BIOCHEMICAL ANALYSIS OF BT COTTON HYBRIDS IN RELATION TO SUCKING PESTS TOLERANCE

by

Mr. NEHETE SACHIN VILAS
(Reg. No. 09/233)

A thesis
Submitted to the

**MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI –413 722DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA**

In Partial Fulfillment of the Requirements for the degree

of

MASTER OF SCIENCE

(AGRICULTURE)

in

BIOCHEMISTRY

**DEPARTMENT OF BIOCHEMISTRY
POST GRADUATE INSTITUTE,
MAHATMA PHULE KRISHI VIDYAPEETH,
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2012

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part

thereof has not been submitted

by me or any other person

to any other University

or Institute for

Degree or

Diploma

Place : MPKV, Rahuri.
Dated : / / 2012 *(Mr. S.V. Nehete.)*

Prof. P. K. LOKHANDE
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Department of Biochemistry,
Post Graduate Institute,
MPKV, Rahuri – 413 722,
Maharashtra (India)

CERTIFICATE

This is to certify that the thesis entitled **"Biochemical analysis of Bt cotton hybrids in relation to sucking pest tolerance.**" submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (M.S.) in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) in BIOCHEMISTRY** embodies the results of bonafide research work carried out by **Mr. NEHETE SACHIN VILAS** under my guidance and supervision. The results embedded in this thesis have not been submitted to any other university or institute for the award of Degree or Diploma. The assistance and help received during the course of this investigation have been duly acknowledged.

Place: MPKV, Rahuri. **(Prof. P.K. Lokhande)**

Date : / / 2012 Research Guide

Dr. R. S. Patil,
Associate Dean,
Post Graduate Institute,
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Maharashtra (India).

CERTIFICATE

This is to certify that the thesis entitled **"Biochemical analysis of Bt cotton hybrids in relation to sucking pests tolerence"**, submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (M.S.) in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **BIOCHEMISTRY** embodies the results of bonafide research work carried out by **Mr. NEHETE SACHIN VILAS** under the guidance and supervision of **Prof**. **P. K . Lokhande,** Associate Professor Department of Biochemistry, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar and that no part of the thesis has been submitted for any other Degree or Diploma.

Place : MPKV, Rahuri (**Dr. R. S. Patil)**

Date : / / 2012 **Associate Dean**

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Place : M.P.K.V., Rahuri

Date : / / 2012 **(Nehete S.V.)**

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LIST OF ABBREVIATIONS

%	:	Per cent
µg	:	Microgram
µmol	:	Micromole
β -ME	:	Beta- mercaptoethanol
^{O}C	:	Degree centigrade (Celsius)
et al	:	et alli (and other)
Fig	:	Figure

Fr. wt	:	Fresh Weight
G	:	gram
ha	:	Hectare
H	:	Hour
i.e	:	That is
kDa	:	Kilo Dalton
Kg	:	Kilogram
M	:	Molar
mg	:	Milligram
min.	:	Minute
ml	:	Millilitre
mM	:	Millimolar
$NADP^+$	:	Nicotinamide adenine dinucleotide phosphate oxidized
NADPH	:	Nicotinamide adenine dinucleotide phosphate reduced
nm	:	Nanometer
OD	:	Optical density
RPM	:	Revolution per minute
Sec	:	Second
BSA	:	Bovine serum albumin
U	:	Unit
V	:	Volt
v/v	:	Volume by volume
viz	:	Videlicet (namely)
w/v	:	Weight by volume
M. ha	:	Million hactare

ABSTRACT

BIOCHEMICAL ANALYSIS OF BT COTTON HYBRIDS IN RELATION TO SUCKING PESTS TOLERANCE

by

Mr. NEHETE SACHIN VILAS

A candidate for the degree of

MASTER OF SCIENCE (AGRICULTURE)

in

Biochemistry

MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413722.

Research Guide	:	**Prof P. K. Lokhande**
Department	:	**Biochemistry**

The Present investigation entitled “Biochemical analysis of Bt cotton hybrids for sucking pest tolerance” was mainly focused for analyzing the biochemical constituents and the level of sucking pest tolerance at different growth stages. The total polyphenol content in the leaves of Bt cotton hybrids ranged from 2.56 to 6.05 mg g^{-1} fr.wt. at 30 DAS. with maximum of 6.05 mg g^{-1} fr.wt. being present in Dyna and the minimum of 2.56 mg g^{-1} fr.wt. in Tulasi. At 60 DAS the content of polyphenol ranged from 4.14 mg g^{-1} fr.wt. to 7.91

mg g^{-1} fr.wt. with the maximum of 7.91 mg g^{-1} fr.wt. being present in NHH-44 and the minimum of 4.14 mg g^{-1} fr.wt. in Tulasi. with At 90 DAS the content of polyphenol ranged from 5.50mg g^{-1} fr.wt. to 8.41 mg g^{-1} fr.wt. with maximum of 8.41 mg g^{-1} fr.wt. in Tulasi and the minimum of 5.50 mg g^{-1} fr.wt. in Rasi-2.

The tannin content in leaves of eight Bt cotton hybrids ranged from 1.67 to 3.98 mg g^{-1} fr.wt. at 30 DAS . The maximum of 3.98 mg g^{-1} fr.wt. being present in Dhuv and the minimum of 1.67 mg g^{-1} fr.wt. in NHH-44, Bt cotton hybrid and at 60 DAS ranged from 1.77 to 8.74 mg g^{-1} fr.wt. at 60 DAS. The maximum of 8.74 mg g^{-1} fr.wt. being present in Monsoon and the minimum of 1.77 mg g^{-1} fr.wt. in NHH-44 and at 90 DAS ranged from 3.21 to 8.35 mg g^{-1} fr.wt.

with maximum of 8.35 mg g^{-1} fr.wt. being present in Monsoon and the minimum of 3.21 mg g^{-1} fr.wt. in NHH-44

The chitinase activity in leaves of Bt cotton hybrids ranged from 0.79 to 4.75 mg N-acetylglucosamine released g^{1} fr.wt.h^{-1} at 30 DAS. The maximum chitinase activity of 4.75 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1} being present in Dyna and the minimum of 0.79 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1} in Mallika. At 60 DAS the activity

ranged from 1.34 to 4.98 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1}. The maximum of 4.98 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1} being present in Rasi-2 and the minimum of 1.34 mg N-acetylglucosamine released g^{-1} fr.wt.h^{-1} in Tulasi and at 90 DAS it ranged from 2.93 to 6.65 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1} with maximum of 6.65mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1} being present in Dhuv and the minimum of 2.93 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1} in Bunny.

The peroxidase activity in leaves of eight Bt cotton hybrids ranged from 0.94 to 2.24 U g^{-1} fr. wt. at 30 DAS. The maximum of 2.24 U g^{-1} fr. wt. being present in Dhuv and the minimum of 0.94 U g^{-1} fr. wt. in Dyna. Bt cotton hybrid and at 60 DAS the activity ranged from 1.04 to 2.65 U g^{-1} fr. wt. The maximum of 2.65 U g^{-1} fr. wt. being

present in Dyna and the minimum of 1.04 U g^{-1}. fr.wt. in Dhuv. At 90 DAS peroxidase activity ranged from 2.13 to 5.00 U g^{-1} fr. wt.. The maximum of 5.00 U g^{-1} fr. wt. being present in Monsoon and the minimum of 2.04 U g^{-1} fr. wt. in Tulasi.

The polyphenol oxidase activity in leaves of eight Bt cotton hybrids ranged from 0.85 to 2.22 U g^{-1} fr. wt. at 30 DAS. The maximum of 2.22 U g^{-1} fr. wt. being present in

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Dyna and the minimum of 0.85 U g^{-1} fr. wt. in Monsoon and at 60 DAS the PPO activity ranged from 1.03 to 2.56 U g^{-1} fr. wt. The maximum of 2.56 U g^{-1} fr. wt. being present in Dyna and the minimum of 1.03 U g^{-1} fr. wt. in Monsoon. At 90 DAS ranged from 1.03 to 3.22 U g^{-1} fr. wt. The maximum of 3.22 U g^{-1} fr. wt. being present in Dyna and the minimum of 1.03 U g^{-1} fr. wt. Dhuv which is moderately resistant against the sucking pest infestation had higher biochemical constituents at different growth stage.

The sucking pest infestation is negatively correlated with total polyphenol, tannin, chitinase and peroxidase, polyphenoloxidase at different growth stages.

1. INTRODUCTION

Cotton in India is grown in varied soils, climates and agricultural practices under irrigated and rainfed situations. Approximately 65% of India's cotton is produced under rainfed conditions and 35% on irrigated lands. It is cultivated in three distinct agro-ecological zones (north, central and south) of the country. The northern zone is almost totally irrigated, while the %age of irrigated area is much lower in the central (23%) and southern zones (40%). Under the rainfed growing conditions rainfall ranges from 400 to 900 mm coupled with aberrant precipitation patterns over the years leading to large-scale fluctuations in production. Cotton in North India is grown in about 1.5 M ha in the thee states, Punjab, Haryana and Rajasthan. Cotton area in Gujrat increased from 1.54 M ha in 2000 to 2.6 M ha in 2010. About 36% of the area (3.9 M hectares) under

cotton is in Maharashtra, primarily under rainfed conditions with only 3-4% under irrigation. In 2010, Madhya Pradesh had 0.65 M ha under cotton. Karnataka grew cotton on 0.52 M ha, Tamilnadu on 0.13 M ha and Andhra Pradesh on 1.74 M ha (Anonymous, 2010).

Bt cotton area in India rose from 0.03 M ha in 2002-03 to l.62 M ha in 2005-06 accounting for 18 % of the total cotton area in the country (Anonymous, 2006). Cotton production has influenced the economic development of many nations around the world including India. Insect pests are the major constraint in profitable cotton production throughout the world. More than 166 insect pests have been reported to damage cotton from seedling stage to lint formation. The most harmful of these being the sucking pest, next only to bollworm complex which causes severe losses. (Dhawan *et al.*, 2004).

Bt cotton hybrids are substantially affected by sucking pests *viz.*, jassids, aphids, thrips and white flies. These sucking pests are causing economical damage and are responsible for low yield of cotton. Cotton jassids, whitefly and thrips are important sucking pests in cotton. There was no significant difference in population densities of these pests in Bt and non Bt cotton when nothing was sprayed. However, insecticide application effectively controlled these pests in both Bt and non Bt cotton. There is no difference in transgenic Bt and non Bt cotton for jassid, whitefly and thrips attack and

application of suitable insecticide is required to these pests on transgenic cotton (Arshad and Suhail, 2010).

Sucking pests have become quite serious from seedlings stage to harvesting and their heavy infestation at times reduces the crop yield to a great extent. The estimated loss due to sucking pests is up to 21.20% (Dhawan *et al.*, 1988). Sole reliance on insecticides is not only ecologically unsustainable but is also becoming economically unviable. Host plant resistance is one of the major components of IPM as it is ecofriendly, sustainable and easy to adopt. Looking to the severity of the problem and present need of Bt cotton hybrids with high degree of tolerance to sucking pests needs to be undertaken in field condition (Biradar and Vennila, 2008).

Bt cotton would still succumb to yield loss due to the blend of sub feeders such as jassids, aphids and thrips spread throughout the growing season, right from seedling emergence to harvest. As the biotic potential of sucking pests is high they are the potential threat to Bt cotton. The proposed work on biochemical analysis of Bt cotton hybrids developed by various private or public sector companies for sucking pest tolerance has therefore been undertaken with some specific following objectives.

1. To estimate the levels of total polyphenols and tannin from the leaves of Bt cotton plants at different growth stages, and

2. To evaluate the level of chitinase, peroxidase and polyphenoloxidase enzymes from the leaves of Bt cotton plants at different growth stages.

2. REVIEW OF LITERATURE

Bt cotton is one of most important commercial crops grown in India and Maharashtra. Bt cotton spuns the largest area in Maharashtra among all others crops but comparatively its yield is the lowest. The main cause of loss in production and productivity of this crop is the infestation of sucking pests. In this context, the relevant research work carried out earlier is briefly reviewed here.

2.1 Total Polyphenol :

Hosagoudar and Chattannavar (2009) carried out biochemical analysis of non Bt cotton genotype *viz.*, Laxmi, Abhadita, DCH-32 and Bt cotton genotypes *viz.*,RCH-2Bt. Non Bt cotton genotypes recorded high amount of total phenol (18.39 to 22.07 mg/g fr.wt) compared to Bt cotton genotypes. At 90 DAS among genotypes JKCH-1 Bt recorded highest phenol content in healthy (4.023 mg/g fr.wt) and in infected condition (2.740 mg/g fr.wt) and differed significantly over other genotypes at 120 DAS

among genotypes Laxmi recorded highest phenol content in healthy (5.452 mg/g fr.wt) and RCH-2 Bt, recorded highest phenol content infested condition (3.360 mg/g fr.wt).

Praveen *et al* (2001) considering the role of phenolics in plant defense some cotton varieties were investigated for their total phenol at various growth stages, change in phenolics as a result of insect exposure and their effect on growth and survival of cotton bollworm larva. The more resistant variety Ravi showed 1.00 to 1.20 mg/100 g D.W. phenolics than susceptible varieties at all growth stages.

Daniel *et al* (1990) reported the increased phenolics in infested cotton leaves than uninfested leaves against bollworm. The polyphenol content in healthy and spotted bollworm infected leaves of verities of cultivated sp. ranged from 12.44 to 14.49 mg 100 g^{-1} and 9.81 to 11.41 mg 100 g^{-1} respectively at 58 DAS. However, the total polyphenol content in the uninfested wild species for spotted bollworm ranged from 16.91 to 19.11 mg 100 g^{-1} (Patil and Kale, 2005). Raju and Reddy (1989) studied 25 cotton cultivars *viz.*, *Gossypium hirsutum* (16 cultivars*) G. herbacium* (3 cultivars) *G.arboreum* (3 cultivars) and *G. barbadnse* (3 cultivars) against cotton jassids. The highest amount of total phenol of leaf sample in resistant cultivar were recorded by MSER-14 (3.211 mg/g fr.wt) and least amount of total phenol of susceptible cultivar were recorded by Stonville – 603 (1.101 mg/g fr.wt.)

Balsubramanian and Gopalan (1978) reported that the variety HB 69 of cotton, resistant to *Amrasca biguttula* had a higher total phenol content than the susceptible variety PRS 72. However, when these varieties were infested with *A. biguttula*, the susceptible variety PRS-72 and resistant variety HB 69 showed a greater reduction in orthodihydroxyphenols than the tolerant one, GS 23. Butter *et al* (1992) tested sixteen cotton cultivars for incidence of whitefly and content of biochemical constituents of cotton during vegetative and reproductive stages. It was observed that the tannin, flavanoids, phenols and *O-dihydroxyphenols* were negatively correlated with whitefly population. Further, it was reported that cultivars having greater total phenolics and *O-dihydroxyphenolic* contents in leaves supported fewer whitefly eggs.

Parameswaran (1983) reported that total phenols in resistance cotton cultivar act as oviposition deterrent to stem weevil, *Pempherulus affinis*. The phenolic compounds have been reported to act as feeding deterrent for the green peach aphid *Myzus persicae* (Montgomery and Arn, 1974)

Rai *et al*. (2011) showed that Fusarium wilt tolerant cultivars of tomato *viz.*, FEB-2, FEB-4 and Floradade and NF-31 had significantly higher phenol content (1.4 to 1.6 mg/g tissue) than susceptible (1.1 to 1.2 mg/g tissues).

Crough and Ericisli (2010) showed that the seven genotypes without insect damage had significantly greater total phenol content in its leaves compare to insect

damaged, *Rosa canina* plants this clearly indicated that total phenolic content in *R.canina* that had no galling insect damage may have an important part of chemical defense of *R.canina* plants against *Diplolepis sp*. The total phenolic contents in the leaves of tolerant Rosa sp were found to range from 57-152 mg/g D.W. (Nowak and Gawlik, 2007).

Banerjee and Kallo (1989) and Singh *et al*. (2002) showed that a resistant variety had higher level of phenolics than susceptible variety of crop plant. The total phenol content in leaves of different tomato accession increased from 1.5 to 3 fold due to white fly damage the systemic induction was highly pronounced in accession *viz.*, LE-19 (18.9 mg/g fr.wt.) followed by LE-14 (13.57 mg/g fr.wt.) (Srinivasan and Uthamasamy, 2004).

The phenolic compounds, *viz.*, phenolic acids and flavonoids and enzymes related to their metabolism *viz.*, peroxidases, polyphenoloxidases and phenylalanine ammonia lyases are widely implicated in resistance to infestation by plant to different insects, pathogenic bacteria, fungi or viruses (Panda, 1979 ; Jambunathan *et al.*, 1986).

Nag and Nath (1993) reported that the total phenol were negatively correlated with plant infestation and cutworm population. Murkute *et al*. (1993) observed that the total polyphenols were higher in resistant pigeonpea varieties to pod borer.

2.2. Tannin :

Bhat *et al.* (1981) reported that positive and significant correlation between total nymphal population and tannin indicating high amount of tannin in tolerant cotton varieties in relation to jassids.

Chakravaraty and Sahni (1972) also observed positive relationship between tannin content and jassid resistance in cotton.

Chan *et al* (1978) reported the condensed tannins exhibited a very potency against all pest species of cotton.

Srinivasan and Uthamasamy (2004) showed that the tannin content in leaves of different tomato accession increased from 1.4-3 fold due to white fly damage the systemic induction was highly pronounced in accession *viz.*, LE-591 (8.73 mg/g fr.wt.) followed by LE-19 (8.47mg/g fr.wt.). Sharma *et al* (1983) observed free phenol negative correlation coefficients between free phenol and with spotted bollworm damage.

Raju and Reddy (1989) screened 25 hirsutum cotton cultivars of 4 genotype *viz.*, *Gossypium hirsutum* (16 cultivars) *G. herbacium* (3 cultivars) *G.arboreum* (3 cultivars) and *G.barbadense* (3 cultivars) against cotton jassids showed that the highest amount of leaf tannin content in resistant cultivar were recorded by *Stonville* 603 (3.461 mg/g fr.wt.) and least amount of tannin of susceptible cultivar were recorded by MSER-14 (0.636 mg/g fr.wt.).

Zummo *et al.* (1983) reported a negative relationship between tannin and bolloworm damage in cotton.

The tannin content in leaf of cotton were found negatively correlated with whitefly population densities (Butter *et al*. 1992).

Chan *et al*. (1978) reported that tannin increases the resistance of cotton against bolloworm *Heliothis* spp. Feeding growth rate and survival were decrease by high tannin content.

Sharma *et al.* (1982) observed that the tannin content in cotton ball was significantly and negatively correlated with adult emergence of spotted bolloworm. The pigmented genotypes of cotton were rich in tannins compared to the non pigmented ones.

Singh (1987) analyzed eighteen genotypes of cotton for phytochemicals in relation to incidence of spotted bollworm. Among the secondary plant metabolites *viz.*, tannin, silica and gossypol etc. only silica had significant negative correlation with the pest attack. However, tannin were significantly higher in squares and bolls of tolerant genotypes in comparison to susceptible ones.

Hanny (1980) analyzed gossypol flavonoid and condensed tannin content in five cotton cultivars in relation to incidence of *Heliothis virescens*. It was observed that higher gossypol present in yellow anthers than the cream anthers, might be involved in growth suppression of *Heliothis virescens* larvae. Further it was reported that condensed tannin and flavonoid content in yellow anthers

were probably not involved in growth suppression of *Heliothis virescens*.

Waiss *et al.* (1981) studied diverse classes of organic components exhibiting growth inhibitory activity against lepidopterous larvae that was isolated from maize, cotton tomato, sunflower and soyabean plants. They reported that tannin were inhibitory compounds and involved in plant defence against insects.

Guinn and Eidenbock (1982) examined that the condensed tannin have antibiotic activity and have been shown to decrease the feeding, growth rate and survival of helianthus sp.

Leafhopper resistant varieties of okra had higher amount of tannin in the leaves (Singh and Agarwal.,1988).

Reese *et al.* (1982) showed the tannin content reduces growth of *Helithis zea* by inhibiting ingestion. Nutritional and growth rate experiments on *H.armigera* on diet incorporated with tannic acid clearly indicated that tannic acid easily passes though the membrane in *H.arimigera* causing deleterious effect (Ananthakrishnan, 1994).

Mohite and Uthamasamy (1998) also indicated that tannin were negatively associated with weight and survival of larvae and population of *H.armigera*.

Lane and Schuster (1981) also reported that tannin is a factor in resistance to spider mites.

Condensed tannins exhibited a very high potency against all the species of pests (Chan *et al.* 1978).

2.3. Chitinase Activity :

Chaiyawat and Klemika (2008) showed that the resistance of pepper to *F. oxysporum* attack corresponded to the expression of 70 kDa chitinase band (chi-3) in the intracellular fluid. Such chitinase could possibly be used a protein marker to identify the tolerant line.

Tomato plants that have been fed upon by Bemisia showed increased level of chitinase activities over those observed in uninfested plants. The chitinase activity in healthy tomato accessions ranged from 0.75 to 1.80 mol $min^{-1}.g^{-1}$ fr.wt. and in infested plant ranged from 1.80 to 4.40 mol $min^{-1}.g^{-1}$ fr.wt. (Srinivasan and Uthamasamay, 2004).

Jimenez *et al.* (1995) reported first time,the PR proteins resulting from feeding by Bemisia. Mayer *et al.* (1996) reported that Bemisia feeding would induce several known PR protein and they found the induction of chitinase P2 & P4 resulting from Bemisia feeding which was very similar to that observed in tomato a poplastic fluids caused by infection of *C.fulum* (Joosten and De wit, 1989 and Joosten *et al.*, 1990). Inbar *et al.*(1999) observed the feeding by leaf miners and whiteflies induced local and systemic production of defensive protein i.e. chitinase, peroxidase. They also reported that the magnitude of the induction for each defensive protein varied between species. Mayer *et al.*

(1996) had also shown that plant varieties also influenced the type and degree of response to insect herbivory.

Saliva of some sucking insects had been shown to contain pectin estreases and polygalactoronases which was thought to play a part in generating oligo sacchide signals that activate plant defensive mechanism (Ryan, 1987).

Tomato plants that have been feed upon by Bemisia showed increased levels of chitiniase activities over those observed in uninfested plants. The specific activity enzymes increased in from 1.5 to 3.5 fold in infested plants compared to uninfested plants. However, bean class I chitinase protected tobacco plant against *Rhizoctonia solani*, the agent that causes damping off disease in tobacco (Broglie *et al*. 1991)

Deborah *et al*. (2001) inoculated rice leaf sheaths with *Rhizoctonia solani* (pathogen) and *Pestalatia palmarum* (non-pathogen) and found that there was an accumulation of pathogenesis related proteins and resulted in a marked increase in activities of chitinase.

Giri *et al*. (1998) reported that chitinase activity in the chickpea seeds of resistant cultivars varied from 2.11 to 2.63 U g^{-1} seed powder, whereas it was in the range of 1.5 to 1.6 U g^{-1} seed powder in the susceptible cultivar. During soaking of seeds, the chitinase activity decreased from 2.24 (0 h) to 1.50 U g^{-1} seed powder (48 h) in resistant cultivar, whereas in a susceptible cultivar the decrease was from 1.73 (0 h) to 1.31 U g^{-1} seed powder (48 h). The resistant

and susceptible cultivars of chickpea when grown in a soil inoculated with *F.oxysporum* f. sp. *ciceri* race 1, higher chitinase activity in root portions of challenged plants was observed. Further, it was observed that increase in activity of root portions was more in challenged plants (2.06 and 5.18 U g^{-1} powder at 10 and 18 DAG, respectively) than the control plants (1.16 and 3.90 U g-1 powder at 10 and 18 DAG, respectively) in resistant cultivar. Xue *et al*. (1998) inoculated bean hypocotyl with a nonpathogenic binucleate *Rhizoctonia* (BNR) species and studied various enzymes along with chitinase in relation to resistance and protection against *Rhizoctonia solani* and *Colletrotrichum lindemuthianum*. They reveled that endochitinase activity increased from 0.19 µmol mg^{-1} protein h^{-1} (control) to 0.47 µmol mg^{-1} protein h^{-1} after 2 h of BNR inoculation. However with the *Rhizoctonia solani* infestion, it increased upto 4.05 µmol mg^{-1} protein h^{-1} in cotyledons.

Tyagi *et al.*(2001) observed that the resistant wheat lines infected with *Alternaria tritici* resulted in rapid rise in chitinase activity than the susceptible line. The enzyme activity of susceptible and resistant wheat lines was 49 and 111 % higher, respectively than their respective controls on the 40^{th} day. Saikia *et al*. (2005) reported that chitinase activity significantly increased by two fold in chickpea seedlings inoculated with the pathogen, *Fusarium oxysporum* f. sp. *ciceri* over the control. Two isoforms of chitinases were detected in induced chickpea plant. The

molecular mass of the purified chitinases was 31 and 62 kDa.

2.4. Peroxidase (EC 1.11.1.7)

Peroxidase (EC 1.11.1.7, donor:hydrogen Peroxidase oxideo-reductase) plays an important role in the biosynthesis of plant cell wall components *viz.*, lignin suberin and cross linked extension (Lamport 1986). Peroxidase also play an important role in one of the earliest observable event of plants defense response i.e. oxidative burst (Walfszek, 1997).

Rai *et al.* (2011) showed the peroxidase (PO) activity in samples of all the seven cultivars of tomato was significantly higher in the resistant cultivars as compared to the susceptible ones. The maximum peroxidase activity was recorded in the resistant cultivar flora dade (0.141U g^{-1} fr.wt.). The result revealed that peroxidase activity was higher (0.229 U g^{-1} fr.wt.) in infected leaf tissues than uninfected leaf tissues (0.045 U.g^{-1}fr.wt.) and that increased considerably with the increase in progression of infection (Senthil *et al.*, 2010). Increased peroxidase activity in host tissues in response to infection by the pathogen (Dutt and Chatterjee, 2000). Patil and Kale (2005) showed that peroxidase activities in healthy leaves of cultivated species ranged from 10.33 to 24.44 U g^{-1} of fr.wt and spotted bolloworm the infested leaves of peroxidase ranged from 2.91 to 12.93 U· g^{-1} of fr.wt. at 58 DAS. However in wild species the infestation of spotted bolloworm was not

observed and peroxidase ranged from 20.13 to 31.30 U $min^{-1.}$ g^{-1} of fr.wt.

Sharma and Sharma (1997) reported that peroxidase activity of uninoculated seedlings of wheat sampled on different days showed a gradual increase from 1.4 to 5.4 O.D. min^{-1} mg^{-1} protein. In comparison to leaf rust, inoculated samples exhibited much higher activity ranging from 1.4 to 7.0 O.D. min^{-1} mg^{-1} protein. The activity of many phenoloxidizing enzymes such as peroxidase, polyphenol oxidase and others are known to be generally higher in the infected tissue of resistant varieties than in the infected susceptible ones which indicates it's utility as screening parameters in breeding for disease resistance programme (Chandniwala, 1996).

Bhite *et al.* (1997) observed that decrease in peroxidase activity in healthy leaves of sterility mosaic susceptible, moderately resistant and resistant cultivars of pigeonpea at 60 DAS. Further, they found that the healthy leaves of moderately resistant varieties had higher peroxidase activity (10.2 U g^{-1} fr.wt.) than the susceptible (9.9 U g^{-1} fr.wt.) and resistant (9.38 U g^{-1} fr.wt.) cultivars. In sterility mosaic inoculated plants, higher peroxidase activity was observed in susceptible cultivars (20.75 U g^{-1} fr.wt.) followed by resistant (11.58 U g^{-1} fr.wt.) and moderately resistant (9.3 U g^{-1} fr.wt.) of pigeonpea cultivars.

Chakraborty *et al.* (2002) reported that the peroxidase activity in healthy tea leaves was in the range of 5.6 to 11.2

Δ O.D. g-1 tissue min-1 whereas in blister blight infected leaves, it was 12.0 to 17.6 Δ O.D. g-1 tissue min-1. Peroxidase enzyme showed a significant increase after infection. The peroxidase isozyme pattern showed four isozymes in healthy tea leaves and five in blister blight infected leaves.

Lodha *et al.* (1993) reported that the healthy leaves of susceptible cultivar of clusterbean showed the high peroxidase activity in the range of 17.4 to 23.4 ΔA $min^{-1}mg^{-1}$ protein than the resistant cultivar which was in the range of 10.9 to 14.2 ΔA min^{-1} mg^{-1} protein. After infection with the bacterial light, the peroxidase activity in resistant cultivar increased upto 22.9 and 153.2 % in 40 and 80 days after planting, respectively,whereas in susceptible cultivar, it was increased upto 12.6 and 180.3 % in 40 and 80 days after planting respectively.

Gowda *et al.* (1989) reported that the peroxidase activity in healthy leaves of all sorghum lines tested was very low, ranging from 15 to 26 U mg^{-1} protein and did not alter significantly up to 60 h. After infection with *Peronosclerospora sorghi*, the resistant cultivars showed high activity from 60 to 250 U mg^{-1} protein in 15-60 h interval whereas in susceptible cultivars, the activity ranged from 65 to 75 U mg^{-1} protein in 15-60 h interval.

Jagdale (1999) reported that the peroxidase activity in healthy leaves of powdery mildew resistant cultivar of mung bean had the higher peroxidase activity (1.480 and 1.516 ΔA

min^{-1} mg^{-1} soluble protein) than the susceptible cultivar (1.001 and 1.14 ΔA min^{-1} mg^{-1} soluble protein). After infection, the higher activity was observed in a susceptible cultivar (2.690 to 6.280 ΔA min^{-1} mg^{-1} soluble protein) than the resistant cultivar (1.710 and 1.820 ΔA min^{-1} mg^{-1} soluble protein).

Xue *et al*. (1998) inoculated bean hypocotyls with a nonpathogenic binucleate *Rhizoctonia* (BNR) *species* and peroxidase activity was observed 1.6 to 2.0 fold and 2.0 to 2.4 fold higher when bean seedlings were protected with BNR prior to their challenge with *Rhizoctonia solani* and *Colletotrichum lindemuthianum*, respectively. Nagy *et al*. (2004) found that peroxidase activity increased from 1 to 2.8 μkatal mg^{-1} protein in root of Norway spruce when infected it Rhizoctonia compared with control (1 to 0.9 μkatal mg^{-1} protein). The enzymes related to phenolic metabolism widely implicated for resistance in plant against incidence to different insects (Panda, 1979).

The resistance of cotton variety to *Alternaria macrospore* was reported to be due to higher levels of peroxidase activities in it (Bhaskaran *et al*. 1975). Aluko and Oghadu (1986) analyzed five varieties of brinjal and found that round green fruits were best for storage and resistant to injury and diseases having high peroxidase activity.

2.5. Polyphenol oxidase (EC 1.10.3.1)

Polyphenol oxidase(PPO) (EC 1.10.3.2) or (EC 1.14.18.1) catalyzing the oxygen dependent oxidation of phenols to quinines are ubiquitous among angiosperms and assumed to be involved in plant defense against pest and pathogen (Yedidia *et al.*, 2003). A number of studies have indicated that phenoloxidizing enzymes such as polyphenol oxidase or peroxidase (POD) may participate in defense reactions and hypersensitivity in resistant plant to viruses, bacteria and fungi. These enzymes are also involved in reactions culminating in wound induced tissue browning and erecting physical barriers against parasites.

Increased polyphenoloxidase and peroxidase activities and accumulation of phenols have been correlated with disease resistance in plants. These include potato/*Phytophthora*, sweet potato/*Ceratostomella fimbriuta*, cotton/*Verticillium*, feba bean (*Vicia faba*)/ *Uromyces faba* and bean (*Vigna sinensis*)/*Psedocercospora* (Leina.*et al.* 1996).

Rai *et al.* (2011) showed that polyphenol oxidase activity higher in the resistance cultivars of tomato to *Fusarium oxysporium Lycopersicon viz.*, FEB-2, FEB-4, Flora Dade and NF-31 as compared to susceptible cultivar *viz.,* Sel-7, Sel-18 and Punjab Chhuhara.The polyphenol oxidase activity was higher (0.459 $U.g^{-1}$ fr.wt.) in infected leaf tissues than uninfected tissues (0.054 $U.g^{-1}$fr.wt.) and that increased considerably with the increase in progression of infection (Senthil *et al.*,2010). Increase the activity of polyphenoloxidase in host tissues in response to infection by the pathogen (Dutt and Chatterjee, 2000).

Patil and Kale (2005) reported the polyphenol oxidase activities in healthy leaves of cultivated species ranged from 5.26 to 7.88 U g^{-1} fr.wt. and in spotted bollworms infested leaves ranged from 5.04to 7.26 U g^{-1} fr.wt. at 58 DAS. However in the wild species the infestation of spotted bollworms was not observed and polyphenol oxidase ranged from 6.10 to 8.38 U g^{-1} fr. wt.

Studies on peroxidase and polyphenol oxidase activities in resistant and susceptible cultivars to *Fusarium graminerarum* of the wheat heads at flowering, milky, dough and ripening stages following inoculation at anthesis, showed that peroxidase specific activity increased in resistant and susceptible wheat cultivars during milky stage as compared with non-inoculated control plants. The activity of PPO in wheat heads reached maximum during the milky stage that was thee times higher in the resistant cultivars than the non-inoculated control (Mojtaba and Kazemi, 2002).

Sharma and Sharma (1997) evaluated the role of peroxidase and polyphenol oxidase interrelation to leaf rust of wheat. It was observed that the resistant lines showed higher POD and PPO activity as compared to susceptible cultivar and revealed the involvement of POD and PPO in conferring varying levels of resistance in terms of infection type by different leaf rust (Lr) genes at different development stages. The activity of PPO in uninoculated samples varied from 0.2 to 0.6 O.D. min^{-1} mg^{-1} protein, whereas in inoculated samples activity was increased up to 10 to 45 %.

Polyphenoloxidase activity in healthy tea leaves ranged from 3.8 to 5.8 Δ O.D. g^{-1} tissue min^{-1} whereas in blister blight infected leaves, it was in the range of 4.6 to 6.8 Δ O.D. g^{-1} tissue min^{-1}. Polyphenol oxidase activity significantly increased after infection. However, an increase of PPO activity was not very significant as compared with peroxidase enzyme activity (Chakraborty *et al*. 2002).

Gowda *et al*. (1989) reported similar fluctuation in PPO activity in healthy leaves of both resistant and susceptible cultivars of sorghum. However, after inoculation with *Peronosclerospora sorghi*, PPO activity gradually increased over a period of time without fluctuation. The greater increase was observed in resistant cultivars (2.2 to 2.9 U mg^{-1} protein) than the susceptible ones (1.9 U mg^{-1} protein).

Jagdale (1999) reported that powdery mildew resistant cultivars of mung bean had comparatively higher PPO activity (0.911 to 0.944 ΔA min^{-1} mg^{-1} soluble proteins) than the susceptible ones (0.747 to 0.688 ΔA min^{-1} mg^{-1} soluble proteins). After the invasion of the pathogen, in resistant cultivars PPO activity decreased and it was in the range of 0.765 to 0.881 ΔA min^{-1} mg^{-1}soluble proteins. However in susceptible cultivars, it was increased and was in the range of 0.959 to 0.923 ΔA min^{-1} mg^{-1} proteins. The enzymes related to phenolic metabolism are widely implicated for resistance in plant against incidence to different insects (Panda, 1979).

The resistance of cotton variety to *Alternaria macrospore* was reported to higher levels of polyphenoloxidase activities in it (Bhaskaran *et al.*, 1975). Murkute *et al.* (1993) reported higher polyphenoloxidase activity in resistant pigeonpea varieties resistant to pod borer attack.

3. MATERIAL AND METHODS

3.1 Material:

3.1.1 Seed material and field trial :

Table 1. The Bt cotton hybrids collected from the All India Co-ordinated Cotton Improvement Project, MPKV, Rahuri and were used for the present investigation.

Sr. No.	Name	Bt-cotton Hybrids	Name of Company or Organization
1	Bunny	Cry1AC MON531 event	M/S Nuziveedu Seeds Ltd, Secunderabad.
2	Dhruv	Cry1Ab and Cry1AC (CGFMCry1A)	M/S Zuari Seeds Ltd, Bangalore.
3	Monsoon	Cry1AC	M/S Yashoda Hybrids Seeds Ltd, Wardha
4	NHH-44	Cry1AC	Central Institute of Cotton Research (CICR), Nagpur.
5	Rasi-2	Cry1AC MON531 event	M/S Rasi Seeds Ltd, Attar
6	Dyna	Cry1AC MON531 event	M/S Vibha Seeds Ltd, Hyderabad
7	Mallika	Cry1AC MON531 event	M/S Nuziveedu Seeds Ltd, Secunderabad.
8	Tulasi	Cry1AC MON531 event	M/S Tulasi Seeds Ltd, Guntur.

The seeds of above Bt cotton hybrids were sown on 31st may, 2010 in the field of at AICRP on cotton, M.P.K.V., Rahuri. The leaves of these species were collected at 30th day, 60 day and 90th day after sowing (DAS) of the Bt cotton hybrids from replicated experimental fields. Sample were analyzed for polyphenol, tannin and chitinase activity, peroxidase and polyphenol oxidase activity. Cotton jassid incidence was recorded at 30 DAS intervals from thee leaves one each from top, middle and bottom region of randomly selected plants per plot. (Table 2) The grouping of cultivars

was made into four classes viz., Susceptible, moderately susceptible, moderately resistant and resistant on the basis of critical differences value obtained after statistical analysis as shown below

Table 2. Jassids population on leaves of Bt cotton cultivars.

		Jassids Population/3 leaves			
Sr. No.	**Name**	**30**	**60**	**90**	**Mean**
1	Bunny	7.1	9.16	6.86	7.70
2	Dhuv	4.9	9.3	5.3	6.50
3	Mansoon	**11.0**	10.1	5.1	**8.73**
4	NH-44	8.04	**6.00**	7.1	7.04
5	Rasi-2	7.1	7.13	6.63	6.95

6	Dyna	**4.02**	8.2	5.8	**6.00**
7	Mallika	8.09	**10.3**	**7.6**	8.66
8	Tulasi	9.3	9.00	**1.03**	6.44
	Range	4.02-11.0	6.0-10.30	1.03-7.6	6.00-8.70
	Mean	7.44	8.63	6.30	6.92
	SE ±	1.65	2.95	1.48	
	CD @ 5%	3.84	7.41	4.29	

Categories	**Pest population (Jassid/3 leaves)**
Resistant	0 – 4.5
Moderately resistant	4.8 – 7.5
Moderately susceptible	7.8 – 10.5
Susceptible	>10.5

3.1.2 Weather data recorded during experimental period

The daily weather data of various of metrological parameters observed during the experimental period i.e. from the month of May to September 2010 are given in Table 3.

Table 3. Weather data recorded during experimental period.

Month	**Met week**	**Temp.**		**Humidity**		**Rainfall (mm)**
		Max	**Min .**	**Morn.**	**Even.**	

May 10	18	37.74	21.03	50.29	26.57	8.20
	19	41.26	23.13	45.00	20.86	-
	20	41.74	24.59	50.71	23.14	-
	21	39.96	21.61	48.96	21.53	-
	22	39.17	24.19	55.00	31.00	-
June 10	23	36.17	23.49	68.00	37.86	-
	24	33.14	23.40	79.14	60.71	67.70
	25	32.94	22.90	76.74	53.57	37.80
	26	34.03	23.67	79.71	53.57	16.40
July 10	27	30.60	22.74	83.29	63.14	47.80
	28	32.10	23.45	78.33	51.33	0.00
	29	31.23	23.12	82.50	64.67	61.00
	30	29.67	22.42	84.33	69.17	9.00
August 10	31	29.97	22.12	76.85	63.85	3.40
	32	27.62	21.52	76.85	53.71	29.80
	33	26.62	20.45	86.14	77.14	5.60
	34	27.51	18.62	83.71	60.00	4.00
	35	29.54	22.21	85.85	89.42	221.84
Sept 10	36	28.82	22.25	81.71	83.00	151.00
	37	27.31	17.54	79.42	55.57	0.00
	38	31.48	22.74	78.71	57.57	106.60
	39	31.50	21.23	85.23	62.43	85.60

3.1.3 Chemicals

All the chemicals used in the present investigation were of analytical grade and procured from Glaxo Laboratories, Mumbai. E. Merck (India) Ltd, Mumbai, S.D.

Fine Chemicals Ltd., Mumbai, Sisco Research Laboratories, Mumbai and Sigma Aldrich Corporation, Missouri-USA.

3.2 Methods:

3.2.1 Total Polyphenols

Total polyphenol content was determined by using Folin Denis reagent as described by Swain and Hills (1959).

Regents

1. Folin-Denis reagent : Sodium tungstate (100 g) and phosphomolybdic acid (20 g) were dissolved in 750 ml water, to which 50 ml of conc. phosphoric acid was added. The contents were refluxed for 2 h and the volume made to 1000 ml.
2. Alkaline reagent : This was prepared by dissolving 350 g of sodium carbonate in 1000 ml water at 80^0C. The solution was allowed to stand overnight and filtered though glass wool.
3. Standard tannic acid solution (0.1%, w/v) : One hundred mg of tannic acid was dissolved in 100 ml of distilled water. Then 10 ml of this solution was diluted to 100 ml with distilled water. One ml of this solution contained 100 µg of tannic acid.

Extraction

One g of plant sample of each species was separately macerated in a mortar and pestle in 10 ml of methanol and phenols were extracted by boiling in hot water bath. The contents were centrifuged at 10,000 rpm. The extraction

was repeated two more times and the supernatant was diluted to 10 ml.

Colour development

One ml of extract was mixed with 7 ml distilled water and 0.25 ml Folin-Denis reagent. After 10 min, 1 ml of the alkaline reagent was added in each tube and the contents were mixed thoroughly. After 20 min, the contents were made to 10 ml and the extinction measured at 650 nm on a Spectronic- 20.

Calibration of a standard curve

It was prepared by using 0,10,20,40,80, and 100 µg of tannic acid concentrations corresponding to 0,0.1,0.21, ... 1 ml of standard solution in separate test tubes in duplicate. The colour was developed in a total volume of 10 ml by the same procedure as described above for colour development. The concentration of total phenolics was calculated from a standard curve (Fig.1.) and expressed as mg g^{-1} fr. wt. basis.

3.2.2 Tannin

The tannins in the above methanolic extract were determined by Vanillin-HCl method of Burns (1971).

Reagents :

1. Vanillin, 1 % in water.
2. Hydrochloric acid, 8 %.

The vanillin- HC1 reagent was prepared fresh by mixing two reagent 1:1, v/v.

3. Catechin standard solution : Catechin, 100 mg was dissolved in 100 ml ethanol.

Colour development

Appropriately diluted supernatant (1 ml) was mixed with 5 ml of vanillin reagent (8 % HC1+ 1% vanillin, 1:1) and absorbance was recorded at 740 nm after 30 min.

Standard curve

A standard solution containing 0 to 1 mg catechin were taken in test tubes and final volume made to 1 ml with water. The colour was developed as described above and standard curve was prepared (Fig.2). Tannins were calculated from this curve and expressed as mg^{-1} g fr. wt. of leaf sample.

3.2.3 Chitinase activity :

The chitinase actvity was estimated according to the methods described by Giri *et al.* (1998).

Reagents

1. 0.1 M Sodium citrate buffer (pH 5.0) : Seventy ml of 0.1 M citric acid and 130 ml of 0.1 M sodium citrate were mixed together.
2. Colloidal chitin : Chitin (1.83 mg) was dissolved in 1 ml of sodium acetate buffer (pH 5.2)

3. 0.1 M sodium acetate buffer (pH 5.2) : 2.1 ml of 0.1 M acetic acid and 0.1 M sodium acetate 7.9 ml were mixed together.

Procedure

Fresh leaf samples of 0.5 g was macerated with 6 ml of 0.1 M sodium citrate buffer in pre-cooled mortar and pestle. The homogenate was centrifuged at 10,000 x g for 10 min at 10^0C and the supernatant was used as crude source of chitinase. For the assay, 1 ml of crude enzyme extract, 4 ml of chitin suspension containing 15 mg of BSA were incubated in water bath at 37^0C for 3 h. One ml water and 1 ml of reaction mixture was boiled in glass marble-covered centrifuge tube for 10 min and subsequently was centrifuged. An aliquot of 0.5 ml was taken for the estimation of N-acetyl glucosamine as per the method of Nelson-Somogyi (Somogyi, 1952).

The chitinase activity was expressed in terms of mg of N- acetyl glucosamine released g^{-1} fr.wt.h^{-1}.

3.2.4 Peroxidase assay

The peroxidase activity was assayed by the method of Kumar and Khan (1982).

Reagents

1. 0.1 M phosphate buffer (pH 7.0) : 47.8 ml of 0.2 M NaH_2PO_4, 76.3 ml of 0.2 M Na_2HPO_4 solution were

mixed, the pH was adjusted to 7.0 and the final volume made to 250 ml.

2. Pyrogallol reagent (0.01 M) : This was prepared by dissolving 0.126 g of pyrogallol in 100 ml of distilled water.
3. Hydrogen peroxidase solution (0.005 M). This was prepared by mixing 0.15 ml of 30 % of hydrogen peroxidase in 100 ml of distilled water.

Procedure

A known quantity (0.5 g) each of green leaves, squares and bolls was macerated separately with 10 ml of 0.1 M phosphate buffer in prechilled mortar and pestle. The homogenate was filtered through four layers of muslin cloth and centrifuged at 10,000 rpm at 4^0C for 30 min. The supernatant was diluted to 10 ml and was used as the enzyme source. To 3.6 ml of 0.1 M phosphate buffer (pH 7.0), 0.1 ml of 0.005 M hydrogen peroxide, 0.1 ml of 0.01 M pyrogallol and 0.2 ml of diluted enzyme extract were added. The absorbance was read at 420 nm on Spectronic-20 for every 30 sec, up to 3 min.

Calculation

One unit of enzyme activity was determined as an increase in OD by 1.0. The enzyme activity was calculated for one g of sample and was expressed as U g^{-1} fr. wt. sample.

3.2.5 Polyphenoloxidase

The polyphenoloxidase activity was assayed by the method of Kumar and Khan (1982).

Reagents

1. 0.1 M phosphate buffer (pH 7.0): 47.8 ml of 0.2 M NaH_2PO_4, 76.3 ml of 0.2 M Na_2HPO_4 solution was added, the pH was adjusted to 7.0 and the final volume made to 250 ml.
2. Pyrogallol reagent (0.01 M) : This was prepared fresh by dissolving 0.126 g of pyrogallol in 100 ml of distilled water.

Procedure

The enzyme extract was prepared as descried under the assay of peroxidase and was used as the enzyme source. To 3.7 ml of 0.1 M phosphate buffer (pH 7.0), 0.1 ml of 0.01 M pyrogallol reagent and 0.2 ml of enzyme extract were added. The absorbance was read at 420 nm on Spectoronic-20 after every 30 sec.

Calculation

One unit of enzyme activity was determined as the change in absorbance by 0.01. The enzyme activity was calculated for one g of sample and was expressed as U g^{-1} fr.wt.

4. RESULTS AND DISCUSSION

Bt cotton is one of the important commercial crops in India, which is infested by a maximum number of pests. Crop protection plays a vital role in agricultural production. In cotton sucking pest is affecting the cotton productivity. Development of resistant verities of cotton is one of the strategies to overcome this problem. Therefore it is necessary to have information on the biochemical composition of the plant, their interactions and effects on insect pests leading to resistance.

The present study was undertaken in order to understand the defense mechanism against the pest. The leaves of cotton were collected at 30, 60 and 90 DAS and were analyzed for various biochemical parameters. The results obtained in this investigation are presented and discussed below.

4.1 Total Polyphenol

The data on total polyphenol content in leaves of Bt cotton hybrid is shown in Table 4. The total polyphenol content in leaves of eight Bt cotton hybrids ranged from 2.56 to 6.05 mg g^{-1} fr.wt. at 30 DAS. The maximum of 6.05 mg g^{-1} fr.wt. being present in Dyna and the minimum of 2.56 mg g^{-1} fr.wt. in Tulasi Bt cotton hybrid. The mean of polyphenol content in leaves of different Bt cotton hybrids was found to be 4.05 mg g^{-1} fr.wt. at 30 DAS. The total polyphenol content in leaves of eight Bt cotton hybrids ranged from 4.14 mg g^{-1} fr.wt. to 7.91 mg g^{-1} fr.wt. at 60 DAS. The maximum of 7.91 mg g^{-1} fr.wt. being present in NHH-44 and the minimum of 4.14 mg g^{-1} fr.wt. in Tulasi. The mean of polyphenol content in leaves of different Bt cotton cultivars was found to be 5.74 mg g^{-1} fr.wt. at 60 DAS. The total polyphenol content in eight Bt cotton hybrids ranged from 5.50 mg g^{-1} fr.wt. to 8.41 mg g^{-1} fr.wt. at 90 DAS. The maximum, of 8.41 mg g^{-1} fr.wt. in Tulasi and the minimum of 5.50 mg g^{-1} fr.wt. in Rasi-2

The mean of total polyphenol content at 90 DAS in different eight Bt cotton hybrids content was found to be 6.67 mg g^{-1} fr.wt.

The value of correlation coefficient between sucking pest population and total polyphenol was negatively correlated at 30 and 90 DAS there by indicating that the population of jassids decrease with the increase in the total polyphenols contents in the leaves of Bt cotton hybrids (Fig 4).

The reported values of total polyphenol content in Bt cotton were in the range of 18.39 to 22.07 mg g^{-1} fr.wt. (Hasahoudar and Chattannavar, 2009),12.44 to 14.49 mg g^{-1} fr.wt. (Patil and Kale, 2009) and 1.10 to 3.21 mg g^{-1} fr.wt. (Raju and Reddy, 1989) and in tomato 1.4 to 1.6 mg g^{-1} fr.wt. (Rai *et al.*, 2011) and 13.57 to 18.90 mg g^{-1} fr.wt. (Srinivasan and Uthamasamy, 2004).

The results on total polyphenol content are in close conformity with the results of earlier investigation.

Table 4. Total polyphenol content in leaves of Bt cotton

Sr. No.	**Name/DAS**	**Total polyohenol (mg g^{-1} fr. Wt.)**			
		30	**60**	**90**	**Mean**
1	Bunny	4.63	6.32	6.81	5.92
2	Dhuv	3.16	6.43	6.70	5.43
3	Monsoon	3.92	5.18	6.32	5.14
4	NHH-44	5.18	**7.91**	7.96	**7.02**
5	Rasi-2	4.03	4.80	5.50	**4.78**
6	Dyna	**6.05**	6.16	6.47	6.22
7	Mallika	2.89	7.52	7.46	5.95
8	Tulasi	2.56	4.14	**8.41**	5.04
	Range	2.56-6.05	4.14-7.91	5.50-8.41	4.78-7.02
	Mean	4.05	5.74	6.67	5.69
	SE $\pm$	0.69	0.91	0.84	

	CD @ 5%	2.11	2.76	2.24	
	Correlation	-0.4296	0.1297	-0.3021	

4.2 Tannin

The data on tannin content in the leaves of Bt cotton hybrid is shown in Table 5. The tannin content in leaves of eight Bt cotton hybrids ranged from 1.67 to 3.98 mg g^{-1} fr.wt. at 30 DAS . The maximum of 3.98 mg g^{-1} fr.wt. being present in Dhuv and the minimum of 1.67 mg g^{-1} fr.wt. in NHH-44 Bt cotton hybrid. The mean tannin content in leaves of different Bt cotton hybrids was found to be 2.85 mg g^{-1} fr.wt. The tannin content in leaves of eight Bt cotton hybrids ranged from 1.77 to 8.74 mg g^{-1} fr.wt. at 60 DAS. The maximum of 8.74 mg g^{-1} fr.wt. was present in Monsoon and the minimum of 1.77 mg g^{-1} fr.wt. in NHH-44. The mean tannin content in leaves of Bt cotton hybrids was found to be 4.75 mg g^{-1} fr.wt.at 60 DAS. The 90 DAS tannin content in eight Bt cotton hybrids ranged from 3.21 to 8.35 mg g^{-1} fr.wt. the maximum of 8.35 mg g^{-1} fr.wt. being present in Monsoon and the minimum of 3.21 mg g^{-1} fr.wt. in NHH-44 the mean tannin content in leaves of Bt cotton hybrids was 5.63 mg g^{-1} fr.wt. at 90 DAS.

The value of correlation coefficient between sucking pest population and tannin was negatively correlated at 60, 90 DAS. This showed that the tannin contents in Bt

cotton leaves against the sucking pest population increased therefore the presence of tannin in Bt cotton hybrids leaves make them resistance to sucking pest (Fig. 5).

The result of present investigation with respect to tannin are in agreement with the results reported by Srinivasan and Uthamasamy (2004), Mohite and Uthamasamy(1998), Raju and Reddy (1989).

Table 5. Tannin content in leaves of Bt cotton

Sr. No.	**Name/DAS**	**Tannin (mg g^{-1} fr. Wt.)**			
		30	**60**	**90**	**Mean**
1	Bunny	1.92	4.24	6.10	4.08
2	Dhuv	**3.98**	6.04	7.74	5.92
3	Mansoon	3.47	**8.74**	**8.35**	6.85
4	NHH-44	1.67	1.77	3.21	2.21
5	Rasi-2	3.21	2.82	6.00	4.01
6	Dyna	2.51	3.60	4.62	3.57
7	Mallika	2.05	4.75	4.88	3.89
8	Tulasi	3.93	4.60	6.30	4.94
	Range	1.67-3.98	1.77-8.74	3.21-8.35	2.21-6.85
	Mean	2.85	4.75	5.63	4.43
	SE $\pm$	0.27	1.05	0.81	
	CD @ 5%	0.84	3.20	2.47	
	Correlation	0.1019	-0.5706	-0.3887	

4.3 Chitinase activity

The data on chitinase activity in leaves of Bt cotton hybrid is shown in Table 6.

The chitinase activity in leaves of Bt cotton hybrids ranged from 0.79 to 4.75 mg N-acetylglucosamine released g^{1} fr.wt.h^{-1} at 30 DAS. The maximum of 4.75 mg N-acetylglucosamine released g^{-1} fr.wt.h^{-1} being present in Dyna and the minimum of 0.79 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1} in Mallika. The mean of chitinase activity in leaves of different Bt cotton hybrids was found to be 1.94 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1}. The chitinase activity ranged from 1.34 to 4.98 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1} at 60 DAS. The maximum of 4.98 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1} being present in Rasi-2 and the minimum of 1.34 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1} in Tulasi. the mean of chitinase activity leaves of Bt cotton hybrids was found to be 3.13 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1} at 60 DAS. The chitinase activity 90 DAS ranged from 2.93 to 6.65 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1}.the maximum of 6.65 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1}. being present in Dhuv and the minimum of 2.93 mg N- acetylglucosamine released g^{-1} fr.wt. h^{-1} in Bunny. the mean of chitinase activity content in leaves in Bt cotton

hybrids was found to be 4.20 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1} at 90 DAS.

The value of correlation coefficient between sucking pest population and chitinase activity was negatively correlated at 30, 60 DAS (Fig. 6).

The result obtained in the present investigation are similar with the results reported by Chaiyawat *et al.*(2008), Giri *et al.,* (1998), Deborah *et al.* (2001) and Srinivasan and Uthamasamy (2004).

Table 6. Chitinase activity in leaves of Bt cotton

Chitinase activity (mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1})					
Sr. No.	**Name/DAS**	**30**	**60**	**90**	**Mean**
1	Bunny	2.21	2.21	2.93	2.45
2	Dhuv	1.99	4.03	**6.65**	4.22
3	Monsoon	0.87	3.33	3.33	2.51
4	NHH-44	1.98	2.48	3.08	2.51
5	Rasi-2	1.34	**4.98**	4.51	3.61
6	Dyna	**4.75**	3.88	3.80	4.14
7	Mallika	0.79	2.77	6.25	3.27
8	Tulasi	1.58	1.34	3.08	2.00
	Range	0.79-4.75	1.34-4.98	2.93-6.65	2.00-4.22
	Mean	1.94	3.13	4.20	3.09
	SE $\pm$	0.58	0.44	0.69	
	CD @ 5%	1.77	2.43	2.12	
	Correlation	-0.7556	-0.1703	0.2725	

This showed that the chitinase activity in Bt cotton leaves at various crop growth stage and the sucking pest population slightly increases.

4.4 Peroxidase activity :

The data on peroxidase activity in leaves of Bt cotton hybrid is shown in Table 7. The peroxidase activity in leaves of eight Bt cotton hybrids ranged from 0.94 to 2.24 U g^{-1} fr. wt. at 30 DAS. The maximum of 2.24 U g^{-1} fr. wt. being present Dhuv and the minimum of 0.94 U g^{-1} fr. wt. Dyna. Bt cotton hybrid. The mean of peroxidase activity in leaves of different Bt cotton hybrids was found to be 1.45 U g^{-1} fr. wt. At 60 DAS peroxidase activity in leaves of eight Bt cotton hybrids ranged from 1.04 to 2.65 U g^{-1} fr. wt. The maximum of 2.65 U g^{-1} fr. wt. being present in Dyna and the minimum of 1.04 U g^{-1}. fr.wt. in Dhuv. The mean peroxidase activity was found to be 1.53 U g^{-1} fr. wt. at 60 DAS. At 90 DAS peroxidase activity ranged from 2.04 to 5.00 U g^{-1} fr. wt. The maximum of 5.00 U g^{-1} fr. wt. being present in Monsoon and the minimum of 2.04 U g^{-1} fr. wt. in Tulasi. The mean of peroxidase activity in leaves in Bt cotton hybrids was found to be 3.02 U g^{-1} fr. wt. at 90 DAS. The value of correlation coefficient between sucking pest population and peroxidase activity was negative correlated at 30, 60, 90 DAS (Fig. 7). The result showed that the peroxidase activity in Bt cotton leaves against the sucking pest population increases and that may sensible for resistance to sucking pest.

Table 7. **Peroxidase activity in leaves of Bt cotton**

Sr. No.	Name/DAS	Peroxidase (U g^{-1} fr. wt.)			
		30	**60**	**90**	**Mean**
1	Bunny	1.57	1.63	3.84	2.34
2	Dhuv	**2.24**	1.04	2.44	1.90
3	M0nsoon	1.38	1.47	**5.00**	2.61
4	NHH-44	1.16	1.33	2.13	1.54
5	Rasi-2	1.70	1.27	3.09	2.02
6	Dyna	0.94	**2.65**	3.18	2.26
7	Mallika	1.29	1.42	2.50	1.73
8	Tulasi	1.13	1.45	2.04	1.54
	Range	0.94-2.24	1.04-2.65	2.04-5.00	1.54-2.61
	Mean	1.45	1.53	3.02	1.99
	SE $\pm$	0.26	0.26	0.42	
	CD @ 5%	0.80	0.80	0.98	
	Correlation	-0.2346	0.4425	-0.1669	

The reported values of peroxidase activity in Bt cotton hybrids ware in the range of 10.33 to 24.44 U g^{-1} fr. wt. (Patil and Kale, 2005), 1.4 to 5.4 O.D. min^{-1} g^{-1} protein (Sharma and Sharma, 1997),9.38 to 10.2 U g^{-1} fr. wt. (Bhite *et al.*, 1997) and 15 to 26 U mg^{-1} protein (Gowda *et al.*, 1989).

Thus the values of peroxidase activity observed in the present investigation are also with in the range of values reported by earlier researches.

4.5 Polyphenoloxidase activity :

The data on polyphenol oxidase activity content in leaves of Bt cotton hybrid is shown in Table 8. The polyphenol oxidase activity in leaves of eight Bt cotton hybrids ranged from 0.85 to 2.22 U g^{-1} fr. wt. at 30 DAS. The maximum of 2.22 U g^{-1} fr. wt. being present Dyna and the minimum of 0.85 U g^{-1} fr. wt. Monsoon. Bt cotton hybrid. The mean of polyphenol oxidase activity was found to be 1.46 U g^{-1} fr. wt.At 60 DAS the polyphenol oxidase activity ranged from 1.03 to 2.56 U g^{-1} fr. wt. The maximum of 2.56 U g^{-1} fr. wt. being present in Dyna and the minimum of 1.03 U g^{-1} fr. wt. in Monsoon. The mean of Polyphenol oxidase activity leaves of Bt cotton hybrids was found to be 1.69 U g^{-1} fr. wt. and At 90 DAS polyphenol oxidase activity in eight different Bt cotton hybrids ranged from 1.06 to 3.22 U g^{-1} fr. wt. The maximum of 3.22 U g^{-1} fr. wt. being present in Dyna and the minimum of 1.06 U g^{-1} fr. wt. in Dhuv. The mean of polyphenol oxidase activity was found to be 2.20 U g^{-1} fr. wt. at 90 DAS.

The value of correlation coefficient between sucking pest population and polyphenol oxidase activity was negative correlated at 30, 60, 90 DAS (Fig. 8).

The increase polyphenol oxidase activity decrease the infestation of sucking pest. The results obtained in the present investigation are quite agree with results reported

by Senthil *et al*. (2010), Patil and Kale (2005), Chakraborty *et al*. (2002) and Gowda *et al*. (1989).

Table 7. Polyphenoloxidase activity in leaves of Bt cotton

Sr. No.	Name/DAS	Polyphenoloxidase (U g^{-1} fr. wt.)			
		30	**60**	**90**	**Mean**
1	Bunny	1.73	1.98	2.19	1.96
2	Dhuv	0.93	1.03	1.06	1.00
3	Monsoon	0.85	1.03	2.24	1.37
4	NHH-44	1.54	1.82	1.99	1.78
5	Rasi-2	1.63	2.25	2.75	2.21
6	Dyna	**2.22**	**2.56**	**3.22**	2.66
7	Mallika	1.50	1.59	1.60	1.56
8	Tulasi	1.28	1.30	2.60	1.72
	Range	0.85-2.22	1.03-2.56	1.06-3.22	1.00-2.66
	Mean	1.46	1.69	2.20	1.78
	SE $\pm$	0.29	0.29	0.26	
	CD @ 5%	0.87	0.89	0.80	
	Correlation	-0.5509**	-0.2582	-0.2266	

** Significant at 1 % level * Significant at 5%level

5. SUMMARY AND CONCLUSION

Bt cotton is one of the important fibre crops in Maharashtra and even in India. But its productivity is reduced due to increasing problems of sucking pest attack. Among these, sucking pest is a commonly occurring disastrous pest which causes severe damage to Bt cotton crop resulting in the yield losses. The knowledge of plant biomolecules synthesized in response to sucking pest attack makes it possible to understand the mechanism of pest resistance. However, scanty information is available on the biochemical analysis of Bt cotton hybrid in relation to sucking pest tolerance. Earlier reports have indicated the involvement of biochemical constituents such as polyphenols, tannins chitinase activity and the level of PPO and POD in offering resistance. In this context, the present investigation was undertaken to analyze eight Bt cotton hybrids. The results obtained on the biochemical analysis of leaves of Bt cotton hybrids at 30, 60 and 90 DAS have been summarized and briefly concluded below:

1. The total polyphenol contents in leaves increased at different growth stages. The highest total polyphenol content 6.054 mg g^{-1} fr.wt. was recorded in Dyna at 30 DAS. In Monsoon at 60 DAS (7.916 mg g^{-1} fr.wt). and in Tulasi at 90 DAS (8.418 mg g^{-1} fr.wt.)

2. Tannin contents in leaves increased at each growth stage. The highest tannin content was observed at 30 DAS in Dhuv (3.98 mg g^{-1} fr.wt) and in Monsoon at 60 DAS (8.74 mg g^{-1} fr.wt) and at 90 DAS (8.350 mg g^{-1} fr.wt).

3. Chitinase activity in leaves of different Bt cotton hybrids was found increasing with different growth stages. Chitinase activity observed highest at 30 DAS in Dyna (4.75 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1})at 60 DAS Rasi2 (4.98 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1}) and at 90 DAS Dhuv (6.65 mg N-acetylglucosamine released g^{-1} fr.wt. h^{-1}).

4. Peroxidase activity at each stage of growth of Bt cotton leaves was found increasing in different hybrids. Peroxidase activity observed highest at 30 DAS in Dhuv (2.24 U g^{-1} fr. wt.), at 60 DAS Dyna (2.65 U g^{-1} fr. wt.) and at 90 DAS Monsoon (5.00 U g^{-1} fr. wt.).

5. Polyphenol oxidase activity in leaves of different Bt cotton hybrids was increase with different growth stage. The highest activity polyphenol oxidase observed in Dyna at 30 DAS (2.22 U g^{-1} fr. wt.) at 60 DAS (2.56 U g^{-1} fr. wt.) and also at 90 DAS (3.22 U g^{-1} fr. wt.).

Thus from these results, it is concluded that a biochemical constituents may be involved in resistance against sucking pests in eight different Bt cotton hybrids. However, the various biochemical constituents in eight different Bt cotton hybrids showing different interaction with higher level total polyphenol, tannin, chitinase activity and POD, PPO is probably responsible for offering resistance against the sucking pest attack at 30 , 60 and 90 DAS.

Amongst eight different Bt cotton hybrids, Dyna showed better resistance against sucking pest at 30,60 and 90 DAS. Dhruv and Monsoon are also showing resistance to sucking pest followed by Dyna at 30,60 and 90 DAS.

Initial growth period of crop is more vulnerable to jassids. Cotto Bt hybrid Dyna showed less jassids population with higher levels of total polyphenol and content with higher tannin, chitinase, peroxidase and plyphenoloxidase activity.

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7. VITA

Mr. Sachin Vilas Nehete

A candidate for the diegree

of

MASTER OF SCIENCE (AGRICULTURE)

in

BIOCHEMISTRY

2012

Title of Thesis ☛ "Biochemical analysis of Bt cotton hybrids in relation to sucking pest tolerence"

Major field ☛ Biochemistry

Biographical information

Personal

- Born on 14July, 1987 at Khiroda Tal. Raver, Dist.Jalgaon, Son of Shi. Vilas Jagannath Nehete and Sau.Pratibha Vilas Nehete.

Educational

- Attended primary school education at Khiroda.
- Passed S.S.C Examination from Dhanaji Nana Vidyalaya Khiroda in 2003 Dist. Jalgaon
- Passed H.S.C. from Dhanaji Nana Junior college, Khiroda Dist.Jalgaon in 2005 Dist. Jalgaon.
- Received Bachelor of science (Agriculture) degree from College of Agriculture, Dhule in 2009

Others

- Successfully completed National Services Scheme camp in 2006 at Boarwihir Dist. Dhule.

  Represent the inter university Cricket tournament held at Surat (Gujarat) in 2010-11.

  Represent the inter university Hockey tournament held at Jaipur (Rajasthan)

in 2008-09.

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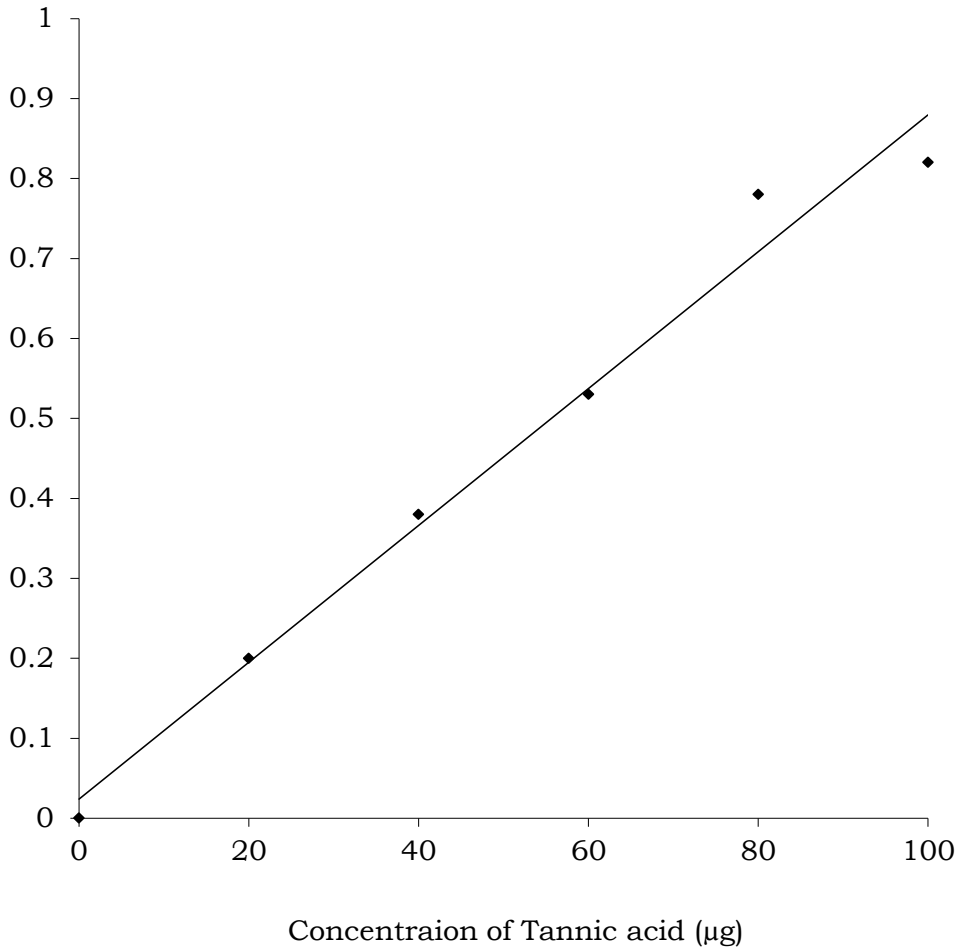

Fig. 1. Calibration of a standard curve of total polyphenol (µg)

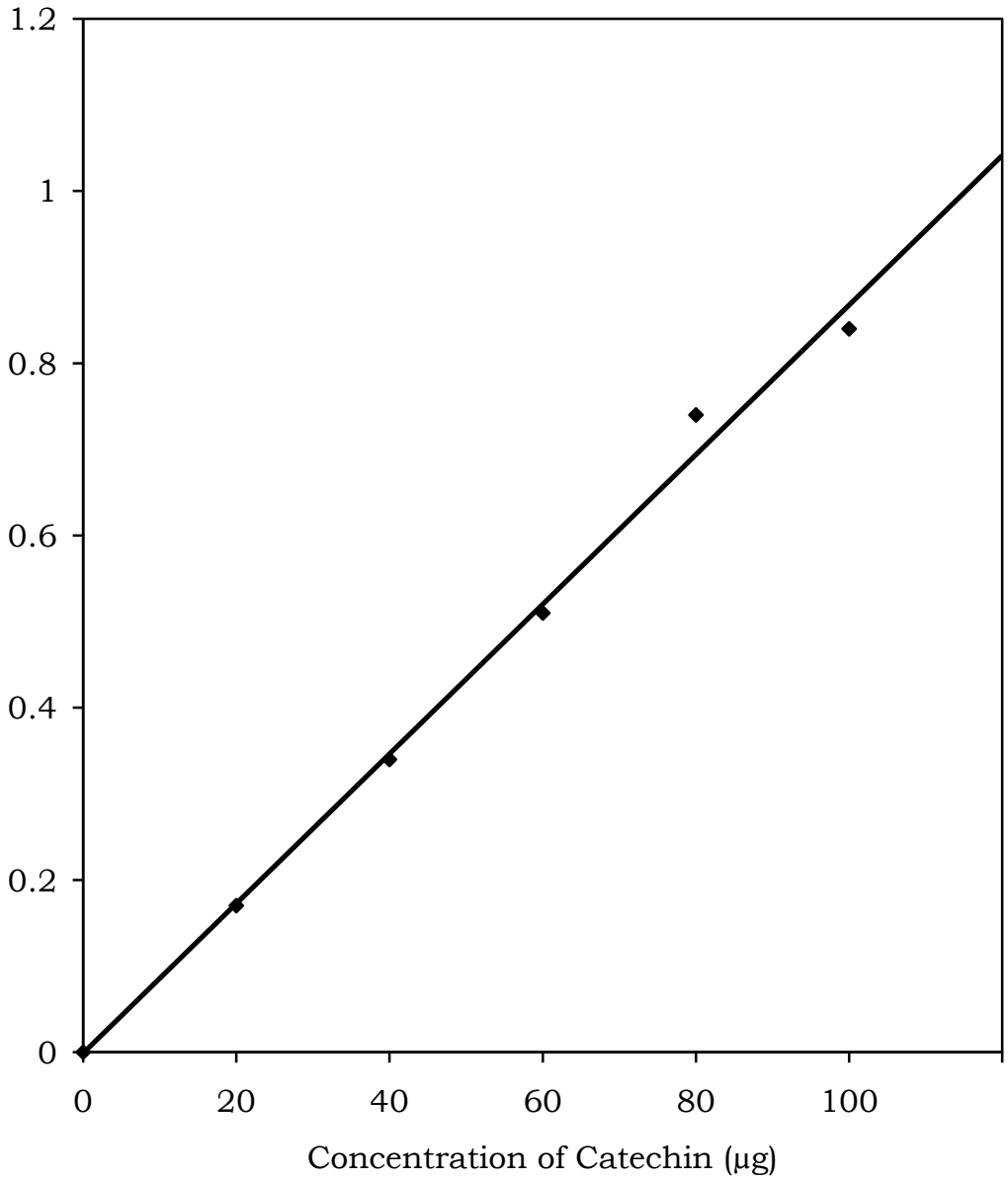

Fig. 2. Calibration of a standard curve of estimation of tannin

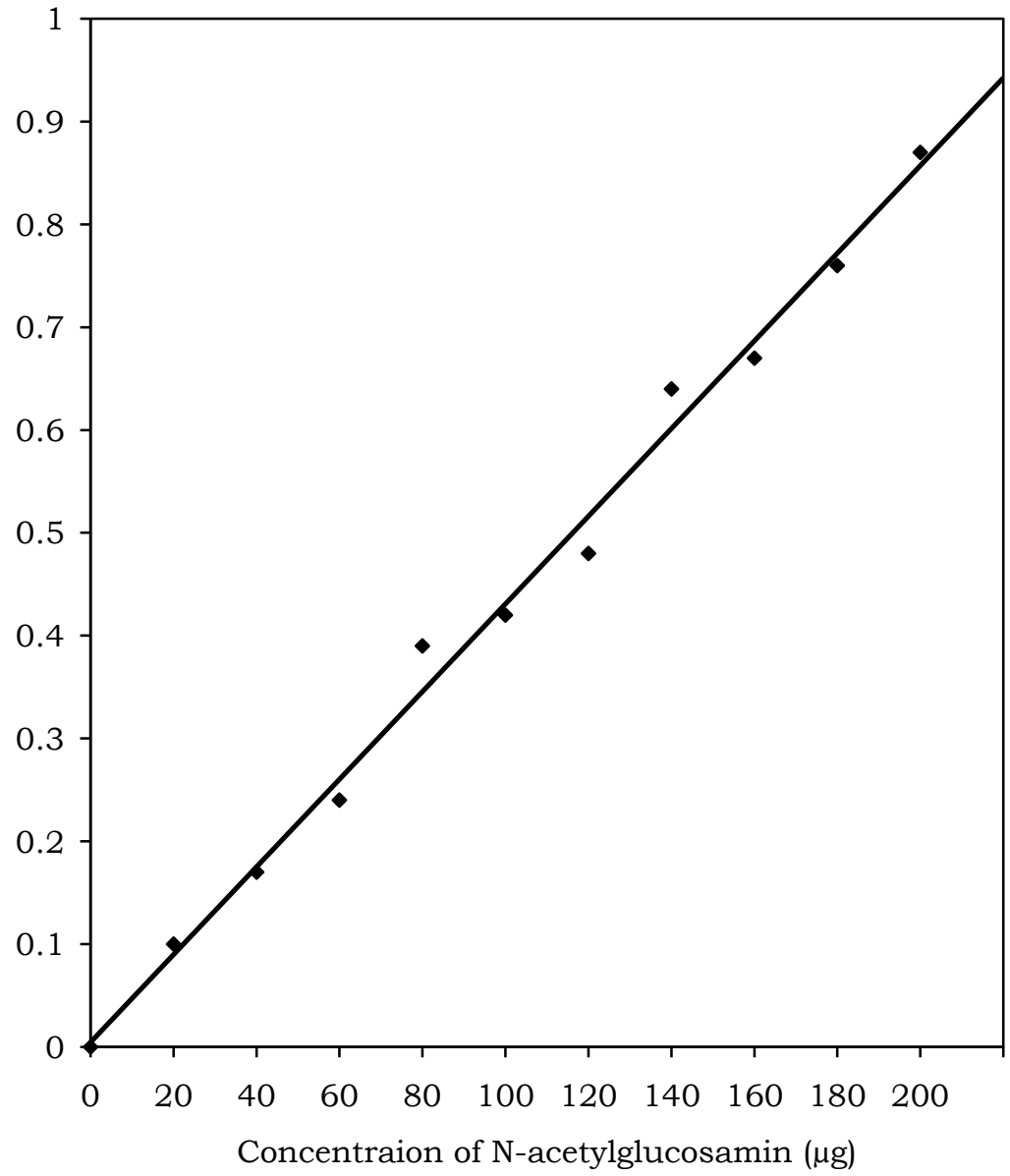

Fig. 3. Calibration of a standard curve of estimation of chitinase activity

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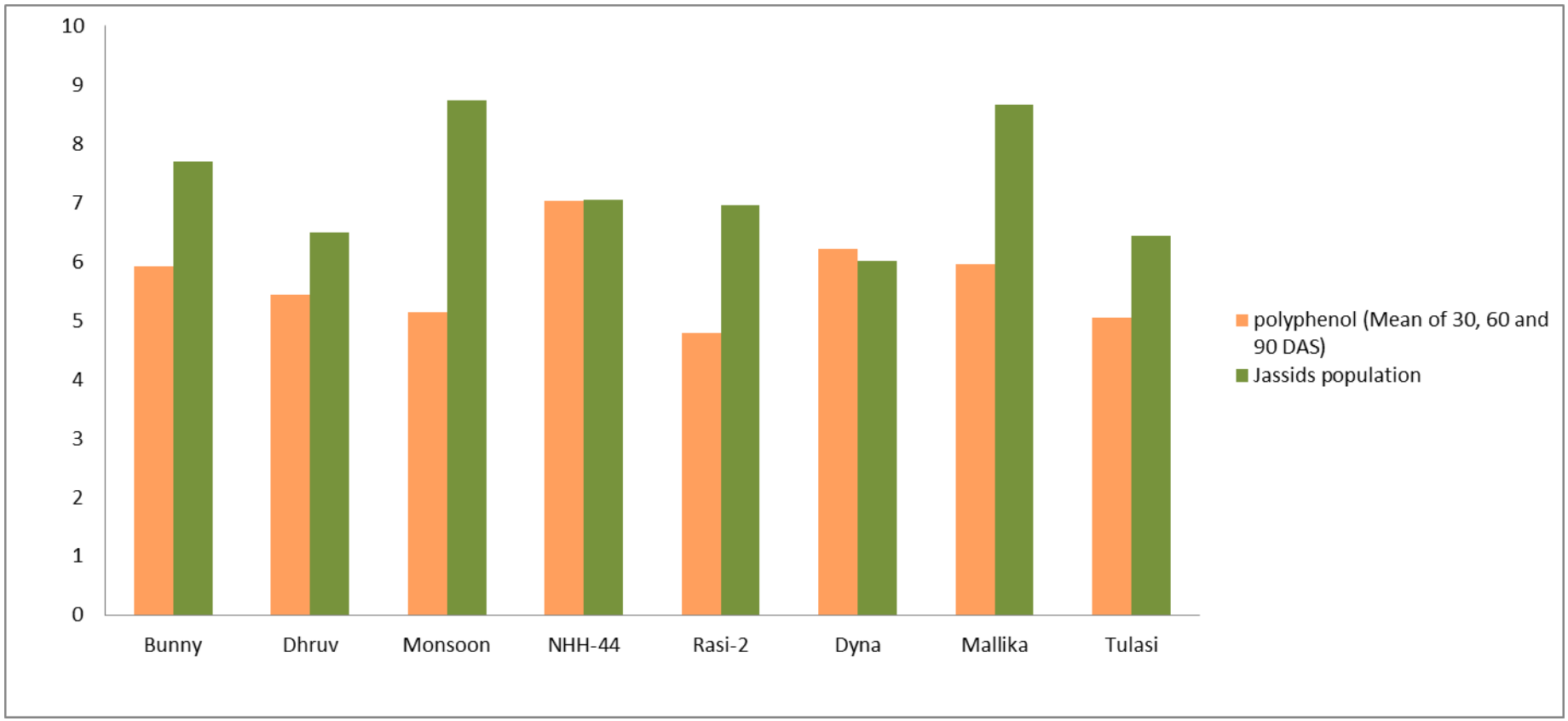

Fig 4. Correlation between total popyphenol content in leaves of Bt cotton and jassids population.

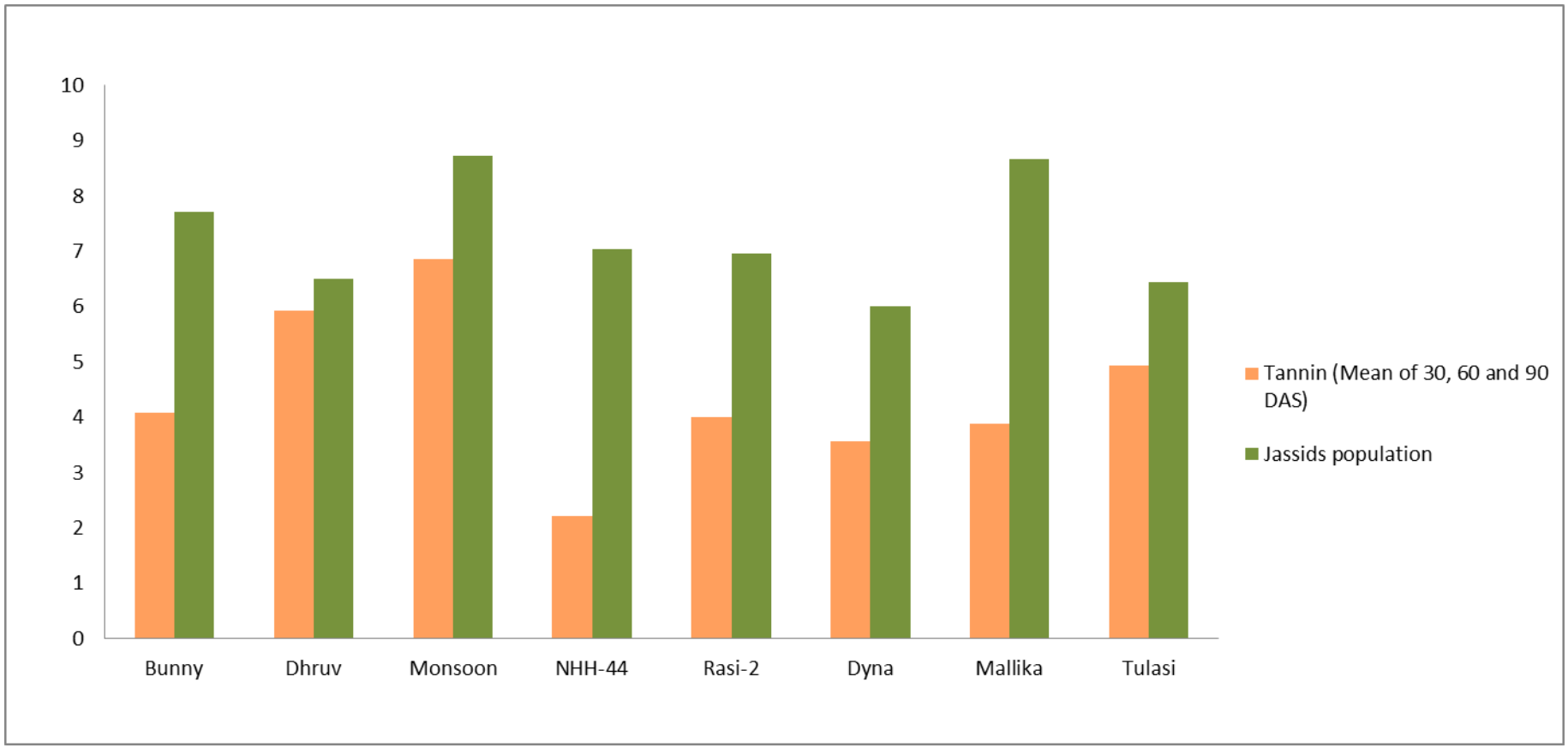

Fig 5. Correlation between tannin content in leaves of Bt cotton and jassids population.

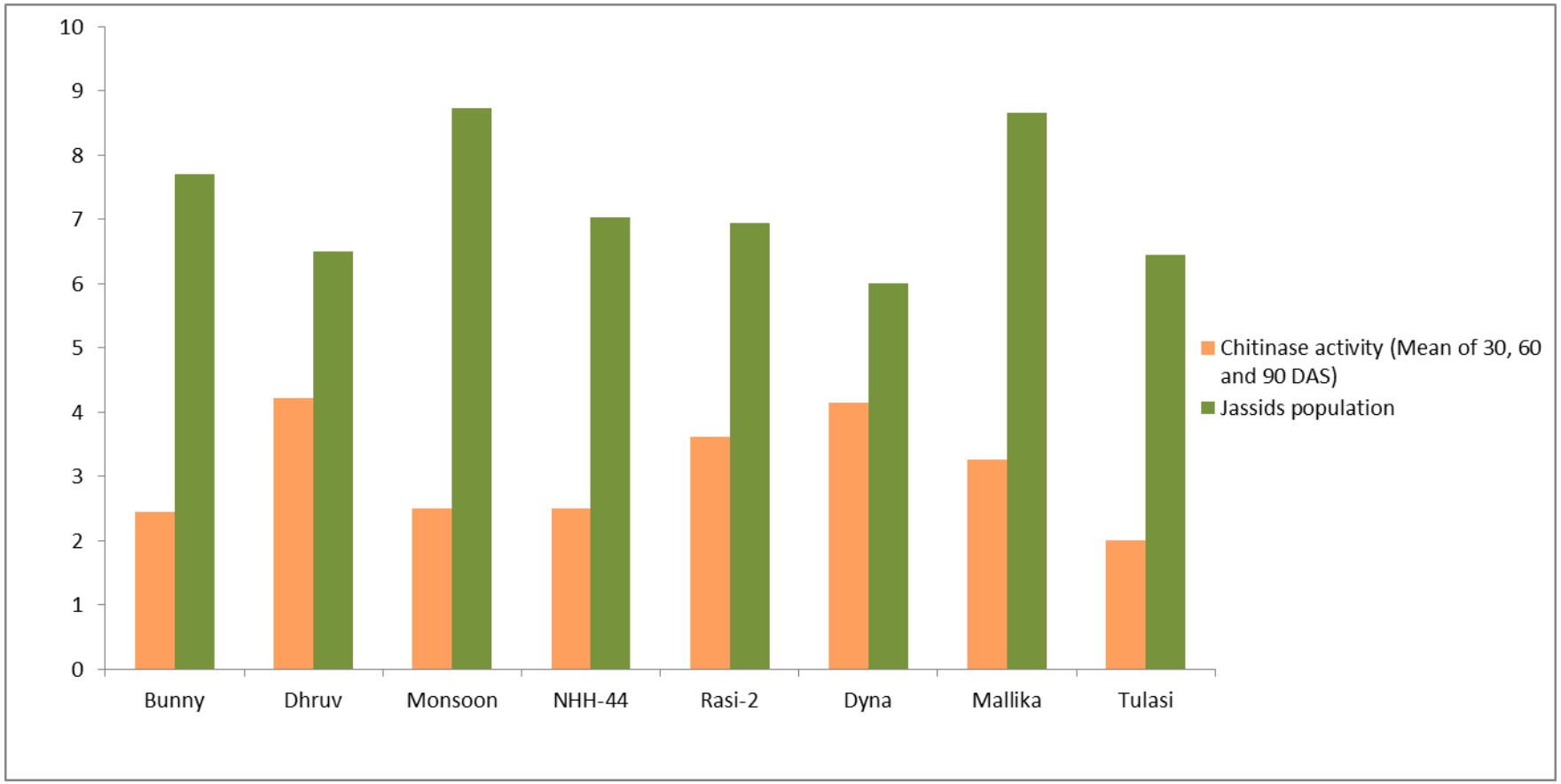

Fig 6. Correlation between chitinase activity in leaves of Bt cotton and jassids population

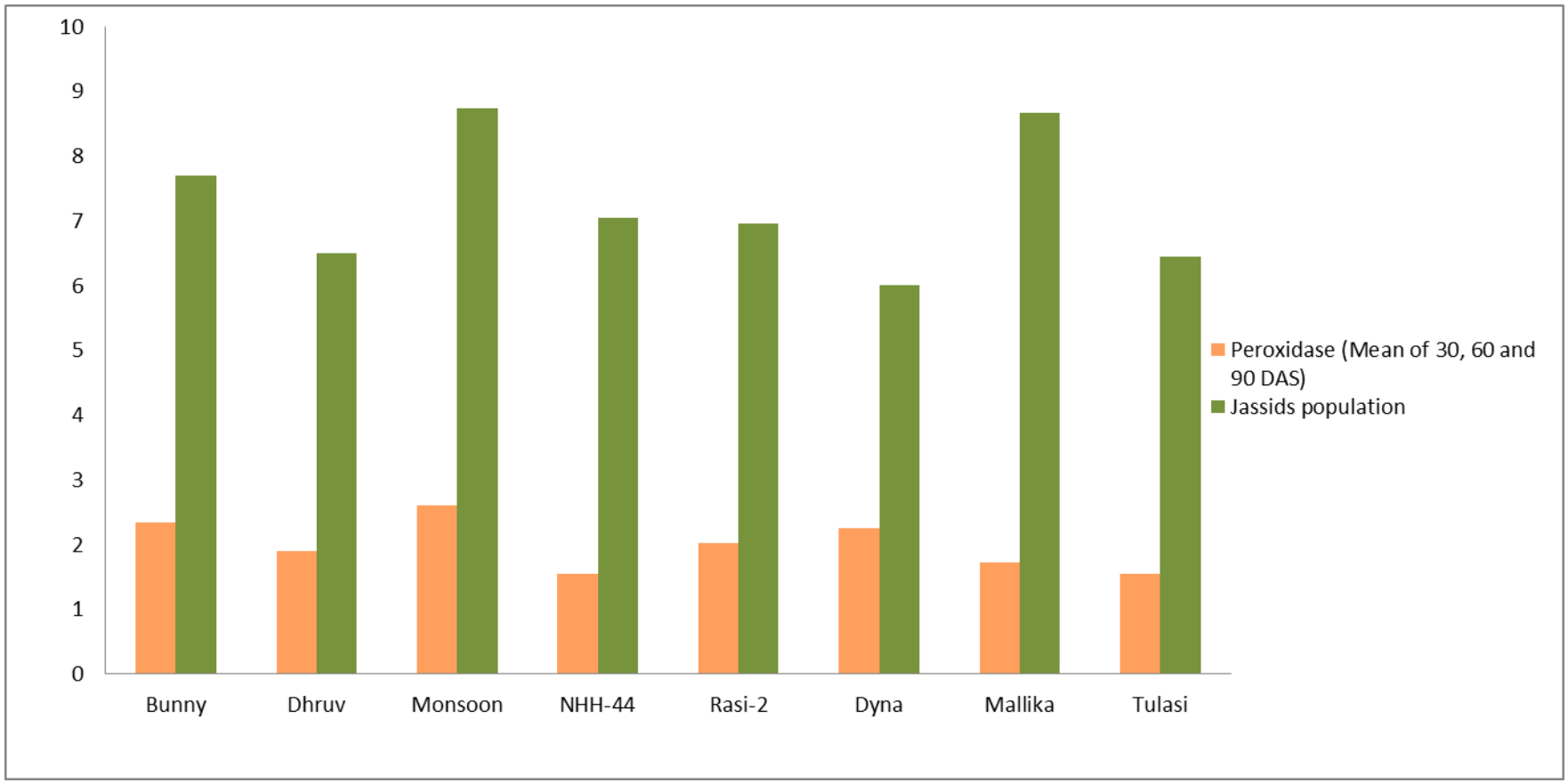

Fig 7. Correlation between peroxidase activity in leaves of Bt cotton and jassids population.

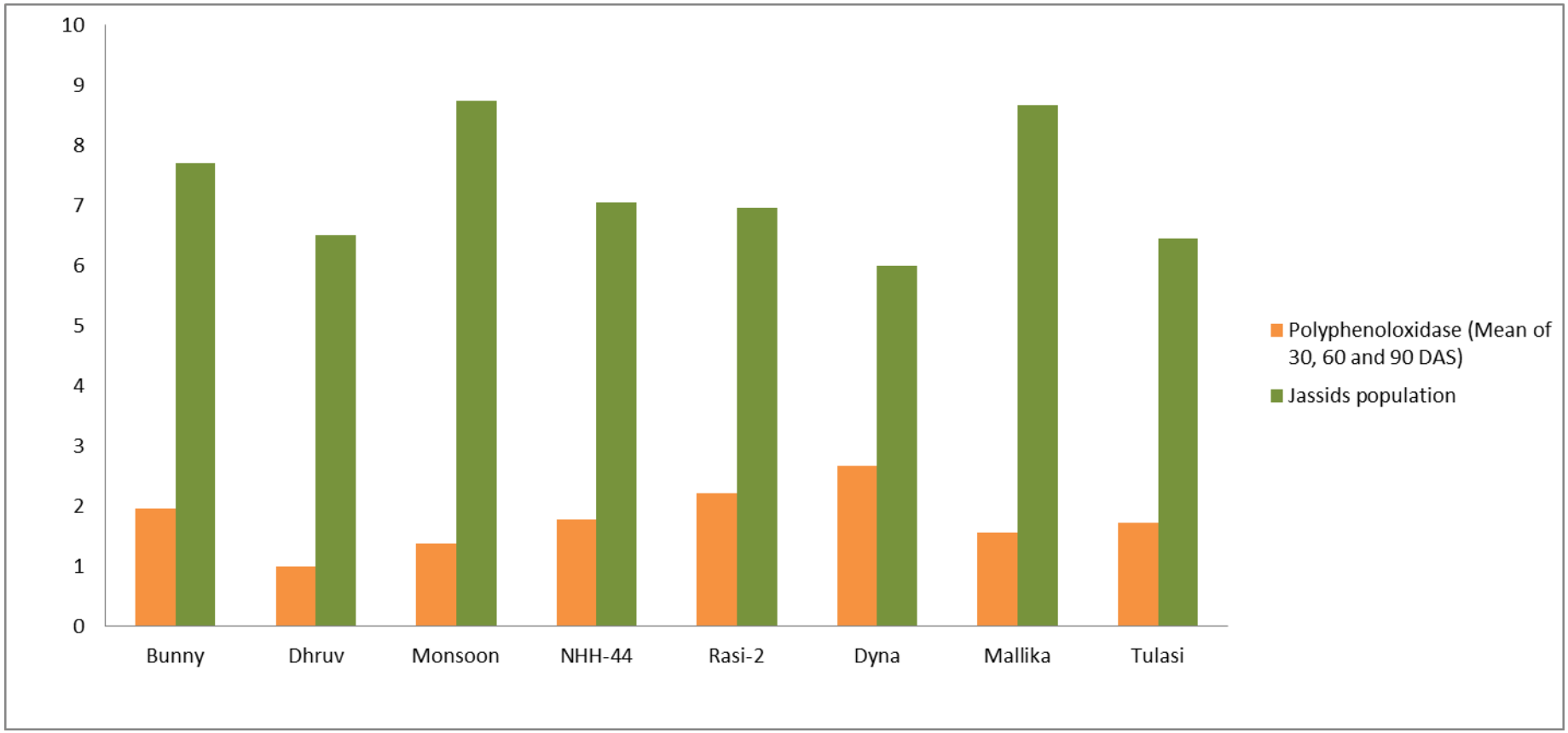

Fig 8. Correlation between polyphenoloxidase activity in leaves of Bt cotton and jassids population.