andhra Pradesh, during *kharif* 2014-15. The study includes assessing the damage potential of *S. litura* on BG II cotton, influence of cotton hybrids containing Cry1Ac (BG I) and Cry1Ac + Cry 2Ab (BG II) of Jaadoo, Mallika and their corresponding non *Bt* versions on larva of *S. litura* with respect to per cent mortality, weight of survival larvae, per cent pupation, per cent adult emergence and leaf area consumed. The results of this study are presented in this chapter.

4.1 ASSESSING THE DAMAGE POTENTIAL OF *S. litura* ON BOLLGARD II *Bt* COTTON

4.1.1. Leaf area consumption of *S. litura* larvae on BG II cotton hybrid at different days after sowing

4.1.1.1. Fifty days after sowing

There was a significant difference in leaf damage between treatments, which received varied number of larvae (Table 4.1, fig 4.1). The leaf damage area ranged from zero in completely protected plant (T1) to 55.77 cm^2 where 21 larvae per plant were released (T8) at 3 days after release (DAR). The plant which received 6 and 9 larvae per plant were on par with each other and differed significantly with treatments which received 12, 15, 18 and 21 larvae per plant. The treatments which received 12 and 15 larvae per plant were on par with each other and differed significantly with treatments which received 18 and 21 larvae per plant. At 7 DAR the leaf area consumption was 9.40 cm^2 for 3 larvae release per plant to 60.07 cm^2 for 21 larvae release per plant. The treatments which received 3 and 6 larvae per plant are on par with each other and differed significantly which received 12, 15, 18 and 21 larvae per plant and the treatments which received 12, 15 and 18 larvae per plant on par with each other. The total cumulative leaf area consumption after 11 DAR was 25.97 cm^2 for 3 larvae released per plant followed by 48, 69.12, 96.10, 115.67, 144.80 and 171.63cm^2 for the plant received 3, 6, 9, 12, 15, 18 and 21 larvae per plant respectively. From this results the leaf area consumption was statically similar in treatment which received 6 & 9 larvae/plant, 12 & 15 larvae/plant and the higher number of larvae of 18 and 21/plant.

4.1.1.2. Eighty days after sowing

Similarly in larvae released at 80 days after sowing (DAS), the leaf damage area varied from zero in complete plant protection to 55.13 cm^2 in 21 larvae released per plant at 3 DAR. The plant which received 3 and 6 larvae per plant are on par with each other and differed significantly with treatments which received 9, 12, 15, 18 and 21 larvae per plant and the plant which received 9 and 12 larvae per plant are on par with each other and differed significantly with the plants which received 18 and 21 larvae per plant. At 7 DAR the leaf area damage varied from 10.37 cm^2 for 3 larvae released per plant to 65.53 cm^2 for 21 larvae released per plant and the plant which received 3, 6 and 9 larvae per plant were on par with each other and differed significantly with the plants which received 12, 15, 18 and 21 larvae per plant and the plant which received 15, 18 and 21 larvae per plant were on par with each other. And the cumulative total leaf area consumption after 11 DAR was 29.11 cm^2 followed by 53.03, 79.87, 108.76, 133.07, 156.53 and 181.47cm^2 which received 3, 6, 9, 12, 15, 18 and 21 larvae per plant, respectively. The treatments which received 15, 18 & 21 larvae per plant were stastically similar in influencing the leaf damage. (Table 4.1)

4.1.1.3. One hundred and ten days after sowing

Leaf damage area ranged from zero in complete plant protection to 58.67 cm^2 in the treatment which received 21 larvae per plant after 3 DAR. The plant which received 3 and 6 larvae per plant were on par with each other and differed significantly to other treatments and the treatments which received 6 and 9 larvae per plant were on par with each other and the treatments which received 9 and 12 larvae per plant were on par with each other at 3 DAR. At 7 DAR the leaf damage area ranged from 12.47 cm^2 for the 3 larvae released per plant to 67.17cm^2 for release of 21 larvae per plant. The treatments which received 3 and 6 larvae per plant were on par with each other and the treatments which received 12, 15, 18 and 21 larvae per plant were on par with each other at 7 DAR (Table 4.1). The total cumulative leaf area consumption was 33.20 cm^2 followed by 56.99, 79.27, 112.17, 134.03, 160.70 and 189.77 cm^2 which received 3, 6, 9, 12, 15, 18 and 21 larvae per plant respectively at 11 DAR. The leaf area consumption was increased by 21.77% for 3 larvae released/plant, 15.77 % for 6 larvae released/plant, 12.80% for 9 larvae released/plant, 14.32% for 12 larvae released/plant, 13.70% for 15 larvae released/plant, 9.89% for 18 larvae released/plant and 9.56% for 21 larvae released/plant compared to 50 DAS larvae released crop. (Table 4.5)

4.1.1.4. One hundred and forty days after sowing

The leaf damage area was more at 140 DAS compared to earlier crop stages. The leaf damage area ranged from zero with no larvae released under complete protection to 70.40 cm^2 where 21 larvae were released per plant at 3DAR and the treatments which received 3 and 6 larvae per plant were on par with each other and the treatments which received 9 and 12 larvae per plant are also on par with each other and the treatments which received 18 and 21 larvae per plant are on par with each other (Table 4.1). At 7 DAR the leaf damage area ranged from 16.53 cm^2 for 3 larvae release per plant to 73.77 cm^2 where, 21 larvae were released per plant and the treatments which received 3 and 6 larvae per plant were on par with each other. The treatments which received 9 and 12 larvae per plant were on par with each other and differ from treatments which received 18 and 21 larvae per plant. The total cumulative leaf damage varied from 46.40 cm^2 followed by 68.13, 102.87, 127.43, 159.27, 187.33 and 219 cm^2 which received 3, 6, 9, 12,15,18 and 21 larvae per plant respectively. The leaf area consumption was increased by 44.02% for 3 larvae released/plant, 29.55 %

for 6 larvae released/plant, 32.81% for 9 larvae released/plant, 24.59% for 12 larvae released/plant, 27.38% for 15 larvae released/plant, 22.70% for 18 larvae released/plant and 21.68% for 21 larvae released/plant compared to 50 DAS larvae released crop. This results showed that the per cent leaf consumption was increased nearly double at 140 DAS when compared to 110 DAS crop (Table 4. 5).

4.1.2. Squares damage by *S. litura* larvae on BG II cotton hybrid at different days after sowing

4.1.2.1. Fifty days after sowing

Even though *S. litura* is leaf eating caterpillar, it damages the fruiting structures of the cotton plant. The square damage ranged from 1.38 to 3.67 squares per plant at 3 DAR (Table 4.2, fig 4.2), and there was no significant difference between the treatments which received zero, 3 and 6 larvae released per plant, and the treatments which received 12,15,18 and 21 larvae per plant were on par at 3DAR. At 7 DAR the number of squares damaged ranged from 1.52 to 4.33 per plant and the treatments which received 3, 6, 9 and 12 larvae per plant were on par with each other and differ significantly treatments which received 15, 18 and 21 larvae per plant which are on par with each other at 7 DAR. The total cumulative square damage after 11 DAR was 3.33 followed by 3.67, 4.67, 6.67, 8.33, 9.67 and 12.00 squares damaged by 3, 6, 9, 12, 15, 18 and 21 larvae per plant, respectively.

4.1.2.2. Eighty days after sowing

Similarly, in larvae released at 80 DAS, the square damaged ranged from 1.00 to 4.00 squares per plant by 3 to 21 larvae per plant at 3DAR and there was no significant difference between the treatment which received zero and 3 larvae per plant and other treatments which received 9, 12, 15, 18 and 21 larvae per plant were on par with each other at 3DAR (Table 4.2). At 7 DAR the number of squares damaged varied from 1.33 to 4.67 per plant which received 3 to 21 larvae per plant. The treatments which received 3 and 6 larvae per plant were on par with each other and these treatments significantly differed from rest of the treatments. The treatments which received 6, 9, 12, 15 and 18 larvae per plant were on par with each other. The total cumulative squares damaged after 11 DAR was 3.33 followed by 5, 7, 8, 9.67, 12 and 13 squares which received 3, 6, 9, 12, 15, 18 and 21 larvae per plant respectively.

4.1.2.3. One hundred and ten days after sowing

The square damaged ranged from 1.33 to 5.67 squares per plant by receiving 3 to 21 larvae per plant, respectively. The treatments which received 3, 6 and 9 larvae per plant were on par with each other and these treatments significantly differed from rest of the treatments at 3DAR and the treatments which received 9, 12, 15 and 18 larvae per plant were on par with each other (Table 4.2). At 7 DAR the squares damaged varied from 1.67 to 6.00 squares pre plant which received 3 to 21 larvae per plant respectively. The treatments which received 3 and 6 larvae per plant were on par with each other and these treatments significantly differed from rest of the treatments. The treatments which received 6, 9 and 12 larvae per plant were on par with each other at 7 DAR. The total cumulative squares damaged after 11 DAR was 4.33 followed by 5.33, 9.67, 11.33, 13.00, 15.33 and 17 squares per plant which received 3, 6, 9, 12, 15, 18 and 21 larvae per plant respectively.

4.1.2.4. One hundred and forty days after sowing

The number of squares damaged is more at this stage of the crop compared to early stage. The squares damaged ranged from 1.67 to 6.33 squares per plant which received 3 to 21 larvae per plant, respectively. The treatments which received 3 and 6 larvae per plant were on par with each other and these significantly differed from rest of the treatments at 3 DAR. The treatments which received 12, 15, 18 and 21 larvae per plant were on par with each other at 3 DAR (Table 4.2). At 7 DAR the number of squares damaged varied from 1.33 to 7.00 squares per plant which received 3 to 21 larvae per plant. The treatments which received 3 and 6 larvae per plant showed no significant difference from each other and these treatments differed from rest of the treatments. The treatments which received 9, 12 and 15 per plant are on par with each other at 7DAR. The total cumulative square damage was 4.33 followed by 7.67, 10.67, 13.67, 16.00, 18.67 and 19.67 squares which received 3, 6, 9, 12, 15, 18 and 21 larvae per plant respectively.

4.1.3. Boll damage by *S. litura* larvae on BG II cotton hybrid at different days after sowing

4.1.3.1. Fifty days after sowing

There was no boll formation at 50 DAS, hence boll damage could not be recorded.

4.1.3.2. Eighty days after sowing

The bolls damaged ranged from 0.67 to 3.00 bolls per plant (Table 4.3, fig 4.3) among the treatments which received 3 to 21 larvae per plant and in the treatments which received zero and 3 larvae per plant there was no significant difference from each other and significantly differed from rest of the treatments. Treatments which received 3, 6, 9 and 12 larvae per plant and the treatments which received 15, 18 and 21 larvae per plant were on par with each other at 3 DAR. After 7 DAR in the treatments which received 3, 6, 9 and 12 larvae per plant there was no significant difference from each other and differed significantly from rest of the treatments at 7 DAR. The total cumulative bolls damaged were 2, 3, 3.67, 4.67, 6, 7.33 and 9 bolls per plant which received 3, 6, 9, 12, 15, 18 and 21 larvae per plant, respectively at 11 DAR.

4.1.3.3. One hundred and ten days after sowing

Bolls damaged were more compared to early stages of crop. The boll damage ranged from 0.33 to 4.00 bolls per plant in the treatments which received 3 to 21 larvae per plant. The treatments which received zero and 3 larvae per plant were on par and also the treatments which received 6, 9 and 12 larvae per plant were on par and significantly differed from each other at 3DAR (Table 4.3). At 7 DAR the bolls damaged ranged from 0.67 to 4.33 bolls per plant which received 3 to 21 larvae per treatment. The treatments which received zero, 3 and 6 larvae per plant were on par with each other and these treatments differed significantly from rest of the treatments and the treatments which received 9, 12, 15 and 18 larvae per plant were on par with each other at 7 DAR. The total cumulative bolls damaged was 1.33 followed by 2.67, 4.67, 6.33, 8.00, 9.00 and 11.33 bolls per plant which received 3, 6, 9, 12, 15, 18 and 21 larvae per plant respectively.

4.1.3.4. One hundred and forty days after sowing

The bolls damaged varied from 0.33 to 4.33 bolls per plant which received 3 to 21 larvae at 3 DAR. The treatments which received 3, 6 and 9 larvae per plant were on par with the treatment under complete protection and significantly differed from rest of the treatments and in the treatments which received 15, 18 and 21 larvae per plant there were no significant difference at 3 DAR (Table 4.3). The bolls damaged ranged from 0.33 to 4.67 bolls pre plant, where 3 to 21 larvae were released per plant at 7 DAR. The treatments which received zero and 3 larva per plant were on par and these treatments differed significantly from rest of the treatments and the treatments which received 6, 9 and 12 larvae per plant were at par with no significant difference from each other at 7 DAR. The total cumulative number of damaged bolls per plant was 1.00 followed by 4.00, 5.33, 6.33, 9.00, 10.33 and 12.00 bolls which received 3, 6, 9, 12, 15, 18 and 21 larvae per treatment respectively.

4.1.4. Seed cotton yield and per cent yield reduction over control

4.1.4.1. Larvae were released fifty days after sowing

The yield was more due to less inflicted damage by *S. litura*. In control the yield recorded was 293.33 g/plant without any damage to leaf, squares and bolls (Table 4.4, fig 4.4). In the treatment which received 3 larvae per plant the yield was 276 g/plant because of 25.97 cm^2 leaf damage and 3.33 square damage due to this the per cent yield reduction over control was 5.91%. When 6 larvae were released per plant the yield was 271 g/plant, and the yield reduction over control was 7.38 % because of 48.00cm^2 leaf damage and 3.67 damaged squares per plant. The treatments which received 9 larvae per plant, recorded 270 g/plant and yield reduction over control was 7.95 % due to 69.12 cm^2 leaf damage and 4.67 damaged squares. In the treatment which received 12 larvae per plant the yield recorded was 263.33g/plant and the per cent yield reduction over control was 10.23 % because of 96.10 cm^2 leaf damage and 6.67 damaged squares by *S. litura.* The treatments which received 15 larvae per plant recorded 253.33g/plant and the per cent yield reduction over control was 13.64% due to 115.67 cm^2 leaf damage and 8.33 damaged squares. The treatments which received 18 larvae per plant, recorded 246.67g/plant and the per cent yield reduction over control was 15.91 % because of 144.80 cm^2 leaf and 9.67 squares damage. Treatments which received 21 larvae per plant, recorded 230g/plant and the per cent yield reduction over control was 21.59% due to 171.63cm^2 leaf damage and 12 squares damage.

4.1.4.2. Larvae were released eighty days after sowing

In control yield recorded was 276.67g/plant without any damage to leaf, squares and bolls. In treatment which received 3 larvae per plant the yield was 249.67 g/plant because of 29.11 cm^2 leaf, 3.33 damaged squares and 2 damaged bolls per plant due to this the per cent yield reduction over control was 9.76 %. When 6 larvae were released per plant the yield was 235 g/plant, with yield reduction over control of 15.06 % because of 53.03cm^2 leaf damage and 5 damaged squares and 3 damaged bolls. The treatments which received 9 larvae per plant, recorded yield 221.67 g/plant and the yield reduction over control was 19.88 % due to 79.87 cm^2 leaf damage, 7 damaged squares and 3.67 damaged bolls per plant. The treatment which received 12 larvae per plant recorded yield of211.67g/plant and the per cent yield reduction over control was 23.49 % because of 108.76 cm^2 leaf damage, 8damaged squares and 4.67 damaged bolls by *S. litura.* The treatments which received 15 larvae per plant, recorded 202.67g/plant and the per cent yield reduction over control was 26.75% due to 133.07 cm^2 leaf damage, 9.67 damaged squares and 6 damaged bolls per plant. The treatments which received 18 larvae per plant, record of 178.33g/plant and the per cent yield reduction over control was 35.54 % because of 156.53cm^2 leaf damage, 12 damaged squares and 7.33 damaged bolls per plant. Treatments which received 21 larvae per plant, recorded lowest yield of 166.67g/plant and per cent yield reduction over control was 39.76% due to 181.47cm^2 leaf, 13 damaged squares and 9 damaged bolls per plant (Table4.4)

4.1.4.3. Larvae were released one hundred and ten days after sowing

Gradual decrease in yield was observed with increase in crop age. In control the yield recorded was 275g/plant without any damage to leaf, squares and bolls. When the treatment received 3 larvae per plant the yield recorded was 245.33 g/plant because of 33.20 cm^2 leaf damage, 4.33 damaged squares and 1.33 damaged bolls per plant due to this the per cent yield reduction over control was 10.79 %. When, 6 larvae were released per plant the yield was 233.33 g/plant and the yield reduction over control was 15.15% because of 56.99 cm^2 leaf damage and 5.33 damaged squares and 2.67 damaged bolls. The treatments which received 9 larvae per plant recorded 202.67 g/plant with yield reduction over control of 26.30 % due to 79.27 cm^2 leaf damage, 9.67 damaged squares and 4.67 damaged bolls per plant. When the treatment received 12 larvae per plant the yield recorded was 187.67g/plant and the per cent yield reduction over control was 31.76 % because of 112.17 cm^2 leaf, 11.33 damaged squares and 6.33 damaged bolls by *S. litura.* In treatments which received 15 larvae per plant the yield was 168g/plant and the per cent yield reduction over control was 38.91% due to 134.03 cm^2 leaf damage, 13 damaged squares and 8 damaged bolls per plant. The treatments which received 18 larvae per plant, recorded 154.33 g/plant and the per cent yield reduction over control was 43.88 % because of 160.70cm^2 leaf damage, 15.33 damaged squares and 9 damaged bolls per plant. Treatments which received 21 larvae per plant, recorded yield of 135g/plant and per cent yield reduction over control was 50.91% due to 189.77cm^2 leaf, 15.33 damaged squares and 11.33 damaged bolls per plant. The per cent yield reduction was increased at 110 and 140 DAS compared to 50 DAS larvae released crop. At 110 DAS larvae released crop, the per cent yield reduction was increased by 11.11 for 3 larvae released/plant, 14.11 % for 6 larvae/plant, 24.94% for 9 larvae released/plant, 28.73% for 12 larvae released/plant, 33.68% for 15 larvae released/plant, 37.43% for 18 larvae released/plant and 41.30% for 21 larvae/plant compared to 50 DAS larvae released crop. (Table 4.6)

4.1.4.4. Larvae were released one hundred and forty days after sowing

In control yield recorded was 270.33g/plant without any damage to leaf, squares and bolls. When the treatment received 3 larvae per plant, yield was 245.33 g/plant because of 46.40 cm^2 leaf damage, 4.33 damaged squares and 1.00 damaged bolls per plant due to this the per cent yield reduction over control was 9.25 %. When 6 larvae were released per plant the yield recorded was 212.67 g/plant with the yield reduction over control of 21.33% because of 68.13cm^2 leaf damage, 7.67 damaged squares and 4 damaged bolls per plant. The treatments which received 9 larvae per plant recorded yield of 194.33 g/plant with yield reduction over control of 28.11 % due to 102.87 cm^2 leaf damage, 10.67 damaged squares and 5.33 damaged bolls per plant. When the treatment received 12 larvae per plant the yield recorded was 170.33g/plant and the per cent yield reduction over control was 36.99 % because of 127.43 cm^2 leaf, 13.67 damaged squares and 6.33 damaged bolls by *S. litura*. In treatment which received 15 larvae per plant the yield recorded was 145g/plant and the per cent yield reduction over control was 46.36% due to 159.27 cm^2 leaf damage, 16 damaged squares and 9 damaged bolls per plant. The treatments which received 18 larvae per plant the yield recorded was 126.33 g/plant and the per cent yield reduction over control was 53.27 % because of 187.33cm^2 leaf, 18.67 damaged squares and 10.33 damaged bolls per plant. Treatments which received 21 larvae per plant recorded yield of 119.33g/plant and the per cent yield reduction over control was 55.86% due to 219.13cm^2 leaf, 19.67 damaged squares and 12.00 damaged bolls. At 140 DAS larvae released crop, the per cent yield reduction was increased by 11.11 for 3 larvae released/plant, 21.72% for 6 larvae/plant, 28.03% for 9 larvae released/plant, 35.32% for 12 larvae released/plant, 42.76% for 15 larvae released/plant, 48.79% for 18 larvae released/plant and 48.12% for 21 larvae/plant when compared to 50 DAS larvae released crop. (Table 4.6)

From this results, it can be summarized that there was a significant difference of leaf, square and boll damage in BG II cotton hybrid at different days of release of *S. litura.* The leaf damage area was less at 50 and 80 DAS larvae released crop as compared to 110 and 140 DAS larvae released crop this may be due to expression of Cry 2Ab toxin in leaves, which was effective against *S. litura* damage. At 110 and 140 DAS crop leaf damage was more may be due to decrease in protein expression of Cry 2Ab. The present findings derive support from Li *et al*. (2006), Bheemanna *et al*. (2008) and Pradeep (2011) who reported that the percentage of feeding damage on BG II cotton was less. El-Sherif *et al.* (1992) reported that the losses of 1 or 50% leaf area at the vegetative growth, budding, flowering, initial fruiting and full fruiting stages decreased the cotton yield. Dhir *et al.* (1992) reported that at seedling stage of ground nut, one larva per plant consumed about 54.7% leaf area and reduced pod yield by 25.8%.

The square and boll damage was more at 110 and 140 DAS crop due to decrease in Cry 2Ab protein. The total number of damaged square and damaged bolls was significantly influenced by *S. litura* density at different stages of the crop. As the density of larvae increased, more damage was observed and this was confirmed by (Luttrell *et al*. 1999), Santos *et al*. (2010) and Katayama *et al.* (1989) who reported that as the defoliation rate increased, the number of pods and the weight of grains decreased in red bean, due to *S. litura.* Qin HouGuo *et al*. (2000) reported that a single larva of *S. litura* during its life span can consume 3.61 cotton leaves, 0.97 square, 0.37 flower and 0.27 boll. The damage increases in exponential function with the rise of larval numbers.

4.2 STUDIES ON THE INFLUENCE OF BOLLGARD II *Bt* COTTON (Cry 1Ac + Cry 2Ab) PLANTS AGAINST *S. litura*

4.2.1. Effect of Test Hybrids on Larval Mortality

Mortality per cent of *S. litura* larvae of different instars reared on leaves and squares of 70 - 80 days old crop of different cotton hybrids are presented in table 4.7, fig 4.5.

4.2.1.1. Leaves

First instar

The larval mortality of *S. litura* was maximum on Jaadoo BGII and Mallika BG II compared to other test hybrids. Mortality of first instar larvae fed on leaves of Mallika BG II and Jaadoo BG II cotton hybrids were 52.50 and 47.50 per cent, while it was 7.50 and 2.50 per cent when fed on Jaadoo BG I and Mallika BG I and 5.00 and 2.50 per cent fed on Mallika non-*Bt* and Jaadoo non-*Bt* hybrids at 3 days after release. The mortality on Jaadoo BGII and Mallika BG II cotton hybrids were 52.50 and 47.50 per cent with significant differences from rest of the hybrids with cumulative mortality of 100 % at 7 days after release , while the total mortality was 17.50 per cent on BG I and 12.50 per cent on non-*Bt* hybrids at 11 day after release.

Second instar

The mortality of 2nd instar larvae fed on leaves of 70-80 days old crop at 3 days after release was 40.00 per cent on Mallika BG II hybrid and 25.00 per cent on Jaadoo BGII, 7.50 per cent mortality on BG I cotton hybrids and 5.00 per cent mortality on non-*Bt* cotton hybrids at three day after release, whereas at 7 days after release higher mortality rates of 47.50 and 40.00 per cent were recorded on Jaadoo BG II and Mallika BG II cotton hybrids respectively, while it was 2.50 per cent on BG I hybrids and non-*Bt* hybrids. The total larval mortality was 92.50 per cent on leaves of both Jaadoo BG II and Mallika BG II hybrids at 11 days after release, while it was 10.00 per cent in the BG I and 7.50 per cent in non-*Bt* hybrids at 11 days after release. (Table 4.7)

Third instar

With regard to 3rd instar larvae, the mortality was nil on leaves of 70-80 days old crop of BG I and non *Bt* hybrids at 3, 7 and 11 days after release, while mortality was recorded on the stacked *Bt* (BG II) hybrids. The mortality at 3 days after release was 22.50 and 17.50 per cent with significant difference on

Jaadoo BG II and Mallika BG II hybrids respectively. The mortality was 15.00 per cent and 12.50 per cent on leaves of both the stacked *Bt* (BG II) hybrids at 7 days after release, while the total mortality was 35.00 and 32.50 per cent with significant difference when fed on leaves of Jaadoo BG II and Mallika BG II hybrids respectively at 11 days after release.

Fourth and fifth instar

The 4^{th} and 5^{th} instar larvae recorded no mortality on leaves of 70 – 80 days old crop of Jaadoo BG II and Mallika BG II and their corresponding BG I , non *Bt* cotton hybrids at 3, 7, and 11 days after release. The stacked *Bt* (BG II) hybrids were found more resistant to fourth and fifth instars as no mortality was recorded on both the stacked *Bt* (BG II) hybrids at 3, 7 and 11 days after release. There is no significant difference between Jaadoo BG II and Mallika BG II cotton hybrids and their corresponding BG I and non- *Bt* hybrids. (Table 4.7)

4.2.1.2. Squares

The mortality of larvae on squares of Jaadoo BG II and Mallika BG II hybrids was seen only up to third instar and decreased gradually with increase in age. (Table 4. 8 and Fig 4.6)

First instar

The larval mortality of *S. litura* was maximum on Jaadoo BG II and Mallika BG II compared to other test hybrids. Mortality of first instar larvae fed on squares of both Jaadoo BG II and Mallika BG II cotton hybrids was 92.50 per cent, while it was 5.00 per cent when fed on Jaadoo BG I and Mallika BG I and 5.00 and 2.50 per cent fed on Mallika non-*Bt* and Jaadoo non-*Bt* hybrids at 3 days after release. The mortality on both Jaadoo BG II and Mallika BG II cotton hybrids was 7.50 per cent with no significant differences from rest of the hybrids at 7 days after release. The total mortality on stacked *Bt* cotton hybrids was cent per cent, while it was 15.00 per cent on BG I and 10.00per cent on non-*Bt* hybrids at 11 day after release.

Second instar

The mortality of 2nd instar larvae fed on squares of 70- 80 days old crop at 3 day after release was 22.50 per cent on Jaadoo BG II hybrid and 17.50 per cent on Mallika BG II and 7.50 per cent mortality on both BG I cotton hybrids and 2.50 per cent mortality on non-*Bt* cotton hybrids at three day after release, whereas at 7 days after release higher mortality rates of 55.00 and 40.00 per cent were recorded on Jaadoo BG II and Mallika BG II cotton hybrids respectively, while it was 2.50 per cent on both BG I hybrids and non *Bt* hybrids. The total mortality was 87.50 per cent on squares of Jaadoo BG II and 75.00 per cent on Mallika BG II hybrids at 11 days after release, while it was 10.00 per cent in the BG I and 5.00 per cent in non *Bt* hybrids at 11 days after release (Table 4.8).

Third instar

With regard to 3rd instar larvae, the mortality was nil on squares of BG I and non *Bt* hybrids at 3, 7 and 11days after release, while mortality was recorded on the stacked *Bt* (BG II) hybrids. The mortality at 3 days after release was 12.50 per cent on both Jaadoo BG II and Mallika BG II hybrids. The mortality was 12.50 per cent on both the stacked *Bt* (BG II) hybrids at 7 days after release, while the total mortality was 25.00 per cent with significant difference when fed on squares of Jaadoo BG II and Mallika BG II and BG I and non *Bt* hybrids at 11 days after release.

Fourth and fifth instar

The 4th and 5th instar larvae recorded no mortality on squares of Jaadoo BG II and Mallika BG II and their corresponding BG I , non *Bt* cotton hybrids at 3, 7, and 11 days after release. The stacked *Bt* (BG II) hybrids were found more resistant to fourth and fifth instars as no mortality was recorded on both the stacked *Bt* (BG II) hybrids at 3,7 and 11 days after release. No significant difference between Jaadoo BG II and Mallika BG II cotton hybrids and their corresponding BG I and non- *Bt* hybrids at all the observations.

4.2.2. Effect of test hybrids on larval weight

The mean weight of larvae that survived beyond 3, 7 and 11 days after release on leaves and squares of 70-80 days old crop from all the six test hybrids was recorded for first, second, third and fourth instars and presented in table 4.9 and 4.10

4.2.2.1 Leaves

The minimum larval weight gain was recorded when fed on leaves of Jaadoo and Mallika BG II hybrids. Jaadoo and Mallika BG II hybrids with least increase in larval weight was significantly superior over other test hybrids (Table4.9 and Fig 4.7). The mean larval weight of first instar larvae was minimum (0.79 mg/larva, 1.10mg/ larva) in both Jaadoo and Mallika BG II hybrids at 3 days after release on leaves. The maximum larval weight (6.85 mg/ larva) was recorded on Jaadoo *Bt* followed by Mallika non- *Bt* (5.32 mg/ larva), Mallika *Bt* (4.76mg/ larva), Jaadoo non *Bt* (4.29 mg/ larva) at 3 days after release on leaves. All the BG I and non *Bt* hybrids are statistically on par and significantly inferior over the Jaadoo and Mallika BG II hybrids. There is no surviving larvae in Jaadoo and Mallika BG II hybrid, due to 100 per cent mortality, and the maximum weight gain (99.21 %) at 11days after release was recorded in Jaadoo BG I hybrid followed by Mallika BG I (99.08), Mallika non *Bt* (99.00%), and Jaadoo non-*Bt* (98.88%). All these are statistically on par with each other.

The mean larval weight of second instar larvae was minimum (38.95 mg/ larva) in Mallika BG II hybrid and (41.08 mg/ larva) in Jaadoo BG II the maximum larval weight (58.45 mg/larvae) was recorded on Mallika non *Bt* followed by Jaadoo non *Bt* (55.45 mg/ larva), Mallika BG I (50.40 mg/ larva), and Jaadoo BG I (50.03 mg/ larva) at 3 days after exposure to leaves. All these hybrids are statistically on par and significantly inferior over the Jaadoo and Mallika BG II hybrids. The per cent weight gain was lowest in Mallika BGII hybrid (80.01%) and in Jaadoo BG II hybrid (80.84 %). The maximum weight gain (92.27 %) at 11 days after exposure was recorded in Mallika non *Bt* hybrid followed by Jaadoo non *Bt* (45.68%), Mallika BG I (87.35), and Jaadoo BG I (85.08%). All the BG II hybrids superior over BG I and non *Bt* hybrids with each other.

The mean larval weight of third instar larvae fed on leaves was minimum in Mallika (146.56 mg/larva) and Jaadoo (150.17 mg/larva) BGII hybrids at 3 days after feeding compared to other test hybrids. The maximum weight gained was on Mallika non-*Bt* (283.75 mg/larva) followed by Jaadoo non-*Bt* (201.65 mg/larva), Mallika BG I (178.80 mg/larva) and Jaadoo BG I (175.38 mg/larva).

At 7 days after release maximum weight gain was on Mallika non-*Bt* (602.22 mg/larva) followed by Mallika BG I (559.25mg/larva), Jaadoo non *Bt* (529.50 mg/larva), Jaadoo BG I (528.87 mg/larva) and minimum weight gain was in Jaadoo BG II (452.12 mg/larva) followed by Mallika BG II (470.12 mg/larva). It means Jaadoo BG II and Mallika BG II on a par and recorded lowest weight gain compared to other hybrids. The per cent weight gain after 7 days after feeding was maximum in Mallika non *Bt* (90.30 %) followed by Mallika BG I (89.56 %) , Jaadoo non *Bt* (88.97 %), Jaadoo BG I (88.96 %) and the per cent weight gain during third instar was lowest (87.08 %) in Jaadoo BG II, followed by (87.58 %) in Mallika BG II which were statistically superior over other hybrids.

The mean larval weight of fourth instar larvae after feeding for 3 days was maximum in Mallika non-*Bt* (537.10 mg/larva) followed by Jaadoo non-*Bt* (519.93 mg/larva) Mallika BG I (480.00 mg/larva) and Jaadoo BG I (466.23 mg/larva) and minimum in Jaadoo BGII hybrid (389.23 mg/larva) followed by Mallika BG II (389.78mg/larva). After three days of feeding the per cent weight gain was low (53.73 %) in Jaadoo BG II and Mallika BG II (53.79%) compared to other test hybrids. The maximum weight gain was noticed in Mallika non-*Bt* (66.47%) followed by Jaadoo non *Bt* (65.36%), Mallika BG I (62.48%), and Jaadoo BG I (61.37%) All these hybrids are statistically on par and significantly different from Jaadoo and Mallika BGII hybrids. The decreased mortality, lower larval and pupal weight growth of *S. litura* on Jaadoo and Mallika BGII observed in the present studies are in conformity with the results of Soujanya (2011), Naik, *et. al* (2013).

4.2.2.2 Squares

The growth of larvae fed on Jaadoo and Mallika BG II squares was minimum with less weight gain in all the five instars *viz.*, first, second, third, fourth and fifth instars tested. The Jaadoo and Mallika BG II hybrids was superior over the other hybrids tested in having less preference for feeding by *S. litura* (Table 4.10 and Fig 4.8).

The mean larval weight of first instar larvae was minimum (3.17 mg/larva) in Mallika BG II hybrid and (3.60 mg/larva) in Jaadoo BG II hybrid at 3 days after release on squares. The maximum larval weight (35.75 mg/larva) was recorded on Mallika BG I, followed by Mallika non- *Bt* (31.53mg/larva), Jaadoo non *Bt* (31.15 mg) and Jaadoo BG I (28.85mg) at 3 days after release on squares. All these hybrids are statistically on par and significantly inferior over the Jaadoo and Mallika BG II hybrid. The per cent weight gain could not be recorded in Jaadoo and Mallika BG II hybrid, due to 100 per cent mortality. The maximum weight gain (98.58 %) at 11days after release was recorded in Mallika non-*Bt* hybrid followed by Jaadoo non-*Bt* (98.29 %), Jaadoo BG I (98.28%), and Mallika BG I (98.16%). All these are statistically on par with each other.

The mean larval weight of second instar larvae was minimum (42.68 mg/larva) in Jaadoo BG II hybrid and (42.93 mg/larva) in Mallika BG II the maximum larval weight (61.13 mg/larva) was recorded on Jaadoo non-*Bt*, followed by Mallika non *Bt* (60.68 mg/larva), Mallika BG I (57.70 mg/larva), and Jaadoo BG I (54.20 mg/larva) at 3 days after exposure to leaves. All these hybrids are statistically on par and significantly inferior over the Jaadoo and Mallika BG II hybrids. The per cent weight gain was lowest (84.49 %) in Jaadoo BGII hybrid and (85.58 %) in Mallika BG II hybrid. The maximum weight gain (91.24 %) at 11 days after exposure was recorded in Mallika non *Bt* hybrid followed by Jaadoo non *Bt* (91.02%), Mallika BG I (87.46), and Jaadoo BG I (87.01%).

The mean larval weight of third instar larvae fed on leaves was minimum in Mallika (128.81 mg/larva) and Jaadoo (164.42 mg/larva) BGII hybrids at 3 days after feeding compared to other test hybrids. The maximum weight gain was on Mallika BG I (350.40 mg/larva) followed by Jaadoo BG I (297.00 mg/larva), Jaadoo non-*Bt* (268.95mg/larva) and Mallika non-*Bt* (239.80mg/larva). (Table 4.10)

At 7 days after release maximum weight gain was on Mallika non-*Bt* (565.73 mg/larva) followed by Jaadoo BG I (502.88mg/larva), Jaadoo non-*Bt* (488.58 mg/larva), Mallika BG I (451.33 mg/larva) and minimum weight gain was in Jaadoo BG II (314.55 mg/larva) followed by Mallika BG II (366.43 mg/larva). It means Jaadoo BG II and Mallika BG II significantly differ in larval weight gain compared to other hybrids. The per cent weight gain after 7 days after feeding was maximum in Mallika non *Bt* (84.90 %) followed by Jaadoo BG I (83.08 %) , Jaadoo non *Bt* (82.52 %), Mallika BG I (81.08 %) and the per cent weight gain during third instar was lowest (72.85 %) in Jaadoo BGII , followed by (76.69 %) in Mallika BG II which are statistically superior over other hybrids.

The mean larval weight of fourth instar larvae after feeding for 3 days was maximum in Mallika non-*Bt* (537.10 mg) followed by Jaadoo non-*Bt* (527.43 mg) Jaadoo BG I (486.93 mg) and Mallika BG I (480.00 mg) and minimum in Mallika BG II hybrid (339.78 mg) followed by Jaadoo BG II (389.23). Both staked *Bt* cotton hybrids are significantly different from other hybrids. After three days of feeding the per cent weight gain was low (50.47 %) in Mallika BG II and Jaadoo BG II (56.76%) compared to other test hybrids. The maximum weight gain was noticed in Mallika non-*Bt* (68.67%) followed by Jaadoo non *Bt* (68.09%), Jaadoo BG I (65.44%), and Mallika BG I (64.94%), All these hybrids are statistically on par and significantly different from Jaadoo and Mallika BG II hybrids.

4.2.3. Effect of test hybrids on pupation

The per cent pupation of surviving larvae of *S. litura* on leaves and squares of different test cotton hybrids. (Table 4.11 and Fig 4.9)

4.2.3.1 Leaves

The Jaadoo and Mallika BG II hybrids were resistant to *S. litura* attack and therefore the per cent pupation of survived larvae fed on leaves of this hybrid was low compared to other test hybrids and they are statistically superior over other test hybrids. The first instar larvae fed on leaves of Jaadoo and Mallika BG II hybrids died completely before pupation. Only larvae of second to fifth instar fed on Jaadoo and Mallika BG II hybrids survived up to the end of the larval stage and pupated (Table 4.11). The per cent pupation in second instar larvae was very less 7.50% in both Jaadoo BG II and Mallika BG II hybrids. Whereas in BG I cotton hybrids 90.00% pupation and in non-*Bt* hybrids 95.00% pupation was observed. There was 67.50% pupation in third instar in Mallika BG II and 65.00% pupation in Jaadoo BG II cotton hybrids and 100.00% in fourth and fifth instar larvae when fed on Jaadoo and Mallika BG II hybrids and this is significantly superior over other hybrids. More than 85% of the survival first and second instar larvae of *S. litura* fed on leaves of test hybrids except Jaadoo and Mallika BG II hybrids entered into pupation. Hence the per cent pupation in these hybrids was more compared to Jaadoo and Mallika BG II hybrids. In fourth and fifth instars larvae, there was no mortality, hence all the larvae pupated at the end on all the hybrids. In Jaadoo and Mallika BG II hybrids also 100.00% pupation was recorded in fourth and fifth instar larvae.

4.2.3.2. Squares

The per cent pupation of larvae when fed on squares of Jaadoo and Mallika BG II hybrids were less in first, second and third instars compared to other test hybrids and are statistically superior over other hybrids. The first instar larvae when fed on Jaadoo and Mallika BG II hybrid squares resulted in complete mortality before pupation .The pupation per cent in other test hybrids varied between 80.00 to 90.00 % and all are statistically on par. In second instar, the pupation per cent was 12.50% in Jaadoo BG II and 25.00 % in Mallika BG II and > 90.00% in other hybrids. The per cent pupation of third instar larvae fed on squares of Jaadoo BG II and Mallika BG II was 75.00% and it was 100.00% in all the hybrids in third, fourth and fifth instars. Table 4.11, fig 4.10

4.2.4 Effect of test hybrids on pupal weight

The mean pupal weight of *S. litura* reared on different test hybrids was given in (Table 4.12).

4.2.4.1. Leaves

The maximum mean pupal weight was recorded, when first instar larvae were reared on Jaadoo non *Bt* (269.96 mg/pupa) followed by Mallika non *Bt* (233.6 mg/pupa) and Jaadoo BG I (219.76 mg/pupa). In Jaadoo and Mallika BG II hybrid there was no pupation of first instar larvae. The pupal weights of second, third, fourth and fifth instar larvae fed on leaves of Jaadoo and Mallika BG II hybrids was less compared to other test hybrids. The maximum pupal weight of 149.94/pupa mg was recorded, when second instars larvae were fed on leaves of Jaadoo non-*Bt* and it is statistically an par with other hybrids except Jaadoo and Mallika BG II hybrids (Table 4.12). The third instar larvae fed on leaves of Jaadoo non *Bt* recorded 224.13 mg/pupa weight followed by Mallika non-*Bt* (220.90 mg/pupa), Mallika BG I (210.55 mg/pupa) and Jaadoo BG I (206.53 mg/pupa) hybrids and all are statistically on par with each other except Jaadoo and Mallika BG II hybrids with only (167.27 and 166.75 mg/pupa) of mean pupal weight respectively. The maximum pupal weight of 281.75 mg/pupa was recorded, when fourth instars larvae were fed on leaves of Jaadoo BG I hybrid and it is statistically on par with other hybrids except Jaadoo and Mallika BG II hybrids (Table 4.12). The fifth instar larvae fed on leaves of Mallika non *Bt* recorded 268.25 mg/pupa of pupal weight followed by Jaadoo non-*Bt* (261.05 mg/pupa), Jaadoo BG I (256.58 mg/pupa) and Mallika BG I (242.75 mg/pupa) hybrids and all are statistically on par with each other except Jaadoo and Mallika BG II hybrid with only 210.90, 220.43 mg/pupa of mean pupal weight respectively. BG II cotton hybrids were statistically significant over the BG I and non-*Bt* hybrids.

4.2.4.2 Squares

The different instars fed on squares exhibited similar trend in pupal weight as those fed on leaves. The larvae of *S. litura* fed on Jaadoo and Mallika BG II hybrids recorded no pupation for first instar larvae. The first instar larvae fed on Mallika non-*Bt* squares recorded the maximum pupal weight (198.35 mg/pupa) and is on par with Jaadoo non *Bt* (183.55 mg/pupa), Mallika BG I (176.39 mg/pupa) and Jaadoo BG I (171.35 mg/pupa). The mean pupal weight has increased gradually with increase in age of larvae on Jaadoo and Mallika BGII hybrids. The maximum pupal weight (268.75 mg/pupa) was recorded on Mallika non *Bt* hybrid and it was statistically on par with Jaadoo BG I (268.25 mg/pupa), Mallika BG I (260.23 mg/pupa), Jaadoo non-*Bt* (260.6 mg/pupa) where as Jaadoo BG II (243.58 mg/pupa) and Mallika BGII hybrid recorded lowest (223.10 mg/pupa) pupal weight when fifth instars larvae were reared on Mallika BG II hybrid.

4.2.5. Effect of different test hybrids on adult emergence

The per cent adult emergence of *S. litura* was recorded on different test hybrids (Table 4.13 and Fig 4.11).

4.2.5.1. Leaves

None of the first instar larvae fed with leaves of Jaadoo and Mallika BG II hybrids reached adult stage. The second instar larvae reared on Jaadoo and Mallika BG II hybrids recorded 5.00% and third instar larvae recorded 62.50, 65.00% adult emergence respectively. The per cent adult emergence when first and second instar larvae reared on other test hybrids was more than 85.00% and all are statistically on par with each other except Jaadoo BG I which is recorded 72.50 %. In fourth and fifth instar there was 100 per cent adult emergence in all the test hybrids (Table 4.13 and Fig 4.11).

4.2.5.2. Squares

None of the first instar larvae fed with squares of Jaadoo and Mallika BG II hybrids reached adult stage. The adult emergence was very poor when second instars were fed on squares of Jaadoo and Mallika BG II hybrids. There was only 10.50% in Jaadoo BG II and 15.00 % in Mallika BG II adult emergence. These hybrids are least preferred by the *S. litura*. The other test hybrids recorded > 90.00% adult emergence when second instar larvae were reared on those hybrids. For, third instar 70% adult emergence was recorded on both Jaadoo and Mallika BG II hybrids. Where as in other hybrids 100.00% adult emergence was recorded and all these are on par. Adult emergence was 100% for fourth and fifth instars reared on the all test hybrids. (Table 4.13 and Fig 4.12.)

The results from the present investigation also clearly suggest that the BG I cotton hybrids which contains Cry1Ac protein, offer marginal influence on 1st and 2nd instar and did no effect on mortality against other instars larvae of *S. litura* and not useful for the management of *S. litura* on cotton. These findings are in close association with the results of Zeng *et al*. (1998) who reported that the development of *S. exigua* on BG I cotton was normal. The BG I cotton hybrids which were efficient against *H. armigera* as revealed from the references of Kranthi *et al.* (2005) and Seshamahalakshmi (2007) have not offered any efficacy against *S. litura* during the 70-80 days old crop.

The Stacked *Bt* (BG II) cotton hybrids with Cry 1Ac + Cry 2Ab dual proteins exhibited higher mortality for first and second instars of *S. litura* than BG I and non *Bt* cotton hybrids. The present findings derive support from Allen *et al.* (2000), Greenplate *et al.* (2000), Johnson *et al.* (2000), Ridge *et al.* (2000), Adamczyk *et al.* (2001), Marchosky *et al*. (2001), Stewart *et al.* (2001), Chitkowski *et al.* (2003), Adamczyk *et al*. (2003), Adamczyk *et al*. (2004), Santos (2007), Adamczyk *et al*. (2008c) and Buntin (2008) who reported that Bollgard II cultivars exhibited far lower number of beet armyworm, *S. exigua*than BG I and non *Bt* varieties. The present findings are also in conformity with Sivasupramaniam *et al*. (2003), Donglin *et al*. (2006), Bheemanna *et al.* (2008), Sivasupramaniam *et al.* (2008), Tindall *et al*. (2009), Greenberg *et al*. (2010), Govindan *et al.* (2010), Hallad *et al.* (2011), Soujanya *et al*.(2011), Naik *et al.* (2013), Peche *et al.* (2013), Saini *et al*. (2013) and Hallad *et al*. (2014) Who found higher mortality of *S. litura* on stacked *Bt* (BG II) cotton over non *Bt* hybrids. Henneberry *et al*. (2001) studied the effects of BG II transgenic cotton on cabbage looper, tobacco budworm, beet armyworm and the mortality was 95%, 100% and 57% respectively. Guifen *et al.* (2006) studied the development of *S. litura* on NuCOTN 33B cotton hybrid which contain fused Cry1Ac/Ac protein and found decreased survival rate.

The mortality of early larval instars was higher than later instars on leaves of stacked *Bt* (BG II) cotton hybrids. The mortality rates were in the order of 1st>2nd>3rd instar larvae which is in accordance with the findings of Soujanya (2009) who reported that the mortality of early larval instars was higher than later instars on leaves of stacked *Bt* (BG II) hybrids of TCH-4 and TCH-117.

The duration of mortality of *S. litura* on leaves of all the test hybrids was studied for all the larval instars during 70 to 80 days old crop. In BG II hybrids, the 1st instar larvae showed 100 per cent mortality at 7 days after release. In case of 2nd instar, there was decrease in mortality rate compared to 1st instar and took longer time for mortality and recorded more than 92.00 per cent mortality. Pertaining to 3rd instar larvae, there was 35.00 per cent mortality of larvae at 7 days after release. The mortality of 4th and 5thinstar larvae were zero. The findings clearly suggest that BG II cotton hybrids can effectively check the 1st& 2nd instar larvae only which was highly economical in cotton ecosystem as the pest can be controlled in initial stages itself (Naik, 2009).

The BG I and non *Bt* cotton hybrids did not effect any larval instar at 70-80 days crop. Hence, it was evident from the results that BG I cotton hybrids which were resistant and showed higher mortality to *H. armigera* (Vennila *et al*., 2006; Jeyakumar *et al.*, 2007; Basavaraja *et al.*, 2008) cannot offer any protection to *S. litura*.

It was clear from the results that larval or pupal weights of all the five larval instars were maximum when fed on leaves of non *Bt* and BG I cotton hybrids. The present findings derive support from Sparks and Norman (2002) and Basavaraja *et al.* (2008) who reported that larval weights were significantly higher on normal cotton leaves than transgenic *Bt* cotton leaves.

The results from the data also revealed that the larvae survived after exposure to stacked *Bt* (BG II) leaves weighed less and developed into smaller pupae than the larvae fed on BG I and non *Bt* hybrids (Plate No.7). The present findings are in conformity with Camberos *et al.* (2000), Donglin *et al.* (2006), Fitt *et al.* (2000) and Seshamahalakshmi (2007) who reported significant reduction in the larval and pupal weights when fed with stacked *Bt* (BG II) cotton leaves.

The results of the BG II hybrids on the age of larvae revealed that the larval weight of 1st instar larvae was lower than 2nd instar larvae on leaves of stacked *Bt* (BG II) cotton hybrids compared to BG I and non *Bt* cotton hybrids, which was in accordance with the findings of soujanya (2009) and Naik (2009) who reported that the weight of early larval instars was higher than later instars on leaves of stacked *Bt* (BG II) hybrids than on BG I and non *Bt* cotton hybrids.

4.2.6. Leaf area consumption by *S. litura* larvae on stacked *Bt* (BG II), BG I and non-*Bt* cotton

First instar

The larvae consumed very less leaf area on BG II hybrids compared to BG I and non *Bt* cotton hybrids at 70-80 days old crop (Table 4.14, fig 4.13). First instar leaf area consumption (Scrapping area) recorded based on grading score, on BG II hybrids score I (1-10 %), BG I hybrids score III (21-30%) and non *Bt* hybrids score IV (31-40%) (plate No. 6) was recorded after 24 h of release. After7 days after release the leaf area consumption on Jaadoo BG II cotton was 11.90 cm^2 and on Mallika BG II 14.50 cm^2. Due to Cry toxin (BG II) all larvae died before 11days after release. In contrary, the larvae that were fed on leaves of BG I were able to survive which resulted in high leaf area consumption of 43.60 cm^2 on Jaadoo BG I, 40.13 cm^2 on Mallika BG I, 58.33 cm^2 on Jaadoo non *Bt* and 61.03 cm^2 on Mallika non *Bt* at 7 days after release which were significantly differed with BG II, BG I and non *Bt* cotton hybrids. At 11 days after release the leaf area consumption of Jaadoo BG I is 77.98 cm^2 in Mallika BG I 77.38 and in Jaadoo non *Bt* 95.38 cm^2, Mallika non *Bt* 92.35 cm^2. The total leaf area consumption is 121.58 on Jaadoo BG I, 117.50 on Mallika BG I, and 153.70 cm^2 on Jaadoo non *Bt*, 153.38 cm^2 on Mallika non *Bt.* which differed significantly from each other.

Second instar

The leaf area consumed by 2nd instar larvae was minimum in both BG II cotton hybrids at 11 days after release. The leaf area consumed was only 36.83 and 38.84 cm^2 on Jaadoo BG II and Mallika BG II cotton hybrids respectively, whereas 212.03 cm^2 leaf area consumption was noticed in Jaadoo non *Bt* followed by Mallika non *Bt* (207.90 cm^2), Mallika BG I (191.20 cm^2), and Jaadoo BG I (184.85 cm^2) however BG II, BG I and non *Bt* hybrids differ significantly with each other. (Table 4.14, Fig 4.15).

Third instar

The amount of leaf area consumed by 3rd instar was higher than 1st and 2nd instar larvae on stacked *Bt* (BG II) hybrids which recorded 137.86 and 143.57 cm^2 on Jaadoo BG II and Mallika BG II hybrids, respectively while there was no significant difference between BG I and non *Bt* hybrids. The amount of leaf area consumed was 196.58 and 188.48 cm^2 on Mallika BG I and Jaadoo BG I, while it was 198.73 and 203.70 cm^2 on Jaadoo non *Bt* and Mallika non *Bt* at 11 days after release (Table 14).

Fourth instar

The leaf area consumption on BG II cotton hybrids was less (137.43 and 138.63 cm^2 on Jaadoo BG II and Mallika BG II) compared to BG I and non *Bt* cotton hybrids which ranged from 163.15 cm^2 to 178.03 cm^2 at 7 days after release and there was no significant difference between BG I and non *Bt* cotton hybrids with regard to leaf area consumption. (Table4.14 and Fig. 4.13).

From the results, it can be summarized that there was no significant difference in the leaf area consumed by *S. litura* on leaves of BG I and non *Bt* cotton hybrids at 2^{nd}, 3^{rd}, 4^{th} and 5th instars during the 70-80 days crop. These findings were comparable to the results of Adamczyk *et al.* (1998), Zeng *et al.* (1998), Janakiraman and Gupta (2002), Chitkowski *et al*. (2003), Radhika *et al*. (2004), Yueshu *et al.* (2004), Vennila *et al.* (2006), Jeyakumar *et al*. (2007) and Basavaraja *et al.* (2008) who reported that fall armyworms exposed to leaves of BG I and non *Bt* cotton hybrids did not show any difference in mortality.

The leaf area consumed by early instars of *S. litura* on non *Bt* cotton was higher compared to BG II cotton hybrids at 70-80 DAS crop and indicates that stacked *Bt* (BG II) cotton hybrids were highly effective in suppressing the larval feeding. The low leaf area consumption on BG II might have been due to expression of Cry2Ab toxin in leaves which was effective against *S. litura*, whereas the presence of Cry1Ac in BG I was not able to suppress feeding of *S. litura*. These findings are in concurrence with Greenplate (1999), Li *et al*. (2006), Bheemanna *et al.* (2008) and Tindall *et al*. (2009) who reported that the per centage of feeding damage on stacked *Bt* (BG II) was very low compared to non *Bt* cotton hybrids.

The first instar larvae of *S. litura* were heavily effected by BG II cotton hybrids which showed 100 per cent mortality within 7 days after release and consumed very small amount of leaf area. From the findings it can be inferred that the BG II cotton hybrids which express Cry 2Ab in addition to Cry1Ac very effectively controlled the 1^{st} instar larvae at 70- 80 DAS crop (Naik, 2009). From the investigation, it can be summarized that the BG II cotton hybrids were able to suppress the larvae effectively at first and second instars compared to 3^{rd}, 4^{th} and 5^{th} instars.

The biotechnological advancement in the form of BG II cotton hybrids containing Cry1Ac + Cry2Ab incorporated cry proteins had recorded higher mortality with very less feeding in first and second instars and offer advantage to cotton growing farmers for managing *S. litura* infestation apart from

H. armigera and *P. Gossypiella*, with limitations as there is chance of migration of third, fourth and fifth instars from neighbour host crops or weeds which may not able to manage through BG II cotton as evident from this study.

Chapter VI

SUMMARY AND CONCLUSIONS

The present investigation on "**DAMAGE POTENTIAL, GROWTH AND DEVELOPMENT OF *Spodoptera litura* (Fab.) ON DUAL TOXIN TRANGENIC *Bt* COTTON**" was conducted at Regional Agricultural Research Station, Lam, Guntur, during *kharif*, 2014-15. The results of the present investigations are summarized below.

There was a significant difference in leaf, square and boll damage between treatments, which received varied number of *S. litura* larvae. The area of leaf damage was less at 50 and 80 DAS crop compared to 110 and 140 DAS crop, this may due to high levels of expression of Cry 2Ab toxin in leaves at early stage which was effective against *S. litura* damage. At 110 and 140 DAS crop leaf damage was more which may due to decrease in protein expression of Cry 2Ab.

Leaf area consumption at 50 DAS larvae released crop was compared with 110 and 140 DAS larvae released crop in which the percentage leaf area consumption was gradually increased at 110 DAS larvae released crop and it was double the percentage of leaf area consumption at 140 DAS larvae released crop, which shows that *S. litura* larvae is having more damage potential at later stages of the crop due to decrease in Cry 2Ab toxin in the plant.

Though *S. litura* is leaf eating caterpillar, it damages squares and bolls but damage on bolls was less compared to squares, showing that larvae of *S. litura* prefer leaf more compared to squares and bolls.

Gradually yield decreased with increasing larval numbers/plant and yield was more when larvae were released at 50 and 80 DAS crop compared to 110 and 140 DAS larvae released crop. The per cent yield reduction over control was increasing where larvae were released at later stages of the crop. When compared the yield of 50 DAS released crop with 110 and 140 DAS released crop the percent yield reduction increased this shows that the damage potential was more at later stages of the BG II cotton crop and cause economic losses to farmers.

The bioassay of all the five instars of *S. litura* (first, second, third, fourth and fifth) larvae was conducted on tender leaves and squares of BG II, BG I and their corresponding non *Bt* version of Jaadoo and Mallika hybrids at 70 – 80 days old crop. The experiment was laid out in a Completely Randomized Design (CRD) with four replications. The data was recorded at 3, 7 and 11 days after release of respective larvae.

In the present investigation, growth and development of all the five instars of *S. litura* showed varied mortality rates on BG II hybrids, whereas the mortality was more or less similar in BG I and non *Bt* cotton hybrids. The BG II cotton hybrids exerted higher mortality. This might be due to expression of high levels of dual toxin at early stages of plant development.

The mortality of early larval instars of *S. litura* was higher than the later instars on the leaves of both the BG II cotton hybrids and the 1st instar larvae showed highest mortality followed by 2nd & 3rd with no mortality in 4th and 5th instar larvae. The mortality of *S. litura* was higher on leaves compared to squares for all the larval instars in Jaadoo and Mallika BG II hybrids whereas the mortality was nil in *Bt* and non *Bt* hybrids.

The mean leaf area consumed by all the five instars of *S. litura* larvae fed on BG II cotton leaves were found to be significantly lower than larvae fed on BG I and non *Bt* cotton leaves. The mean leaf area consumption of later instars of *S. litura* was higher than the early instars on the leaves of both the BG II cotton hybrids, whereas it was more or less similar in BG I and non *Bt* cotton hybrids.

The mean larval weight of larva fed on BG II cotton leaves was found to be significantly lower than that of the larva fed on BG I and non *Bt* leaves. The mean larval weight of later instars of *S. litura* was higher than the early instars on the leaves of both the BG II cotton hybrids, whereas it was more or less similar in BG I and non *Bt* versions.

The exposure of late instar larvae to plant parts of Jadoo and Mallika BG II hybrids exhibited adverse effect on the growth and development such as reduced larval weight, prolonged larval development period, reduced pupation, formation of small pupae with less weight, reduced adult emergence and low growth.

Conclusions

- Leaf, square and boll damage due to *S. litura* on BG II cotton was low in 50 and 80 days crop because of Cry 2Ab toxin expression was more at early stages of the crop.
- The yield was more when the larvae were released at early stages of the crop than at later stages.
- The per cent yield reduction was more when larvae were released at 110 and 140 DAS crop when compared to 50 and 80 DAS larvae released crop.
- In BG II cotton hybrids, there were higher larval mortality rates, lower larval/pupal weights and leaf area consumption for *S. litura*
- BG I cotton hybrids marginal influence on survival and feeding behavior of *S. litura* larvae.
- BG II cotton hybrids were more toxic to 1^{st} instar larvae followed by 2^{nd}, 3^{rd} and 4^{th} instars larvae.
- In BG II cotton hybrids, larval/pupal weight and leaf area consumption were lower in the order of $1^{st}<2^{nd}<3^{rd}<4^{th}<5^{th}$instar larvae.
- The efficacy of BG II cotton hybrids on *S. litura* was high at 70- 80 days old crop.

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