

**EFFECT OF CALCIUM, BORON AND
SALICYLIC ACID ON TOLERANCE OF
BLACKGRAM (*Vigna mungo* (L.)Hepper) TO
YELLOW MOSAIC VIRUS**

**By
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B.Sc. (Agri.)

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DECLARATION

I, **Ms. MENGAJE RUPALI NILKANTH.**, hereby declare that the thesis entitled “**EFFECT OF CALCIUM, BORON AND SALICYLIC ACID ON TOLERANCE OF BLACKGRAM (*Vigna mungo* (L.) Hepper) TO YELLOW MOSAIC VIRUS**” submitted to the **Acharya N. G. Ranga Agricultural University** for the degree of **Master of Science in Agriculture** in the major field of **Crop physiology** 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.

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CERTIFICATE

Ms. MENGAJE RUPALI NILKANTH has satisfactorily prosecuted the course of research and that thesis entitled “**EFFECT OF CALCIUM, BORON AND SALICYLIC ACID ON TOLERANCE OF BLACKGRAM (*Vigna mungo* (L.) Hepper) TO YELLOW MOSAIC VIRUS.**” 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 her for a degree of any university.

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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.

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LIST OF TABLES

Table No.	Title	Page No.
3.1	Weather data during crop growth period	
3.2	Soil characteristics of the experimental field	
4.1	Effect of Calcium, Boron and Salicylic acid application on YMV disease incidence (%) in Blackgram	
4.2	Effect of Calcium, Boron and Salicylic acid application on white fly count of YMV infected Blackgram	
4.3	Effect of Calcium, Boron and Salicylic acid application on Number of branches in YMV infected Blackgram	
4.4	Effect of Calcium, Boron and Salicylic acid application on leaf dry matter (g plant^{-1}) in YMV infected Blackgram	
4.5	Effect of Calcium, Boron and Salicylic acid application on stem dry matter (g plant^{-1}) in YMV infected Blackgram	
4.6	Effect of Calcium, Boron and Salicylic acid application on total dry matter (g plant^{-1}) in YMV infected Blackgram	
4.7	Effect of Calcium, Boron and Salicylic acid application on total phenols content ($\text{mg}/100\text{mg}$) in leaf of YMV infected Blackgram	
4.8	Effect of Calcium, Boron and Salicylic acid application on peroxidase activity ($\Delta \text{ Abs}/\text{min}/\text{g}$) in leaf of YMV infected Blackgram	
4.9	Effect of Calcium, Boron and Salicylic acid application on phenylalanine ammonia lyase (η moles of transcinnamic acid/ min/g) in leaf of YMV infected Blackgram	
4.10	Effect of Calcium, Boron and Salicylic acid application on membrane injury index (%) of YMV infected Blackgram	
4.11	Effect of Calcium, Boron and Salicylic acid application on chlorophyll-a content (mg g^{-1}) in leaf of YMV infected Blackgram	
4.12	Effect of Calcium, Boron and Salicylic acid application on chlorophyll-b (mg g^{-1}) content in leaf of YMV infected Blackgram	
4.13	Effect of Calcium, Boron and Salicylic acid application on carotenoids (mg g^{-1}) content in leaf of YMV infected Blackgram	

4.14	Effect of Calcium, Boron and Salicylic acid application on calcium content (mg g^{-1}) in leaf of YMV infected Blackgram	
4.15	Effect of Calcium, Boron and Salicylic acid application on potassium content (%) in leaf of YMV infected Blackgram	
4.16	Effect of Calcium, Boron and Salicylic acid application on boron content (mg kg^{-1}) in leaf of YMV infected Blackgram	
4.17	Effect of Calcium, Boron and Salicylic acid application on yield and yield components of YMV infected Blackgram	
4.18	Effect of Calcium, Boron and Salicylic acid application on yield and yield components on YMV infected Blackgram	
4.19	Effect of calcium, boron and salicylic acid application on yield (kg ha^{-1}) of YMV infected blackgram	

LIST OF FIGURES

Table No.	Title	Page No.
3.1	Weekly temperature data during crop growth period	
3.2	Weekly rainfall and mean relative humidity data during crop growth period	
3.3	Layout plan of experimental field	
4.1	Effect of Calcium, Boron and Salicylic acid on YMV Disease incidence in YMV infected Blackgram	
4.2	Effect of Calcium, Boron and Salicylic acid on whitefly count of YMV infected Blackgram	
4.3	Effect of Calcium, Boron and Salicylic acid on number of branches plant ⁻¹ in YMV infected Blackgram	
4.4	Effect of Calcium, Boron and Salicylic acid on leaf dry matter (g plant ⁻¹) in YMV infected Blackgram	
4.5	Effect of Calcium, Boron and Salicylic acid on stem dry matter (g plant ⁻¹) in YMV infected Blackgram	
4.6	Effect of Calcium, Boron and Salicylic acid on total dry matter (g plant ⁻¹) in YMV infected Blackgram	
4.7	Effect of Calcium, Boron and Salicylic acid on total phenols in leaf of YMV infected Blackgram	
4.8	Effect of Calcium, Boron and Salicylic acid on peroxidase activity in leaf of YMV infected Blackgram	
4.9	Effect of Calcium, Boron and Salicylic acid on phenylalanine ammonia lyase activity in leaf YMV infected Blackgram	
4.10	Effect of Calcium, Boron and Salicylic acid on membrane injury index in YMV infected Blackgram	
4.11	Effect of Calcium, Boron and Salicylic acid on Chlorophyll a content in leaf of YMV infected Blackgram	
4.12	Effect of Calcium, Boron and Salicylic acid on Chlorophyll b content in leaf of YMV infected Blackgram	
4.13	Effect of Calcium, Boron and Salicylic acid on carotenoids in leaf of YMV infected Blackgram	

4.14	Effect of Calcium, Boron and Salicylic acid on calcium in leaf of YMV infected Blackgram	
4.15	Effect of Calcium, Boron and Salicylic acid on potassium content in leaf of YMV infected Blackgram	
4.16	Effect of Calcium, Boron and Salicylic acid on boron content in leaf of YMV infected Blackgram	
4.17	Effect of Calcium, Boron and Salicylic acid on yield components in YMV infected Blackgram	
4.18	Effect of Calcium, Boron and Salicylic acid on yield components in YMV infected Blackgram	
4.19	Effect of Calcium, Boron and Salicylic acid on Yield (Kg ha^{-1}) of YMV infected Blackgram	

LIST OF SYMBOLS AND ABBREVIATIONS

a.i.	:	Active ingredient
ANOVA	:	Analysis of variance
@	:	at the rate of
⁰ C	:	Degrees Celsius
%	:	Per cent
CD	:	Critical difference
cm	:	Centimetre
CV	:	Coefficient of variation
DAS	:	Days after sowing
DAE	:	Days after emergence
dia.	:	Diameter
<i>et al.</i>	:	and other co-workers
Fig.	:	Figure
g	:	gram (s)
h	:	hour
ha ⁻¹	:	Per hectare
<i>i.e.</i>	:	that is
kg	:	kilo gram
L	:	litre
m	:	Metre
M	:	Molar

mg	:	milli gram (s)
ml	:	milli litre
mm	:	millimeter
mM	:	millimoles
η	:	Nano
N	:	Normality
nm	:	Nanometre
PAL	:	Phenylalanine ammonia lyase
POD	:	Peroxidase
pH	:	Hydrogen ion concentration
ppm	:	parts per million
RH	:	Relative humidity
rpm	:	Revolutions per minute
SA	:	Salicylic acid
SEm	:	Standard Error of mean
t	:	Tonnes
μg	:	Microgram
viz.,	:	Namely

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LIST OF CONTENTS

Chapter No.	Title	Page No.
I	INTRODUCTION	
II	REVIEW OF LITERATURE	
III	MATERIAL AND METHODS	
IV	RESULTS AND DISCUSSION	
V	SUMMARY AND CONCLUSIONS	
	LITERATURE CITED	

ABSTRACT

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The field experiment was carried out at Agricultural College Farm, Bapatla during *Rabi* season 2016-17 to “study of the effect of calcium, boron and salicylic acid on tolerance of blackgram (*Vigna mungo* (L.) Hepper) to yellow mosaic virus”. The experiment was laid out in Randomized Block Design with ten treatments viz., T₁ : Control ; T₂ : Gypsum @ 300 kg ha⁻¹ + Boron @ 1kg ha⁻¹; T₃ : 0.3 % Calcium (CaNO₃.4H₂O) spray ; T₄ : 0.25 % Boron (Borax) spray ; T₅ : 50ppm Salicylic acid spray ; T₆ : Complete nutrient solution spray ; T₇ : (T₂ + T₅) ; T₈: (T₃ + T₅) ; T₉ : (T₄ + T₅) ; T₁₀: (T₆ + T₅) in three replications.

The Observations were recorded on percent incidence of YMV disease, whitefly count, morphological, physiological and biochemical parameters ; nutrient composition (Ca, K, B) in leaf and yield components.

Seven days delay and significant reduction in YMV disease incidence was observed with application of calcium, boron and SA. The reduction in disease incidence ranged from 1.7 to 5.8 percent at 30 DAE and 15.1 to 30.5 percent at 45 DAE, high with T₂, T₆, T₇ and T₈ (30.5, 29.2, 28.3 & 26.1 % respectively).

Whitefly population reduced ranging from 12.4 to 35.6 percent at 15 DAE, 4.5 to 36.9 at 30 DAE and 8.1 to 21.9 at 45 DAE, high with T₈, T₂ and T₇.

The increase in branch number was 7.0 to 14.0 percent, high in gypsum 300kg + boron 1 kg ha⁻¹) and T₇ (gypsum 300 kg + boron 1 kg ha⁻¹ + 50ppm SA spray).

Leaf dry matter accumulation plant⁻¹ with calcium, boron and SA application increased ranging from 14.2 to 133.9 percent at 30 DAE, 0.5 to 82.1 percent at 60 DAE and 32.2 to 111.7 percent at harvest, high with gypsum 300 kg + boron 1 kg ha⁻¹ alone and with 50ppm SA sprays and low with sprays of 0.3% calcium + 50ppm SA.

Dry matter accumulation in stem increased by 6.3 to 14.3 folds at 30 DAE, 1.6 to 2.8 folds at 60 DAE and 1.1 to 1.2 folds at harvest over control.

Total dry matter production increased with application of calcium, boron and SA over control, ranged from 14.5 to 176.3 percent at 30 DAE, 7.5 to 76.0 percent at 60 DAE and 0.7 to 42.0 percent at harvest, high in T₇ (42.0 %), T₂ (40.4 %), T₁₀ (34.4 %) and T₆ (29.8 %).

Total phenols content was found higher in gypsum 300 kg + boron 1 kg ha⁻¹ in both healthy and diseased leaves [62.2 % at 15 DAE, 97.9 % in healthy and 38.0 % in diseased leaves at 30 DAE and 80.6% in healthy and 49.3 % in diseased leaves at 45 DAE]. The percent increase in total phenol was recorded high in healthy than in diseased leaves.

POD activity in healthy plants was high with T₅ and T₇ before incidence of YMV, T₂ and T₇ just after incidence and T₂ at later stages. While in diseased plants, just after incidence at stages POD activity was high with T₇ and T₂. In both healthy and diseased plants less increase in POD activity was noticed in 0.25% boron spray in healthy and complete nutrient solution + 50ppm SA in diseased plants.

The PAL activity was found high with combination of gypsum 300 kg + boron 1 kg ha⁻¹ + 50ppm SA (At 15 and 30 DAE) and with gypsum 300 kg + boron 1 kg ha⁻¹ (at 45 DAE). The diseased plants showed the high value of PAL activity than that of healthy ones. The increase in PAL activity ranged from 27.7 to 64.8 percent at 15 DAE, 27.8 to 63.9 percent in healthy and 12.9 to 40.8 percent in disease infected leaves at 30 DAE, 19.4 to 97.2 percent in healthy and 2.7 to 19.8 percent at 45 DAE in diseased ones at 45 DAE.

All treatments showed the significant reduction in membrane injury index, high was observed with sprays of 0.3 % Calcium + 50 ppm SA and gypsum 300 kg + boron 1 kg ha⁻¹. The percent reduction in membrane injury index ranged from 2.7 to 7.9 at 15 DAE, 5.6 to 8.5 at 30 DAE and 5.0 to 10.8 percent at 45 DAE.

Chlorophyll-a, chlorophyll-b and carotenoids content increased due to application of calcium, boron and salicylic acid, high was recorded with T₂ and T₁₀, T₆ and T₆ & T₁₀ respectively.

The increase in calcium content of leaf was high with T₂, T₃, T₇ and T₈ in both healthy and diseased plants before and after YMV incidence and low with T₄ and T₉.

Potassium content in leaf increased, ranging from 1.1 to 1.7 folds at 15 DAE, 1.2 to 1.8 folds in healthy and 1.3 to 3.1 folds in disease infected plants at 30 DAE and 1.2 to 1.6 folds in healthy and 1.6 to 2.7 folds in disease infected plants at 45 DAE, high with gypsum 300 kg + boron 1 kg ha⁻¹ in both healthy and disease infected plants before and after YMV incidence.

Boron content in leaf raised by 14.8 to 46.7 percent at 15 DAE, 14.2 to 46.9 percent in healthy and 39.7 to 131.0 percent in disease infected plants at 30 DAE and 22.4 to 46.3 percent in healthy and 51.9 to 92.7 percent in disease infected plants at 45 DAE, high with T₂ and T₇ before YMV incidence and T₂ after incidence.

The extent of increase in percentage was 1.8 to 12.3 in number of pods plant⁻¹ (high in T₁₀), 20.6 to 28.1 in pod length (high in T₅)13.5 to 21.1 in number of seeds pod⁻¹ (high in T₂), 9.3 to 30.6 in 100 seed weight (high T₁₀ , T₂ & T₇). Seed yield in YMV infected blackgram by 16.7 to 40.7 percent. Seed yield obtained high with sprays of complete nutrient solution + 50ppm SA (40.7 %) followed by gypsum 300 kg + boron 1 kg ha⁻¹ (37.7 %), 0.3% calcium spray (35.4 %) and by gypsum 300 kg + boron 1 kg ha⁻¹ + 50ppm SA (34.6 %).

Chapter I

INTRODUCTION

Blackgram (*Vigna mungo* L.), popularly known as *urad*, is one of the most important pulse crop grown across India. It contains 24% protein, 3.2% minerals and 59.6% carbohydrate. It also contains 154 mg calcium, 9.1 mg iron and 38 mg β -carotene per 100g of split dal (Bakr *et al.*, 2004). It also contains 78% to 80% nitrogen in the form of albumin and globulin. Like other leguminous crops, blackgram plays a vital role in maintaining the nitrogen balance in the soil. It possesses nodules on its roots, containing symbiotic *Rhizobium* sp which fixes nitrogen in association with the plant and contribute a significant amount for plant growth and development.

Blackgram is a major pulse crop of Andhra Pradesh grown in an area of 5.03 lakh ha producing 2.59 lakh tonnes with a productivity of 514.91 kg/ha (Agropedia). The coastal Andhra region in Andhra Pradesh is famous for blackgram after paddy. The Guntur District ranks first in Andhra Pradesh for the production of black gram.

Mungbean yellow mosaic virus (MYMV) is a serious constraint in the cultivation of blackgram in recent years. Estimates on the incidence of the disease are highly variable; 3 to 26 % and in severe cases 100 % (Biswas *et al.*, 2009). It can cause 100 % yield loss, if infection occurs at seedling stage (Varma *et al.*, 1992 and Ghafoor *et al.*, 2000). The disease is caused by the geminivirus - MYMV (mungbean yellow mosaic virus) which has bipartite genome (Honda *et al.*, 1983 ; Mandal and Verma 1996 ; Karthikeyan *et al.*, 2009). The virus is transmitted by white flies (*Bemisia tabaci*). Initial symptoms of the disease appear as small yellow specks along the veins which spread over the leaf. In severe infections the entire leaf may become chlorotic which later turns into necrotic regions (Qazi *et al.*, 2007).

Several attempts to manage YMV, made mostly focussing on the vector, *Bemisia tabaci* management, were reasonably successful over a considerable period (Kumar *et al.*, 2006; Ganapathy and Karuppiah, 2004). However in recent years, management of MYMV through vector management was ineffective for unknown reasons. There by sources of resistance for the virus were identified (Gupta *et al.*, 2001 and Singh and Awasthi, 2004) their use is limited by undesirable characters.

Nutrient management improves the plant health, enables the plant to tolerate the incidence and herbivory of sucking as well as chewing pests. Mineral nutrients applied either in combination or in sequence were found to decrease MYMV incidence and severity in blackgram.

Application of nutrients not only contributes to host plant resistance but also increases yield. The study on the variation in mineral composition of blackgram cultivars for tolerance to yellow mosaic virus revealed that the resistant cultivar PU-31 maintain in higher ratio of Ca/K throughout its growth. Boron concentration in leaves of blackgram cultivars varied from 3.1-7.9 ppm, which lies in the range of deficient to low according to Epstein's interpretive criteria of plant tissue analysis (Vijaya Santhi, 2014).

Micronutrients can control or reduce pathogen damage to plant by either direct toxicity to the pathogen or by promoting induced systemic resistance. Reuveni *et al.* (2008) reported that B can release calcium from cell wall through cation exchange. The Ca^{2+} cations are believed to interact with endogenous SA and intensify SAR mechanisms that reduce the disease severity (Reuveni and Reuveni., 1998). Salicylic acid (SA) is an important component in the signal transduction pathway leading to systemic acquired resistance (SAR) to the entire spectrum of plant pathogens: bacteria, fungi, and viruses (Durner *et al.*, 1997).

Alleviation of the effects of virus infection with a view to limiting the losses could be an alternative strategy for managing the disease. Exogenous application of systemic acquired resistance (SAR) elicitors like salicylic acid (Radwan *et al.*, 2008; Chandra and Bhatt, 1998) and attenuated virus preparations (Nakazono-Nagaoka *et al.*, 2009 and Lin *et al.*, 2007) was shown to alleviate the effects of infection by some plant viruses.

Since the established management practice through vector control is not effective and resistant varieties do not enjoy agronomic preference, the present investigation, based on the hypothesis that blackgram plants could be alleviated of MYMV infection by offsetting its effects, was undertaken with the following objectives:

1. To study the effect of Calcium, Boron and Salicylic acid individual foliar spray on YMV disease incidence, growth and yield in Blackgram.
2. To study the combined effect of Calcium, Boron and Salicylic acid foliar spray on YMV disease incidence, growth and yield in Blackgram.
3. To study the effect of complete nutrient solution spray on YMV disease incidence growth and yield in blackgram.
4. To compare the effect of soil applied Calcium and Boron with effect of foliar sprays on YMV disease incidence, growth and yield in blackgram.

Chapter II

REVIEW OF LITERATURE

A field experiment entitled “Effect of calcium, boron and salicylic acid on tolerance of blackgram (*Vigna mungo* (L.) Hepper) to yellow mosaic virus” was conducted during *rabi* 2016-17 at Agricultural College Farm, Bapatla. The response of blackgram to calcium, boron and salicylic acid was analyzed through changes in the morphological, physiological, biochemical and nutrient parameters along with yield and its components.

A review of related experimental work carried out by earlier researchers is presented in the following paragraphs.

DISEASE INCIDENCE AND SEVERITY

Nutrients are important for growth and development of plants as well as microorganisms and they are important factors in disease control (Agrios., 2005). All the essential nutrients can affect disease severity (Huber and Graham., 1999). Nutrients can affect the disease tolerance or resistance of plants to pathogens by affecting their rate of growth and their state of readiness to defend themselves against pathogen attack (Linus-Muriithi and Irungu, 2004).

Nutrient management improves the plant health, enables the plant to tolerate the incidence and herbivory of sucking as well as chewing pests. Mineral nutrients applied alone either successively or alternated with each other were found to decrease MYMV incidence and severity in blackgram. Application of nutrients not only contributes to host plant resistance but also increases yield. The study on the variation in mineral composition of blackgram cultivars for tolerance to yellow mosaic virus revealed that the resistant cultivar PU-31 maintain in higher ratio of Ca/K throughout its growth. Boron concentration in leaves of blackgram cultivars studied varied from 3.1-7.9 ppm, which lies in the range of deficient to low according to Epstein’s interpretive criteria of plant tissue analysis (Vijaya Santhi, 2014). Calcium is important nutrient that affects the susceptibility to diseases in two ways. First, Ca is important for the stability and function of plant membranes and when there is Ca

deficiency there is membrane leakage of low molecular weight compounds, e.g. sugars and amino acids, from the cytoplasm to the apoplast, which stimulate the infection by the pathogens. Second, Ca is an important component of the cell wall structure as calcium polygalacturonates are required in the middle lamella for cell wall stability. When Ca concentration drops, there is an increased susceptibility to fungi which preferentially invade the xylem and dissolve the cell walls of the conducting vessels, which leads to wilting symptoms. In addition, plant tissues low in Ca are much more susceptible than tissues with normal Ca levels to parasitic diseases during storage (Marschner, 1995).

Ca also plays a vital role in counteracting the adverse effect of virus and in promoting local lesion formation and development. Ca concentration in plant tissue affects gene expression of pectolytic enzymes especially polygalacturonase, produced by pathogen as a virulent factor result in resistance to disease (Volpin and Elad., 1991).

Eraslan *et al.*, (2007) also reported that, Ca plays a vital role in counteracting the adverse effect of virus and in promoting local lesion formation and development. As for the Ca-dependent changes in the resistance to disease, it was suggested that the concentration of Ca in plant tissue may regulate the level of resistance. ToMV symptoms can be reduced through nutrition by foliar Ca spray. The results may be of help in the integrated control of ToMV in tomato plants. Foliar Ca spray depressed the severity of ToMV in tomato plants, and the depression was more pronounced especially after the second and third foliar spray (15 and 30 days after inoculation, respectively) and all Ca sources ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{CaNO}_3 \cdot 4\text{H}_2\text{O}$, $\text{Ca}(\text{C}_2\text{H}_3\text{O}_2)_2$ and $\text{CaNO}_3 \cdot 4\text{H}_2\text{O} + 0.05 \text{ M Na EDTA}$) significantly reduced the virus concentration. The average reduction in ToMV concentration 15 days after the third foliar Ca spray was 32, 33, 38 and 29% respectively in comparison with control. A general decrease in number of local lesions was observed in Ca treated plants.

According to Yamazaki *et al.*, (2000), disease development was not affected by the Ca concentration applied before inoculation, but only when Ca was applied after inoculation Ca increased the resistance of tomato to bacterial wilt and decreased the population of pathogen in xylem. Berry *et al.* (1988) reported that, reduction in symptoms and virus concentration caused by Ca application is attributed to the

increased resistance of the tissue cells to virus. A significant reduction in fresh and dry weights at zero Ca reflects the rapidity at which plants succumbed to the virus disease when they were Ca deficient. On the other hand, proper nutrition maximizes the inherent resistance of plants. Tissue Ca concentration was increased by foliar-applied Ca.

Tomato plants grown in low Ca (0.5 mM) treatment began to wilt 4 days after inoculation and disease progress was very rapid and all plants were dead 14 days after inoculation, than the plants grown in medium Ca level (5 mM) solution. Medium Ca plants began to wilt 4 days after inoculation and their disease severity rating was 77.1% at 20 days after inoculation. Plants in high Ca (25 ml) began to wilt 6 days after inoculation and disease progress was slow i.e., the disease severity was only 56.8% at 20 days after inoculation (Jia-Feng *et al.*, 2013). Increase in Ca nutrition near to level of physiological sufficiency significantly reduced the severity of tomato wilt.

Ali *et al.* (2014) conducted field experiment on management of chocolate spot disease (*B. faba*) in *Vicia faba* with Ca, B, Mo, Blight stop and Kocide 101 (as foliar spray) and reported that maximum reduction of disease severity in comparison with control at 55 and 70 DAS was recorded by Blight Stop (32.60 % and 36.61 %), followed by Boron 2 ppm (12.97, 12.79), molybdenum 4 ppm (13.58, 16.49) and calcium (calciven) 6 mL⁻¹ (13.30 % and 15.04 %) respectively. It was also observed that Boron @ 6 ppm was most effective in reducing pathogen growth after Kocide 101 and Blight stop than Ca at 6 ppm, which had no effect on linear growth of *B. fabae* and suggested that fungicides can be replaced with boron at 2 ppm or calcium as calciven at 6 mL⁻¹ or Mo at 4 ppm as foliar spray for minimizing faba bean chocolate spot disease and maximizing yield of faba bean plants.

Foliar application of CaCl₂ was also reported to delay ripening and control mould disease in many fruits and vegetables (Conway *et al.*, 1994 and Cheour *et al.*, 1991). Calcium ions can form salt bridge cross-links and make cell wall less accessible to the enzymes and control ripening as well as plant diseases (Sams *et al.*, 1993). Beresford *et al.* (1996) reported that the spraying of calcium hydroxide (slacked lime) 1.6 kg/100 liters of water alone.

Atim *et al.*, (2013) studied the application of potassium (78, 158 391, 783, 1565 and 3913 mg L⁻¹), calcium (12, 24, 60, 121, 241 and 603 mg L⁻¹) and nitrogen (84, 168, 420, 841 and 1682 mg L⁻¹) on the susceptibility of four banana cultivars to Banana Xanthomonas wilt in in-vitro conditions. For each of the nutrients BXW incidences were significantly reduced, and incubation periods (time from inoculation to appearance of first disease symptoms) increased with increasing nutrient concentration in the medium. The time taken for symptomatic plantlets to die was significantly affected by nutrient concentration only for Ca but not for K and N.

Esh (2005) reported that calcium compound could reduce the severity of *Cercospora* leaf spot disease of sugar beet. In vitro studies showed that high levels of Ca solutions significantly inhibited mycelia growth, sporulation and cercosporin production (Chung, 2003 and Esh and El-Kholi, 2006). At two weeks after infection, *cercospora* leaf spot disease severity of sugar beet plants treated with two calcium compounds (calcium chelates and calcium chloride) @ 1200 ppm significantly reduced compared to untreated plants. Calcium choride spray resulted in 34.23 % reduction in disease severity, with calcium chelate resulted 52.25 % reduction. At 4 weeks after infection, the reduction of disease severity with calcium chelate was 50 % and with calcium chloride was 43.34 % (El-Kholi and Esh, 2011).

Kumar *et al.* (2015) studied the effect of CaSO₄, KCl, ZnSO₄ and Na₂B₄O₇.10H₂O on disease progression of alternaria blight in musturd variety Varuna and reported that minimum average apparent infection rate on leaf was observed in CaSO₄ at 0.5% (0.616) followed by at CaSO₄ at 1.5% (0.633) and Na₂B₄O₇.10H₂O at 0.75% (0.638) in comparison to check.

Pramanik and Ali (2001) reported that single or combined application of boron (2 kg/ha) and molybdenum (1.5 kg/ha) significantly reduced the severity of yellow mosaic in winter mungbean and increased the grain yield. Islam *et al.* (2002) studied the effect of nutrients and weeding on the incidence of mungbean mosaic and they reported that the disease incidence, severity and mosaic leaves were minimum in B + Zn + S applied plots.

A basal application of borax, sulphur and ZnSO₄ followed by spray of *Pseudomonas fluorescences* was found most effective to reduce the alternaria blight intensity by 19.06 % and 20.7 % on leaves and by 5.83 and 15.1 % on pods,

respectively (Singh et al., 2014). Kumar *et al.* (2014) and Tewari (1991) revealed that soil or foliar application of calcium compounds resulted in systemic induced resistance against alternaria blight of rapeseed mustard.

Foliar spray of 20 ppm B and 20 ppm Cu significantly reduced the number of diseased (fungal infected) plants (24.3 %) compared to control (29.65 %) in rice. This indicates that there is suppression of fungal infestation up to a certain limit and rice plants with minute amount of B and Cu treatments sustain and produce high yield even without fungicidal treatments (Liew *et al.*, 2012)

Boron has close relationship with calcium in both plant and soil, because calcium increases the boron requirement of plants due to similarity in function and reduces the availability of boron in soil, perhaps due to the formation of a calcium metaborate complex. Reuveni *et al.* (2008) reported that B can release calcium from cell wall through cation exchange. The Ca^{2+} cations are believed to interact with endogenous SA and intensify SAR mechanisms that reduce the disease severity (Reuveni and Reuveni, 1998).

B has a direct function in cell wall structure and stability and has a beneficial effect on reducing disease severity. Effect of iron, sulphur, boron, and weeding on incidence of MYMV was studied. The disease was reduced with application of iron, sulphur and boron along with weeding as compared to control. When iron, sulphur and boron were applied to mungbean without weeding the disease severity and incidence increased as compared to treatment having nutrient application along with weeding (Islam *et al.*, 2002).

Meena *et al.* (2011) reported that calcium sulphate, borax and zinc sulphate were found effective against the incidence of alternaia blight of Indian mustard. Boron has shown to reduce tobacco mosaic virus in bean and tomato yellow leaf curl virus in tomato (Graham and Webb, 1991).

Application of borax (10.6 % B), Zinc sulphate (24 % Zn) and gypsum (18 % sulphur and 29.2 % Calcium) @ 2,2 and 20 kg ha⁻¹, respectively showed minimum 21 and 26 % infection at 40 and 60 DAS. There was slight variation in mosaic leaves percentage between boron, zinc and gypsum plots. The influence of these treatments was found reduced when weeding was not performed. (Islam *et al.*, 2002). Boron and zinc application in urd bean reduced the disease severity of urd bean leaf crinkle virus by 18.31 percent (Zeshan *et al.*, 2012).

Ali *et al.*, (2014) reported that, commercially available nutrient solution like NPK solution (Fashion) and B and Zn solution (Classic) @ 5 ml L⁻¹ minimized the okra yellow mosaic virus disease incidence percentage most effectively. They also observed the reduction in disease incidence with the use of plant extract and suggested it can be included as a component of an integrated disease management package as it is eco-friendly.

Foliar spray of boron (0.1 gL⁻¹), zinc (4 gL⁻¹) reduced the rust occurrence in wheat next to effect of fungicide tilt (2.5 ml L⁻¹) both under green house and field conditions. The reduction in leaf rust severity was higher after 45 days than after 60 days (Somaya and Seham, 2015).

Incidence of TMV reduced by two third in tomato plants with spray of 0.05% boric acid. Boron deficiency is also said to encourage the spreading of tobacco mosaic virus and exacerbate the symptoms of yellow mosaic virus (Bergmann 1992).

Qin *et al.* (2010) revealed that B strongly inhibited spore germination, germ tube elongation and mycelia spread of *Botrytis cinera* in culture medium. Application of B at 1% caused appearance of abnormal spores. Furthermore boron led to the leakage of cellular constituents (soluble protein and carbohydrate) from hyphae of *B. cinera*. Reduction in disease severity has been reported in crops after single foliar application of 0.005M H₃BO₃, 0.0025M CuSO₄ and 0.0025M MnCl₂ or KMnO₄ on upper surface of the first true leaf of cucumber plants 2 h before inoculation with a conidial suspension of powdery mildew, which provided induced systemic protection against powdery mildew in leaves 2 and 3 without causing any damage on induced leaf (first leaf), (Reuveni *et al.*, 1997) and also suggested that applications of nutrients such as B, Cu and Mn can exchange and release Ca²⁺ cation from cell walls, which interact with salicylic acid and activate systemic acquired resistance mechanisms.

Foliar spray with H₃BO₃, CuSO₄, MnCl₂ or KMnO₄ separately induced systemic protection against powdery mildew in cucumber plants. Similar results were found in wheat, where application of B, Mn and Zn separately increased the resistance of plants to tan spot (Simoglou and Dordas, 2006).

Asif khan (2016) reported that, in LBG-623 the reduction in disease incidence with gypsum, boron and gypsum + boron application was 3.3 to 24 percent at 30 and 45 DAS and maximum reduction obtained with 300 kg gypsum + boron 1 kg ha⁻¹.

Brecht *et al.* (2004) concluded that the influence of calcium silicate alone, chlorothalonil and calcium silicate + chlorothalonil to St. Augustine grass reduced the gray leaf spot by 7, 65 and 68% at site one; 28, 34 and 59% at site two and 41, 55 and 68% at site three, respectively, when compared with the untreated control. However, when disease severities were analyzed weekly the calcium silicate treatment significantly reduced the percentage of disease at week 2, 6 and 8 at site one and weeks 3 to 8 at site three when compared with control. Foliar spray of boric acid (0.53 %) gave 20 to 64 percent disease control of *Alternaria* blight of mustard (Vishwnath, 1987).

SA is proposed to be a translocatable signal compound in systemic Induced Resistance and interacts with intercellular Ca²⁺ in the induction of chitinase in carrot suspension culture. Application of cations such as Mn, Cu and B can increase the Ca²⁺ cations, and interact with SA and activate systemic Induced Resistance (Reuveni and Reuveni, 1998; Yalpani *et al.*, 1991; and Reuveni *et al.*, 1997).

Radwan *et al.* (2006) reported that spraying of pumpkin leaves with salicylic acid led to recovery from the undesirable effects of ZYMV infection. Salicylic acid treatment increased plant resistance against bean yellow mosaic virus (BYMV) in *V. faba*. This was observed through induction of chloroplast number, reduction in percentage of infected plants, decrease in disease severity and virus concentration of plants treated with SA prior to the BYMV inoculation (Radwan *et al.*, 2008).

Plants exhibiting systemic acquired resistance (SAR) show a decreased level of susceptibility to many types of pathogen. In tobacco treated with solutions of SA, CMV infection is not prevented but the systemic movement of the virus is inhibited and the progress of disease induction is markedly slowed down. SA-induced resistance to viruses in tobacco and *A. thaliana* is mediated in part by a pathway that appears to involve signals transduced through changes in redox or reactive oxygen species (ROS) in the mitochondria (Singh and Awasthi 2004).

It is believed that a signal is generated at the primary infection site and is subsequently translocated to distal plant tissues to activate defence mechanisms. Salicylic acid (SA) is an important component in the signal transduction pathway leading to systemic acquired resistance (SAR) to the entire spectrum of plant pathogens: bacteria, fungi, and viruses (Durner *et al.*, 1997). SAR responses are associated with the accumulation of salicylic acid (SA) and the transcriptional up regulation of a number of genes, including those encoding pathogenesis-related (PR) proteins such as PR1, PR2, and PR5 in both local and systemic tissues.

Radwan *et al.* (2008) demonstrated that harmful effects of bean yellow mosaic virus infection in faba bean could be reduced by spraying SA. It was also shown to alleviate growth inhibition caused by Zucchini Yellow Mosaic Virus (ZYMV) in *Cucurbita pepo* (Radwan *et al.*, 2006). Reduction in okra yellow vein mosaic virus infection was found to be reduced by barium chloride, acetyl salicylic acid and SA (Pun *et al.*, 2000). Mayers *et al.* (2005) recorded a lowered Cucumber Mosaic Virus (CMV) infection in squash and *Arabidopsis thaliana* with SA application.

INCIDENCE OF WHITEFLY

Nutrient management improves plant health, which enables the plant to tolerate the incidence of herbivores, sucking as well as chewing insect pests. So there is an opportunity of changing the preference of insects by meeting optimal plant nutritional requirement via altering the fertilizer or nutritional management. The insect plant relationship may be affected by the application of micro/macronutrients to crop plants (Abro *et al.*, 2004) as nutrient deficient plants are weak and vulnerable to incidence of plant diseases and insect pest attack (Huber and Thompson, 2007 and Marschner, 1995).

Yadav and Singh (2006) reported that the appearance of whitefly in mungbean from second week after sowing. Variation in nutrient level in plants could influence physiological performance of the plants (William *et al.*, 2002), could also change plant-pest relationship especially with herbivorous insect pests (Teja and Bradford, 2000) or alter pest population (Zurina *et al.*, 2010). The average population of cotton leaf curl virus disease vector, whitefly (*Bemisia tabaci*) on twelve cultivars studied ranged from 2.8 to 4.6 per leaf throughout the growth season (Ashraf and Zafar, 1999).

Khattak (2000) studied the defence mechanism of cabbage plant (*Brassica* var. *capitata*) against phloem sucking aphid (*Brassica oleracea* var. *capitata*). Aphid feeding significantly increased the uptake of Ca^{2+} and K^+ , while the other measured elements were markedly decreased.

Gogi *et al.* (2012) reported that the addition of boron in the nutrient management schedule of non-Bt cotton resulted in population fluctuation of whitefly upto 83.1 %. Control plots receiving no nutrient had maximum population of whitefly and nutrient applied plot had minimum. It is therefore recommended that nutrient management should be integrated with other IPM tools.

Rasdi *et al.* (2013) reported that when whitefly non infested, whitefly pre-infested and aphid pre-infested brinjal plants were supplied with nutrient mixture containing 46% calcium nitrate, 0.072% boric acid along with other nutrients N, K, Mg, P, Zn, Cu, S, Mo, Mn, Fe the population of whiteflies and aphids reduced both in non infested and pre infested plants.

Abro *et al.* (2004) reported that the application of micronutrient (Bonus) on cotton plants showed no significant effect on population development of whitefly (0.49leaf^{-1}) compared to control (0.50 leaf^{-1}). Shahzad *et al.* (2014) reported that application of micronutrient on spotted bollworm, *Earias vittella*, significantly reduced the bollworm infestation and maximized the boll volume and opening of bolls and yield. Ahsan *et al.* (2015) suggested that by using micro and macro nutrients, it is possible to develop resistance to wheat aphid in wheat crop.

Caroline (2004) stated that boric acid and borates (naturally occurring compounds containing boron) are recommended as least toxic pesticide for killing insects, mites, algae and fungi by acting as a stomach poison and also as an abrasive on the outer surface of insect and absorbs waxes from surface of the insect causing them to dry out. Application of boric acid 85%, borax 80% @ 1g L^{-1} and 5g L^{-1} on strawberry showed reduction in motile stages of aphids *Tetranychus urticae*, compared to control (Habashy *et al.*, 2010).

Reddy and Rao (1989) reported that optimum nutrient content of the host plants is associated with plant resistance against whitefly. Modification of nutrient management could play a role in regulating the whitefly populations.

Rajaratnam and Hock (1975) studied the effect of boron nutrition on the susceptibility of young tenera oil palm seedlings to red spider mite. Highest population of mites were recorded in seedlings which had received little or no boron in the growing media. Boron is required for the biosynthesis of cyanidin and related polyphenols which are involved in resistance against these insects.

Plants have multitude of biochemical and physical defences, both preformed and wound induced, that protect them from consumption by insect herbivores. Calcium oxalate, the most abundant and wide spread insoluble mineral found in plants of 200 plant families (Whittaker, 1970 and Franceschi and Horner, 1980), could play important role in defence (act against feeding insects), serves as a means of regulating internal Ca stores, sink for oxalic acid and maintain ionic balance (Webb, 1999 and Jauregui-Zuninga *et al.*, 2005).

Asif Khan (2016) reported that, the percent reduction in whitefly population with application of gypsum, boron and gypsum + boron at different level ranged from 8.0 to 84.9, 13.4 to 56.8, 11.7 to 58.3 and 19.8 to 86.2 at 15, 30, 45 and 60 DAS respectively.

GROWTH AND BIOMASS OF DISEASE INFECTED PLANTS

Application of nutrients and growth regulators having role in plant defense mechanism, can affect the disease tolerance or resistance of plants to pathogen affecting their rate of growth. Singh (1981) reported the disease to cause considerable reduction in growth, upto 38.2 percent reduction in plant height of mungbean. Yuri *et al.* (2006) noticed significant positive relationship between calcium treatments and plant height, pre-inoculation mortality rate and treatments solution conductivity, while there was no association between infection severity and calcium treatment.

According to Kumar *et al.* (2015), there was no significant effect on plant height due to the foliar spray of CaSO_4 (0.5, 1.0 1.5 %), $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ (0.25, 0.5, 0.75 %), K_2SO_4 (0.5, 1.0 & 1.5 %) and KCl (0.5, 1.0, 1.5 %) in mustard infected with alternaria blight. But CaSO_4 at 1 % resulted in more plant height (2.07 m) followed by $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ at 0.25 % (2.06 m) over check (1.89 m).

In a study effect of Ca nutrition on resistance of tomato against bacterial wilt induced by *Ralstonia solanacearum*, Jiang *et al.* (2013) noticed that the plant biomass in low Ca (0.5 mM) treatment was significantly lower (3.87 g) than medium (5.0 mM) and high Ca (25 mM) treatments, which were 4.71 and 5.10g respectively. Although biomass values continued to increase as Ca increased, there was no significant difference in biomass between the medium and high Ca treatments, suggesting that plant physiological sufficiency was met by the high Ca rate.

Ali *et al.*, (2014) studied the response of faba bean (infected with chocolate spot disease) to the foliar application of nutritional elements and fungicides and found that the highest plant height was obtained by the application of fungicide Blight Stop followed by Ca at 6 ml L⁻¹, then boron 2 ppm and molybdenum 4ppm. Higher levels of boron (4 and 6 ppm) and lower levels of Ca (2 ml L⁻¹) had no significant effect on faba bean plant height compared to check. Number of branches plant⁻¹ was more with blight stop followed by boron 2 ppm than calcium 6 ml L⁻¹.

Asif Khan (2016) reported that, total dry matter production increased by 9.1 to 44.0 percent with application of gypsum alone. Boron alone increased it by 19.4 to 24.2 percent and combined application of gypsum + boron showed 20.5 to 62.5 percent increase in accumulation of total dry matter.

Reduction in plant height was minimal with application of borax (10.6 %), zinc sulphate (24 % Zn) and gypsum (29.2 % Ca, 18 % S) @ 2, 2 and 20 Kg ha⁻¹ respectively in YMV infected mungbean compared to control. This was attributed to lower disease ensured by plant nutrients (Islam *et al.*, 2002).

While studying management of chocolate spot disease in broad bean (*Vicia faba*) through optimal application of nutrients Abeer *et al.* (2014) observed that nutrients differed in plant height incremental effect in the foliar spray of B @ 2 ppm > Ca 6 mL⁻¹ > Mo @ 4 ppm.

YIELD OF DISEASE INFECTED PLANTS

MYMV virus causes more than US\$ 300 million loss every year in different leguminous crops (Varma *et al.*, 1992). Yield losses due to this disease vary from 5 to 100% depending upon disease severity, susceptibility of cultivars and population of whitefly (Rathi, 2002).

Early infected plants had more severe symptoms than the later infected ones. Quantum of loss varied with the age at which blackgram plants were infected; loss could be 100% when plants were infected upto three weeks age (Nene, 1972). Singh *et al.* (1982) and Sharma and Varma (1984) also reported that yield loss in mungbean varied with time of infection.

The yields were reduced by 23-74% in MYMV affected *V. aconitifolia* (Vir, 1984). MYMV caused 76 %, 52 % and 15 % reduction in yields of soybean when infected at the pre-bloom, bloom and post bloom stages of growth, respectively (Dhingra and Chenulu, 1985). They also observed that MYMV caused 61 % reduction in the number of nodules. In soybean MYMV caused 37 % yield loss (Gill and Ratual, 1988). Haq *et al.* (1992) also reported that there was significant decrease in plant height, number of branches, number of pods/plant, number of seeds/pod, 100 seed weight and yield. Over a broad geographic range, the yield reductions between 10% and 85% have been reported (Varma *et al.* 1992 and Khattak *et al.* 2000).

It has been demonstrated that plant nutrition reduced the infection and the incidence of papaya ring spot virus and increased the production of papaya (Ray *et al.*, 1999). Foliar spray of calcium as calceiven @ 6 ml L⁻¹ on chocolate spot disease plant infected faba bean gave the highest values for number of pods, 100 seed weight and seed yield followed by Blight spot and then the boron @ 2 ppm compared to control (Ali *et al.*, 2014).

Jia-Feng (2013) reported that the reduction in grain yield ranged from 39.9 to 51.5 % due to MYMV. Winter urdbean varieties are highly susceptible to yellow mosaic and leaf crinkle, showed 67-100 % loss of grain yield in the severely infected plants (Bashir *et al.*, 2006).

Pramanik and Ali (2001) reported that the single application of boron significantly reduced the severities of yellow mosaic in winter mungbean and increased the grain yield (24.8 to 30.6%) by increasing pods plants⁻¹ and 100 seed weight. This is due to the balanced fertilization which reduced the severity and incidence of disease by increasing the metabolic activity. Similar findings were reported by Zaman *et al.* (1996).

Application of borax (10.6 % B), zinc sulphate (24 % Zn) and gypsum (29.2 % Ca, 18 % S) @ 2, 2 and 20 kg ha⁻¹ reduced the yellow mosaic virus disease incidence in mungbean to 21 to 26% and favoured by the crop to produce better with minimum reduction in pod length, 100 seed weight and seed yield compared to control. Lower percentage of disease severity give rise to minimum reduction in yield parameters of mungbean due to YMV disease, which is because of lower disease severity insured by plant nutrient (Islam *et al.*, 2002). Plots that received the treatment, combined application of B + Zn+ gypsum shown the highest number of pods plant⁻¹, number of seed pod⁻¹, 100 seed weight and seed yield (Islam *et al.*, 2002)

Morsy (2012) found that Ca, Zn and Mn treatments induced resistance in faba bean plants against rust disease (*Uromyces viciae fabae*) and improved the plant growth and yield over control by increasing pod number plant⁻¹ and 100 seed weight.

Chand and Verma (1983) reported that mungbean cultivars might suffer 66.6 percent decrease in plant yield and 25.7 percent decrease in 1000 seed weight due to MYMV.

Bimal *et al.*, (2001) studied the effects of soil application of boron and molybdenum under field conditions on the severity and incidence of MYMV and yield in winter mungbean during 1996-97 and 1997-98. The single or combined application of boron (2kg ha⁻¹) and molybdenum (1.5kg ha⁻¹) significantly reduced the severity of MYMV and increased the yield (24.8- 30.6 %).

Warncke (2005) reported that boron application at a concentration of 0.5 g L⁻¹ ameliorate the internal black spot in cranberry bean seed and showed remarkable effect on development of pods. Asif khan (2016) reported that in blackgram (LBG-623), gypsum addition improved yield by 2.3 folds, boron by 1.6 to 1.7 folds and gypsum + boron by 1.8 to 2.9 folds.

Abd El-Hai *et al.* (2009) found that, seed presoaking in combination of salicylic acid with citric acid caused significant increase in plant height, number of leaves per plant and plant yield in sunflower plant infected with *Rhizoctonia solani* and *Macrophomina phaseolina*.

PHYSIOLOGY OF PLANT DEFENCE

Multiple lines of evidence generated by various researchers in different crops suggested a central role of calcium in recognition of plant defensive response to pathogens. When plants are challenged by pathogens and pathogen derived elicitor compounds, cytosolic free Ca concentration increases, calcium binding sensor molecules are activated, specific responses including oxidative burst are initiated leading to hypersensitive response and expression of defence associated genes (Yuri *et al.*, 2006).

In tomato plants inoculated with *R. solanacearum* (Bacterial wilt) treated with three levels of calcium: 0.5, 5 and 25 mM, representing low, medium and high concentration respectively, peroxidase (POD) activity was influenced by Ca rate and increased to the highest point in first 24h after inoculation. POD activity was lowest with the low Ca, intermediate in the medium Ca and highest with the high Ca (40.70, 72.35, 99.09 $\mu\text{g FW}^{-1}$ or 86 %, 306 % and 307 % greater than it was before inoculation, respectively). Polyphenol oxidase (PPO) activity increased sharply during first 12h after inoculation and remained high compared to before inoculation. PPO activity with low, medium and high Ca at 12h after inoculation was 77.45, 88.29 and 107.24 $\mu\text{g FW}^{-1}$ (109 %, 27 % and 316 %) higher than it was before inoculation. A negative correlation was found between Ca concentration, level of H_2O_2 , POD and disease severity (Jia-Feng *et al.*, 2013).

Tobias *et al.* (1993) reported that most of Ca (60%) present in cells accumulate in the region of middle lamella by forming ionic bonds inside and between pectin polymers and reduce the capacity of some fungal enzymes to degrade cell wall components and thus increase the resistance of cell wall.

Foliar spray of calcium as calciven @ 4 ml L^{-1} gave the highest values of chlorophylls a and b followed by boron 2 ppm, then molybdenum 4 ppm in *Vicia faba* infected with chocolate spot disease (Ali *et al.*, 2014). Dantre *et al.* (1996) working with biochemical changes in resistant and susceptible cultivars of soybean to yellow mosaic virus reported that, chlorophyll (a, b and total chlorophyll) reducing sugar, non reducing sugar and total sugar were decreased while total amino acid, total phenol and ribonucleic acid (RNA) were increased in infected leaves of resistant and susceptible cultivars.

Mali *et al.* (2000) found reduction in chlorophyll ('a' and 'b') in diseased plants of moth bean genotype infected with yellow mosaic virus. The TMV infection led to a decrease in chlorophyll a+b, total phenols and soluble protein by the rate of 47.89, 7.89 and 61.35% in infected leaves. As well as increase in the phenolic antioxidant compounds in infected leaves was observed.

Michael and Krishnaswamy (2009) studied the effect of YVMV infection on PS II efficiency and thylakoid membrane protein changes in bhendi and found a reduction in photosynthetic pigments, soluble proteins, nitrate reductase activity and ribulose - 1, 5 biphosphate carboxylase activity thereby a marked inhibition of PS II activity.

Working with effect of uniconazole on polyphenol metabolism in the leaves infected with MYMV in resistant and susceptible cultivars, Jindal *et al.* (1998) found that there was significant increase in peroxidase, polyphenol oxidase and phenylalanine ammonia lyase in susceptible cultivar compared to resistant cultivar. They also reported that tyrosine ammonia lyase increased in treated healthy leaves of both cultivars and phenols, orthodihydroxy phenols, flavonols and sugars increased in treated healthy plants indicating the resistance mechanism against MYMV.

Haque *et al.* (2005) reported that ZYMV infection reduced chlorophyll a, chlorophyll b, total chlorophyll, calcium, magnesium, potassium, and iron content of pumpkin leaves, while organic carbon, nitrogen, protein and phosphorus content were increased compared to healthy ones. They also reported that infected leaves showed a decreased rate of photosynthesis and an increased rate of respiration.

Mali *et al.* (2000) reported significant decrease in levels of soluble carbohydrates, starch, chlorophyll and orthodihydroxy phenols in mothbean genotypes infected with MYMV. They also reported that the levels of these biochemical constituents were more in susceptible cultivars than in resistant ones. Gill and Singh (2000) established that there was reduced amount of chlorophyll in the leaves of MYMV infected mungbean cultivar ML-267, affecting photosynthetic activity. Lehto *et al.* (2003) reported that tobacco infected with TMV shows strong yellow and green mosaic symptoms in developing leaf, yellowing of fully expanded leaves, and distinct malformation of chloroplast. They also established that chlorosis

in mature leaves was accompanied by depletion of the entire photosystem II (PSII) core complex.

Rust infected wheat plants when applied with B (0.1 g L^{-1}), Zn and Mn @ 4 g L^{-1} and Tilt individually the activity of both peroxidase and polyphenol oxidase of leaves compared increased. Boron showed the highest activity of oxidative enzymes followed by Mn and Zn, whereas, the least enzyme activity was recorded with Tilt. Total phenols were higher in wheat leaves treated with B, Zn and Mn. Boron ranked the best treatment in increasing the concentration of total phenols followed by Zn, Tilt and Mn (Somaya and Seham, 2015).

A rapid accumulation of phenols at the infection site is the first step of defence mechanism in plants which restricts or slows the pathogen growth because of its action as antioxidant, antimicrobial and photoreceptor (Lamba *et al.*, 2008). It is well known that plant phenols are highly effective antioxidants. Ca as Calciven @ 6 ml L^{-1} , boron @ 2 ppm and molybdenum @ 4 ppm as foliar spray increased the total phenol content significantly (Ali *et al.*, 2014) in faba bean infected with chocolate spot disease and also reported that boron @ 2 ppm increased the phytoalexins (wyeron acid) level of faba bean.

Dobermann and Fairhurst (2000) noted the function of B in enhancing lignin formation. The lack of B can cause distortion of cell wall structure and also inhibit biosynthesis of lignin in plants (Marschner, 1995). The function of B in reducing disease susceptibility could be because of its function in cell wall structure, cell membrane permeability, stability, rigidity or its role in metabolism of phenolics or lignin. Lewis (1980) has put forward the hypothesis that boron is involved in the enhanced formation of phenolics phytoalexins in response to microbial infections.

Modifications in the antioxidant status were observed by changing some antioxidant enzymes activities and contents of antioxidant metabolites following BYMV infection and SA treatment (Radwan *et al.*, 2010). Changing antioxidant status and accumulation of some antioxidant metabolites as well as pronounced alterations in the protein composition indicate a kind of plant response against pathogen invasion and in case of SA treatment it was considered a way by which the defence response was activated.

SA increases the phenolic compounds that have ability to interfere and enhance the resistance against pathogen (Vimala and Suriachandraselvan, 2009). SA

also increases the pathogen-related proteins which have antimicrobial activity and thus increase the resistance against viral diseases (Huijsduijnen *et al.*, 1986).

SA-induced resistance to viruses in tobacco and *A. thaliana* is mediated in part by a pathway that appears to involve signals transduced through changes in redox or reactive oxygen species (ROS) in the mitochondria (Singh *et al.* 2004).

Salicylic acid synthesized in cells can move freely in and out of the cells, tissues and organs (Kawano 2004) and this movement is finely regulated by ROS and Ca^{2+} (Chen and Kuc, 1999 and Chen *et al.*, 2001). Supplementation of tobacco cell suspension culture with higher concentration of salicylic acid resulted in a de novo induction of SA excretion across the membrane which was mediated by the generation of ROS and activation of a cascade of Ca^{2+} signalling and protein phosphorylation. However, exogenous supply of lower concentrations of salicylic acid did not require a de novo synthesis of proteins and was found independent of ROS, Ca^{2+} and protein kinases (Chen *et al.*, 2001).

Boron promotes stability and rigidity of the cell wall structure and therefore supports the shape and strength of the plant cell. Furthermore, B is possibly involved in the integrity of the plasma membrane (Marschner, 1995).

Shoal and Bajaj (1993) reported that MYMV infection in mungbean resulted in malfunctioning of polyphenol metabolism by increasing total sugars, free amino acids and total phenols in both resistant and susceptible varieties, whereas orthodihydroxy phenols and flavonoids increased only in the resistant varieties upon MYMV infection.

It has been reported that phytoalexins production in soybean, carrots and potato has been shown to be dependent on the maintenance of an external Ca supply. The performance of plant cell membrane under the stress of pathogen attack might depend on the Ca^{2+} status of the membrane and the availability of Ca^{2+} inside and outside the cell (Gurr *et al.*, 1982).

Wei *et al.* (2002) stated that Ca^{2+} increased the activity of peroxidase and PPO by 0.342 % and 0.022% respectively and enhanced the carnation resistance of leaf spot disease. PPO is normally found in its latent form and Ca has been reported to

modify the conformational states of these enzymes and boost its activity (Ruiz *et al.*, 2003).

Asif Khan (2016) studied the effect of YMV in blackgram reported that calcium and boron nutrition increased the total phenols, activity of peroxidase and PAL content in both healthy and disease leaves and thus induced the defence mechanism.

Ca, B, Mg and Zn maintain the structural integrity and permeability of the cell membrane and prevent pathogen attack (Rice, 2007). The electrolyte leakage was greater in nutrient deficient treatments than in the full strength nutrient solution treatment and it was correlated with the extent of damage due to various stresses including diseases (Shi *et al.*, 2006 : Tamuli *et al.*, 2010 and Kirandeep *et al.*, 2011).

Huber (1980) and Maathuis (2009) reported that Ca, B and Zn have significant roles in maintaining structural integrity and controlling permeability of cell membranes. In addition nutrients are responsible for production of anti microbial compounds like phytoalexins, phenols, flavonoids and other defence compounds.

Arora *et al.* (2009) studied the effect of MYMV on pathogenesis related enzymes in moth bean and reported the increased activity of peroxidase, polyphenols oxidase and phenylalanine ammonia lyase and decreased activity of catalase in infected leaves.

The function that B has in reducing disease susceptibility could be because of the function of B in cell wall structure, cell membrane permeability, stability or function and its role in metabolism of phenolics or lignin (Brown *et al.*, 2002).

Plants respond to pathogen infection by enhancing the activity of defence enzymes like peroxidase, phenylalanine ammonia lyase and polyphenol oxidase. Arora *et al.* (2009) found a sharp rise in the activity of POD in MYMV infected mothbean leaves. Increased PO activity was also shown to be induced by exogenous application of SAR elicitors like SA (Radwan *et al.*, 2008), which contributed to alleviation of the effects of zucchini yellow mosaic virus in *Cucurbita pepo*.

Increase in phenol content of plants and corresponding decrease in symptom expression in SA treated blackgram plants infected with MYMV was observed by Kundu *et al.* (2011).

Salicylic acid application was reported to induce chloroplast number besides resistance in faba bean infected with bean yellow mosaic virus (Radwan *et al.*, 2008). Increased chlorophyll content and photosynthetic activity alongside elevated SAR was observed in SA treated cowpea plants (Chandra and Bhatt, 1998). Kundu *et al.* (2011) found increased chlorophyll content besides elevated levels of SAR in MYMV infected blackgram plants.

PLANT NUTRIENT (K, Ca, B) STATUS

Crop plant receiving a calcium augmented nutrition regime often have higher tissue Ca concentration and experienced reduction in pathogen damage via enhanced resistance to infection or more moderate symptoms expression (Volpin and Elad, 1991 and Yamazaki and Hoshina, 1995).

Among the cotton leaf curl virus susceptible, moderately resistant and resistant cultivars (CIM100 and Cedix) had significantly higher Ca content in their leaves than the other CVs. A marked decrease in K and Ca concentration in diseased leaves in comparison with healthy ones was observed only in S-12, the susceptible cultivar (Asraf and Zafar, 1999). In contrast to this, the diseased leaves of NIAB –krishna (moderately resistance), almost uniform maintenance of K in both healthy and diseased leaves was observed.

Rains and Epstein (1967) and Johansen *et al.* (1968) indicated the cross linkage of pectic substances by divalent cations which is affected more by the ratio of divalent / monovalent cations than by the concentration of any one cation.

Exogenous application of SA has also been found to shift the nutrient status leading to a decreased uptake of phosphate and potassium by roots and this decrease was found to be dependent on pH, suggesting a higher activity of protonated form of SA (Hayat and Ahmad, 2007).

For several plant diseases in which pectic enzymes have been implicated, increase in calcium content of plant tissue correlates with increased resistance (Bateman and Miller, 1966). Virus inoculation significantly decreased the tissue Ca concentration of tomato plants from 38.03 to 36.03 g kg⁻¹. Tissue Ca concentration was significantly increased by foliar application of Ca from four different sources- Ca Cl₂.2H₂O, Ca(C₂H₃O₂)₂ and Ca NO₃.4H₂O + 0.05 M Na EDTA. Higher K concentration was also obtained from Ca (C₂H₃O₂)₂ sprayed plants.

In tomato plants inoculated with *R. solanacearum* (Bacterial wilt), tissue Ca was the lowest for low Ca compared with medium and high Ca treatments (3.58, 12.35, 17.15g kg DW⁻¹ in shoot respectively). Thus, the Ca in plants receiving low Ca was 71.01% (in roots) and 64.84% (in shoot) less than in plants receiving medium level of Ca, but it was significantly higher in plant receiving the highest Ca (38.87% in roots and 25.99% in shoots) (Jia-Feng *et al.*, 2013). They found significant negative correlation between the severity of bacterial wilt and Ca in tomato root. The correlation with shoot Ca was not significant indicating that the reduction in disease severity by Ca was mainly due to increased Ca in tomato roots. Bacterial wilt is a soil born disease where plant roots are infected first and Ca root cells may function in the cell wall to protect the invasion of pathogen. Bacterial wilt resistant tomato cultivars have been characterized by high Ca uptake (Yamazaki *et al.*, 1996 and Yamazaki, 2001). Sugimoto *et al.* (2008) reported that *Phytophthora* stem rot disease reduction in soyabean and was related to the increased Ca uptake by plants.

Asif Khan (2016) conclude that in LBG-623 gypsum + boron application increased the boron content at 15 DAS by 1.2 to 1.5 folds and calcium content increased by 1.4 to 1.5 folds.

According to the finding of Sherwood and Huisingh (1970) from the study of calcium nutrition and resistance of alfalfa to *Ditylenchus dipsaii*, increased nutrient Ca²⁺ concentration resulted in increased Ca²⁺ content, decreased Na⁺ and K⁺ content and unchanged Mg content in buds and cotyledons. Increased nutrient Ca²⁺ resulted in increased divalent / monovalent cation ratios (Ca²⁺ + Mg²⁺ / Na⁺ + K⁺). Resistance to pathogen reproduction was correlated more closely with the divalent / monovalent cation ratio than with Ca²⁺ content of tissue.

Chapter III

MATERIAL AND METHODS

A field experiment was conducted to study the “Effect of Calcium, Boron and Salicylic acid on tolerance of blackgram (*Vinga mungo* (L.) Hepper) to yellow mosaic virus” at Agricultural College Farm, Bapatla during *rabi* season of 2016-17. The methods of conducting experiment, materials and inputs used during the experiment action period are given in detail in this chapter under different heads.

3.1 EXPERIMENTAL SITE

The experiment was conducted in Northern Block, Field No. 26 of Agricultural College Farm, Bapatla, Guntur district, Andhra Pradesh. It is 9 km away from Bay of Bengal in agro-climatic zone III (Guntur, Nellore and prakasam districts of Andhra Pradesh) at an altitude of 5.49 m above mean sea level, 15⁰54' Northern latitude and 80⁰25' Eastern longitude.

3.2 WEATHER CONDITIONS

The weekly meteorological data recorded during the crop growth period *i.e.*, from 01-10- 2016 to 31- 12- 2016 are presented in table 3.1.

3.2.1 Temperature (°C)

The weekly mean maximum and minimum temperatures ranged from 29.5⁰C to 34.4⁰C and 16.5⁰C to 24.8⁰C, respectively, while the average maximum and minimum temperature during the crop growth period were 32.3⁰C and 20.6⁰C, respectively.

3.2.2 Relative Humidity (RH) (%)

The average RH during the crop growth period ranged from 69.2 to 91.4 % (8:30 hrs) and 50.2 to 74.4 % (at 17:30 hrs), averaged relative humidity during crop growth period was 82.0 % (8:30 hrs) and 58.3 % (at 17:30 hrs).

3.2.3 Rainfall (mm)

The average rainfall received was 30.6 mm in three rainy days.

Table 3.1. Weekly meteorological data during the crop growth period

Standard week	Date and month	Temperature(°C)		R.H. (%)		Rainfall (mm)	No. of rainy days
		Max	Min	08:30 hrs	17:30 hrs		
40	01-07 Oct	33.5	24.8	85.2	74.4	19.6	1
41	8-14 Oct	33.7	24.6	81.4	65.7	5.6	1
42	15-21 Oct	34.2	21.1	71.1	50.2	0	0
43	22-28Oct	34.4	20.8	69.2	57.4	0	0
44	29 Oct-04Nov	33.0	23.6	84.0	67.2	0.5	0
45	05-11Nov	33.4	20.4	73.2	50.5	0	0
46	12-18Nov	31.7	22.7	84.4	73.4	0	0
47	19-25Nov	31.2	18.9	87.0	61.1	0	0
48	26 Nov-02Dec	33.0	18.7	84.7	61.2	4.7	1
49	03-09Dec	31.5	19.7	82.4	62.0	0	0
50	10-16Dec	29.5	20.4	84.1	74.0	0.2	0
51	17-23Dec	30.9	16.5	85.4	59.4	0	0
52	24-31Dec	30.5	16.7	91.4	62.5	0	0
Total						30.6	3
Mean		32.3	20.6	82.0	58.3		

3.3 SOIL AND ITS CHARACTERISTICS

Initial soil sample from the experimental field was collected at random from 0 to 30 cm depth and a composite sample was analyzed for physical and physico-chemical properties. The results of the analysis (Table 3.2) indicated that the soil was clay loam in texture, slightly alkaline in reaction (p^H 7.9), high in organic carbon (0.6%), available nitrogen (310.3 kg ha^{-1}), available phosphorus (23.0 kg ha^{-1}), available potassium (244.6 kg ha^{-1}), available calcium (29 meq) and sufficient in boron (0.578 ppm).

3.4 EXPERIMENTAL DETAILS

The experiment was laid out in randomized block design (RBD) with three replications. The field layout is depicted in fig 3.1.

Location	:	Agricultural College Farm, Bapatla
Crop	:	Blackgram (<i>Vigna mungo</i> (L.) Hepper)
Season	:	Rabi 2016-17
Design	:	Randomized block design
Plot size	:	4m x 3m
Spacing	:	30cm x 10cm
Date of sowing	:	22/10/2016
Replications	:	3
Treatments	:	10
T ₁	:	Control
T ₂	:	Gypsum @300 kg ha ⁻¹ + Boron@1kg ha ⁻¹
T ₃	:	0.3% Calcium (CaNO ₃ .4H ₂ O) spray at 10, 20 and 30 DAE
T ₄	:	0.25% Boron (Borax) spray at 10, 20 and 30 DAE
T ₅	:	50ppm Salicylic acid spray at 10, 20 and 30 DAE
T ₆	:	Complete nutrient solution spray (Hoagland solution) at 10, 20 and 30 DAE
T ₇	:	T ₂ +T ₅
T ₈	:	T ₃ +T ₅
T ₉	:	T ₄ +T ₅
T ₁₀	:	T ₆ +T ₅

Table 3.2. Soil characteristics of the experimental field

S. No.	Particulars	Value	Method of Analysis
A)	Mechanical analysis		Bouyoucos Hydrometer method (Bouyoucos, 1962)
	Sand (%)	20.7	
	Silt (%)	38.6	
	Clay (%)	40.7	
	Textural class	Clay loam	
B)	Chemical analysis		
1	pH (1:2.5–soil water suspension)	7.9	Glass electrode method (Jackson, 1973)
2	EC (dS m ⁻¹)	0.29	Conductivity bridge (Jackson, 1973)
3	Organic carbon (%)	0.6	Walkley and Black's modified method (Walkley and Black, 1934)
4	Available nitrogen (kg ha ⁻¹)	310.3	Alkaline permanganate method (Subbaiah and Asija, 1956)
5	Available phosphorus (kg P ₂ O ₅ ha ⁻¹)	23.0	Olsen's extractant method (Olsen <i>et al.</i> , 1954)
6	Available potassium (kg K ₂ O ha ⁻¹)	244.6	Neutral normal ammonium acetate method (Jackson, 1973)
7	Calcium (meq/100 g)	29	Versanate titration method (Jackson,1967)
8	Boron (ppm)	0.578	Hot water extraction (Berger and Troug, 1939)

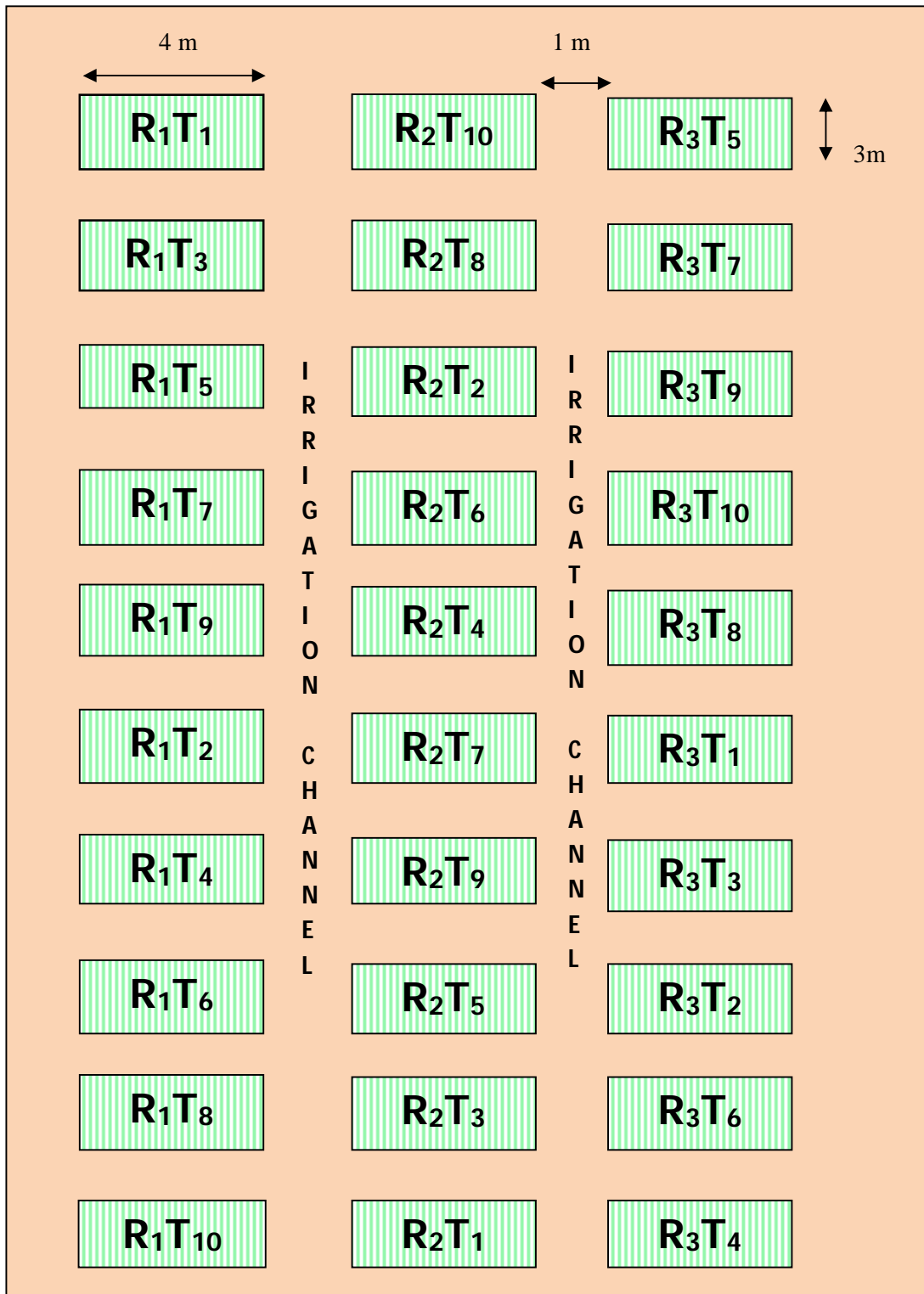
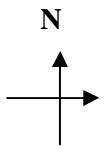


Fig. 3.1 Layout Plan of Field Experiment

Design : RBD
 Replications : Three
 Season : Rabi 2016-17

Spacing: 30 cm x 10 cm
 plot size: 4 m x 3 m
 Treatments: 10

3.5 VARIETY DESCRIPTION

LBG-623

Photo insensitive non season bound variety. Plant height is 20 to 39 cm. Suitable for all seasons. Early maturing determinate bushy variety. Plants are compact in habit with dark green leaves of ovate, lanceolate leaflets. Pods are glabrous. Seeds are bold, black shiny with green tinge. Susceptible to YMV disease. Duration 61-78 days. Yield potential is 16-17.5 q ha⁻¹.

3.6 FIELD OPERATIONS

The following field operations were carried out during the experimentation period.

3.6.1 Land Preparation

The land was prepared by thorough ploughing and harrowing and soil was brought to a fine tilth. Weeds and stubbles were removed, the field was levelled and then laid out into plots as per the design given in Fig 3.1.

3.6.2 Fertilizer Application

Nitrogen @ 20 kg N ha⁻¹ and phosphorus @ 50 kg P₂O₅ ha⁻¹ were applied as basal dose before sowing of crop in the form of urea and single super phosphate, respectively.

3.6.3 Seeds and Sowing

Blackgram variety LBG-623 was selected for study. The seed material was obtained from ARS, Podalakur, SPSR Nellore. Sowing was done by line sowing method on 22/10/2016 with a spacing of 30 cm between the rows and 10 cm between plants within the row.

3.6.4 Thinning

After 10 days of sowing, seedlings were thinned out to maintain the interplant spacing of 30 x 10 cm and one plant per hill.

3.6.5 Hand Weeding

First hand weeding was done at 15DAS followed by second hand weeding at 30 DAS.

3.6.6 Herbicide Application

The post emergence herbicides *viz.*, imazethapyr 10% EC @ 50 g a.i ha⁻¹ was sprayed at 15 DAS using knapsack sprayer fitted with flat fan nozzle at spray volume of 500 L ha⁻¹.

3.6.7 Plant Protection Measures

All the crop protective measures were taken up regularly to prevent the incidence of pests and diseases. Monocrotophos @ 1.5 ml L⁻¹ was sprayed twice at 5 days interval from 30 DAS to prevent the attack of *Spodoptera* and *Heliothes* caterpillars.

3.6.8 Irrigation

Three irrigations were given to the crop *i.e.*, at sowing, 20 DAS and at flowering stage.

3.6.9 Harvesting and Threshing

The crop was harvested at physiological maturity in all plots by pulling out the plants along with roots excluding the border rows. The plants were dried in the sun and later threshed plot wise for separation of seeds from the pods. Seeds from each micro plot were weighed after drying. Yield per net plot was recorded and finally yield ha⁻¹ was computed.

3.7 COLLECTION OF EXPERIMENTAL DATA

Data were collected starting from 15 DAS to harvest by using the methods of non-destructive and destructive growth analysis. Five adjacent uniform sized plants in a single row per each plot (excluding border rows) were selected and tagged for recording the data on morphological parameters. For destructive growth analysis, five

uniform sized plants adjacent in a single row per each plot were selected and dug out with roots to record the observations for dry matter estimation. Yield and yield components were estimated from the sample collected at the time of harvesting.

3.7.1 Morphological Parameters

1 Disease incidence (%)

MYMV incidence was scored by counting the total number of plants infected in each treatment plot and per cent disease incidence was calculated by using the formula given by Alice and Nadarajan (2007).

$$\text{Disease Incidence} = \frac{\text{Number of plants infected in a row}}{\text{Total number of plants in a row}} \times 100$$

2. White fly count

Population of white fly was estimated by the visual count technique (Salam *et al.*, 2009). Whitefly count was taken on top three trifoliolate leaves plant⁻¹ during early hours of day from five randomly selected plants in each replication of all treatments at 15, 30 and 45 DAS and the average was computed.

3. Number of branches

The number of branches per plant was counted for five plants selected and tagged for non-destructive measurement. The mean value was calculated and expressed as number of branches plant⁻¹.

3.7.2 Physiological Parameters

1. Dry matter accumulation (g plant⁻¹)

The total dry matter accumulation and its partitioning was estimated from the five adjacent uniformed sized plants sampled from each treatment in three replications and then separated into roots, stems, leaves and pods. The plant parts were dried to a constant weight in hot-air oven at 80°C for two days and the dry weights were recorded and expressed in g plant⁻¹.

3.7.3 Yield and Yield Components

The observations pertaining to yield components and yield were taken at the time of harvest.

1. Number of pods plant⁻¹

The number of pods from the five plants tagged was counted and the average was expressed as number of pods plant⁻¹.

2. Number of seeds pod⁻¹

The number of seeds per pod was counted for the pods collected from five tagged plants and the mean value was calculated.

3. Pod Weight (g plant⁻¹)

The pods obtained in each of the five plants tagged earlier in each plot were dried in shade to around nine percent seed moisture content and then weighed. The average was worked out and expressed as pod weight in g plant⁻¹.

4. 100 seed weight (g)

A lot of seeds were drawn at random from each treatment in three replications and weighed. Number of seeds constituting the sample was counted and from these values 100 seed weight was computed.

4. Seed yield (g plant⁻¹) or (kg ha⁻¹)

The number of seed collected from the pods obtained from five tagged plants were collected, weighed and expressed as seed yield plant⁻¹ in grams. Harvested plants were kept in the field for drying. After drying the pods, total pods from a plot were threshed and the resulted seed yield was calculated for a net plot area and it was computed to hectare and expressed as kg ha⁻¹.

3.8 BIOCHEMICAL PARAMETERS

3.8.1 Peroxidase activity (PO)

Peroxidase activity was determined according to Hammer Schmidt *et al.* (1982).

Reagents:**a. 0.1M Sodium phosphate buffer, p^H: 6.5.**

1.76g of Na₂HPO₄ and 3.24 g of NaH₂ PO₄ were dissolved in double distilled water, volume was made to 50 ml and p^H was adjusted to 6.5 with NaOH.

b. Pyrogallol 0.05M

1.26 g of Pyrogallol was dissolved in 200ml of distilled water.

c. 1% Hydrogen peroxide

1ml of H₂O₂ is diluted to 100ml with distilled water.

Procedure:

The enzyme extract from leaves of blackgram plants was prepared by homogenizing 1g of sample in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) at 4⁰ C. The homogenate was centrifuged at 10000 rpm at 4⁰ C for 30 min in a cooling centrifuge. The supernatant was used as enzyme source. In a sample cuvette, 1.5 ml of 0.05 M pyrogallol and 100 μl of enzyme extract were taken. In the reference cuvette, 100 μl of inactivated enzyme extract (by boiling) was taken along with 1.5 ml of 0.05 M pyrogallol. The reading for absorbance was adjusted to zero at 420 nm in a spectrophotometer with the reference sample. To initiate the reaction, 100 μl of 1% hydrogen peroxide (v/v) was added to the sample cuvette and the absorbance values were read at 420 nm. The enzyme activity was expressed as changes (Δ) in absorbance min⁻¹g⁻¹ fresh weight.

3.8.4 Phenylalanine Ammonia-Lyase (PAL)

The activity of Phenylalanine Ammonia Lyase activity was determined according to Dikerson *et al.* (1984).

Reagents:**a. 0.1M Sodium borate buffer, p^H 7.0**

38.6g of sodium tetraborate and 6.2g of boric acid were dissolved to 1000ml with distilled water and adjusted the p^H with boric acid.

b. 0.1g of polyvinyl pyrrolidone**c. 12mM L-Phenylalanine**

19.8mg of L-Phenylalanine was dissolved in 10ml of water and p^H was adjusted with 0.1N KOH at 8.7.

d. Borate buffer

4.76 g Boric acid and 2.54 g Borax were dissolved to 1000mL with double distilled water and p^H was adjusted to 8.4 with NaOH.

Procedure:

Blackgram leaves weighing 1 g were homogenized in 5 ml of 0.1 M sodium borate buffer (pH 7.0) containing 0.1 g insoluble polyvinyl pyrrolidone (PVP). The extract was filtered through cheese cloth and the filtrate was centrifuged at 10000 rpm for 30 min. at 4⁰ C. The supernatant was used as an enzyme source. PAL activity was determined as the rate of conversion of L-phenylalanine to transcinnamic acid at 290 nm as described by Dickerson *et al.* (1984). Samples containing 0.4 ml of enzyme extract were incubated with 0.5 ml of 0.1 M borate buffer pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30⁰ C. In the reference cell, 0.4 ml of the inactivated enzyme extract (by boiling) was taken along with 1 ml of borate buffer. PAL activity was determined as the rate of conversion of L-phenylalanine to transcinnamic acid at 290 nm. The amount of transcinnamic acid synthesized was calculated using the extinction coefficient of 9630 M⁻¹cm⁻¹ (Dickerson et al., 1984). Enzyme activity was expressed on a fresh weight basis (η mole of transcinnamic acid min⁻¹g⁻¹).

3.8.5 Estimation of Total Phenols

The total phenols were determined by AOAC colorimetric method (Anonymous, 1965).

Reagents

- a. 95% Ethanol
- b. Foline-ciocalteau Reagent
- c. Saturated Na_2CO_3 .
- d. Standard (100mg Catechol in 100ml water)

Procedure:

One gram of blackgram leaf sample was put in Erlenmeyer flask containing 20 ml of boiling 95% ethanol. The contents were boiled for 8 minutes, cooled, shaken and filtered through Whatman No. 1 filter paper on a Buchner funnel. The filtrate was concentrated to 2-3 ml on a water bath. The concentrated extract was diluted to 20 ml with distilled water.

One ml of the above phenolic extract was taken in a test tube. Two ml of Folin- Ciocalteu's reagent diluted by 2 ml of distilled water followed by 5 ml of saturated sodium carbonate were added to it. The solution was mixed well and the absorbance of the sample was measured after 30 min. at 750 nm by spectrophotometer. A standard curve was prepared by using catechol solution and plotted the absorbance at 750 nm on Y-axis against μg catechol on X-axis. The total phenol contents were calculated with the help of the standard curve and expressed as mg of phenol/ 100 mg tissue on fresh weight basis

3.8.6. Membrane Injury Index

Membrane injury index was determined according to the method of Premachandra *et al.*, (1990).

Leaf discs (0.2g) were thoroughly washed in running tap water and double distilled water and then placed in 20mL. of double distilled water at 56°C for 1 hour in treatment vials. The control vials were placed at room temperature ($18-25^{\circ}\text{C}$). After incubation of vials at 10°C for 24 hours samples were brought to room temperature

and conductivity of medium is measured by electrical conductivity meter and recorded as C_1 for control and T_1 for treatment conductivities. Subsequently, the samples were placed in boiling water bath (100°C) for 15 min, equilibrated to room temperature and the conductivity of all samples was measured and recorded as C_2 and T_2 respectively. Membrane Injury Index (MII) was calculated using the formula:

$$\text{Percent Injury (\%)} = 1 - \frac{(1 - T_1/T_2)}{1 - (C_1 / C_2)} \times 100$$

Where,

T_1 and T_2 = conductivities before and after boiling of treatment vials

C_1 and C_2 = conductivities before and after boiling of control vials

3.8.7. Estimation of Photosynthetic Pigments

The content of photosynthetic pigments in leaves was estimated colorimetrically by non-macerating technique using dimethyl sulphoxide (DMSO) as described by Hiscox and Stam (1979).

The leaf samples were collected, surface cleaned and cut into small pieces. 300 mg of leaf sample was taken and immersed in stoppered test tubes containing 10 mL of pure dimethyl sulphoxide (DMSO). The test tubes containing sample + DMSO were kept in a hot air oven maintained at $60\text{-}65^{\circ}\text{C}$ for 3 hours. The optical densities of the supernatant liquid were taken at 480, 510, 645, 652 and 663 nm separately using Systronics Spectrophotometer (Model 105). The total chlorophyll, chlorophyll a, chlorophyll b and carotenoids were calculated by using the following formulae (Arnon, 1949).

$$\text{Chlorophyll a (mg g}^{-1}\text{)} = 12.7 (\text{D.663}) - 2.69 (\text{D.645}) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b (mg g}^{-1}\text{)} = 22.9 (\text{D. 645}) - 4.68 (\text{D. 663}) \times \frac{V}{1000 \times W}$$

$$\text{Carotenoids (mg g}^{-1}\text{)} = 7.6 (\text{D.480}) - 1.49 (\text{D.510}) \times \frac{V}{1000 \times W}$$

Where,

D = Optical density

V = Final volume of DMSO

W= Fresh weight of sample taken

3.9 LEAF ANALYSIS

The leaves of plants samples collected at different time intervals were washed with tap water followed by distilled water and finally with double distilled water. They were first dried in shade and then in hot air oven at 60⁰C. Oven dried plant samples were ground in a grinder and stored in butter paper covers. Powdered leaf samples were analysed for mineral composition of K, Ca and B. For estimation of the concentration of K and Ca the extract was prepared by wet digestion method.

For wet digestion, 1g of dried and finely powdered leaf material was digested in 10mL of diacid mixture at 9:4 ratio of nitric acid (HNO₃) and perchloric acid (HClO₄). The extract was finally made upto 50 mL with double distilled water and aliquots in required volume were used for estimation of mineral nutrient concentration.

3.9.1 Calcium

Calcium content in leaf samples was determined by versanate titration method (Jackson, 1967).

Reagents

- a. 0.02N EDTA (Ethyelne diamine tetra acetic acid disodium salt) solution.
- b. 16 percent NaOH: 16g of NaOH dissolved to 100 mL of distilled water.
- c. Pattons and reeder's indicator: patton and reeder's powder was mixed with 100g of potassium sulphate (K₂SO₄) and ground well.

Procedure

5 mL of plant extract was taken in porcelain basin and to this 2 to 5 mL of 16 percent NaOH solution was added followed by addition of pinch of Patton and Reeder's reagent. Wine red colour appeared after adding indicator. This was titrated against 0.02 N EDTA until the colour of solution changed to sky blue. The concentration of calcium was worked out by using the factor, 1 mL of 0.02 N EDTA = 0.0004 g of Ca and tissue Ca was expressed as mg g^{-1} dwt.

3.9.2 Potassium

Potassium in diacidic extract of plant samples was determined by direct feeding of extract to flame photometer adjusting the instrument with suitable standards as per the method described by Jackson (1973).

Preparation of standard curve

Different concentrations of potassium solutions were prepared (0, 20, 40, 60, 80 and 100 ppm) by using potassium chloride and then solution was fed to flame photometer. The readings were noted and the standard curve was prepared by taking concentration of K on X-axis and flame photometer readings on Y-axis.

3.9.3 Boron

Boron content in leaf samples was determined by using Azomethine H reagent method (John *et al.*, 1975).

Reagents

- a. Distilled water
- b. Buffer solution

250 g of ammonium acetate (NH_4OAc) and 15 g of EDTA disodium salt were dissolved in 400 ml of distilled water and slowly added 125ml glacial acetic acid to it and mixed.

c. Azomethine – H reagent

0.45 g azomethine –H was dissolved in 100-ml of 1% (1-g/100ml water) L- ascorbic acid solution and stored in refrigerators.

d. Calcium hydroxide suspension

0.4 g Ca(OH)_2 dissolved in 100ml distilled water.

e. 0.1 HCl : 8.3 ml conc. HCl added to 900ml distilled water, allowed to cool to room temperature and made volume 1000ml.

f. Calcium chloride (0.01M) : 1.11 g of anhydrous CaCl_2 was dissolved in 900ml distilled water and volume made to 1000ml.

g. Standard solution : 0.114g of boric acid was dissolved in distilled water and adjusted the volume to 1000ml. 10, 20, 30, 40 and 50ml of stock solution was diluted to 100ml with distilled water to have solutions with B concentration of 2, 4, 6, 8 and 10 $\mu\text{g of B ml}^{-1}$, respectively.

Procedure

Plant digest: 0.5g of plant sample was taken into porcelain dish and 0.5g Ca(OH)_2 was added to it. Sample was ignited in the muffle furnace at 550°C for 4 hours to obtain white grey ash. The dish was cooled and ash was moist with little distilled water and then 5ml of 0.1 N HCl was added. The content was transfer to 25 ml volumetric flask and volume was made.

1ml of aliquot of blank and diluted boron standards were taken into a 10ml polypropylene tube. 2ml buffer solution and 2ml of H-azomethine were added to it and mixed. The mixture was allowed to stand for 30 minutes and read the absorbance on spectrophotometer at 420 nm. With the help of absorbance readings of standard solutions of different concentration of boron, the standard curve was prepared and boron concentration was calculated.

3.10 STATISTICAL ANALYSIS

In the present investigation, data were statistically analyzed following the standard procedures (Gomez and Gomez, 1984).

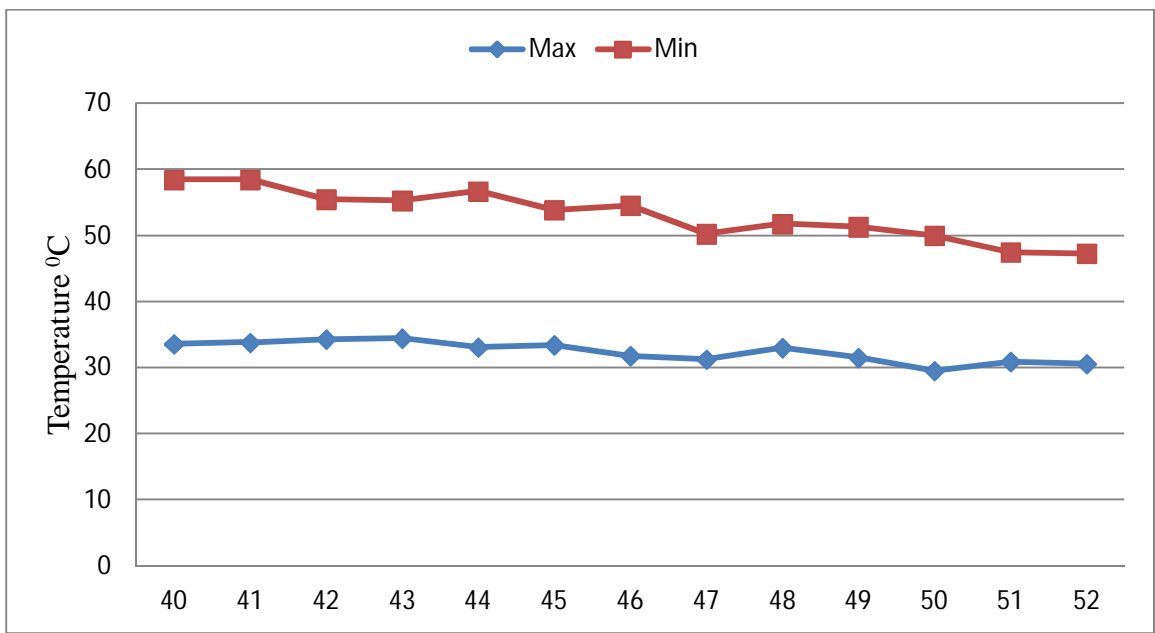


Fig. 3.1. Weekly temperature during crop growth period

Chapter IV

RESULTS AND DISCUSSION

Results obtained in the study on alleviation of mungbean yellow mosaic virus (MYMV) in blackgram through foliar application of Calcium, Boron and Salicylic acid were presented and discussed in this chapter.

4.1 YMV DISEASE INCIDENCE (%)

The data recorded on effect of calcium, boron and salicylic acid on incidence of yellow mosaic virus in blackgram during *rabi* season 2016-17 were presented in Table 4.1. and Fig 4.1.

The first visual symptoms of YMV incidence was observed at 27 DAE in T₁ (control) and T₃ (0.3% calcium spray) and in all other treatments it was observed at 34 DAE. The percentage of disease infected plants gradually increased. Observations revealed significant differences in YMV incidence among treatments (Table 4.1 and Fig. 4.1). All treatments recorded less disease incidence than control. Disease incidence reached to maximum at 45 DAE.

Application of calcium, boron and salicylic acid resulted in decrease in disease incidence and effect of all treatments was significant compared to control (19.4 %) at 30 DAE. Among treatments T₂ to T₁₀, the lowest disease incidence was observed with soil application of 300 kg gypsum + 1kg boron ha⁻¹ (13.6 %), which was on par with 0.25 % boron spray + 50ppm salicylic acid (14.1 %) and complete nutrient solution spray (14.3 %). The remaining treatments also recorded less disease incidence ranging from 14.9 to 17.7 percent.

At 45 DAE, significant difference was observed in disease incidence with all treatments compared to control (72.7 %). Among the treatments T₂ to T₁₀, the lowest disease incidence was recorded in 300 kg gypsum + 1 kg boron ha⁻¹ (40.2 %) followed by 300 kg gypsum + 1 kg boron ha⁻¹ + 50ppm SA spray (44.5 %) and 0.3% calcium spray + 50ppm SA spray (46.6 %). All other treatments (T₃, T₄, T₅, T₉ & T₁₀) also recorded less disease incidence ranging from 48.8 to 57.6 percent.

A delay of seven days in disease incidence was noticed in all treatments T₂ to T₇ except T₃. The reduction in disease incidence with application of calcium, boron and SA ranged from 1.7 to 5.8 percent at 30 DAE and 15.1 to 30.5 percent at 45 DAE and it was high with 300 kg gypsum + 1 kg boron ha⁻¹ and low with 0.25% boron spray. The reduction in disease incidence in T₆, T₇ and T₈, which were on par with T₂ was 29.2, 28.2 and 26.1 percent respectively. These results indicated that disease severity can be reduced through application of calcium, boron and salicylic acid. Delay in disease incidence and reduction in disease severity could be due to reduction in whitefly population with Ca, B and SA application. Calcium, boron nutrition and SA spray increased the total phenol in plant and also the activity of the enzyme peroxidase and phenylalanine ammonia lyase which might be responsible for the reduced disease incidence. Eraslan *et al.* (2007) reported that foliar spray of calcium from four different sources (CaCl₂.2H₂O, Ca(NO₃).4H₂O, Ca(C₂H₃O₂)₂ and CaNO₃.4H₂O +0.05 M Na EDTA) at the time of inoculation, 15 and 30 days after inoculation depressed the severity of ToMV disease by causing reduction in virus concentration in leaf tissue especially after second and third foliar spray. Boron @ 3 kg ha⁻¹ and molybdenum @ 2 kg ha⁻¹ when applied to soil reduced MYMV incidence and severity in mungbean (Jalaluddin *et al.*, 2006). Radwan *et al.* (2008) demonstrated that harmful effects of bean yellow mosaic virus infection in faba bean could be reduced by spraying SA. It was also shown to alleviate growth inhibition caused by Zucchini Yellow Mosaic Virus (ZYMV) in *Cucurbita pepo* (Radwan *et al.*, 2006).

4.2. WHITE FLY COUNT

The observations recorded on number of whiteflies at 15, 30 and 45 DAE were presented in Table 4.2 and Fig. 4.2. Reduction in whitefly population was noticed in all treatments at all days of observation.

At 15 DAE, the plant treated with sprays of 0.3% calcium + 50ppm SA recorded the lowest population of whitefly (2.02 leaf⁻¹) followed by combination of gypsum 300 kg + boron 1 kg ha⁻¹ with 50ppm SA spray (2.04 leaf⁻¹), sprays of complete nutrient solution + 50ppm SA (2.15 leaf⁻¹), gypsum 300 kg + boron 1 kg ha⁻¹ (2.19 leaf⁻¹), 0.25% boron + 50ppm SA sprays (2.27 leaf⁻¹), 0.3% calcium sprays (2.31 leaf⁻¹) and 0.25% boron sprays (2.37 leaf⁻¹). The highest number of whitefly was

noticed with sprays of 50ppm SA (2.75 leaf^{-1}) and it was on par with control (3.14 leaf^{-1}). The significantly least value for whitefly count was observed with application of complete nutrient solution sprays (2.45 leaf^{-1}) compared to control.

At 30 DAE, application of calcium, boron and SA resulted in decreased population of whitefly (2.64 to 3.71 leaf^{-1}) over control (4.19 leaf^{-1}) except T₈ (4.0 leaf^{-1}). Among these treatments (T₂ to T₁₀), the lowest whitefly population was observed with soil application of gypsum $300 \text{ kg} + \text{boron } 1 \text{ kg ha}^{-1}$ (2.64 leaf^{-1}) and the highest was with sprays of $0.3\% \text{ calcium} + 50\text{ppm SA}$ (4.00 leaf^{-1}). The treatments T₃, T₄, T₅, T₆, T₇, T₉ and T₁₀ also reduced whitefly population ranging from 3.39 to 3.62 leaf^{-1} .

At 45 DAE, in all treatments, significant decrease in whitefly population (3.63 to 4.14 leaf^{-1}) was noticed compared to control (4.65 leaf^{-1}) except T₅. Among the treatments, least value of whitefly was observed with gypsum $300 \text{ kg} + \text{boron } 1 \text{ kg ha}^{-1}$ (3.63 leaf^{-1}) followed by sprays of $0.3\% \text{ calcium} + 50\text{ppm SA}$ (3.74 leaf^{-1}), $0.3\% \text{ calcium}$ sprays (3.79 leaf^{-1}) and gypsum $300 \text{ kg} + \text{boron } 1 \text{ kg ha}^{-1}$ with sprays of 50ppm SA (3.93 leaf^{-1}). The highest whitefly count was recorded with sprays of 50ppm SA (4.27 leaf^{-1}) and it was on par with control (4.65 leaf^{-1}). The treatments T₄, T₆, T₉ and T₁₀ recorded reduced whitefly population ranging from 4.03 to 4.14 leaf^{-1} .

The calcium, boron and SA application resulted in reduced whitefly population compared to control ranged from 12.4 to 35.6 percent at 15 DAE, 4.5 to 36.9 at 30 DAE and 8.1 to 21.9 at 45 DAE. It was high with gypsum $300 \text{ kg} + \text{boron } 1 \text{ kg ha}^{-1}$ and low with sprays of 50ppm SA . Compared to sprays of calcium, boron and SA, application of gypsum $300 \text{ kg} + \text{boron } 1 \text{ kg ha}^{-1}$ resulted in more reduction in whitefly population and also compared to control all treatments showed reduction in whitefly population. This suggested that nutrient management reduces the severity of YMV incidence by controlling whitefly population to certain extent. This could be due to increase in plant total phenols which was evident from the results of present investigation. The reduction in whitefly population with inclusion of gypsum (calcium nitrate 46%) and boron ($0.072\% \text{ boric acid}$) in nutrient mixture was reported by Rasdi *et al.* (2013) in brinjal. Gogi *et al.* (2012) reported that the addition of boron in nutrient management schedule of non Bt-cotton resulted in population fluctuation of whitefly upto 83.1% .

4.3 MORPHOLOGICAL PARAMETERS

4.3.1. Number of Branches

The data pertaining to number of branches recorded at 30 DAE, 45 DAE and 60 DAE were presented in Table 4.3 and Fig 4.3. The number of branches increased with advancement of crop age and maximum branch number was recorded at 60 DAE.

At 30 DAE, effect of calcium, boron and salicylic acid on number of primary braches recorded was significant but the difference among the treatments was on par. However, increase in number of branches (4.3 to 5.0 plant⁻¹) was noticed compared to control (4.13). The highest number of branches among the treatments (T₂ to T₁₀) was recorded in 300 kg gypsum + 1 kg boron ha⁻¹ + 50ppm SA sprays (5.0) and the lowest was recorded in 50ppm SA sprays (4.3). All other treatments also showed increase in number of branches ranging from 4.6 to 4.8.

At 60 DAE, number of branches plant⁻¹ increased in all treatments compared to control, but differences were non significant. Among the treatments T₂ to T₁₀, soil application of gypsum 300 kg + boron 1 kg ha⁻¹ recorded the highest number of branches (6.5) and the lowest was recorded in 0.25% boron sprays + 50 ppm SA sprays (5.6). All other treatments also recorded increase in number of branches ranging from 5.9 to 6.4.

At harvest, all treatments showed increase in number of branches compared to control (5.7). The highest number of branches was recorded in gypsum 300 kg + boron 1 kg ha⁻¹ (6.5) and the lowest number of branches was noticed with sprays of complete nutrient solution (6.1). The treatments T₃, T₄, T₅, T₇, T₈, T₉ and T₁₀ also recorded increase in number of branches plant⁻¹ ranging from 6.2 to 6.4.

The increase in number of branches with application of calcium, boron and salicylic acid ranged from 4.9 to 21.9 percent at 30 DAE, 3.7 to 20.4 percent at 60 DAE and 7.0 to 14.0 percent at harvest and it was high in T₂ (gypsum 300kg + boron 1 kg ha⁻¹) and low in T₄ (0.25% boron sprays). The increase in number of branches could be because of lower disease severity ensured by calcium and boron nutrition + SA sprays. Gafoor *et al.*, (2000) reported that in urdbean, YMV infection caused a

reduction in number of primary branches. Ali *et al.*, (2014) reported increase in number of branches plant⁻¹ with boron and gypsum application in faba bean against chocolate spot disease. The increase in number of primary branches in YMV infected winter mungbean with 2 kg boron ha⁻¹ and in combination of boron + molybdenum (1.5 kg ha⁻¹) was reported by Pramanik and Ali (2001).

4.4. DRY MATTER ACCUMULATION

4.4.1. Leaf Dry matter (g plant⁻¹)

The data recorded on amount of dry matter accumulated in leaf (g plant⁻¹) at 30 DAE, 60 DAE and at harvest were presented in Table 4.4 and Fig 4.4. Leaf dry matter increased with advancement in age of crop.

At 30 DAE, effect of all treatments on accumulation of leaf dry matter plant⁻¹ (0.63 to 1.21 g) was significant compared to control (0.56 g) except T₃, T₄, T₅ and T₁₀. Among those treatments, the maximum leaf dry matter (1.31 g) was observed with 0.25% boron in combination with 50ppm salicylic acid (T₉) which was on par with gypsum 300 kg + boron 1 kg ha⁻¹ in combination with sprays of 50ppm salicylic acid (1.21 g) and with gypsum 300 kg + boron 1 kg ha⁻¹ (1.17 g). The treatments T₆ and T₈ also recorded high leaf dry matter 0.86 and 0.95 g plant⁻¹ respectively.

At 60 DAE, significant difference was observed in leaf dry matter accumulated with treatments T₂, T₄ and T₇ (2.48 to 3.15 g) compared to control (1.73 g). The highest leaf dry matter was recorded in 300 kg + boron 1 kg ha⁻¹ with sprays of 50ppm SA (3.15 g) followed by gypsum 300 kg + boron 1 kg ha⁻¹ (3.02 g). The effect of treatments T₃, T₅, T₆, T₈, T₉ & T₁₀ (1.5 to 2.26 g) was found on par with control.

Leaf dry matter recorded at harvest in all treatments (2.71 to 4.34 g) showed significant increase compared to control (2.05 g). Among the treatments T₂ to T₁₀, the highest leaf dry matter was recorded in plants treated with gypsum 300 kg + boron 1 kg ha⁻¹ in combination with sprays of 50ppm salicylic acid (4.34 g plant⁻¹) and it differ significantly with all other treatments except T₂. The lowest value was observed with sprays of 0.3 % calcium + 50ppm SA (2.71 g). The remaining treatments also recorded increase in leaf dry matter ranging from 2.95 to 3.54 g plant⁻¹.

Calcium, boron and salicylic acid application resulted in increase in leaf dry matter plant⁻¹ ranging from 14.3 to 133.9 percent at 30 DAE, 0.6 to 82.1 percent at 60 DAE and 32.2 to 102.0 percent at harvest and it was high in gypsum 300 kg + boron 1 kg ha⁻¹ with 50ppm SA sprays and low in sprays of 0.3 % calcium + 50ppm SA. Soil application of gypsum 300 kg + boron 1 kg ha⁻¹ alone and its combination with 50ppm SA recorded increase in accumulation of leaf dry matter (102.0 and 111.7 % respectively). The application of calcium, boron alone and its combination with 50ppm SA also showed marked effect on leaf dry matter accumulation (67.8, 69.2, 24.3 and 46.3 % respectively).

4.4.2. Stem Dry matter (g plant⁻¹)

The data pertaining to the effect of calcium, boron and SA application on stem dry matter plant⁻¹ recorded at 30, 60 DAE and harvest were presented in Table 4.5 and Fig 4.5. The stem dry matter plant⁻¹ increased with increase in age of the crop.

At 30 DAE, stem dry matter plant⁻¹ obtained with different treatments (0.11 to 0.16 g) except T₅ was significantly higher than control (0.11 g). Among the treatments, plants treated with complete nutrient solution spray in combination with 50 ppm SA (0.18 g) recorded the highest stem dry matter and the lowest was with 0.3 % calcium with 50 ppm SA (0.14 g). All other treatments T₂, T₃, T₄, T₆, T₇ and T₈ showed increase in stem dry matter ranging from 0.16 to 0.20 g plant⁻¹.

At 60 DAE, plants treated with gypsum 300 kg + boron 1 kg ha⁻¹ r (0.33 g) recorded the highest stem dry matter followed by 300 kg gypsum + 1 kg boron in combination with 50ppm SA spray (3.32 g). The lowest stem dry matter recorded with 0.3% calcium spray in combination with 50 ppm SA (0.21 g) followed by 0.25% boron spray (0.25 g), sprays of 0.25% boron + 50ppm SA spray (0.23 g).

At harvest, the highest stem dry matter was recorded in gypsum 300 kg + boron 1 kg ha⁻¹ in combination with 50 ppm SA (1.20 g) and the lowest was recorded with spray of 0.3% calcium (0.74 g). All other treatments showed increased in stem dry matter over control.

The increase in stem dry matter with application of calcium, boron and SA ranged from 1.2 to 1.8 folds at 30 DAE, 1.5 to 1.65 folds at 60 DAE and 1.07 to 1.7 folds at harvest and it was high with gypsum 300 kg + boron 1 kg ha⁻¹ in combination

sprays. Complete nutrient solution spray alone and its combination with 50ppm SA spray showed marked increase in stem dry matter. Calcium and boron alone and its combination with 50ppm SA spray showed no effect on accumulation of stem dry matter.

4.4.3. Total Dry matter (g plant⁻¹)

The data pertaining total dry matter plant⁻¹ recorded at 30, 60 DAE and at harvest were presented in Table 4.6 and Fig. 4.6.

At 30 DAE, data obtained on total dry matter showed that there was significant increase in total dry matter in all treatments compared to control (0.56 g) except T₁₀ (sprays of complete nutrient + 50ppm SA). Maximum amount of total dry matter observed with gypsum 300 kg + boron 1 kg ha⁻¹ (1.52 g plant⁻¹) which was on par with gypsum 300 kg + boron 1 kg ha⁻¹ with 50ppm SA (1.49 g plant⁻¹). Minimum total dry matter content was recorded in complete nutrient solution spray with 50ppm SA (0.63 g) which was on par with control. All other treatments T₃, T₄, T₅, T₆, T₇, T₈ and T₉ also recorded increase in total dry matter ranging from 0.82 to 1.14 g plant⁻¹.

At 60 DAE, gypsum 300 kg + boron 1 kg ha⁻¹ (5.14 g) treated plants showed the highest total dry matter accumulation and it was on par with gypsum 300 kg + boron 1 kg ha⁻¹ in combination with 50 ppm salicylic acid (5.12 g) and sprays of 0.3% calcium with 50ppm SA (5.09 g). Among the treatments, the lowest total dry matter accumulation was recorded in 0.3% calcium spray (3.14 g) followed by sprays of 0.25% boron with 50ppm SA (3.35 g) and complete nutrient solution in combination with sprays of 50ppm SA (3.2 g) which were on par with control (2.92 g). The treatments T₄ and T₆ also recorded increase in total dry matter content 4.15 and 4.5 g plant⁻¹ respectively.

At harvest, the highest total dry matter accumulation was recorded in gypsum 300 kg + boron 1 kg ha⁻¹ in combination with sprays of 50ppm SA (12.35 g plant⁻¹) and it was on par with gypsum 300 kg + boron 1 kg ha⁻¹ (12.22 g), complete nutrient solution spray (11.30g) and combination of complete nutrient solution with sprays of SA (11.17 g plant⁻¹). Among all the treatments 50ppm salicylic acid recorded the lowest total dry matter accumulation (8.76 g plant⁻¹) followed by 0.25% boron sprays

(9.7 g) and combination of 0.3% calcium + sprays of 50ppm SA (9.6 g) which were on par with control (8.7 g). The treatments T₃ and T₉ also recorded increase in total dry matter accumulation 10.2 and 10.3 g plant⁻¹ respectively.

The increase in total dry matter with application of calcium, boron and salicylic acid ranged from 14.5 to 176.3 percent at 30 DAE, 7.5 to 76.0 percent at 60 DAE and 0.7 to 41.95 percent at harvest and it was high with gypsum 300 kg + boron 1 kg ha⁻¹ in combination with sprays of 50 ppm SA and low with 50ppm salicylic acid sprays. Application of gypsum 300 kg + boron 1 kg ha alone and its combination with 50ppm SA showed the marked increase in total dry matter accumulation 40.4 and 41.9 respectively. Calcium spray alone showed the increase (17.2), while its combination with SA spray did not show any effect. Complete nutrient solution spray alone and its combination showed increase in total dry matter (29.8 and 34.4 percent respectively). Application of boron alone did not show any effect, but its combination recorded marked increase in total dry matter accumulation (18.4 %).

4.5. BIOCHEMICAL PARAMETERS

4.5.1. Total Phenols (mg/100mg)

The data pertaining to effect of calcium, boron and salicylic acid on total phenols content of blackgram infected with YMV were presented in Table 4.7 and Fig 4.7.

At 15 DAE (before the incidence of YMV), all treatments were significantly different with control (0.50 mg/100mg). The highest total phenol content was recorded in gypsum 300 kg + boron 1 kg ha⁻¹ treated plants (0.81 mg/100mg) followed by combination of 300 kg + boron 1 kg ha⁻¹ and sprays of 50 ppm SA (0.80 mg/100mg). Among the treatments T₂ to T₁₀, the lowest content of total phenols was recorded in 0.25% boron spray (0.64 mg/100mg) and it was on par with 0.3% calcium spray (0.65 mg/100mg) and sprays of 0.25% boron with 50ppm SA (0.64 mg/100mg). All other treatments T₅, T₆, T₈ and T₁₀ also showed high total phenols compared to control ranging from 0.70 to 0.72 mg/100mg.

At 30 DAE, diseased leaves showed high total phenol content compared to healthy leaves in all treatments except T₇ (gypsum 300 kg + boron 1 kg ha⁻¹ with

sprays of 50ppm SA) where in total phenols content was found high in healthy leaves. All treatments, compared to control, significantly increased the total phenols both in healthy and diseased leaves except T₄ in diseased leaves. Gypsum 300kg + boron 1 kg ha⁻¹ in combination with sprays of salicylic acid showed the highest total phenol content (0.99 mg/100mg) in healthy leaves and in diseased leaves, gypsum 300kg + boron 1 kg ha⁻¹ recorded the highest total phenol content (0.98 mg/100mg). The lowest total phenol content was recorded in 0.25% boron spray in both healthy (0.65 mg/100mg) and disease infected plants (0.76 mg/ 100mg). All other treatments, T₂, T₃, T₅, T₆, T₈, T₉ & T₁₀ also recorded increase in total phenol content compared to control in healthy plants ranging from 0.74 mg/100mg to 0.93 mg/100mg and in disease infected plants, the treatments T₃, T₅, T₆, T₇, T₈, T₉ & T₁₀ showed high total phenol content ranging from 0.80 to 0.91 mg/100mg.

At 45 DAE, all treatments recorded significant increase in total phenol content compared to control in healthy and disease infected plants except T₃ (0.3% calcium spray) in diseased ones and all treatments resulted in high total phenol content in diseased ones compared to healthy plants. Among all T₂ to T₁₀ treatments, gypsum 300 kg + boron 1 kg ha⁻¹ with sprays of 50ppm SA resulted in high total phenol content both in healthy (1.13 mg/100 mg) and disease (1.26 mg/100mg) infected leaves and it was on par with 300 kg gypsum + boron 1 kg ha⁻¹ (1.12mg/100mg in healthy and 1.24 mg/100mg in diseased plants). Among the treatments, the lowest total phenol content was recorded with 0.3% calcium spray (0.76 mg/100mg) in healthy and in disease infected leaves (0.87 mg/100mg). All other treatments T₄, T₅, T₆, T₈, T₉ and T₁₀ also recorded high total phenol content ranging from 0.86 to 0.96 mg/100mg in healthy plants and 0.94 to 1.14 mg/100mg in diseased plants.

Application of calcium, boron and salicylic acid resulted in increase in total phenol content in both healthy and disease infected plants and the increase in total phenol content ranged from 27.8 to 62.2 percent at 15 DAE, 38.3 to 110.6 percent in healthy and 7.0 to 38.0 percent in disease infected plants at 30 DAE and 22.5 to 82.2 percent in healthy and 4.8 to 51.80 percent in diseased plants at 45 DAE. Compared to control, impact of treatments on percent increase in leaf total phenols was more in healthy plants than in diseased plants. In healthy plants, before the incidence of YMV the percent increase in leaf total phenols was high with gypsum + boron soil application and after incidence of YMV it was found high with gypsum + boron soil

application along with 50ppm SA sprays. In diseased plants, just after the incidence (30 DAE) the percent increase in leaf total phenols was high with gypsum + boron soil application and later (45 DAE) it was found high with gypsum + boron soil application along with 50ppm SA sprays.

In the present study, total phenol content in infected blackgram plants had shown an increase and the increase was more in plants that received the treatments than the control plants. Just after the incidence in both healthy and diseased plants, the increase in leaf total phenols content with application of boron, calcium, complete nutrient solution and its combination with SA spray was less than that with SA alone, gypsum + boron soil application and gypsum + boron with SA application. These findings indicated that, the increase in total phenols both in healthy and disease leaves was high with application of calcium, boron and salicylic acid. It is well known that plant phenols are highly antioxidants and rapid accumulation of phenols at infection sight is the first step of defense mechanism in plants which restricts or slows the pathogen growth because of its action as anti oxidant, anti microbial and photoreceptor (Lamba *et al.*, 2008). Dantre *et al.* (1996) in soybean in longbean. reported that increase in phenol content of plants and corresponding decrease in symptom expression in SA treated blackgram plants infected with MYMV was also observed by Kundu *et al.* (2011). Increase in total phenols content with calcium and boron nutrition as foliar spray was reported by Ali *et al.* (2014) in faba bean infected with chocolate spot disease. Somaya and Seham (2015) reported that the phenols were higher in leaves of rust infected wheat treated with boron (0.1 g/l) than those of untreated infected control.

4.5.2. Peroxidase Activity (Δ Abs/min/g)

Data pertaining to effect of calcium, boron and salicylic acid on peroxidase (POD) activity of blackgram plants infected with YMV were presented in Table 4.8 and Fig 4.8.

At 15 DAE, all treatments showed significant increase in POD activity compared to control (0.16 Δ Abs/min/g). Among treatments T₂ to T₁₀, 50 ppm salicylic acid treated plants showed the highest POD activity (0.32 Δ Abs/min/g) and it was on par with gypsum 300 kg and boron 1 kg ha⁻¹ in combination with sprays of SA (0.31 Δ Abs/min/g). The lowest POD activity was recorded in complete nutrient solution sprays (0.21 Δ Abs/min/g) and it was on par with sprays of complete nutrient solution

+ 50ppm SA (0.21 Δ Abs/min/g), 0.25% boron spray (0.22 Δ Abs/min/g) and 0.3% calcium spray (0.22 Δ Abs/min/g). The treatments T₂, T₈ and T₉ also recorded increase in POD activity ranging from 0.25 to 0.27 Δ Abs/min/g.

At 30 DAE, POD activity in healthy leaves was found lower than disease infected leaves. The highest POD activity was recorded with gypsum 300 kg and boron 1 kg ha⁻¹ in combination with sprays of SA (0.35 Δ Abs/min/g) followed by gypsum 300 kg and boron 1 kg ha⁻¹ (0.33 Δ Abs/min/g) in healthy leaves and in disease infected leaves, the highest POD activity was recorded in gypsum 300 kg and boron 1 kg ha⁻¹ in combination with 50ppm SA spray (0.53 Δ Abs/min/g). Among all T₂ to T₁₀ treatments, 0.25% boron (0.24 Δ Abs/min/g) spray recorded the lowest POD activity in healthy plants which was on par with sprays of complete nutrient solution + 50ppm SA (0.26 Δ Abs/min/g), complete nutrient solution spray (0.26 Δ Abs/min/g) and in disease infected plants it was recorded with sprays of complete nutrient solution sprays (0.37 Δ Abs/min/g) which was on par with control. The treatments, T₃, T₅, T₈ and T₉ also recorded increase in POD activity ranged from 0.27 to 0.32 Δ Abs/min/g in healthy plants and the treatments T₂, T₃, T₄, T₅, T₈, T₉ and T₁₀ also recorded increase in POD activity in disease infected plants ranging from 0.38 to 0.49 Δ Abs/min/g.

At 45 DAE, all treatments were significantly different compared to control (0.27 Δ Abs/min/g in healthy and 0.47 Δ Abs/min/g in disease plants). Effect of calcium, boron and salicylic acid on POD was recorded high for disease infected plants compared to healthy plants. The highest POD activity was recorded in gypsum 300 kg and 1 kg boron ha⁻¹ (0.40 Δ Abs/min/g) in healthy plants. In disease infected plants gypsum 300 kg and 1 kg boron ha⁻¹ with sprays of 50ppm SA recorded the highest activity (0.64 Δ Abs/min/g) and it was on par with gypsum 300 kg and boron 1 kg ha⁻¹ (0.61 Δ Abs/min/g). The lowest POD activity recorded in 0.25% boron spray (0.28 Δ Abs/min/g) in healthy and sprays of complete nutrient solution with 50ppm SA (0.49 Δ Abs/min/g) in disease infected plants followed by 0.3% calcium + 50ppm SA (0.52 Δ Abs/min/g) and 0.3% calcium sprays (0.50 Δ Abs/min/g). The treatments T₃, T₅, T₆, T₇, T₈, T₉ and T₁₀ also recorded increase in POD activity ranging from 0.32 to 0.36 Δ Abs/min/g in healthy plants and treatments T₄, T₅, T₆ and T₉ in disease infected plants ranging from 0.53 to 0.59 Δ Abs/min/g.

The increase in POD activity with application of calcium, boron and salicylic acid ranged from 31.2 to 100.0 percent at 15 DAE, 26.3 to 84.2 percent in healthy and 2.7 to 43.2 percent in disease infected leaves at 30 DAE and 3.7 to 48.1 percent in healthy and 4.2 to 36.1 percent in disease infected plants at 45 DAE and it was high with gypsum 300 kg and 1 kg boron ha⁻¹ in healthy and gypsum 300 kg and 1 kg boron ha⁻¹ with 50ppm SA sprays in disease infected leaves. Compared to control, the impact of treatments on percent increase in POD activity was more in healthy plants than in disease plants. In healthy plants, before the incidence of YMV, the percent increase in POD activity was high with SA sprays and gypsum + boron + sprays of SA. Just after incidence (30DAE), it was high with soil application of gypsum 300 kg + boron 1 kg ha⁻¹ alone and its combination with 50ppm SA sprays and later (45 DAE) it was found high with gypsum 300 kg + boron 1 kg ha. In disease plants, just after the incidence and later stages, the percentage increase in POD activity was high with application of gypsum 300 kg + boron 1 kg ha⁻¹ alone and its combination with SA sprays. Results of present investigation was similar with finding of Asif Khan (2016), stated that in healthy leaves of LBG-623 gypsum + boron application increased the POD activity by 1.7 to 3.5 and 1.5 to 2.9 folds at 15 and 45 DAS respectively. In disease leaves it was 1.1 to 1.3 folds.

Plants respond to pathogen infection by enhancing the activity of defence enzymes like peroxidase, phenylalanine ammonia lyase and polyphenol oxidase. Peroxidase participates in oxidative burst, involve in detoxification of ROS and play an active role in plant defense system to pathogen. Arora *et al.* (2009) found a sharp rise in the activity of POD in MYMV infected mothbean leaves. Increased POD activity was also shown to be induced by exogenous application of SAR elicitors like SA (Radwan *et al.*, 2008), which contributed to alleviation of the effects of zucchini yellow mosaic virus in *Cucurbita pepo*. Increase in activity of peroxidase with boron was reported by Somaya and Seham (2015) in rust infected wheat plants. The activity of peroxidase is influenced by rate of calcium supply; Jiang *et al.* (2013) reported increase in POD activity with increase in calcium rate in tomato plants inoculated with *R. solanacearum*.

4.5.3. Phenylalanine Ammonia Lyase Activity (η moles of Transcinnamic acid/min/g)

The data pertaining to PAL activity of blackgram plants infected with YMV under calcium, boron nutrition and salicylic acid treatment were presented in Table 4.9 and Fig 4.9.

As observed in all defense enzymes, phenylalanine ammonia (PAL) lyase activity observed was high in disease infected plants than in healthy plants. At 15 DAE, all treatments recorded significant increase over control (0.54 η moles/min/g). Among all treatments T₂ to T₁₀, gypsum 300 kg and boron 1 kg ha⁻¹ in combination with sprays of SA showed the highest PAL activity (0.89 η moles/min/g). The lowest PAL activity was recorded in 0.25% boron spray (0.69 η moles/min/g) and complete nutrient solution with 50ppm SA (0.69 η moles/min/g). All other treatments (T₂, T₃, T₅, T₆, T₈ & T₉) also recorded increase in PAL activity ranging from 0.73 to 0.86 η moles/min/g.

At 30 DAE, all treatments significantly increased the PAL activity over control in both healthy (0.61 η moles/min/g) and disease infected plants (0.77 η moles/min/g). Among treatments T₂ to T₁₀, the highest PAL activity recorded in gypsum 300 kg and boron 1 kg ha⁻¹ with sprays of 50ppm SA in both healthy (1.00 η moles/min/g) and disease infected plants (1.14 η moles/min/g). The lowest PAL activity was recorded in 0.25% boron spray (0.78 η moles/min/g) in healthy and in disease infected plants (0.87 η moles/min/g). All other treatments T₂, T₃, T₅, T₆, T₈, T₉ and T₁₀ also recorded increase in PAL activity ranged from 0.81 to 0.95 η moles/min/g in healthy and 0.94 to 1.06 η moles/min/g in disease infected leaves.

At 45 DAE, in all treatments significant increase was noticed over control in healthy (0.72 η moles/min/g) and in disease infected plants (1.46 η moles/min/g) except T₆ (complete nutrient sprays) in diseased ones. Gypsum 300 kg + boron 1 kg ha⁻¹ recorded the highest PAL activity among the treatments (1.42 η moles/min/g) in healthy and disease infected plants (1.75 η moles/min/g) but it was on par with 0.25% boron spray (1.72 η moles/min/g) in diseased ones. The lowest PAL activity was recorded in 0.25% boron spray (0.86 η moles/min/g) in healthy plants and in disease infected plants, sprays of complete nutrient solution (1.50 η moles/min/g) recorded

the lowest PAL activity which was on par with 0.3% calcium + 50ppm SA sprays, 0.25% boron + 50ppm SA sprays and control (1.46 η moles/min/g). The treatments T₃, T₅, T₆, T₇, T₈, T₉, and T₁₀ also recorded increase in PAL activity ranging from 0.93 to 1.31 η moles/min/g in healthy and treatments T₃, T₅, T₇ and T₁₀ also induced the increase in PAL activity ranging from 1.56 to 1.69 η moles/min/g in disease infected plants.

The increase in PAL activity with calcium, boron and salicylic acid ranged from 27.7 to 64.8 percent at 15 DAE, 27.8 to 63.9 percent in healthy leaves and 12.9 to 40.8 percent in disease infected leaves at 30 DAE, 19.4 to 97.2 percent in healthy and 2.7 to 19.8 percent at 45 DAE in disease infected plants. PAL activity was high with Gypsum 300 kg + boron 1 kg ha⁻¹ in both healthy and disease infected plants and low with 0.25% boron sprays in healthy and complete nutrient sprays in disease infected plants. In healthy plants before the incidence of YMV, PAL activity was high with soil application of gypsum 300 kg + boron 1 kg ha⁻¹ in combination with 50ppm SA spray. Just after incidence of YMV, in both healthy and diseased plants it was high with same treatment. At later stage, in both healthy and disease ones, the percent increase in PAL activity was high with gypsum 300 kg + boron 1 kg ha⁻¹.

The results of present finding indicate that there was increase in PAL activity with soil application of gypsum 300 kg + boron 1kg ha⁻¹ compared to sprays of calcium, boron and SA but compared to control there was increase in PAL activity with application of calcium, boron and SA in response to YMV incidence, this finding was similar to Asif Khan, (2016) reported that, gypsum + boron application to soil increased the PAL activity in LBG-623 by 7.5 to 40.3 percent at 15 DAS and 54.8 to 72.6 percent at 45 DAS.

Elevated defense enzymes viz. PO, PPO and PAL activity with *P. fluorescens*, alum, sodium dihydrogen orthophosphate and acibenzolar treatments against MYMV in blackgram was demonstrated by Venkatesan *et al.* (2010). Karthikeyan *et al.* (2009) also reported a similar increase with *P. fluorescens* application in blackgram which contributed to decreased severity of urdbean leaf crinkle virus infection.

4.5.4. Membrane Injury (%)

Data pertaining to membrane injury (%) of blackgram in response to YMV disease incidence with application of calcium, boron and salicylic acid were presented in Table 4.10 and Fig 4.10.

Application of calcium, boron and salicylic acid resulted in significant reduction in membrane injury index compared to control (18.08) at 15 DAE. Among the treatments the lowest membrane injury index was recorded in gypsum 300 kg + boron 1 kg ha⁻¹ (10.19) and the highest membrane injury index was recorded in sprays of complete nutrient solution with 50ppm SA (15.34) and it was on par with 50ppm SA sprays (15.30). The treatments T₃, T₄, T₆, T₇, T₈ and T₉ also showed reduction in membrane injury index ranging from 11.18 to 14.22.

At 30 DAE, all treatments recorded significant reduction in membrane injury index over control (21.79). Among the treatments T₂ to T₁₀, gypsum 300 kg + boron 1 kg ha⁻¹ (13.32) showed the lowest membrane injury index followed by complete nutrient solution sprays (13.45), 0.3% calcium spray (13.77) and gypsum 300 kg + boron 1 kg ha⁻¹ with 50ppm SA sprays (14.23) and the highest membrane injury index recorded with sprays of complete nutrient solution + 50ppm SA (16.19) which was on par with T₅, T₉ and T₄.

At 45 DAE, significant reduction in membrane injury index was observed in all treatments compared to control (25.17). Among the treatments T₂ to T₁₀, the lowest membrane injury index was recorded in sprays of 0.3% calcium (14.40) and the highest membrane injury index noticed with sprays of 0.25% boron + 500ppm SA (20.13) and it was on par with sprays of complete nutrient solution with 50ppm SA (20.06). The treatments T₂, T₄, T₅, T₆, T₇ and T₈ also recorded reduction in membrane injury index ranged from 15.35 to 17.99.

The decrease in membrane injury index with application of calcium, boron and salicylic acid ranged from 2.74 to 7.89 percent at 15 DAE, 5.6 to 8.47 percent at 30 DAE and 5.04 to 10.77 percent at 45 DAE and it was high with T₂ and T₃ and low with sprays of T₁₀. The application of calcium, boron and salicylic acid resulted in least membrane injury index and damage due to YMV was more pronounce with higher value of membrane injury index found in untreated plants. The leaves of blackgram not supplied with nutrients and SA have high values of membrane injury index. The balanced nutrition prevented the electrolyte leakage because the nutrients play a major role in the plants ability in maintaining membrane integrity. Ca, B, Mg and Zn maintain the structural integrity and permeability of the cell membrane and prevent pathogen attack (Rice, 2007).

4.5.5. Photosynthetic Pigments

Chlorophyll-a (mg g^{-1})

The data pertaining to the effect of calcium, boron and salicylic acid application on chlorophyll-a content in blackgram infected with YMV were presented in Table 4.11 and Fig 4.11.

At 15 DAE, among the treatments T_2 to T_{10} , gypsum 300 kg + boron 1 kg ha^{-1} showed high chlorophyll a content in leaves (0.580 mg g^{-1}) followed by 50ppm SA spray (0.541 mg g^{-1}), 0.25% boron spray (0.528 mg g^{-1}), spray of complete nutrient solution + 50ppm SA (0.521 mg g^{-1}), 0.3% calcium spray (0.491 mg g^{-1}) and complete nutrient solution spray (0.470 mg g^{-1}). The lowest chlorophyll-a recorded in sprays of 0.3% calcium in combination with sprays of 50ppm SA (0.324 mg g^{-1}) was on par with gypsum 300 kg + boron 1 kg ha^{-1} + spray of 50ppm SA (0.331 mg g^{-1}) and control (0.211 mg g^{-1}).

At 30 DAE, significant increase in chlorophyll-a content was recorded in all treatments of calcium, boron and SA compared to control. In control, due to incidence of disease, chlorophyll-a content at 30 DAE decreased. Among the treatments T_2 to T_{10} , the highest chlorophyll-a was observed with sprays of complete nutrient solution + 50ppm SA (0.599 mg g^{-1}) and the lowest was recorded in sprays of 0.3% calcium with 50ppm SA (0.281 mg g^{-1}) and it was on par with combination of gypsum 300 kg + boron 1 kg ha^{-1} with sprays of 50ppm SA (0.291 mg g^{-1}). The treatments, T_2 , T_3 , T_4 , T_5 , T_6 and T_9 also showed increase in chlorophyll-a content ranging from 0.432 to 0.585 mg g^{-1} .

At 45 DAE, Chlorophyll-a content in all treatments except T_2 and T_6 was found significantly different compared to control. Among the treatments T_2 to T_{10} , sprays of complete nutrient solution with 50ppm SA (0.466 mg g^{-1}) was found superior to all other treatments. Minimum chlorophyll a content was recorded for 300 kg gypsum + boron 1 kg ha^{-1} (0.162 mg g^{-1}) followed by complete nutrient sprays (0.174 mg g^{-1}) and combination of gypsum 300 kg + boron 1 kg ha^{-1} and 50ppm SA sprays. The treatments, T_3 , T_4 , T_5 , T_8 and T_9 also resulted in increase in chlorophyll a ranging from 0.198 to 0.410 mg g^{-1} .

The increase in chlorophyll-a content with application of calcium, boron and salicylic acid ranged from 2.1 to 2.7 folds at 15 DAE, 1.4 to 2.9 folds at 30 DAE and 1.2 to 2.9 folds at 45 DAE and it was high with T₂ and T₁₀ upto 30 DAE and with T₁₀ at 45 DAE. In present investigation it was noticed that combination of sprays of complete nutrient solution with 50ppm SA showed high chlorophyll a content at 30 and 45 DAE compared to application of calcium, boron and SA and low was noticed in sprays 0.25% boron + 50ppm SA (at 15 DAE), 0.3% calcium + 50ppm SA (at 30 DAE) and gypsum 300 kg + boron 1 kg ha⁻¹ + 50ppm SA (at 45 DAE). From this finding, it can be suggested that sprays of complete nutrient solution + 50ppm SA on YMV infected blackgram crop will resynthesize the chlorophyll.

Chlorophyll b (mg g⁻¹)

The data pertaining to the effect of calcium, boron and salicylic acid application on chlorophyll-b content in blackgram infected with YMV were presented in Table 4.12 and Fig 4.12.

At 15 DAE, all treatments were significantly different with control (0.283 mg g⁻¹) in influencing chlorophyll-b content except sprays of 0.3% calcium + 50ppm SA (0.328 mg g⁻¹). Among T₂ to T₁₀ treatments, the highest chlorophyll-b content was noticed in complete nutrient solution treated plants (0.691mg g⁻¹) and the lowest in 0.3% calcium with 50ppm SA (0.328 mg g⁻¹). The second lowest was observed with 0.3% calcium sprays (0.388 mg g⁻¹) which was on par with gypsum 300 kg + boron 1 kg ha⁻¹ (0.401 mg g⁻¹) and 50ppm SA spray (0.400 mg g⁻¹). All other treatments T₄, T₇, T₉ and T₁₀ also recorded increase in chlorophyll-b content ranging from 0.425 to 0.563 mg g⁻¹.

At 30 DAE, chlorophyll-b content of plants in all treatments except T₆ and T₁₀ was found on par with control. Chlorophyll-b recorded was maximum in complete nutrient solution spray (0.581 mg g⁻¹) and it was on par with combination of complete nutrient solution and 50 ppm SA sprays.

At 45 DAE, the chlorophyll b content observed with T₂ to T₁₀ treatments was significantly different compared to control (0.143 mg g⁻¹) in all treatments. Among T₂ to T₁₀ treatments, sprays of complete nutrient solution treated plants showed maximum chlorophyll b content (0.318 mg g⁻¹). The minimum was recorded in

50ppm SA sprays (0.161 mg g^{-1}) followed by gypsum $300 \text{ kg} + \text{boron } 1 \text{ kg ha}^{-1}$ (0.164 mg g^{-1}) and 0.3% calcium sprays. The treatments T₄, T₇, T₈, T₉ and T₁₀ also recorded increase in chlorophyll-b content ranging from 0.176 to 0.286 mg g^{-1} .

The increase in chlorophyll-b content with application of calcium, boron and salicylic acid was 1.4 to 2.4 folds at 15 DAE, 1.5 to 1.7 folds at 30 DAE and 1.1 to 2.2 folds at 45 DAE and it was high with complete nutrient solution sprays. This finding of present investigation suggested that high chlorophyll b content can be maintained with application of complete nutrient solution compared to application of calcium, boron and SA alone or in combination.

Carotenoids (mg g^{-1})

The data pertaining to the effect of calcium, boron and salicylic acid application on carotenoids content in blackgram infected with YMV were presented in Table 4.13 and Fig 4.13.

At 15 DAE, with application of calcium, boron and salicylic acid the highest carotene content was recorded in sprays of complete nutrient solution with 50ppm SA (0.435 mg g^{-1}) followed by complete nutrient solution sprays (0.421 mg g^{-1}) and 0.25% boron + 50ppm SA spray (0.389 mg g^{-1}). The lowest carotenoids content was recorded in 0.25% boron spray (0.275 mg g^{-1}) followed by 0.3% calcium sprays (0.296 mg g^{-1}), 0.3% calcium spray with 50ppm SA (0.292 mg g^{-1}) and 50ppm SA (0.303 mg g^{-1}) and these were on par with control (0.259 mg g^{-1}). The treatments T₂ and T₇ also recorded high content of carotenoids 0.316 and 0.346 mg g^{-1} respectively compared to control.

At 30 DAS, among the treatments combination of complete nutrient solution sprays with 50ppm salicylic acid (0.317 mg g^{-1}) showed maximum carotene content and the lowest carotene content was recorded in 0.3% calcium with 50ppm SA sprays (0.223 mg g^{-1}) followed by gypsum $300 \text{ kg} + \text{boron } 1 \text{ kg ha}^{-1}$ and 50ppm SA (0.227 mg g^{-1}) and 0.3% calcium spray (0.226 mg g^{-1}) and these were on par with control (0.222 mg g^{-1}). All other treatments T₂, T₄, T₅, T₆ and T₉ also recorded increase in carotene content ranging from 0.242 to 0.304 mg g^{-1} .

At 45 DAE, among the treatments complete nutrient solution treated plants showed maximum carotenoids content (0.308 mg g^{-1}). The lowest carotenoids content was recorded in 0.3% calcium sprays (0.083 mg g^{-1}) followed by gypsum $300 \text{ kg} +$

boron 1 kg ha^{-1} (0.084 mg g^{-1}) and it was on par with control (0.079 mg g^{-1}). All other treatments T₄, T₅, T₇, T₈, T₉ and T₁₀ also recorded increase in carotene content ranged from 0.104 to 0.267 mg g^{-1} .

The application of calcium, boron and salicylic acid resulted in increase in carotenoids content by 1.2 to 1.6 folds at 15 DAE, 1.1 to 1.4 folds at 30 DAE and 1.3 to 3.8 folds at 45 DAE and the increase was high with complete nutrient spray solution at 45 DAE and sprays of complete nutrient solution + 50ppm SA (at 15 and 30 DAE). In the present study the reduction in photosynthetic pigment in untreated (control) plants was much higher than in treatments. This could be due to disease reducing effect of treatments coupled with their beneficial effect on photosynthetic pigment synthesis or stability of chloroplasts under stressed conditions as in the case of pathogen infections. Salicylic acid application was reported to induce chloroplast number besides resistance in faba bean infected with bean yellow mosaic virus (Radwan *et al.*, 2008). Increased chlorophyll content and photosynthetic activity alongside elevated SAR was observed in SA treated cowpea plants (Chandra and Bhatt, 1998). Kundu *et al.* (2011) found increased chlorophyll content besides elevated levels of SAR in MYMV infected blackgram plants.

4.6. LEAF ANALYSIS

4.6.1. Calcium (mg g^{-1})

Data pertaining to calcium content in leaf of blackgram in response to YMV disease incidence and application of calcium, boron and salicylic acid were presented in Table 4.14 Fig 4.14.

Application of calcium, boron and salicylic acid resulted in increase in calcium content and effect of all treatments was significant compared to control (13.33 mg g^{-1}) at 15 DAE. Among treatments T₂ to T₁₀, the highest calcium content was observed with soil application of gypsum $300 \text{ kg} + \text{boron } 1 \text{ kg ha}^{-1}$ (20.08 mg g^{-1}) and it was followed by 0.3% calcium spray (19.47 mg g^{-1}) and combination of gypsum $300 \text{ kg} + \text{boron } 1 \text{ kg ha}^{-1}$ with sprays of 50ppm SA (19.84 mg g^{-1}). The lowest calcium content was recorded with 0.25% boron spray (16.07 mg g^{-1}) which was on par with complete nutrient solution spray (16.09 mg g^{-1}), 0.25% boron sprays

with 50ppm SA (16.31 mg g⁻¹), 50ppm SA (16.71 mg g⁻¹) and sprays of complete nutrient solution + 50ppm SA (17.11 mg g⁻¹). The treatment T₈ also recorded increase in calcium content (18.44 mg g⁻¹).

At 30 DAE, maximum value for calcium content recorded in gypsum 300 kg + boron 1 kg ha⁻¹ (19.26 mg g⁻¹) in healthy followed by 0.3% calcium (18.68 mg g⁻¹) and sprays of 0.3% calcium with 50ppm SA (18.41 mg g⁻¹) and in disease infected plants, gypsum 300 kg + boron 1 kg ha⁻¹ plants (22.26 mg g⁻¹) recorded the highest calcium content. The lowest calcium content was recorded in 0.25% boron with 50ppm salicylic acid sprays (15.54 mg g⁻¹) in healthy plants and it was on par with 0.25% boron sprays (16.33 mg g⁻¹). In disease infected plants the lowest calcium content was recorded in 50ppm salicylic acid spray (17.36 mg g⁻¹) followed by sprays of 0.25% boron with 50ppm salicylic acid (17.58 mg g⁻¹) and those were on par with control (16.56 mg g⁻¹). The treatments T₅, T₆, T₇ and T₉ also recorded increase in calcium content ranging from 16.38 to 17.33 mg g⁻¹ in healthy plants and treatments T₃, T₄, T₆, T₇, T₈ and T₁₀ also showed high calcium content ranging from 17.85 to 20.14 mg g⁻¹.

At 45 DAE, application of calcium, boron and salicylic acid resulted in high calcium concentration in disease plants than in healthy plants. All treatments showed significant increase over control in both healthy (except T₄) and disease infected plants. Gypsum 300 kg + boron 1 kg ha⁻¹ was found superior to all other treatments (20.39 mg g⁻¹) in healthy followed by 0.3% calcium sprays (19.74 mg g⁻¹), sprays of 0.3% calcium + 50ppm SA (19.58 mg g⁻¹) and gypsum 300 kg + boron 1 kg ha⁻¹ (22.22 mg g⁻¹) in disease infected plants. The lowest calcium concentration was recorded in 0.25% boron spray in both healthy (16.57 mg g⁻¹) and in disease infected plants (18.25 mg g⁻¹). The remaining treatments T₅, T₆, T₇, T₉ and T₁₀ also showed increase in calcium content ranging from 17.46 to 18.89 mg g⁻¹ in healthy and treatments T₃, T₅, T₆, T₇, T₈, T₉ and T₁₀ also showed increase in calcium content ranging from 18.47 to 21.14 mg g⁻¹ in disease infected plants.

The increase in calcium content was recorded in all treatments compared to control in both healthy and disease infected plants with application of calcium, boron and salicylic acid ranging from 20.5 to 50.6 percent at 15 DAE, 10.9 to 37.4 percent in healthy and 4.8 to 36.9 percent in disease infected plants at 30 DAE and 12.8 to 30.8 percent in healthy and 5.4 to 28.4 percent at 45 DAE. The increase in calcium

content was high with gypsum 300 kg + boron 1 kg ha⁻¹ in both healthy and diseased leaves and low with 0.25% boron sprays and its combination with 50ppm SA. Thus it showed that there was increase in calcium content with application of calcium, boron and SA, maximum in disease infected plants than healthy plants compared to control.

Addition of gypsum + boron to soil and its combination with SA increased calcium content in blackgram leaves at 15 DAE by 50.6 percent and 48.8 percent respectively. The spray of calcium alone and in combination with SA increased the calcium content by 46.0 and 38.3 percent respectively. The spray of boron alone and in combination with SA increased the calcium content by 20.5 and 22.3 percent respectively. Complete nutrient solution alone and its combination with SA increased the calcium content by 20.7 and 28.3 percent respectively. The spray of SA alone increased it by 25.3 percent. This indicates that calcium concentration in leaf increased more by gypsum + boron, calcium spray and its combination with SA spray. After incidence of disease (at 30 and 45 DAE) also, calcium concentration in both healthy and diseased plants increased more by gypsum + boron, calcium spray and calcium + SA spray and moreover it was more in healthy plants. Thus due to these treatments calcium content increased and these might have caused change in defense metabolism which ultimately resulted in delay in disease incidence, reduction in percent disease incidence and improvement in growth and yield. Increase in tissue calcium concentration might have lead to increased divalent/monovalent cation ratio (Ca/K). Higher calcium content observed in gypsum + boron application was similar to that made by Jiang *et al.* (2013), who reported that tissue calcium was lowest in tomato infected by bacterial wilt for low calcium compared with medium and high calcium treatments. Addition of gypsum and boron to soil increased calcium content in leaves of LBG-623 by 1.4 to 1.5 folds before the incidence of YMV and by 1.3 to 1.4 folds after the incidence of YMV (Asif Khan, 2016). Similar results also reported by Volpin and Alad (1991) and Yamazaki and Hoshima (1995). The greater impact of application of calcium and SA is supporting the report of Reuveni *et al.*, (2008) who stated that boron can released calcium from cell wall and release calcium ions act together with salicylic acid to trigger the synthesis of pathogen resistance proteins.

Sherwood and Huisingh (1970) stated that, increased calcium content resulted in divalent/monovalent cation ratios and resistance was correlated more closely with a higher value of divalent/monovalent cation ratio than with calcium content of tissue.

More over cross linkage of pectic substances by divalent cation could influenced more by the ratios of divalent/monovalent cation than by concentration of any other cation (Johnsen *et al.*, 1968; Rains and Epstein 1967).

4.6.2. Potassium (%)

The data pertaining to effect of calcium, boron and SA application on potassium content in leaf of blackgram infected with YMV were tabulated in Table 4.15 and Fig 4.15. Potassium content in leaf increased with advancement in age of crop.

At 15 DAE, all treatments showed significant increase in potassium content compared to control (1.07%) except T₄. Among the treatments T₂ to T₁₀, the highest potassium content was recorded in gypsum 300 kg + boron 1 kg ha⁻¹ (1.82%) followed by 0.3% calcium (1.79%). The lowest potassium content was recorded with 0.25% boron spray (1.14%) which was on par with control, 50ppm SA spray and complete nutrient solution spray. The other treatments T₇, T₈, T₉ and T₁₀ also showed increase in potassium content ranging from 1.38 to 1.57 percent.

At 30 DAE, disease infected plants in all treatments showed maximum potassium content compared to healthy plants except in T₁ and T₉. Gypsum 300 kg + boron 1 kg ha⁻¹ was found superior to other treatments by recording 2.98 percent potassium content in healthy and 3.92 percent in disease infected plants. The lowest potassium content in healthy plants was recorded in complete nutrient solution sprays (1.36%) followed by gypsum 300 kg + boron 1 kg ha⁻¹ with 50ppm salicylic acid (1.55%), which were on par with control (1.48%) and in disease infected plants, sprays of 0.25% boron with 50ppm salicylic acid (1.63%) shown the lowest potassium content. The treatments T₃, T₄, T₅, T₈, T₉ and T₁₀ also recorded increase in potassium content ranging from 1.75 to 2.45 percent in healthy and the treatments T₃, T₄, T₅, T₆, T₈, T₉ and T₁₀ in disease infected plants also showed high potassium content ranging from 1.89 to 3.46 percent.

At 45 DAE, potassium content recorded in all treatments was high in disease plants compared to healthy plants except control. All treatments increased the potassium content significantly except T₅ in healthy plants. The potassium content

recorded was maximum in gypsum 300 kg + boron 1 kg ha⁻¹ (2.84%) in healthy and disease infected plants (3.975) and the lowest potassium was recorded in 50ppm SA (1.79%) in healthy which was on par with control (1.74%) and in disease infected plant combination of gypsum 300 kg + boron 1 kg ha⁻¹ with 50ppm salicylic acid sprays recorded the lowest potassium content (2.39%) and it was on par with complete nutrient solution sprays (2.48%). The treatments T₃, T₄, T₆, T₇, T₈, T₉ and T₁₀ showed the increase in potassium content in healthy plants ranging from 2.01 to 2.64 percent and the treatments T₃, T₄, T₅, T₈, T₉ and T₁₀ in disease infected plants also showed high potassium content ranging from 2.64 to 3.64 percent.

The application of calcium, boron and salicylic acid resulted in increase in potassium content in both healthy and disease infected plants compared to control and disease infected plants showed high potassium content compared to healthy plants. The increase in potassium content ranged from 1.1 to 1.7 folds at 15 DAE, 1.2 to 1.8 folds in healthy and 1.3 to 3.1 folds in disease infected plants at 30 DAE and 1.2 to 1.6 folds in healthy and 1.6 to 2.7 folds in disease infected plants at 45 DAE and it was high with gypsum 300 kg + boron 1 kg ha⁻¹ in both healthy and disease infected plants. Present investigation showed that there was increase in potassium content with application of calcium, boron and salicylic acid and the high potassium content was recorded with soil application of gypsum 300 kg + boron 1 kg ha at 15, 30 and 45 DAE compared to foliar application of calcium, boron and SA. Eraslan *et al.* (2007) stated that higher potassium concentration was noticed from calcium sprayed plants in tomato infected with ToMV.

4.6.3. Boron (mg kg⁻¹)

The data pertaining to effect of calcium, boron and SA application on boron content in leaf of blackgram infected with YMV were tabulated in Table 4.16 and Fig 4.16. Boron content in leaf found increased with advancement in age of crop.

At 15 DAE, application of calcium, boron and SA resulted in increase in boron content and effect of all treatments (8.95 to 4.43 mg kg⁻¹) was found significant compared to control (7.79 mg kg⁻¹). Among treatments T₂ to T₁₀, the highest boron content was recorded in gypsum 300 kg + boron 1 kg ha⁻¹ (11.43 mg kg⁻¹) and it was on par with 0.25% boron spray (11.43 mg kg⁻¹), combination of gypsum 300 kg +

boron 1 kg ha⁻¹ with 50ppm SA spray (11.25 mg kg⁻¹), sprays of 0.25% boron with 50ppm SA (11.02 mg kg⁻¹) and 0.3% calcium sprays (10.69 mg kg⁻¹). The lowest boron content was recorded in 50ppm SA spray (8.95 mg kg⁻¹) which was found on par with complete nutrient solution spray and combination of complete nutrient solution and SA spray. The treatment T₈ also showed increase in boron content (9.88 mg kg⁻¹).

At 30 DAE, there was significant increase in boron content obtained in all treatments compared to control except T₅. Among treatments, the highest boron content was recorded in 0.25% boron spray (14.03 mg kg⁻¹) in healthy followed by gypsum 300 kg + boron 1 kg ha⁻¹ (13.67 mg kg⁻¹), sprays of 0.25% boron + 50ppm SA (13.24 mg kg⁻¹) and in disease infected plants, it was in gypsum 300 kg + boron 1 kg ha⁻¹ (18.75 mg kg⁻¹) which was on par with 0.25% boron sprays (18.40 mg kg⁻¹). The lowest boron content recorded in 50ppm SA (10.23 mg kg⁻¹) in healthy and in disease infected plants (11.35 mg kg⁻¹). The treatments T₃, T₆, T₇, T₈ and T₁₀ also resulted in high boron content ranging from 10.91 to 12.86 mg g⁻¹ in healthy plants and the treatments T₃, T₆, T₇, T₈, T₉ and T₁₀ also recorded increase in boron content ranging from 12.33 to 17.33 mg kg⁻¹ in disease infected ones.

At 45 DAE, all treatments showed significant difference compared to control both in healthy (17.90 mg kg⁻¹) and disease infected plants (19.84 mg kg⁻¹). Gypsum 300 kg + boron 1 kg ha⁻¹ showed the highest boron content (26.2 mg kg⁻¹) in healthy followed by 0.3% calcium spray (24.9 mg kg⁻¹), complete nutrient solution spray (26.13 mg kg⁻¹), gypsum 300 kg + boron 1 kg ha⁻¹ with 50ppm SA sprays (26.05 mg kg⁻¹), sprays of 0.3% calcium with 50ppm SA (26.05 mg kg⁻¹) and 0.25% boron sprays + 50ppm SA (25.8 mg kg⁻¹). In disease infected plants also gypsum 300 kg + boron 1 kg ha⁻¹ (38.2 mg kg⁻¹) recorded the highest boron content followed by 0.3% calcium spray (37.77 mg kg⁻¹) and 0.25% boron spray (35.62 mg kg⁻¹). The lowest boron content was recorded in 50ppm salicylic acid (21.92 mg kg⁻¹) in healthy and complete nutrient solution (30.13 mg kg⁻¹), complete nutrient solution + 50ppm SA (30.27 mg kg⁻¹), in disease infected plants. The treatments T₄ and T₁₀ also recorded increase in boron content 23.24 and 24.15 mg kg⁻¹ respectively in healthy and the treatments T₇, T₈, T₉ and T₁₀ also recorded increase in boron content ranging from 30.27 to 34.17 mg kg⁻¹ in disease infected plants.

The increase in boron content with application of calcium, boron and salicylic acid ranged from 14.81 to 46.7 percent at 15 DAE, 14.2 to 46.9 percent in healthy and 39.7 to 131.0 percent in disease infected plants at 30 DAE and 22.4 to 46.3 percent in healthy and 51.9 to 92.7 percent in disease infected plants at 45 DAE. The boron high was recorded in gypsum 300 kg + boron 1 kg ha⁻¹ in both healthy and disease infected plants. Finding of present investigation showed that boron content was high in disease infected plants compared to healthy plants and low boron content was recorded with alone application of 50ppm SA compared to calcium and boron sprays alone and in combination.

4.7. YIELD AND YIELD COMPONENTS

The data pertaining to the effect of calcium, boron and salicylic acid application on yield components and yield of YMV infected blackgram were presented in Table 4.17, 4.18 and 4.19. Fig 4.17, 4.18 and 4.19.

4.7.1 Number of Pods Plant⁻¹

Impact of treatments T₂ to T₁₀ on number of pods plant⁻¹ was on par with control. However, higher value was observed in sprays of complete nutrient solution and 50ppm SA (6.4 pod plant⁻¹) and the lower in sprays of 0.25% boron + 50ppm SA (5.7 pod plant⁻¹). Other treatments T₂, T₃, T₄, T₅, T₆, T₇ and T₈ resulted in pod number plant⁻¹ ranging from 5.8 to 6.2.

Eventhough it was nonsignificant, number of pods plant⁻¹ obtained with complete nutrient solution + SA spray was 12.3 percent higher than control. The treatments T₂, T₃ and T₇ resulted in 8.8, 7.0 and 5.3 percent increase over control.

4.7.2. Pod Length (cm)

Application of calcium, boron and SA resulted in increase in pod length compared to control and the highest pod length was recorded in 50ppm SA sprays (4.1 cm) followed by sprays of 0.25% boron + 50ppm SA (4.0cm), gypsum 300 kg + boron 1 kg ha⁻¹ (3.93 cm), 0.3% calcium + 50ppm SA sprays (3.93 cm) and 0.25% boron sprays (3.86 cm). the pod length obtained in other treatments was on par with control.

The increase in pod length with application of calcium, boron and salicylic acid ranged from 20.6 to 28.1 percent and it was high recorded in 50 ppm SA and low in combination of gypsum 300 kg + boron 1 kg ha⁻¹ with 50ppmSA spray. The application of complete nutrient solution spray alone and in combination showed no effect on pod length. Calcium spray alone had no marked effect on pod length, while its combination with SA showed increase (22.8%) in pod length. The application of boron and its combination with SA spray showed increase (20.6 and 25.0 % respectively) in pod length. Spray of SA alone also showed 28.1 percent increase in pod length. This finding suggested that application of calcium, boron and SA to YMV infected blackgram plants will improve the pod length.

4.7.3. Number of Seed Pod⁻¹

Calcium, boron and salicylic acid application showed increase in number of seed pod⁻¹ compared to control. The highest number of seed pod⁻¹ was obtained in gypsum 300 kg + boron 1 kg ha⁻¹ (6.3), sprays of 0.25% boron + 50ppm SA (5.9) and complete nutrient solution with 50ppm SA sprays (5.9). The number of seed pod⁻¹ observed in other treatments did not differ with control.

The increase in number of seed pod⁻¹ obtained with application of calcium, boron and salicylic acid ranged from 13.5 to 21.1 percent, high with gypsum 300 kg + boron 1 kg ha⁻¹. Calcium and complete nutrient solution spray alone had no marked effect, while its combination with SA sprays showed marked increase in seed number by 13.5 percent. The increase in seed number pod⁻¹ could be attributed to increase in pod length.

4.7.4. 100 Seed Weight (g)

Among the treatments, sprays of complete nutrient with 50ppm salicylic acid (4.26 g) showed the highest 100 seed weight followed by sprays of 0.25% boron with 50ppm SA (4.0 g), 0.3% calcium sprays (3.93 g) and gypsum 300 kg + boron 1 kg ha⁻¹ (3.93 g) and gypsum + boron with SA spray (3.93 g). The lowest was recorded in 0.3% calcium with 50ppm SA (3.53 g) followed by complete nutrient sprays (3.73 g), 50ppm SA sprays (3.8 g) and 0.25% boron sprays (3.8 g) and these were on par with control (3.26 g).

There was increase in 100 seed weight ranging from 9.3 to 30.6 percent. The application of gypsum + boron to soil alone and its combination showed increase (20.6%) in 100 seed weight. Boron, SA and complete nutrient solution alone had no marked effect on 100 seed weight, but the combination of boron and complete nutrient solution with SA spray showed increase in 100 seed weight (22.7 and 30.7% respectively). Application of calcium alone showed marked effect (20.6%) on 100 seed weight, while its combination had no effect on 100 seed weight.

4.7.5. Pod Weight (g plant⁻¹)

The highest pod weight was recorded in sprays of 0.25% boron + 50ppm SA (7.0 g plant⁻¹) followed by gypsum 300 kg + boron 1 kg ha⁻¹ with 50ppm SA (6.96 g plant⁻¹), sprays of 0.3% calcium + 50ppm SA (6.96 g plant⁻¹), gypsum 300 kg + boron 1 kg ha⁻¹ (6.83 g plant⁻¹) and complete nutrient solution sprays (6.8 g plant⁻¹). The lowest pod weight was recorded in complete nutrient solution with 50ppm SA sprays (6.26g plant⁻¹) followed by 50ppm SA (6.30 g plant⁻¹), 0.25% boron (6.37 g plant⁻¹) and 0.3% calcium sprays (6.53 g plant⁻¹) and these were on par with control (5.46 g plant⁻¹).

There was increase in pod weight in treated plants compared to control ranging from 24.5 to 28.2 percent. Calcium, boron and SA alone application showed no effect on pod weight, while the combination of calcium and boron with SA showed increase (27.4 and 28.2% respectively) in pod weight. Gypsum + boron soil application alone and in combination with SA showed increase (25.0 and 27.4% respectively) in pod weight. Complete nutrient solution spray alone had no marked effect on pod weight, but its combination with SA showed increase (14.6%) in pod weight.

4.7.6. Seed Yield (g plant⁻¹)

Seed yield plant⁻¹ was found high in sprays of complete nutrient solution with 50ppm SA (2.28 g) followed by gypsum 300 kg + boron 1 kg ha⁻¹ (2.23 g), combination of gypsum 300 kg + boron 1 kg ha⁻¹ with 50ppm SA (2.18 g) and 0.3% calcium sprays (2.16 g). The lowest seed yield was recorded with 50ppm SA (1.64 g) followed by 0.25% boron sprays (1.79 g), complete nutrient solution sprays (1.89 g), sprays of 0.25% boron + 50ppm SA (1.92 g) and 0.3% calcium + 50ppm SA (1.98 g) and these were on par with control (1.62 g).

Calcium, boron nutrition and SA application increased the seed yield plant⁻¹ in YMV infected blackgram by 1.2 to 40.7 percent. The increase in seed yield plant⁻¹ with 0.3% calcium spray alone was 33.3 percent, with gypsum + boron and SA spray was 34.5 percent, with gypsum + boron was 37.7 percent and with complete nutrient solution and SA sprays was 40.7 percent.

4.7.7. Yield (kg ha⁻¹)

All treatments showed significant increase in yield compared to control (472.5 kg ha⁻¹) except T₄ and T₅. The highest seed yield was obtained with sprays of complete nutrient solution + 50ppm SA (665.0 kg ha⁻¹) followed by gypsum 300 kg + boron 1 kg ha⁻¹ (650.41 kg ha⁻¹), gypsum 300 kg + boron 1 kg ha⁻¹ with 50ppm SA sprays (635.8 kg ha⁻¹) and 0.3% calcium sprays (630.0 kg ha⁻¹). The lowest yield obtained in 50ppm SA (478.3 kg ha⁻¹) followed by 0.25% boron sprays (522.08 kg ha⁻¹) and these were on par with control (472.5 kg ha⁻¹). The treatments T₆, T₈ and T₉ also recorded increase in yield ranging from 551.2 to 577.5 kg ha⁻¹.

Calcium, boron and SA application increased the seed yield in YMV infected blackgram by 16.7 to 40.7 percent over control. The increase in seed yield with gypsum and boron application to soil and its combination with 50ppm SA sprays was 37.7 and 34.6 percent respectively. This could be attributed to high content of calcium, boron and potassium in leaf, leaf total phenols, POD and PAL activity, then reduction in whitefly incidence, delay and reduction in disease incidence, which further resulted in improvement in growth, total dry matter, seed number pod⁻¹ and 100 seed weight. The calcium spray alone increased the yield over control by 35.4 percent, which could be attribute to increase in calcium, boron and potassium content in leaf, reduction in membrane injury index, reduction in whitefly incidence, delay and reduction of disease incidence, improvement in growth, leaf dry matter and 100 seed weight, while the combination of 0.3% calcium and 50ppm SA spray increased the yield only by 22.2%. 0.25% boron spray alone had no effect, but the combination of boron and SA spray showed 18.5% increase in seed yield. Spray of complete nutrient solution alone increased the yield only by 16.7 percent but its combination with 50ppm SA resulted in greater increase by 40.7 percent. This could be attributed to high content of photosynthetic pigments which might have increased the photosynthesis and accumulation of dry matter and further increase in seed number pod⁻¹, 100 seed weight and seed yield.

From these findings, it was evident that application of complete nutrient solution + 50ppm SA spray, gypsum 300 kg + boron 1 kg ha, 0.3% calcium spray and gypsum 300kg + boron 1 kg ha + 50ppm SA spray improved the yield of blackgram in the situation of YMV incidence.

Chapter V

SUMMARY AND CONCLUSIONS

The field experiment was carried out at Agricultural College Farm, Bapatla during *Rabi* season 2016-17 to “study of the effect of calcium, boron and salicylic acid on tolerance of blackgram (*Vigna mungo* (L.) Hepper) to yellow mosaic virus”. The experiment was laid out in Randomized Block Design with ten treatments viz., T₁ : Control ; T₂ : Gypsum @ 300 kg ha⁻¹ + Boron@1kg ha⁻¹; T₃ :0.3% Calcium (CaNO₃.4H₂O) ; T₄ :0.25% Boron (Borax); T₅:50ppm Salicylic acid spray ; T₆ : Complete nutrient solution spray ; T₇ : gypsum 300 kg + boron 1 kg ha + 50ppm SA ;T₈: 0.3% calcium + 50ppm SA; T₉ :0.25% boron + 50ppm SA; T₁₀:complete nutrient solution + 50ppm SA in three replications. The results of investigation summarized in this chapter.

Observation was recorded on percent incidence of YMV, Whitefly number, morphological, physiological, biochemical parameters and mineral nutrient composition before and after incidence of YMV. Data on yield and yield components were recorded at harvest.

Seven days delay and reduction in YMV incidence was noticed with application of calcium, boron and SA. The reduction in disease incidence ranged from 1.7 to 5.8 percent at 30DAE and 15.1 to 30.5 percent at 45 DAE, high with soil application of 300 kg gypsum + boron 1 kg ha⁻¹ (30.5%), complete nutrient solution spray (29.2%), gypsum + boron + SA (28.3%) and 0.3% calcium + SA spray (26.1%) and low in 0.25% boron spray.

Whitefly population reduced compared to control ranging from 12.4 to 35.6 percent at 15 DAE, 4.5 to 36.9 percent at 30 DAE and 8.1 to 21.9 percent at 45 DAE. The reduction was high in 0.3% calcium + 50ppm SA spray, gypsum 300 kg + boron 1 kg ha⁻¹ alone and its combination with 50ppm SA spray and low in 50ppm SA spray.

The increase in number of branches with application of calcium, boron and salicylic acid ranged from 4.9 to 21.9 percent at 30 DAE, 3.7 to 20.4 percent at 60 DAE and 7.0 to 14.0 percent at harvest, high in T₂ (gypsum 300kg + boron 1 kg ha⁻¹) and T₇ (gypsum 300 kg + boron 1 kg ha⁻¹ + 50ppm SA spray).

Leaf dry matter accumulation plant⁻¹ with calcium, boron and SA application increased ranging from 14.2 to 133.9 percent at 30 DAE, 0.5 to 82.1 percent at 60 DAE and 32.2 to 111.7 percent at harvest, high with gypsum 300 kg + boron 1 kg ha⁻¹ alone and with 50ppm SA sprays and low with sprays of 0.3% calcium + 50ppm SA. The increase in leaf dry matter in other treatments T₃, T₄, T₅, T₆, T₉ and T₁₀ which were on par was 40.4, 40.9, 42.1, 40.7, 31.7 and 43.9 percent respectively.

Calcium, boron and SA application increased the dry matter accumulation in stem over control by 6.3 to 14.3 folds at 30 DAE, 1.6 to 2.8 folds at 60 DAE and 1.1 to 1.2 folds at harvest. The increase in stem dry matter was obtained with T₈, T₉ and T₁₀. Sprays of complete nutrient solution and 50ppm SA showed the maximum increase in stem dry matter and low with 50ppm SA sprays compared to control.

Total dry matter production increased with application of calcium, boron and SA over control, ranged from 14.5 to 176.3 percent at 30 DAE, 7.5 to 76.02 percent at 60 DAE and 0.7 to 42.0 percent at harvest. It was observed high in T₇ (42.0%), T₂ (40.4%), T₁₀ (34.4%) and T₆ (29.8%) and low in SA spray alone (T₅ - 0.7%).

Total phenols content in both healthy and disease infected plants increased ranging from 27.8 to 62.2 percent at 15 DAE, 38.3 to 110.6 percent in healthy and 7.04 to 38.0 percent in disease infected plants at 30 DAE and 22.5 to 82.2 percent in healthy and 4.8 to 51.8 percent in diseased plants at 45 DAE. In healthy plants before and after incidence the increase was high with gypsum + boron soil application alone (T₂) and its combination with SA (T₇) and low with 0.3% calcium spray (T₃). In diseased plants also increase in total phenols was high with same treatments T₂ and T₇ and low with T₃. Compared to control the impact of treatments on percent increase in leaf total phenols was more in healthy plants than in diseased plants.

The increase in POD activity with application of calcium, boron and salicylic acid ranged from 31.2 to 100.0 percent at 15 DAE, 26.3 to 84.2 percent in healthy and 2.7 to 43.2 percent in disease infected leaves at 30 DAE and 3.7 to 48.1 percent in healthy and 4.2 to 36.1 percent in disease infected plants at 45 DAE. In healthy plants the increase was high with T₅ and T₇ before incidence of YMV, T₂ and T₇ just after incidence and T₂ at later stages. While in diseased plants, just after incidence and at later stages POD activity was high with T₇ and T₂. In both healthy and diseased plants

less increase in POD activity was noticed in 0.25% boron spray in healthy and complete nutrient solution + 50ppm SA in diseased plants. The impact was more in healthy plants than in diseased ones.

The extent of percent increase in PAL activity with calcium, boron and SA application was found more in healthy than in diseased plants, ranged from 27.7 to 64.8 percent at 15 DAE, 27.8 to 63.9 percent in healthy leaves and 12.9 to 40.8 percent in disease infected leaves at 30 DAE, 19.4 to 97.2 percent in healthy and 2.7 to 19.8 percent in disease infected plants at 45 DAE. In healthy plants before YMV incidence increase in PAL activity was high with T₇. In both healthy and diseased plants, just after YMV incidence increase in PAL activity was high with T₇ and at later stages with T₂. Less increase in PAL activity was noticed with T₄ in healthy and T₄ (30DAE) and T₆ (45 DAE) in disease infected plants.

Application of calcium, boron and salicylic acid resulted in reduction of membrane injury index to an extent of 2.7 to 7.9 percent at 15 DAE, 5.6 to 8.5 percent at 30 DAE and 5.0 to 10.8 percent at 45 DAE. The reduction in membrane injury index was high with sprays of 0.3% calcium + 50ppm SA, gypsum 300 kg + boron 1 kg ha⁻¹ and low with sprays of 0.25% boron + 50ppm SA and complete nutrient solution.

Chlorophyll-a content increased due to application of calcium, boron and salicylic acid to an extent of 2.1 to 2.7 folds at 15 DAE, 1.4 to 2.9 folds at 30 DAE and 1.2 to 2.9 folds at 45 DAE. The hike in chlorophyll-a high with T₂ and T₁₀ upto 30 DAE and with T₁₀ at 45 DAE, while low was noticed in sprays 0.3% calcium + 50ppm SA (at 15 & 30 DAE), complete nutrient solution spray (at 45 DAE).

Chlorophyll-b content increased with by 1.4 to 2.4 folds at 15 DAE, 1.5 to 1.7 folds at 30 DAE and 1.1 to 2.2 folds at 45 DAE, high with complete nutrient solution sprays and low with 0.3% calcium + 50ppm SA upto 30 DAE and 50ppm SA at 45 DAE.

The raise in carotenoids content due to treatments was 1.2 to 1.6 folds at 15 DAE, 1.1 to 1.4 folds at 30 DAE and 1.3 to 3.8 folds at 45 DAE, high with complete nutrient solution spray (at 45 DAE) and sprays of complete nutrient solution + 50ppm SA (at 15 and 30 DAE).

The increase in calcium content over control was recorded in all treatments in both healthy and disease infected plants, ranging from 20.5 to 50.6 percent at 15 DAE, 10.9 to 37.4 percent in healthy and 4.8 to 36.9 percent in disease infected plants at 30 DAE and 12.8 to 30.8 percent in healthy and 5.4 to 28.4 percent in diseased ones at 45 DAE. The increase was high with gypsum + boron, calcium spray and its combination with SA sprays in both healthy and diseased plants before and after YMV incidence.

Potassium content in both healthy and disease infected plants increased due to application of calcium, boron and SA compared to control, ranged from 1.1 to 1.7 folds at 15 DAE, 1.2 to 1.8 folds in healthy and 1.3 to 3.1 folds in disease infected plants at 30 DAE and 1.2 to 1.6 folds in healthy and 1.6 to 2.7 folds in disease infected plants at 45 DAE. The hike in leaf potassium was high with gypsum 300 kg + boron 1 kg ha⁻¹ in both healthy and disease infected plants before and after YMV incidence.

Boron content in leaf raised with application of calcium, boron and salicylic acid ranged from 14.81 to 46.7 percent at 15 DAE, 14.2 to 46.9 percent in healthy and 39.7 to 131.0 percent in disease infected plants at 30 DAE and 22.4 to 46.3 percent in healthy and 51.9 to 92.7 percent in disease infected plants at 45 DAE. The extent of increase was found higher in healthy than in diseased plants with gypsum + boron alone and its combination with SA before YMV incidence and with gypsum + boron alone after YMV incidence.

Number of pods plant⁻¹ increased with application of calcium, boron and salicylic acid by 1.8 to 12.3 percent, high in sprays of complete nutrient solution with 50ppm SA and low in 0.25% boron + 50ppm SA sprays.

Pod length increased to an extent of 20.6 to 28.1 percent, high with 50 ppm SA and low in gypsum 300 kg + boron 1 kg ha⁻¹ + 50ppm SA. Spray of complete nutrient solution alone and in combination with SA showed no effect on pod length. Calcium spray alone had no marked effect, while its combination with SA showed increase (22.8%) in pod length. Spray of boron and its combination with SA showed increase (20.6 and 25% respectively) in pod length. Spray of SA alone also showed 28.1 percent increase.

Number of seeds pod^{-1} increased ranging from 13.5 to 21.1 percent with application of calcium, boron and SA, high with gypsum 300 kg + boron 1 kg ha^{-1} , calcium and complete nutrient solution spray alone had no marked effect, while its combination with SA sprays showed marked increase in seed number by 13.5 percent.

100 seed weight increased to an extent of 9.3 to 30.6 percent, high with sprays of complete nutrient solution + 50ppm SA (30.7%) and gypsum + boron alone and its combination with SA (20.5%).

Increase in pod weight in treated plants compared to control ranged from 24.5 to 28.2 percent. Spray of calcium, boron and SA alone (T_3 , T_4 & T_5) showed no effect, while the combinations of calcium and boron with SA (T_2 , T_7 , T_8 & T_9) and complete nutrient solution (T_6) showed increase in pod weight.

Calcium, boron and SA application increased the seed yield plant^{-1} in YMV infected blackgram by 1.2 to 40.7 percent, 33.3 percent with 0.3% calcium spray alone, 34.5 percent with gypsum + boron and SA spray, 37.7 percent with gypsum + boron and 40.7 percent with complete nutrient solution and SA sprays.

Calcium, boron and SA application increased the seed yield in YMV infected blackgram over control. Complete nutrient solution + 50ppm SA sprays (40.7%) recorded the highest percent increase in seed yield through increase in number of pods, number of seed pod^{-1} , pod length, 100 seed weight and pod weight. 0.3% calcium spray alone increased the yield by 35.4 percent, but its combination with SA spray increased the yield only by 22.2 percent. Gypsum + boron soil application alone and in combination with SA also recorded marked increase in seed yield (37.7 and 34.6 percent respectively). The treatments T_6 and T_9 which were on par with T_8 also recorded increase in seed yield to an extent of 16.7, 22.2 and 18.5 percent respectively.

Based on the results of present investigation, the following conclusions can be made

1. Calcium, boron and SA application delayed the incidence of YMV disease by seven days. The reduction in percent disease incidence was high with gypsum 300 kg + boron 1 kg ha^{-1} . The reduction in disease incidence may be due to reduction in whitefly population.

2. Application of gypsum 300 kg + boron 1 kg ha⁻¹, complete nutrient solution spray, gypsum 300 kg + boron 1 kg ha⁻¹ + 50ppm SA, 0.3% calcium + 50ppm SA spray reduced the disease incidence.
3. More reduction in whitefly population obtained with 0.3% calcium + 50ppm SA spray, gypsum 300 kg + boron 1 kg ha⁻¹ alone and its combination with SA.
4. Gypsum 300 kg + boron 1 kg ha⁻¹ alone and in combination with 50ppm SA spray, 0.3% calcium spray and 50ppm SA spray increased the number of branches. Leaf dry matter increased more by gypsum 300 kg + boron 1 kg ha⁻¹ and its combination with SA. Total dry matter accumulation was high in T₇, T₂, T₆ and T₁₀.
5. Total phenols content and PAL activity in leaf was high with gypsum + boron alone (T₂) and its combination with SA (T₇). POD activity in leaf was high with T₅ and T₇ before YMV incidence and T₂ and T₇ after incidence. Membrane injury was less in 0.3% calcium + SA 50ppm (T₈), gypsum + boron soil application (T₂). Chlorophyll a, chlorophyll b and carotenoids content increased with T₂ and T₁₀, T₆ and T₆ & T₁₀ respectively.
6. Calcium and potassium content in leaf was high with T₂ before and after YMV incidence. Boron content in leaf was high with treatments T₂ and T₄ before and after YMV incidence.
7. Pod length was high in T₅, T₉, T₂, T₈ and T₄. Seed number pod increased with T₂, T₈ and T₁₀. 100 seed weight increased with treatments T₁₀, T₉, T₂ and T₃. Pod weight was high in treatments T₉, T₈, T₇, T₂ and T₆. Seed yield plant increased with treatments T₁₀, T₂, T₇ and T₃. Seed yield ha⁻¹ was high in treatments T₁₀, T₂, T₃ and T₇ (40.7, 37.7, 35.4 and 34.6 percent respectively).
8. Any of the treatments can be used for integrity disease management.
9. Plants treated with complete nutrient solution and 50 ppm SA recorded highest yield and it might be due to reduction in whitefly incidence increased in leaf, stem and total dry matter which resulted in higher biomass production and thus improved yield.

Future line of work

As high yield was obtained with complete nutrient solution + SA spray was high followed by soil application of gypsum + boron alone and in combination with SA spray and 0.3% calcium spray, the impact of complete nutrient solution having calcium 0.3% and boron 0.25% + 50ppm SA spray on alleviation of YMV disease incidence can be studied.

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Note: The literature is cited as per the “Thesis Guidelines” prescribed by Acharya N. G. Ranga Agricultural University, Guntur.



Plate 1. Overall view of field experiment

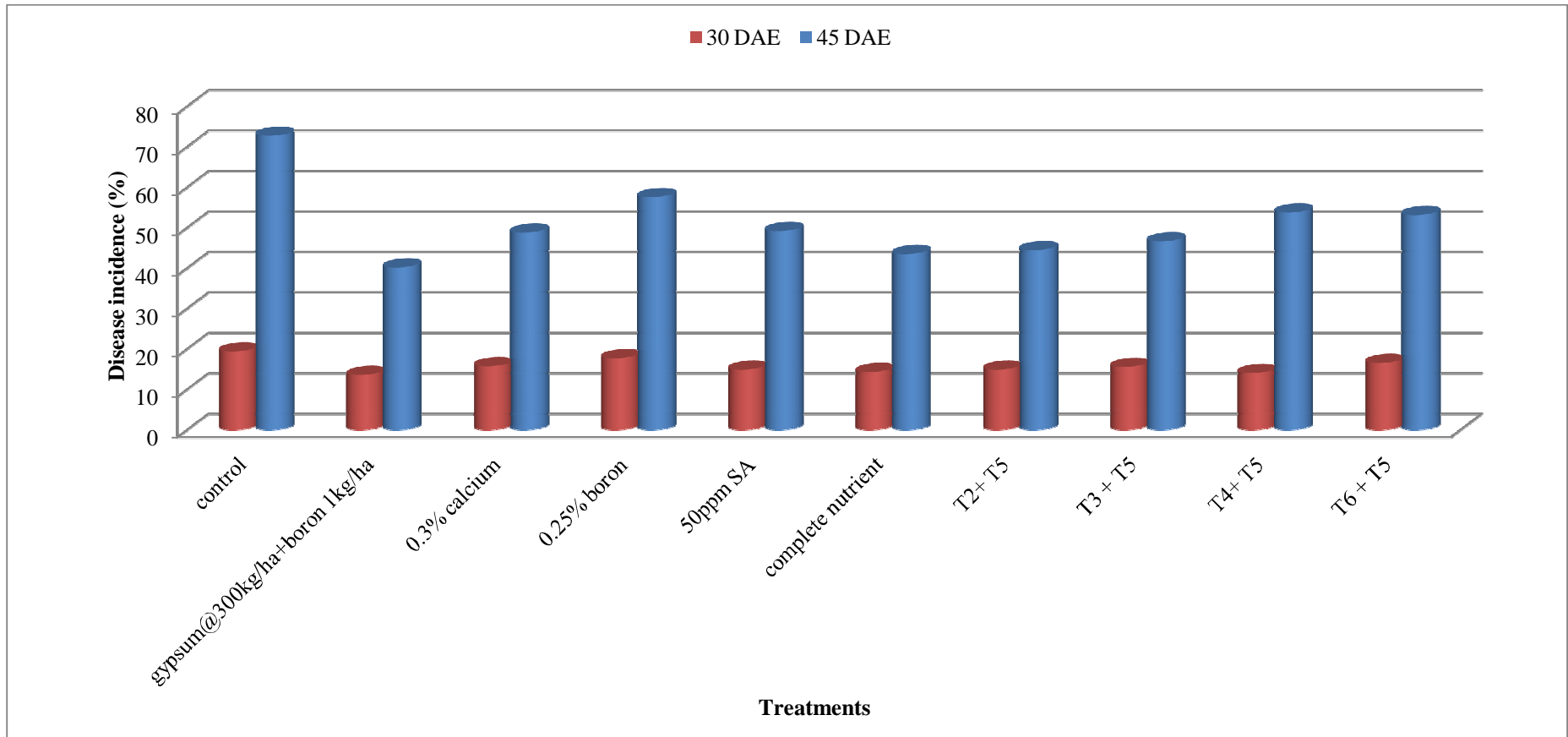


Fig. 4.1. Effect of Calcium, Boron and Salicylic acid on YMV Disease incidence in YMV infected Blackgram

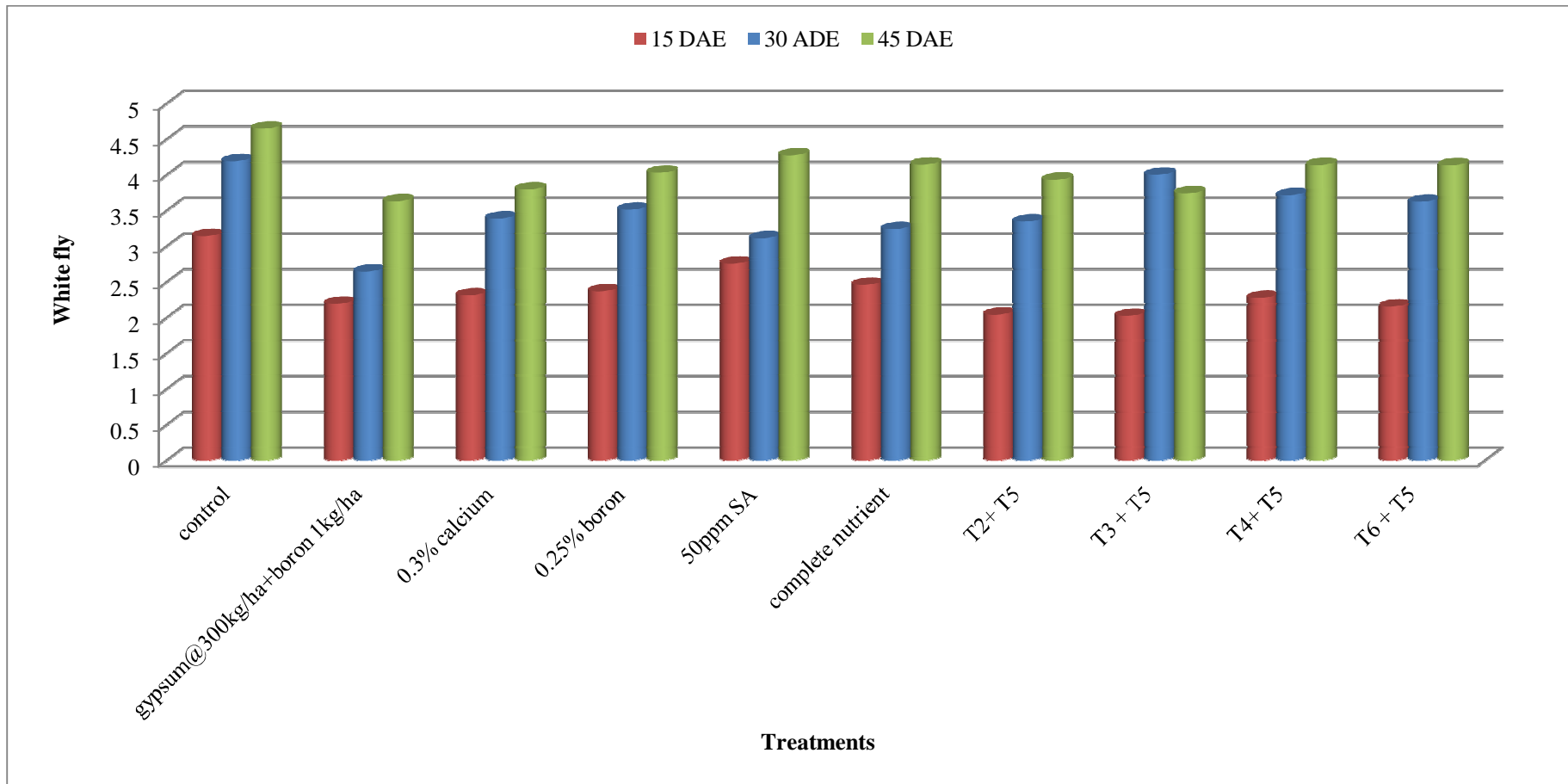


Fig. 4.2. Effect of Calcium, Boron and Salicylic acid on whitefly count of YMV infected Blackgram

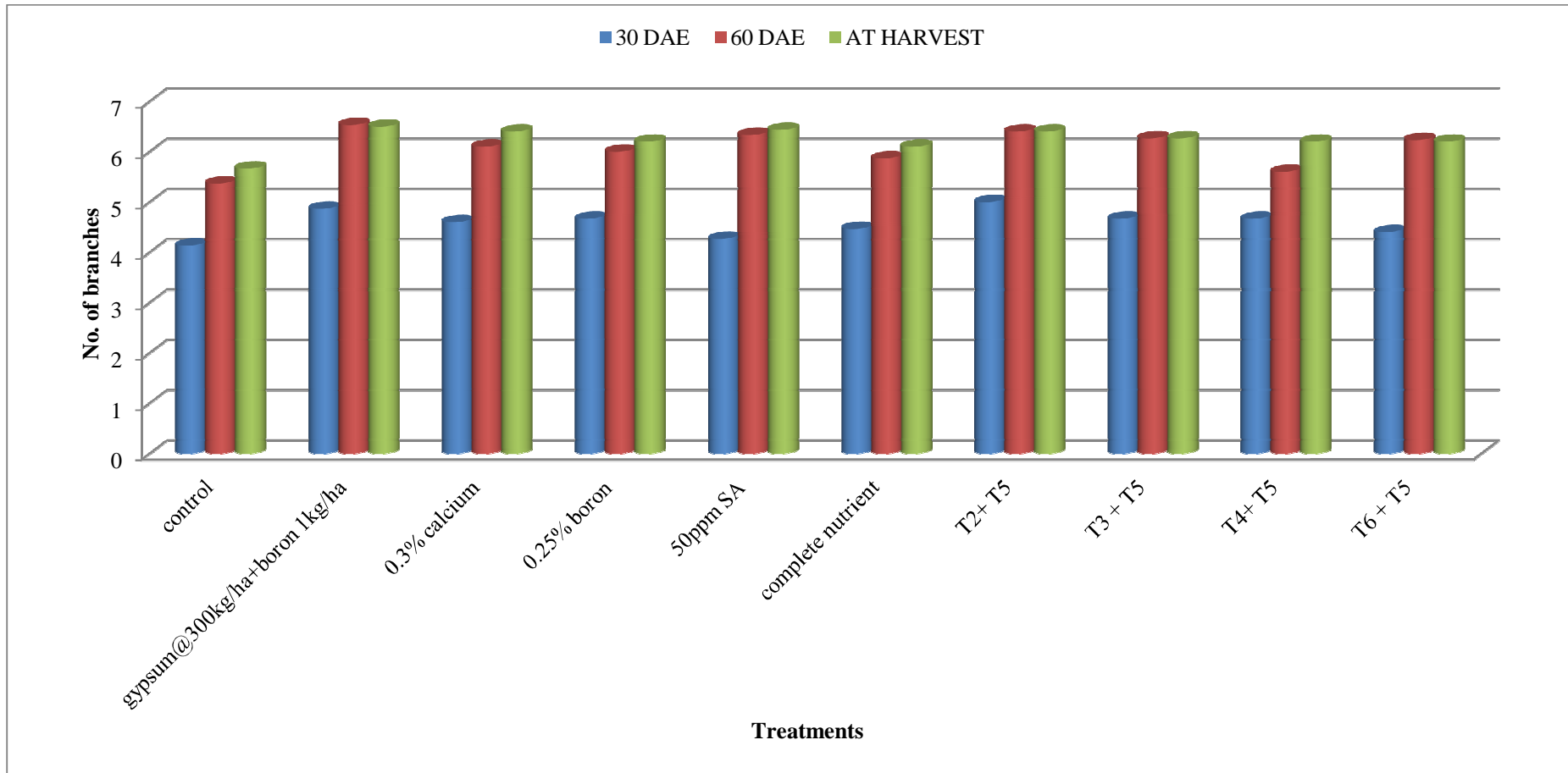


Fig. 4.3. Effect of Calcium, Boron and Salicylic acid on number of branches plant⁻¹ in YMV infected Blackgram

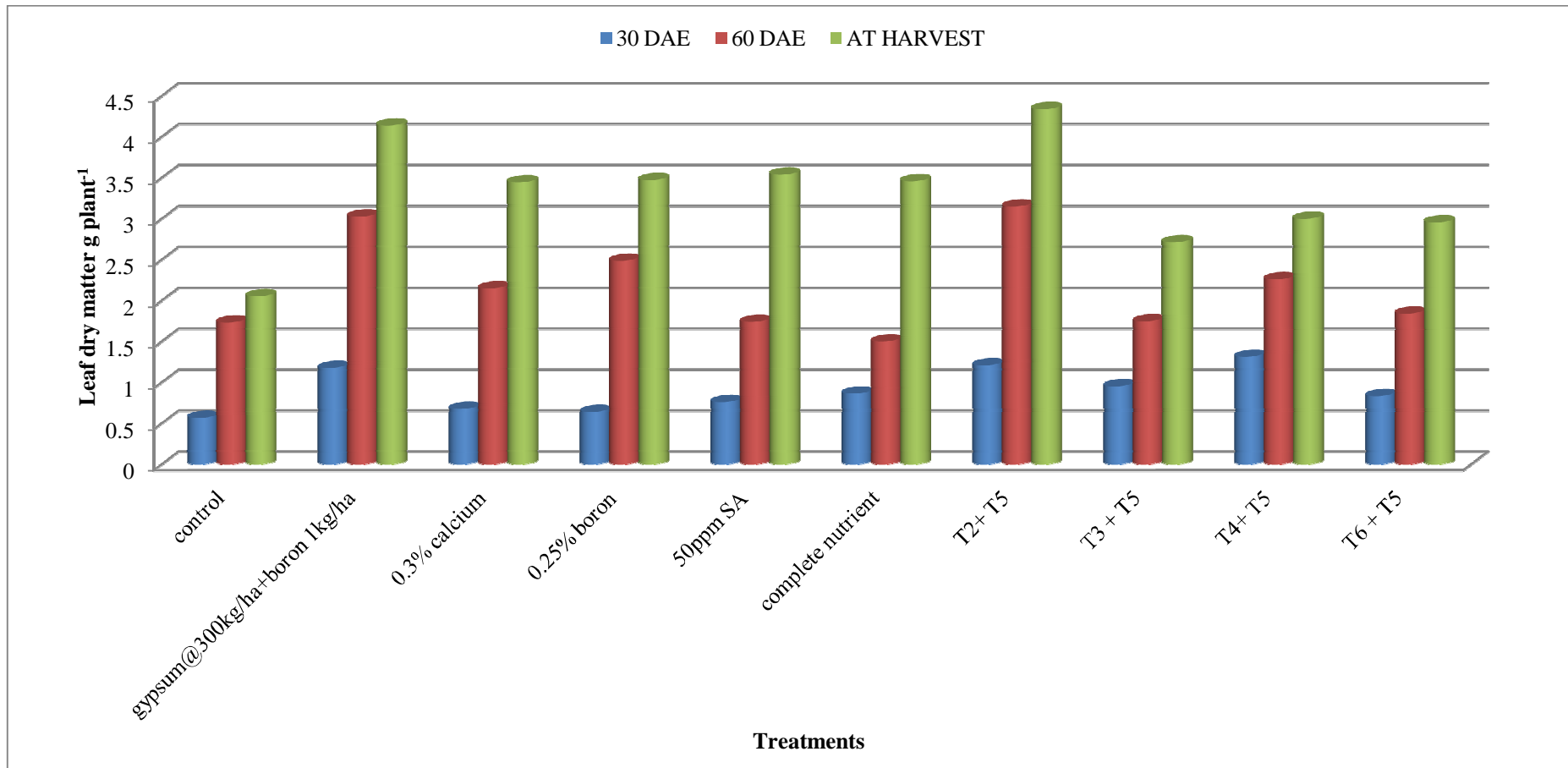


Fig. 4.4. Effect of Calcium, Boron and Salicylic acid on leaf dry matter (g plant⁻¹) in YMV infected Blackgram

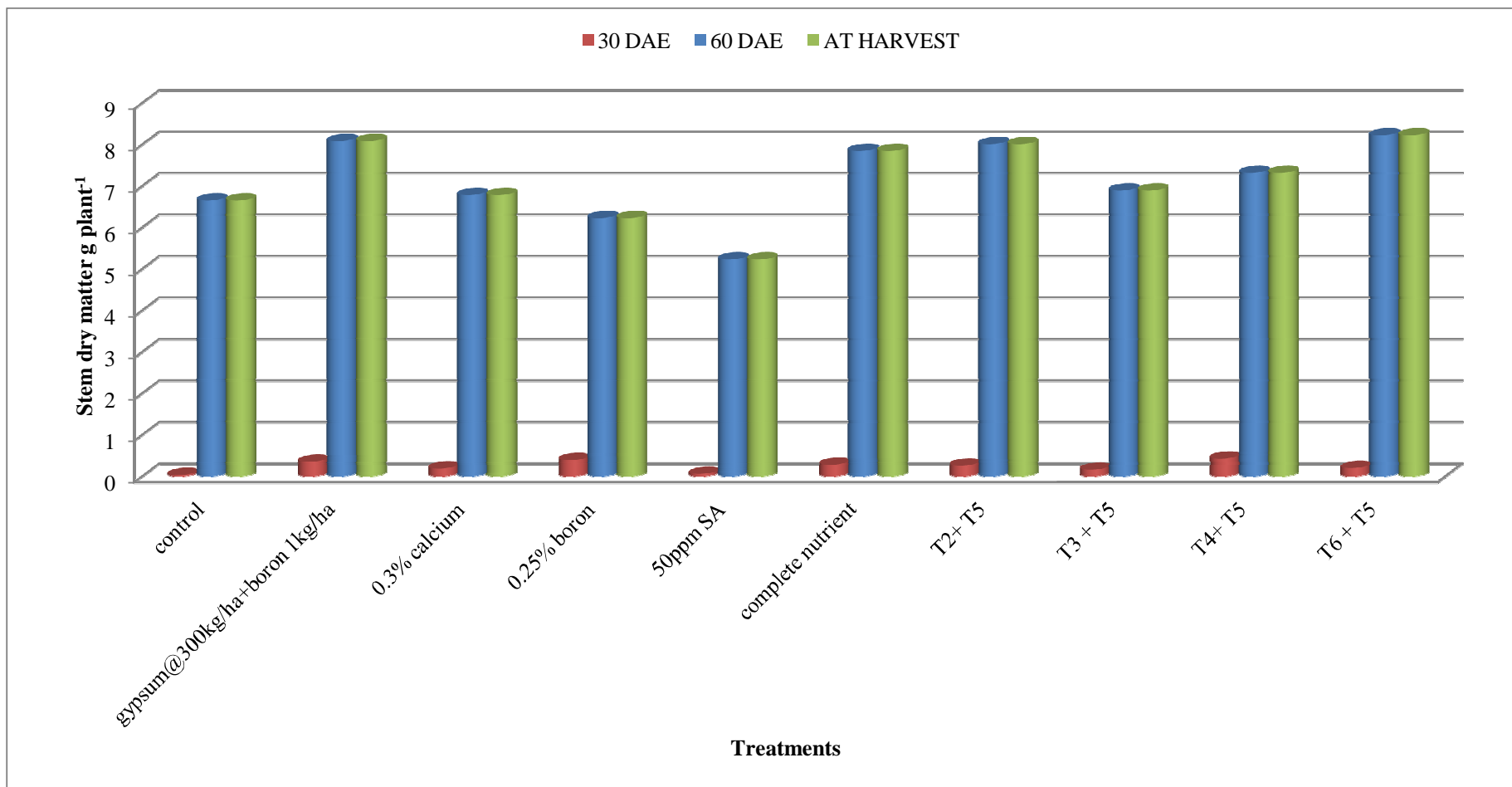


Fig. 4.5. Effect of Calcium, Boron and Salicylic acid on stem dry matter (g plant⁻¹) in YMV infected Blackgram

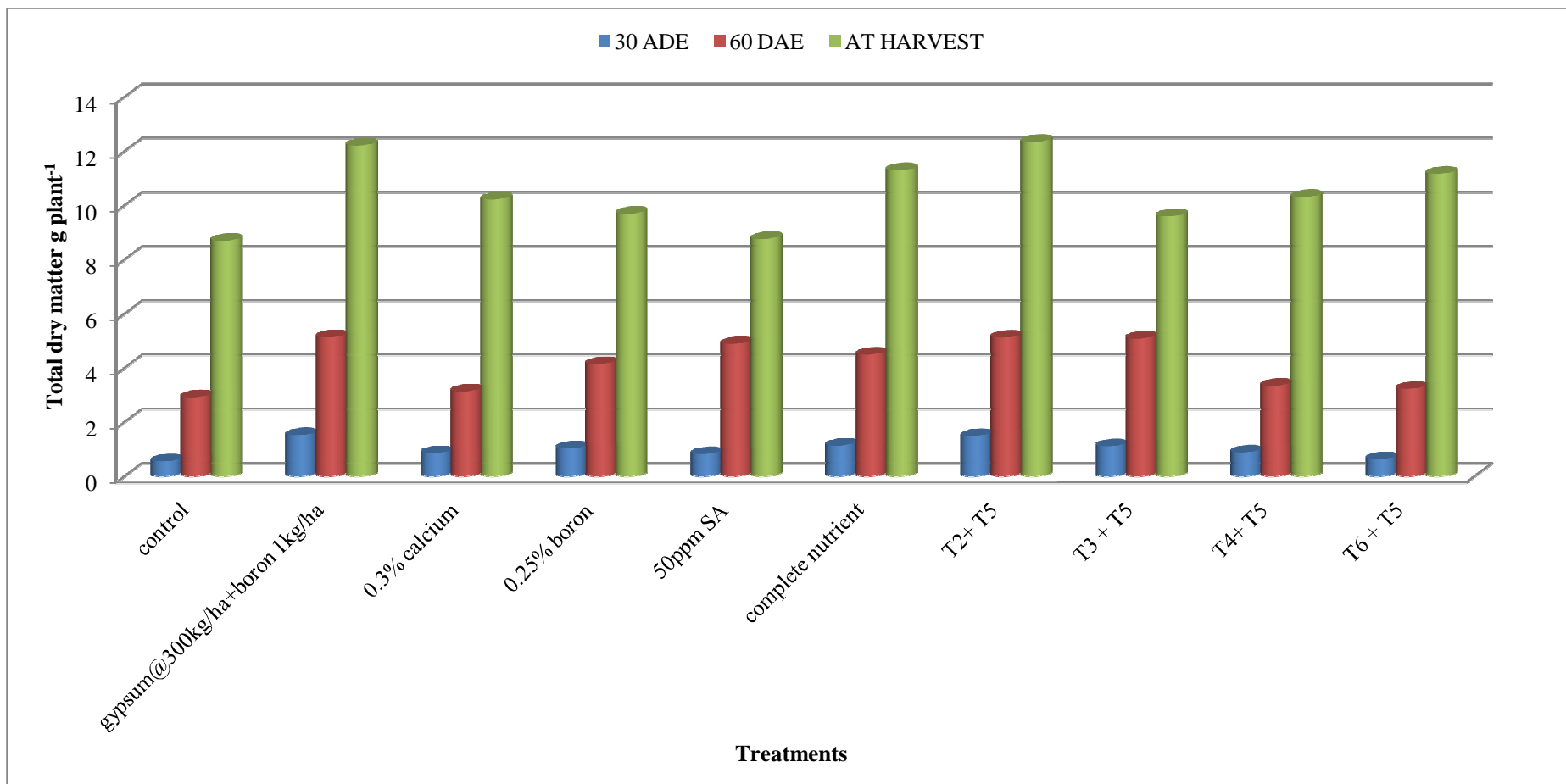


Fig. 4.6. Effect of Calcium, Boron and Salicylic acid on total dry matter (g plant⁻¹) in YMV infected Blackgram

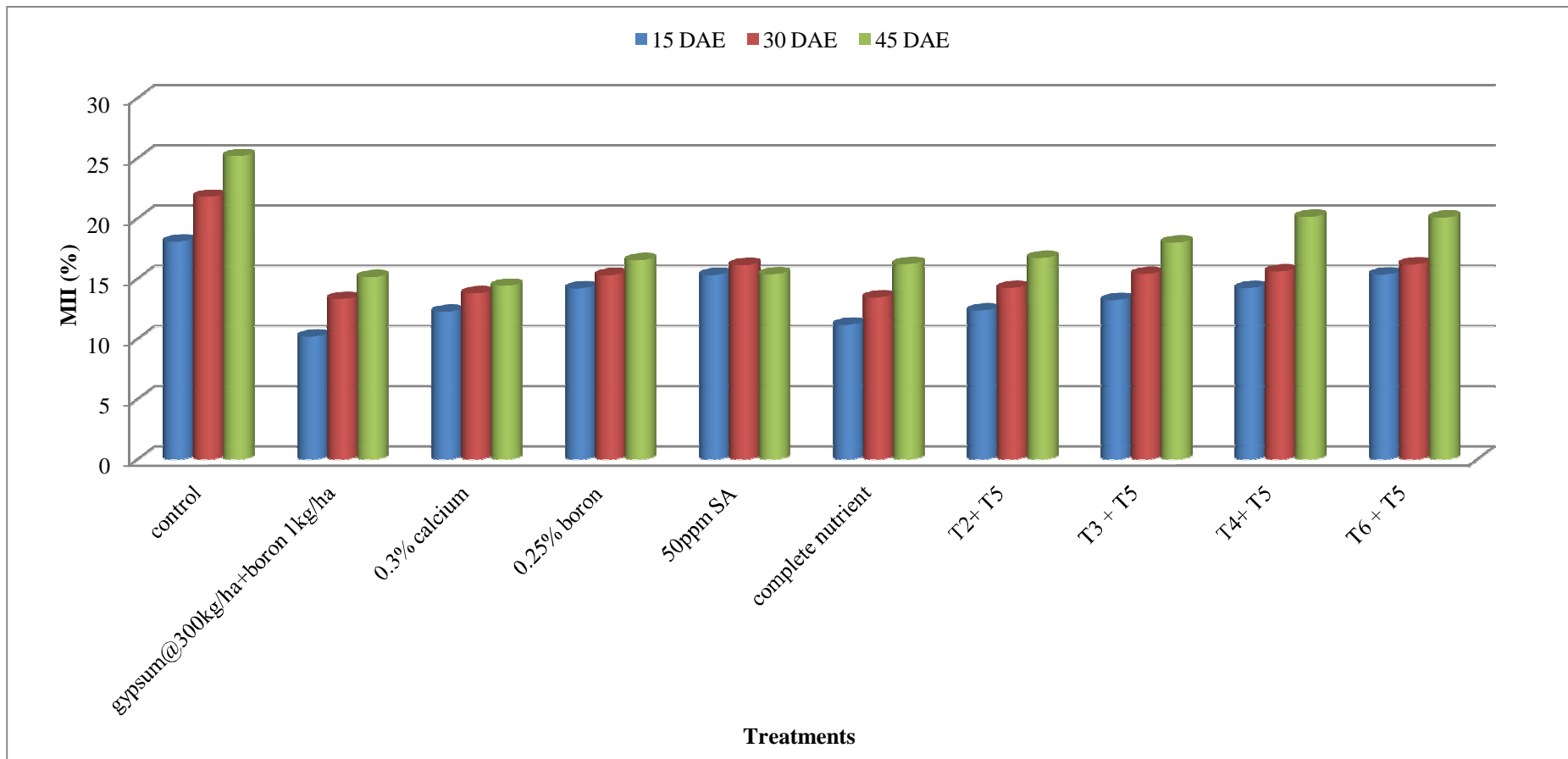


Fig. 4.10. Effect of Calcium, Boron and Salicylic acid on membrane injury index in YMV infected Blackgram

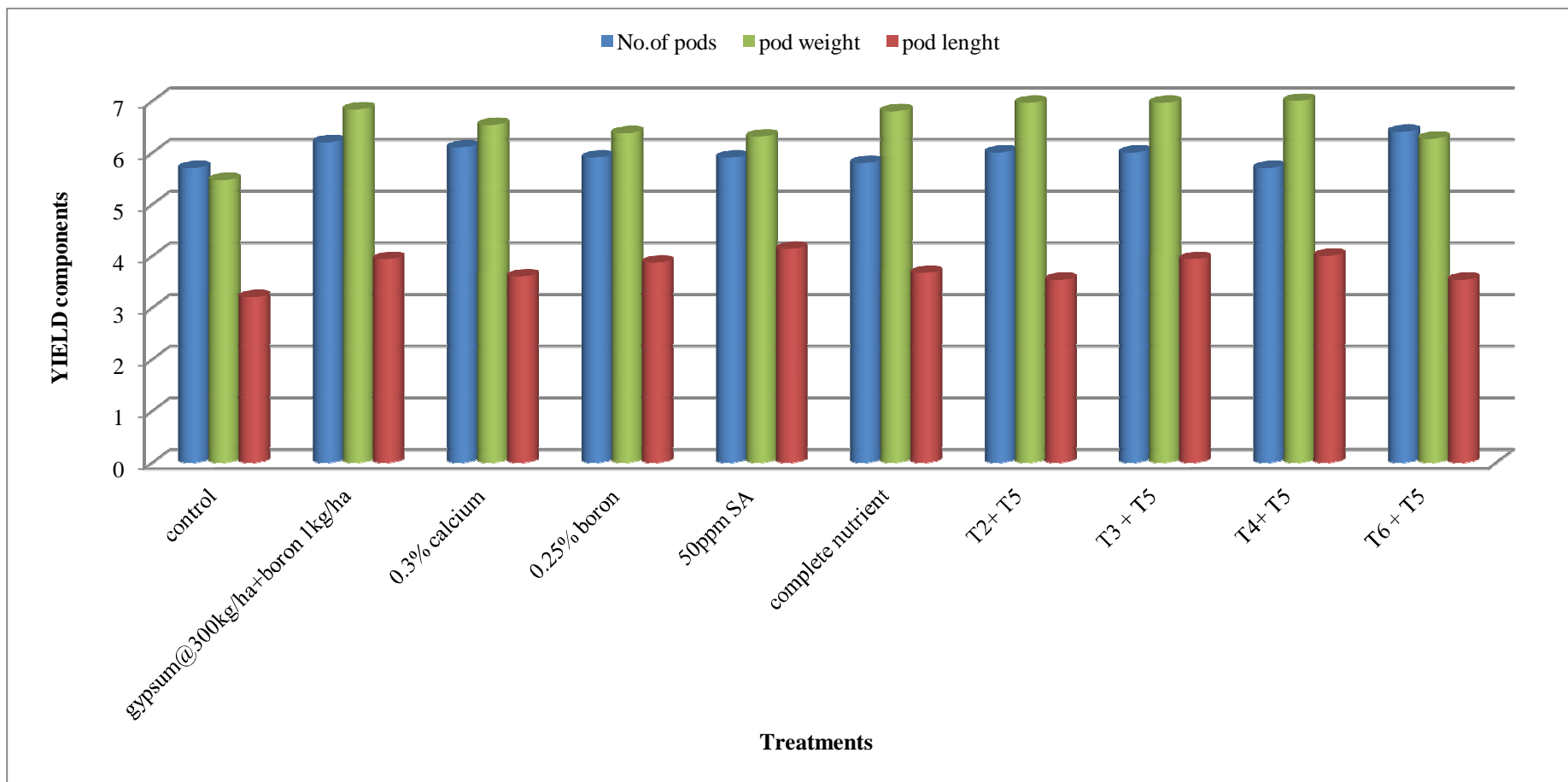


Fig. 4.17. Effect of Calcium, Boron and Salicylic acid on yield components in YMV infected Blackgram

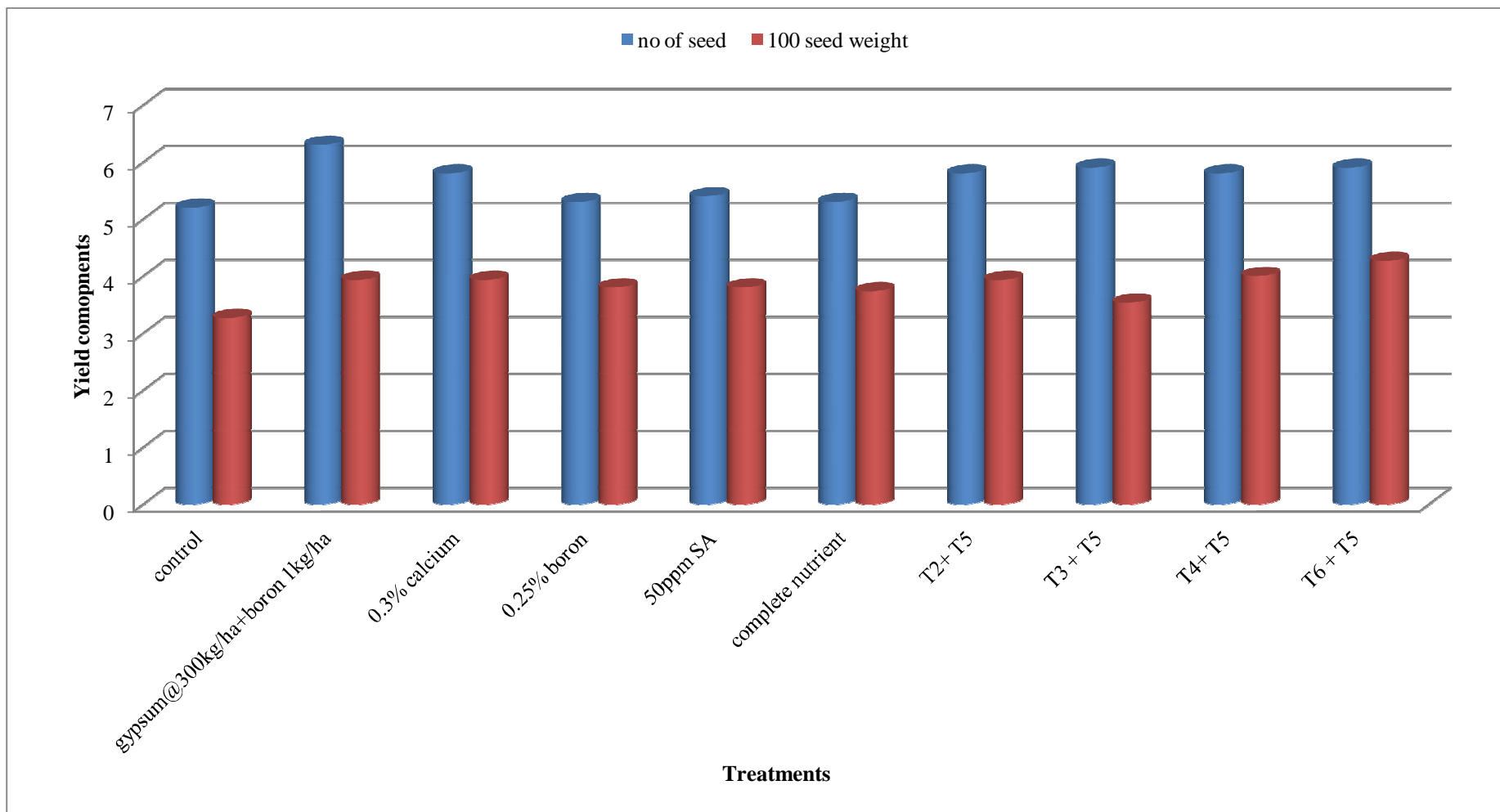


Fig. 4.18. Effect of Calcium, Boron and Salicylic acid on yield components in YMV infected Blackgram

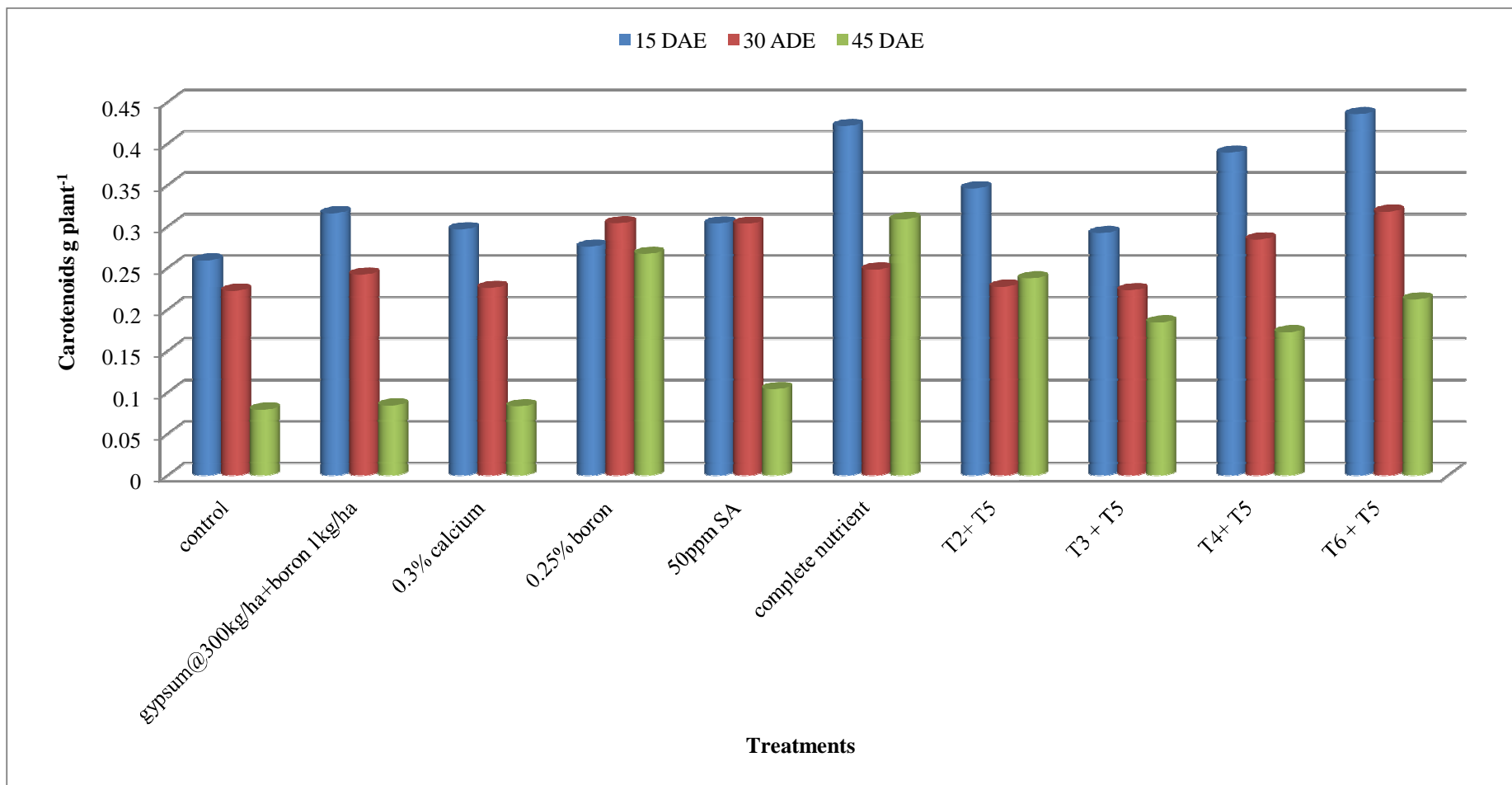


Fig. 4.13. Effect of Calcium, Boron and Salicylic acid on carotenoids in leaf of YMV infected Blackgram

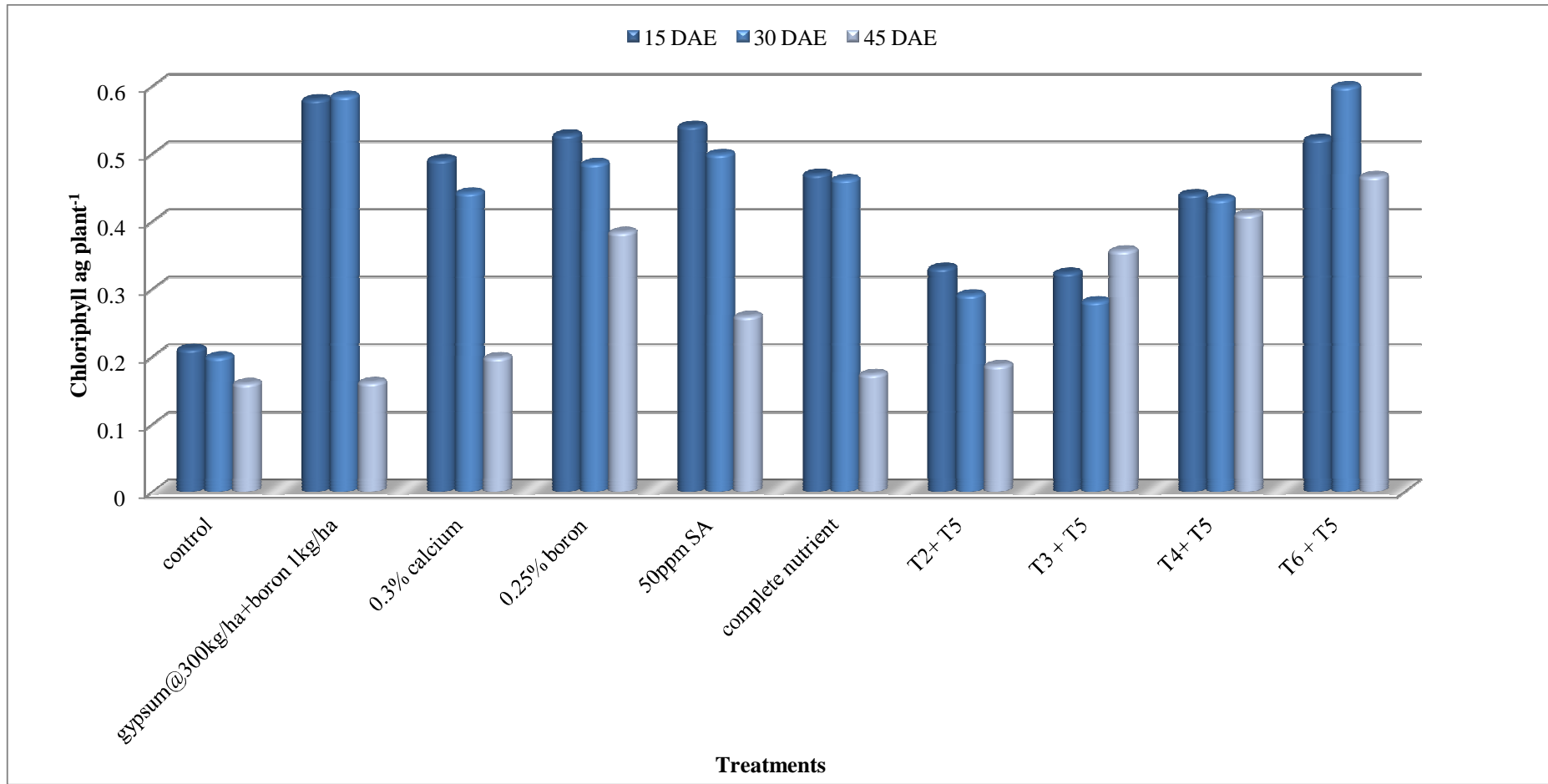


Fig. 4.11. Effect of Calcium, Boron and Salicylic acid on Chorophyll a content in leaf of YMV infected Blackgram

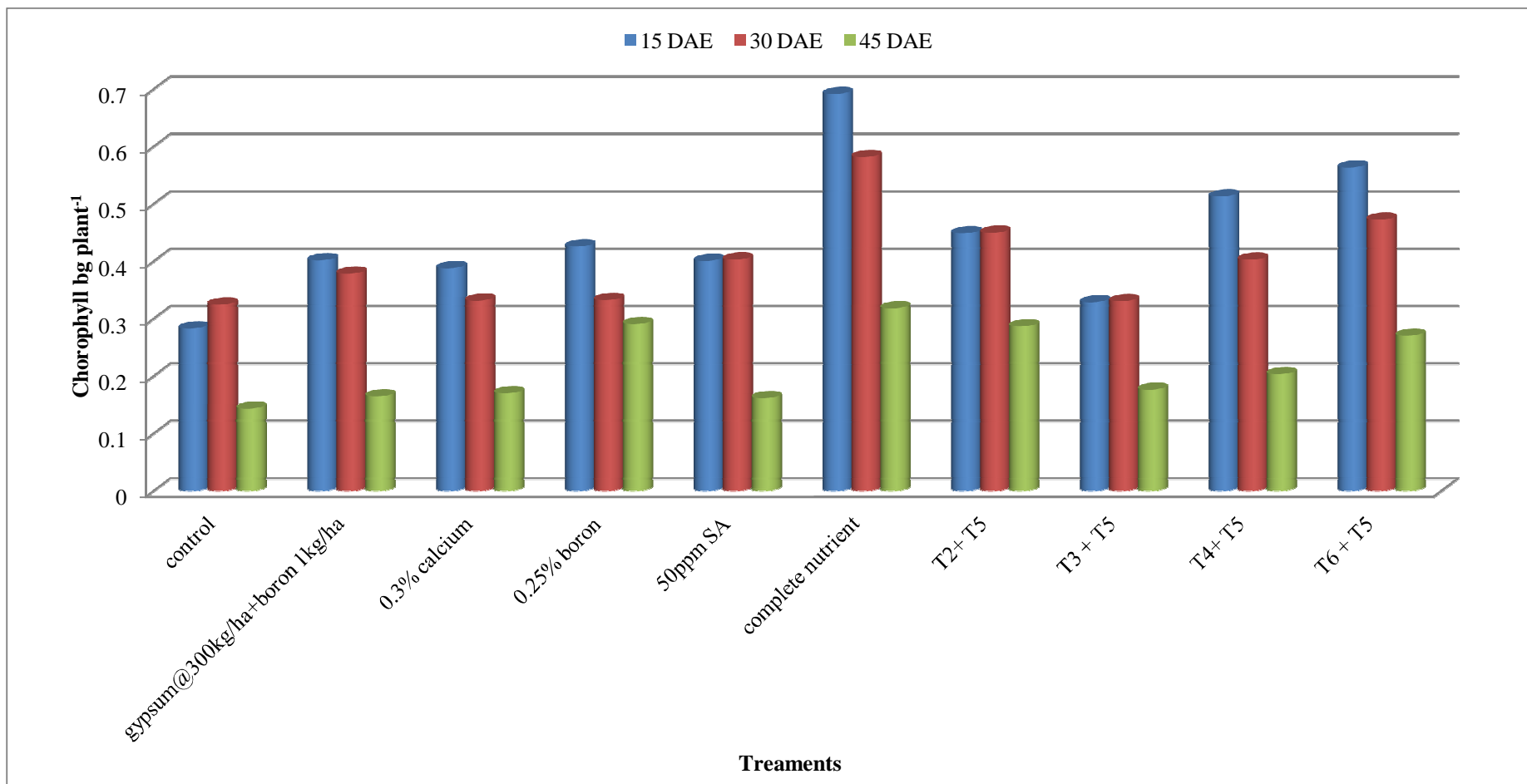


Fig. 4.12. Effect of Calcium, Boron and Salicylic acid on Chlorophyll b content in leaf of YMV infected Blackgram

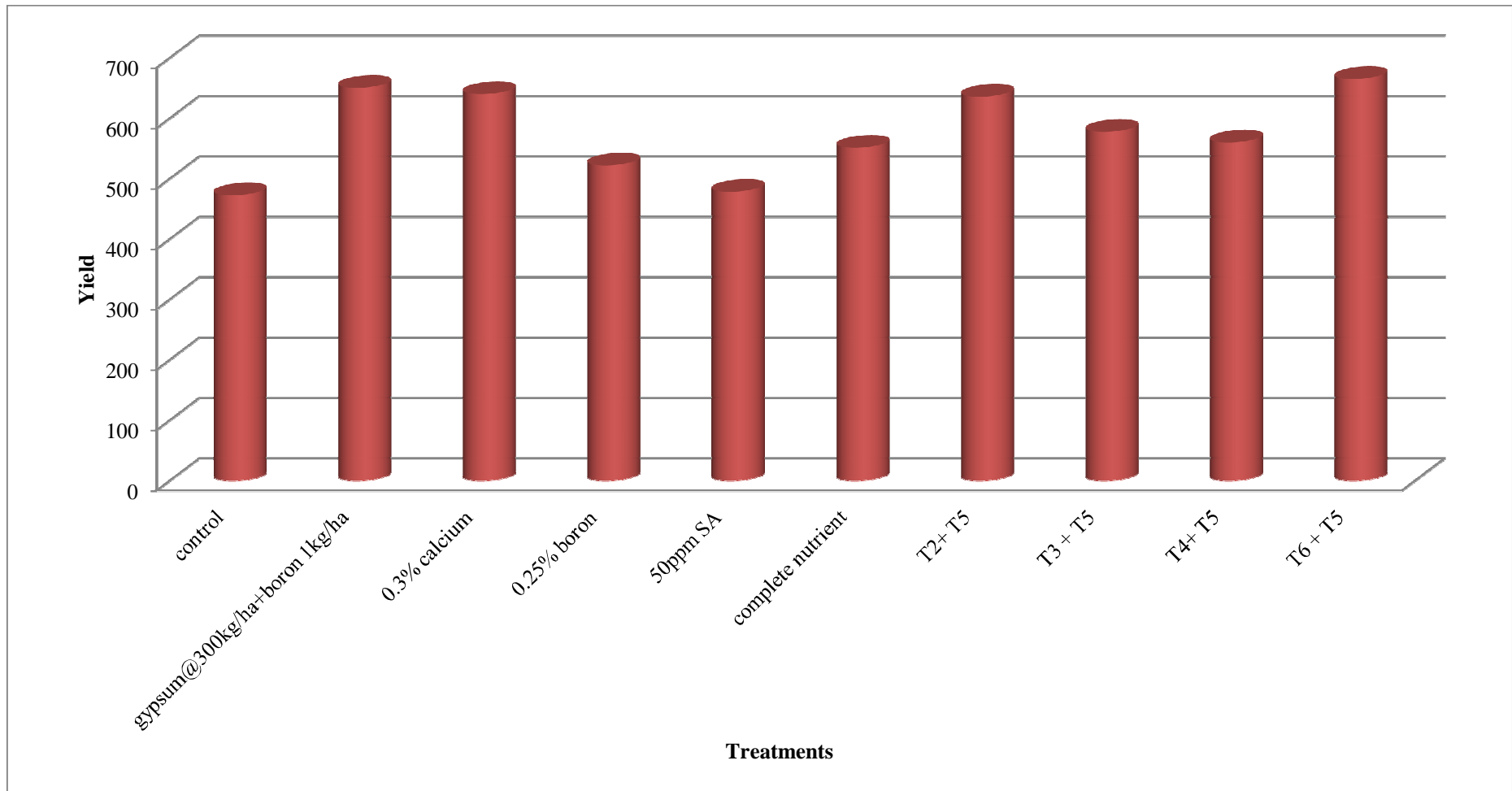


Fig. 4.19. Effect of Calcium, Boron and Salicylic acid on Yield (Kg ha⁻¹) of YMV infected Blackgram

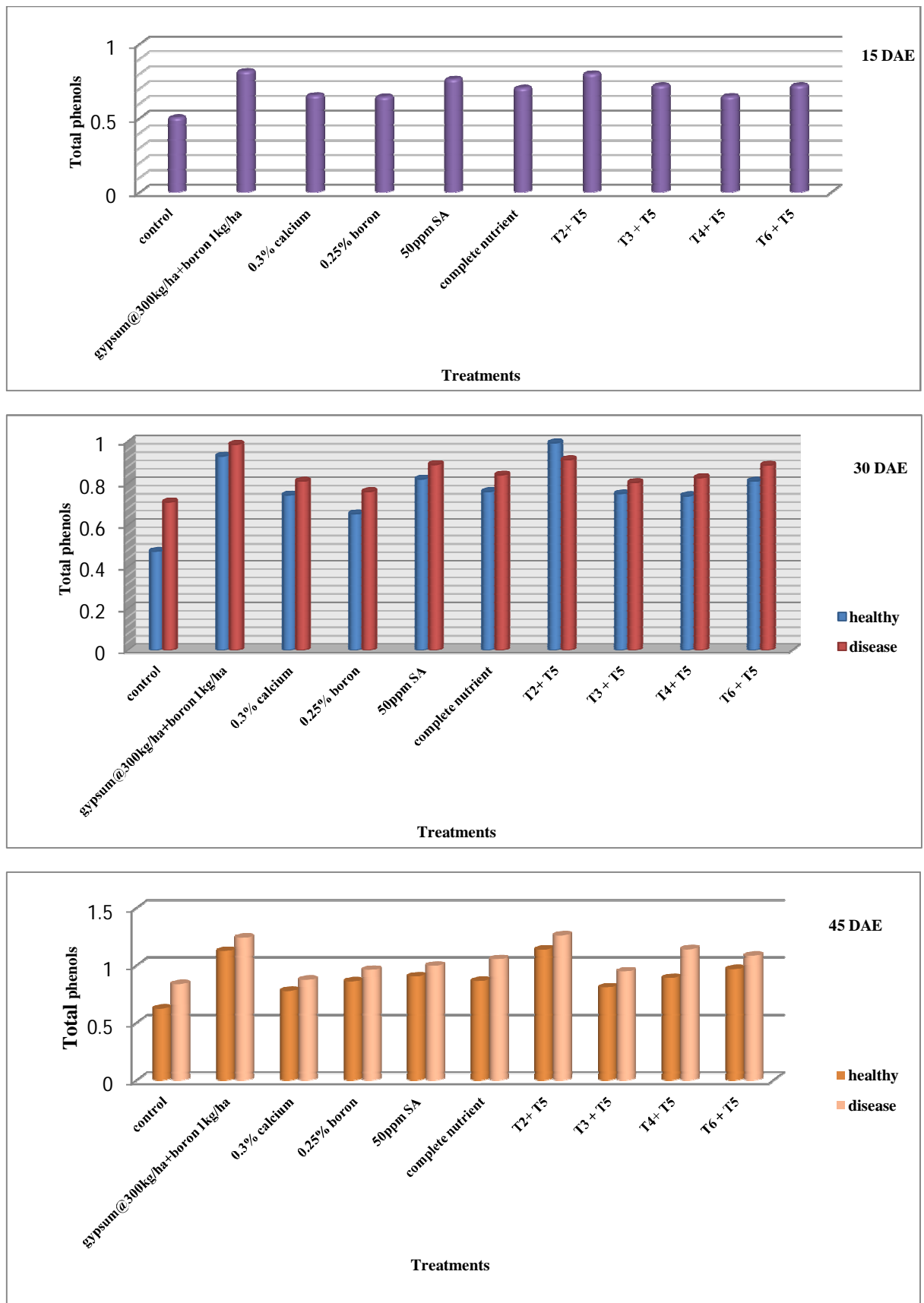


Fig. 4.7. Effect of Calcium, Boron and Salicylic acid on total phenols in leaf of YMV infected Blackgram

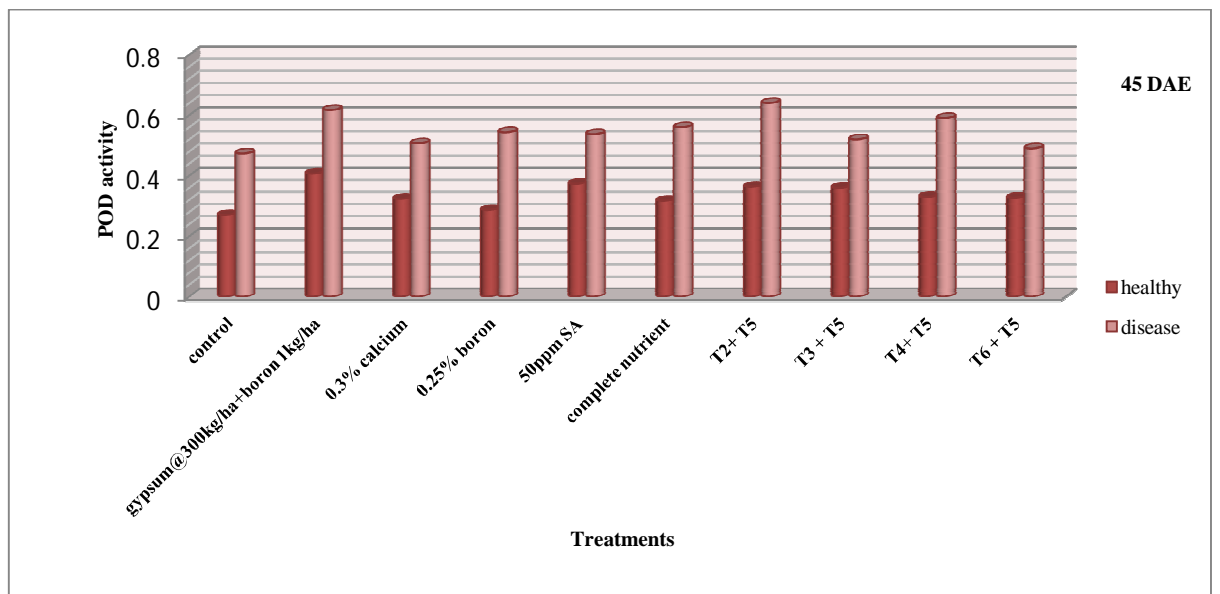
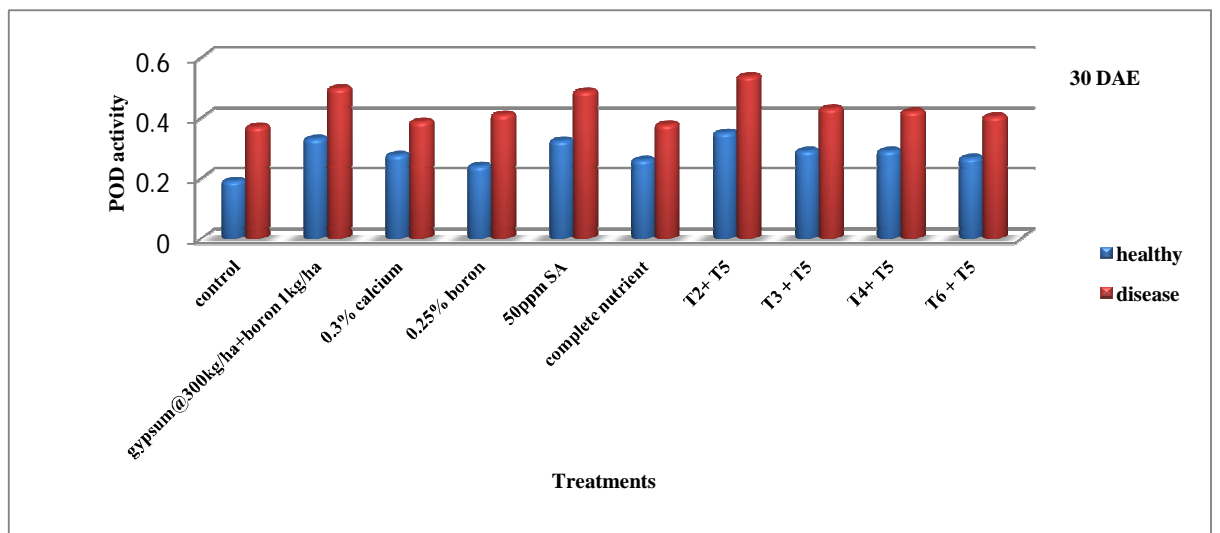
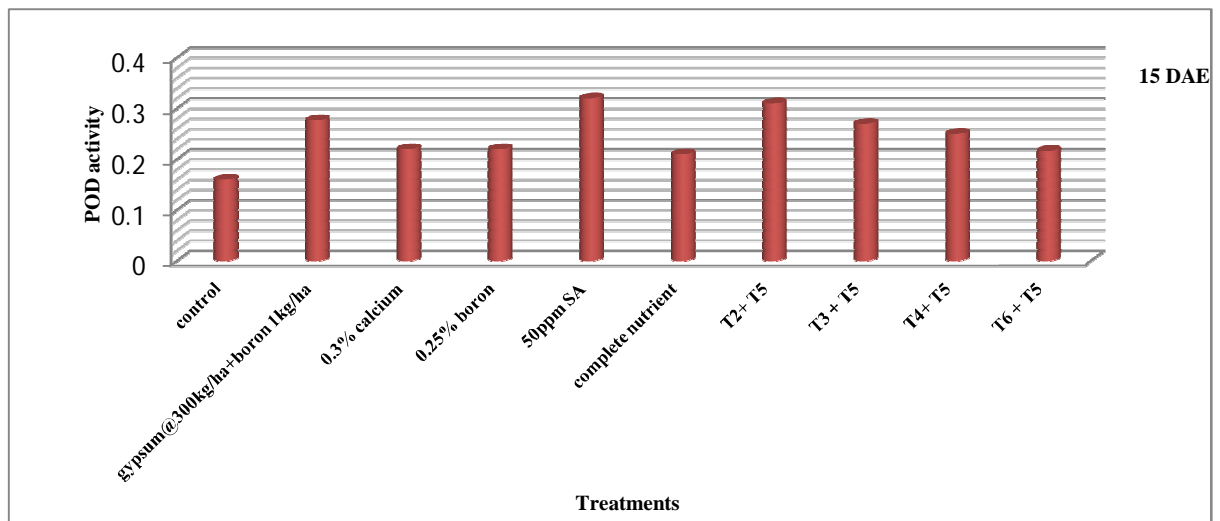


Fig 4.8. Effect of Calcium, Boron and Salicylic acid on peroxidase activity in leaf of YMV infected Blackgram

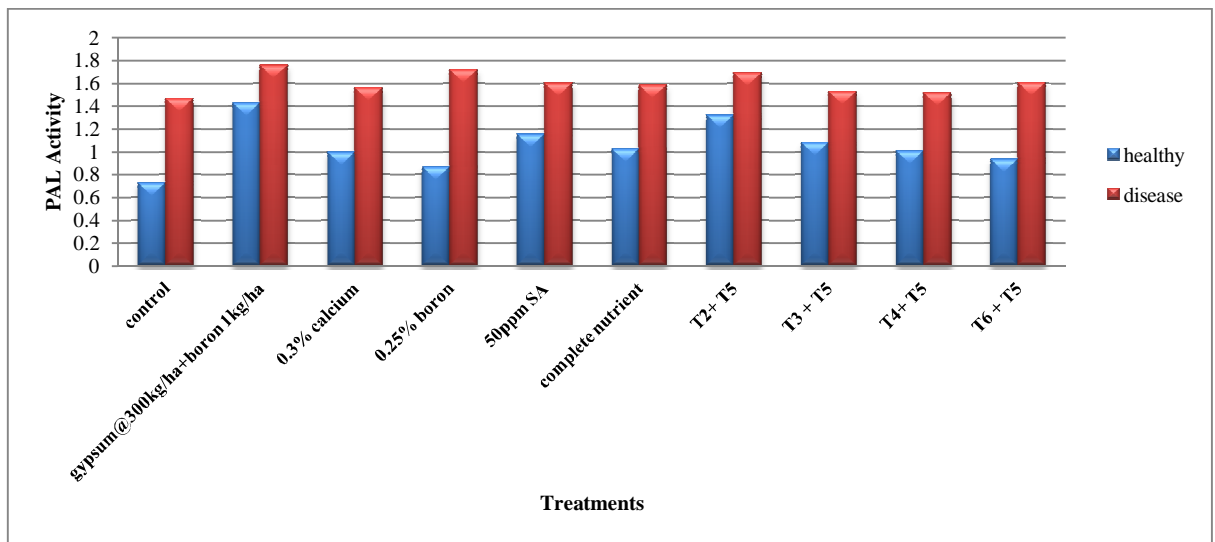
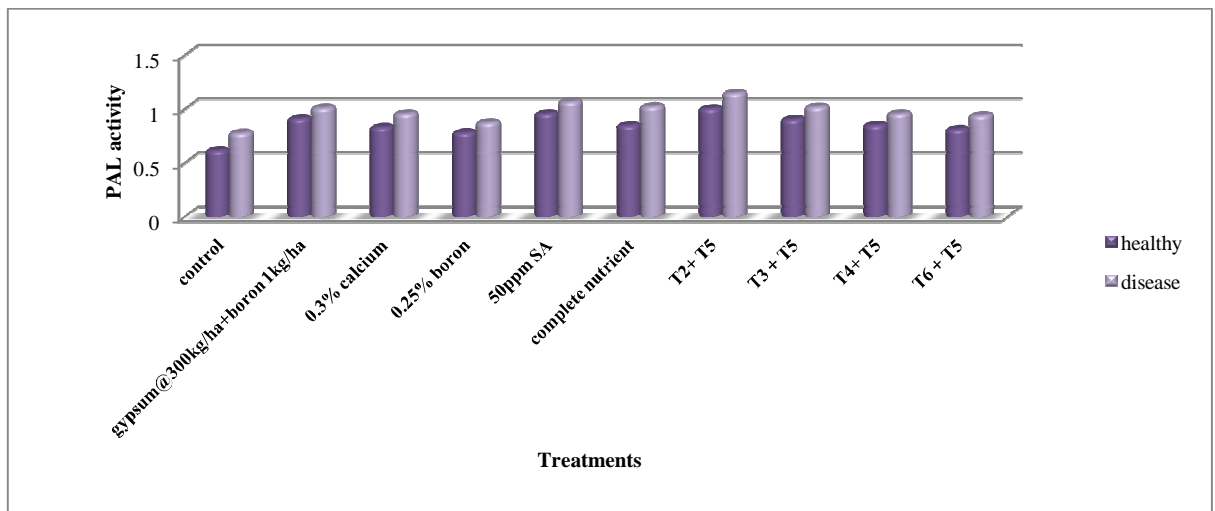
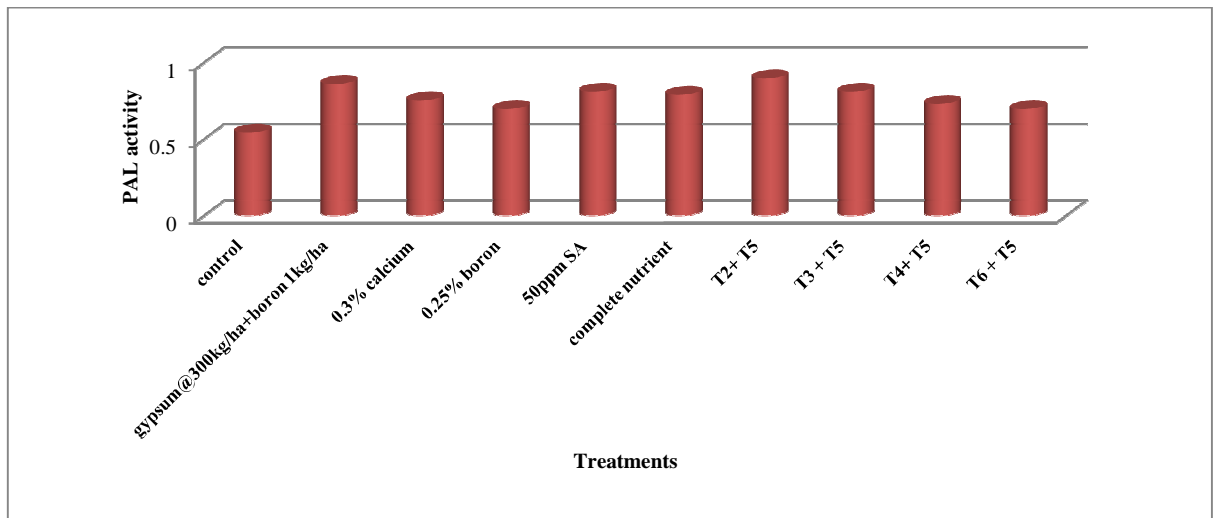


Fig 4.9. Effect of Calcium, Boron and Salicylic acid on phenylalanine ammonia lyase activity in leaf YMV infected Blackgram

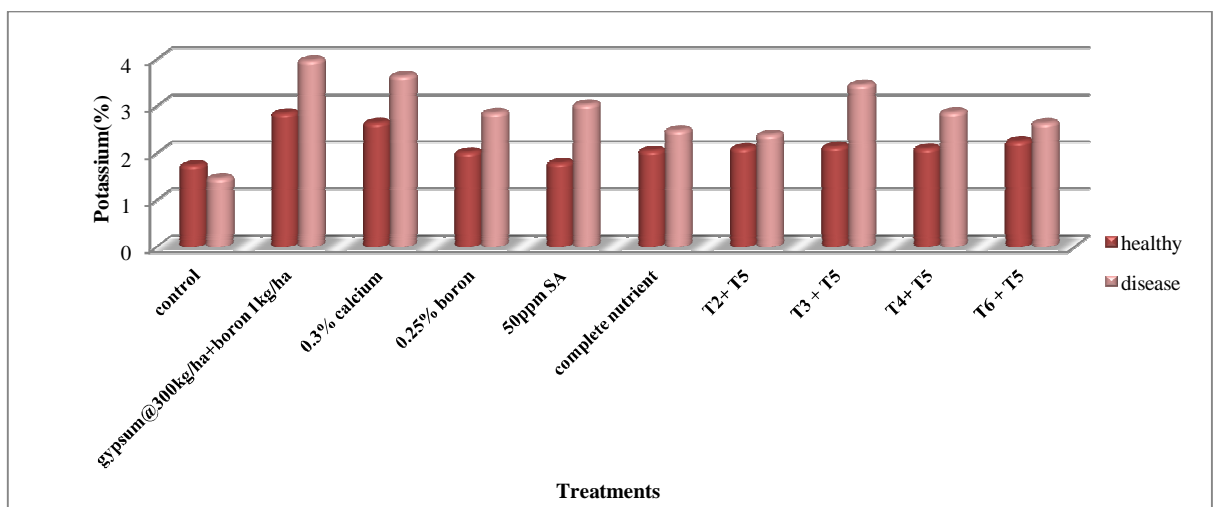
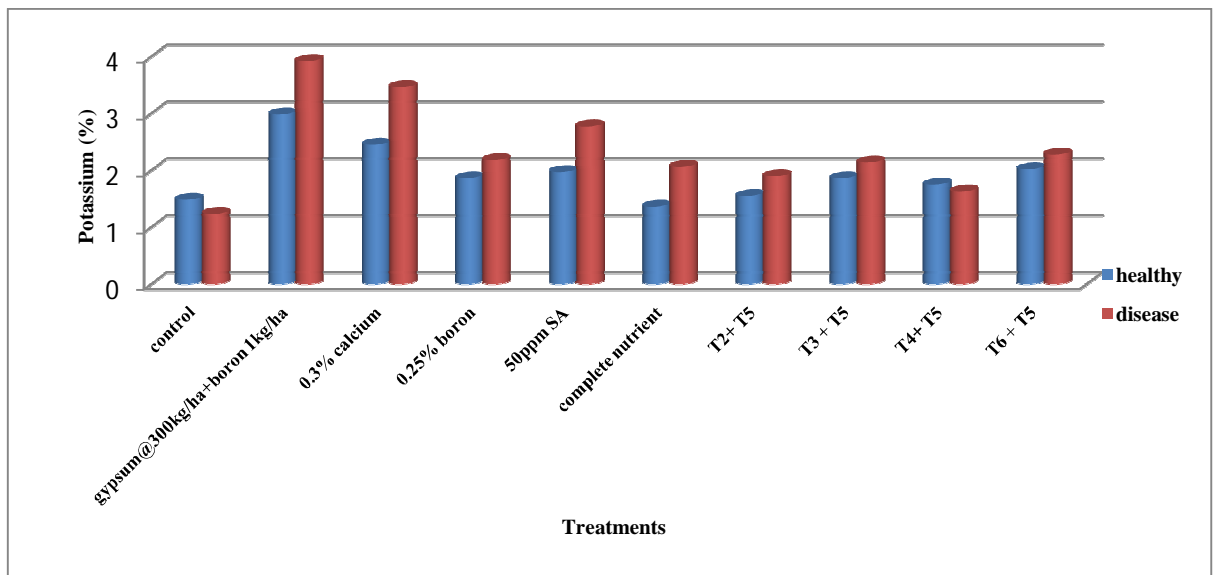
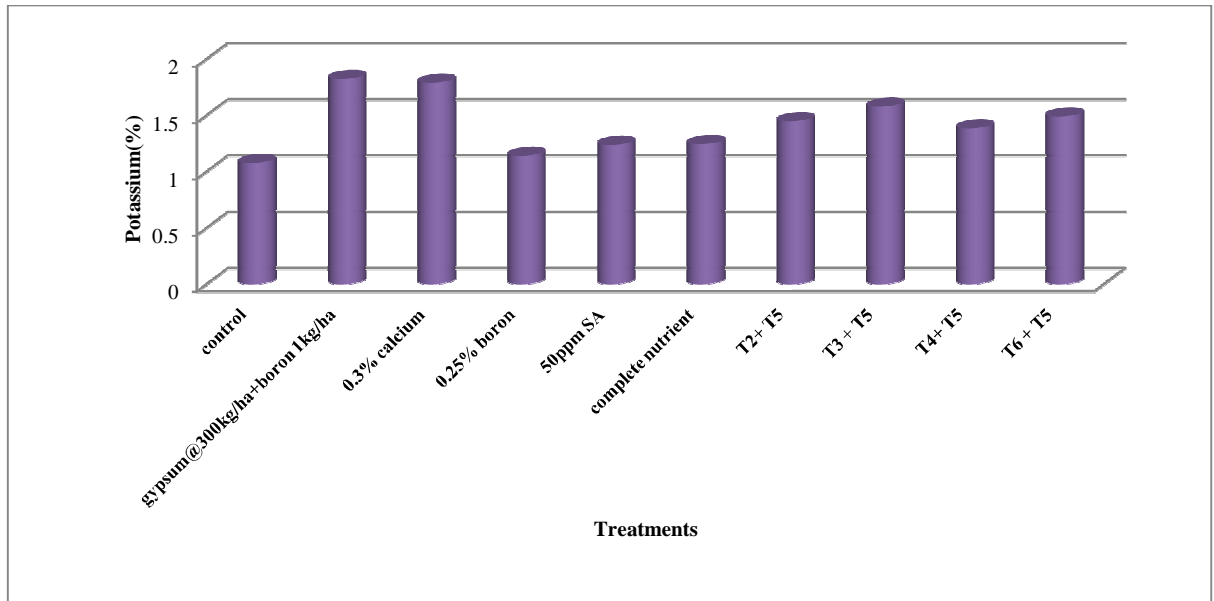


Fig 4.15. Effect of Calcium, Boron and Salicylic acid on potassium content in leaf of YMV infected Blackgram

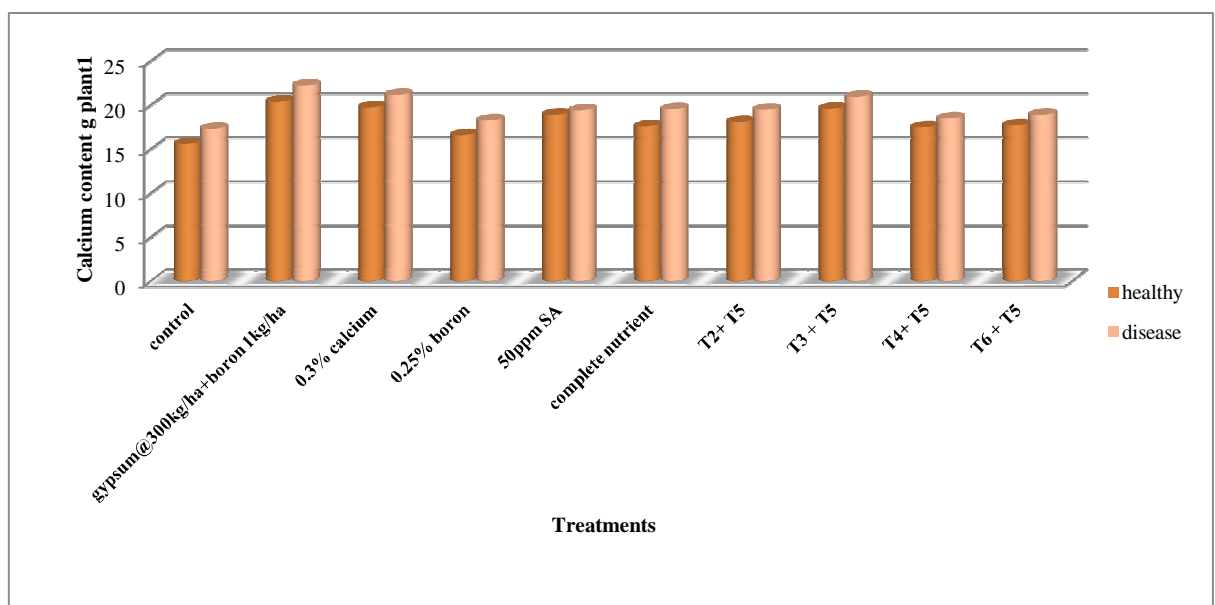
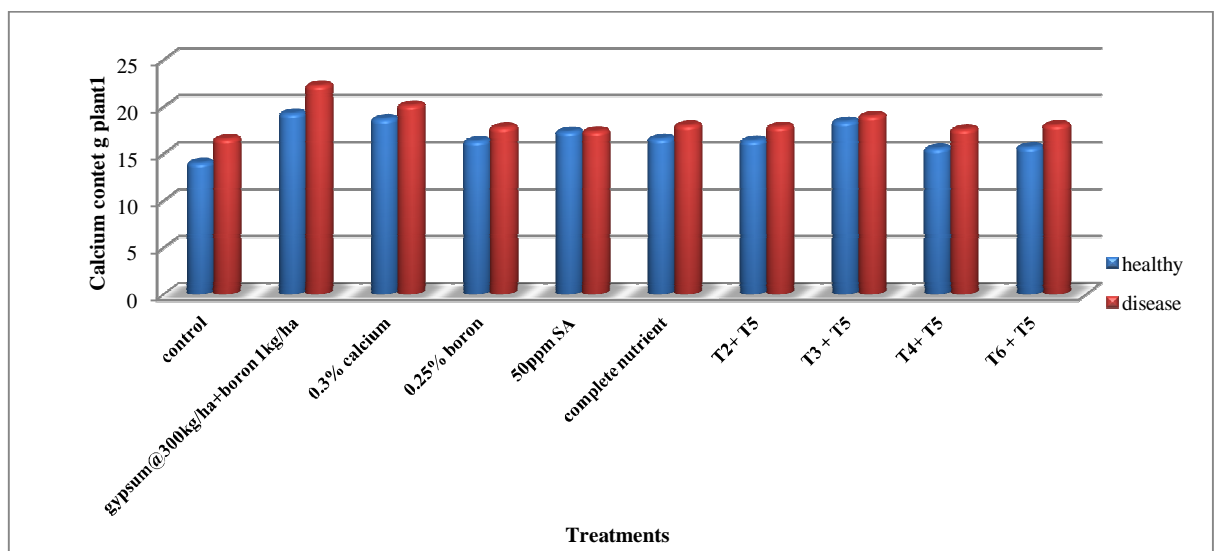
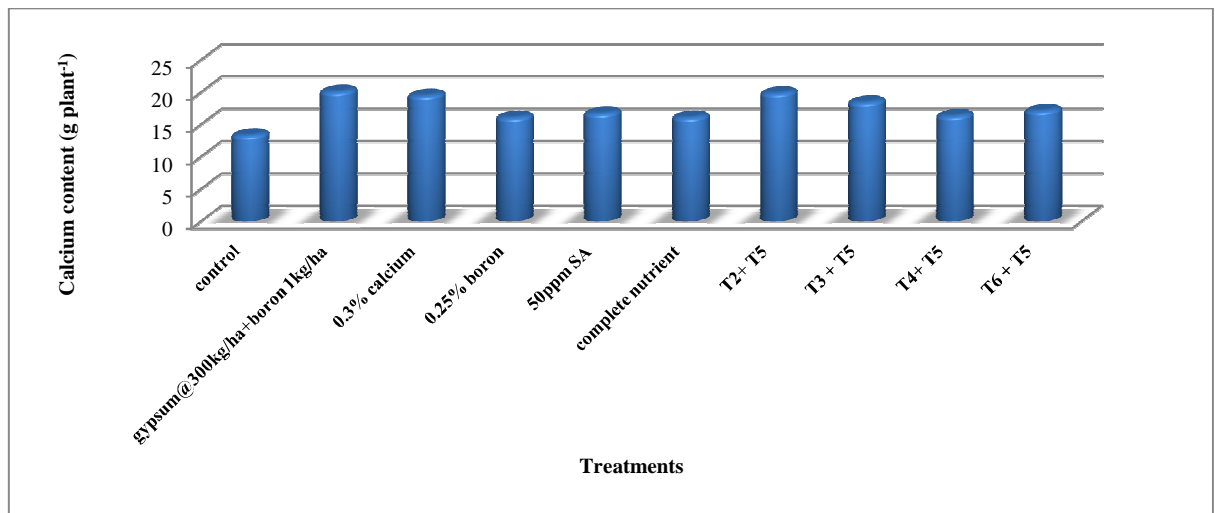


Fig 4.14. Effect of Calcium, Boron and Salicylic acid on calcium in leaf of YMV infected Blackgram

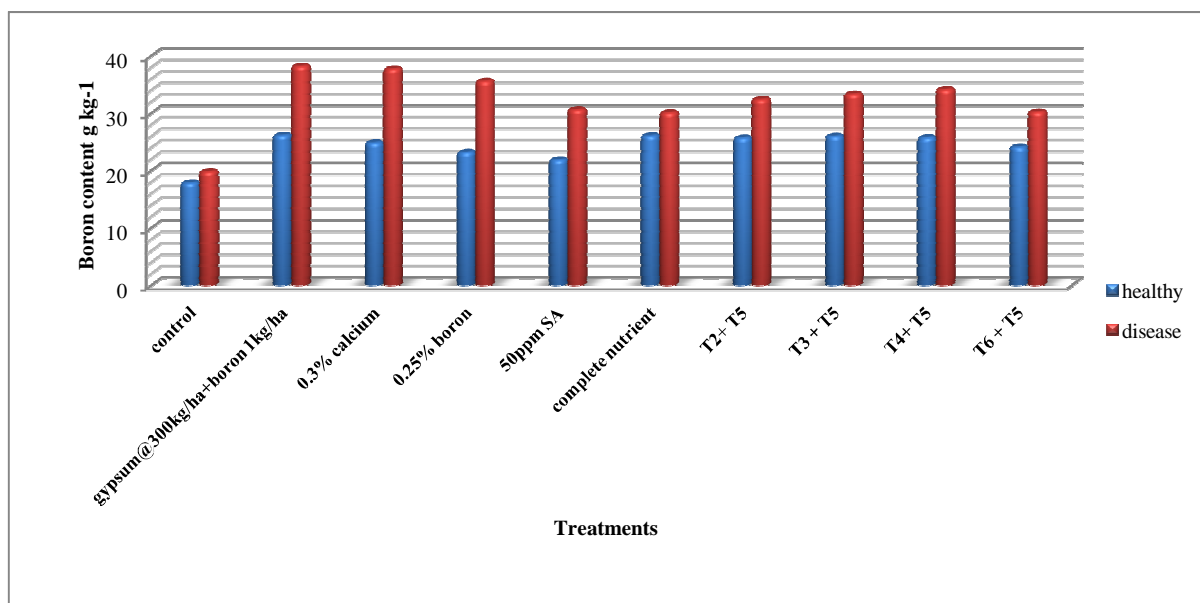
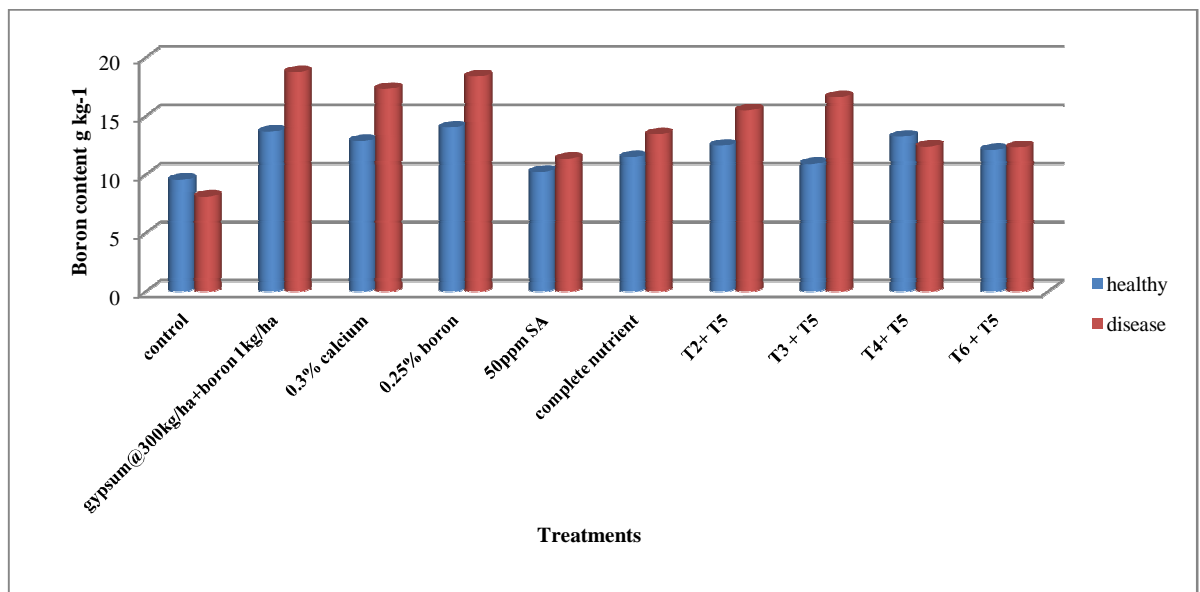
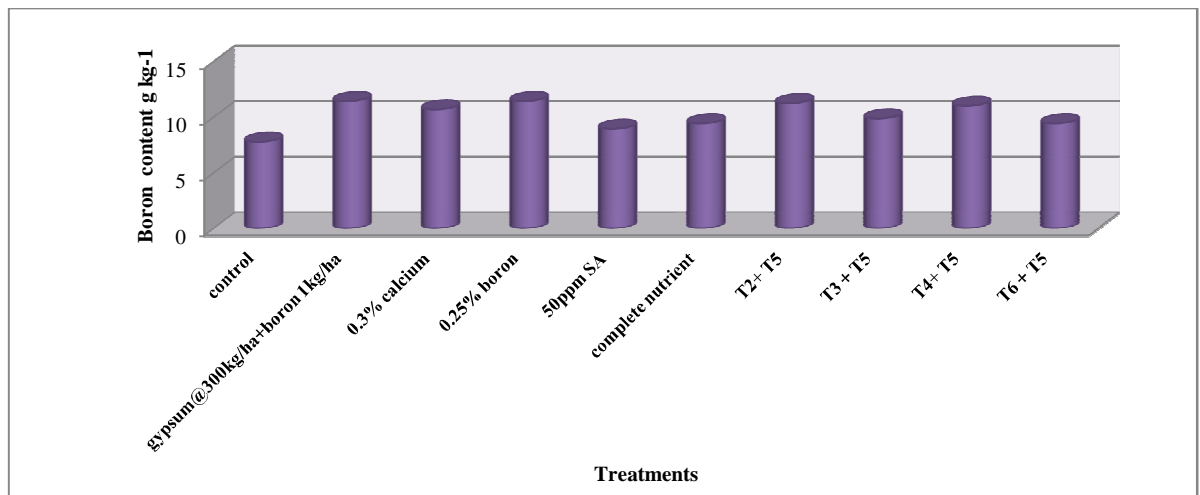


Fig 4.16. Effect of Calcium, Boron and Salicylic acid on boron content in leaf of YMV infected Blackgram



T₁



T₂



T₃



T₄



T₅



T₆



T₇



T₈



T₉



T₁₀

Plate 2. Effect of Ca, B and SA on disease incidence of YMV infected blackgram at 30 DAE



T1



T2



T3



T4



T5



T6



T10

Plate 3. Effect of Ca, B and SA on disease incidence of YMV infected blackgram at 45 DAE

LIST OF PLATES

S.No.	Title	Page No.
1	Overall view of Field Experiment	
2	Effect of Ca, B and SA on disease incidence of YMV infected blackgram at 30 DAE	
3	Effect of Ca, B and SA on disease incidence of YMV infected blackgram at 45 DAE	

Table 4.1. Effect of Calcium, Boron and Salicylic acid application on YMV disease incidence (%) in Blackgram

TREATMENTS	DISEASE INCIDENCE (%)	
	30 DAE	45DAE
T₁ : Control	19.40	72.73
T₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	13.56	40.16
T₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	15.73	48.80
T₄ : 0.25% Boron spray	17.73	57.56
T₅ : 50 ppm salicylic acid spray	14.90	49.16
T₆ : Complete nutrient solution spray	14.33	43.50
T₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	14.90	44.46
T₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	15.56	46.63
T₉ : 0.25% Boron + 50ppm SA spray	14.06	53.73
T₁₀ : Complete nutrient solution + 50ppm SA spray	16.53	53.16
SEm ±	0.61	3.85
CD (P ≤ 0.05)	1.28	8.10
CV (%)	5.69	11.93

Table 4.2. Effect of Calcium, Boron and Salicylic acid application on white fly count of YMV infected Blackgram

TREATMENTS	WHITEFLY COUNT		
	15 DAE	30 DAE	45 DAE
T₁ : Control	3.14	4.19	4.65
T₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	2.19	2.64	3.63
T₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	2.31	3.39	3.79
T₄ : 0.25% Boron spray	2.37	3.51	4.03
T₅ : 50 ppm salicylic acid spray	2.75	3.11	4.27
T₆ : Complete nutrient solution spray	2.45	3.24	4.14
T₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	2.04	3.34	3.93
T₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	2.02	4.00	3.74
T₉ : 0.25% Boron + 50ppm SA spray	2.27	3.71	4.14
T₁₀ : Complete nutrient solution + 50ppm SA spray	2.15	3.62	4.13
SEm ±	0.19	0.18	0.21
CD (P ≤ 0.05)	0.40	0.38	0.44
CV (%)	10.03	6.42	6.47

Table 4.3. Effect of Calcium, Boron and Salicylic acid application on Number of branches in YMV infected Blackgram

TREATMENTS	NUMBER OF BRANCHES		
	30 DAE	60 DAE	AT HARVEST
T ₁ : Control	4.1	5.4	5.7
T ₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	4.9	6.5	6.5
T ₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	4.6	6.1	6.4
T ₄ : 0.25% Boron spray	4.7	6.0	6.2
T ₅ : 50 ppm salicylic acid spray	4.3	6.3	6.4
T ₆ : Complete nutrient solution spray	4.5	5.9	6.1
T ₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	5.0	6.4	6.4
T ₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	4.7	6.3	6.3
T ₉ : 0.25% Boron + 50ppm SA spray	4.7	5.6	6.2
T ₁₀ : Complete nutrient solution + 50ppm SA spray	4.4	6.2	6.2
SEm ±	0.5	0.5	0.3
CD (P ≤ 0.05)	1.0	1.1	0.6
CV (%)	13.8	10.9	6.1

Table 4.11. Effect of Calcium, Boron and Salicylic acid application on chlorophyll-a content (mg g⁻¹) in leaf of YMV infected Blackgram

TREATMENTS	Chlorophyll-a(mg g ⁻¹)		
	15 DAE	30 DAE	45 DAE
T₁ : Control	0.211	0.199	0.160
T₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	0.580	0.585	0.162
T₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	0.491	0.442	0.198
T₄ : 0.25% Boron spray	0.528	0.485	0.384
T₅ : 50 ppm salicylic acid spray	0.541	0.499	0.259
T₆ : Complete nutrient solution spray	0.470	0.462	0.174
T₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	0.331	0.291	0.187
T₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	0.324	0.281	0.356
T₉ : 0.25% Boron + 50ppm SA spray	0.440	0.432	0.410
T₁₀ : Complete nutrient solution + 50ppm SA spray	0.521	0.599	0.466
SEm ±	0.064	0.007	0.012
CD (P ≤ 0.05)	0.134	0.014	0.026
CV (%)	4.60	2.03	5.54

Table 4.12. Effect of Calcium, Boron and Salicylic acid application on chlorophyll-b (mg g^{-1}) content in leaf of YMV infected Blackgram

TREATMENTS	Chlorophyll-b (mg g^{-1})		
	15 DAE	30 DAE	45 DAE
T₁ : Control	0.283	0.324	0.143
T₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	0.401	0.378	0.164
T₃ : 0.3% Calcium ($\text{CaNO}_3 \cdot 4\text{H}_2\text{O}$) spray	0.388	0.331	0.170
T₄ : 0.25% Boron spray	0.425	0.332	0.290
T₅ : 50 ppm salicylic acid spray	0.400	0.403	0.161
T₆ : Complete nutrient solution spray	0.691	0.581	0.318
T₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	0.448	0.449	0.286
T₈ : 0.3% Calcium ($\text{CaNO}_3 \cdot 4\text{H}_2\text{O}$) + 50ppm SA spray	0.328	0.330	0.176
T₉ : 0.25% Boron + 50ppm SA spray	0.513	0.402	0.203
T₁₀ : Complete nutrient solution + 50ppm SA spray	0.563	0.472	0.270
SEm ±	0.022	0.069	0.004
CD ($P \leq 0.05$)	0.046	0.14	0.01
CV (%)	6.13	5.08	2.7

Table 4.13. Effect of Calcium, Boron and Salicylic acid application on carotenoids (mg g⁻¹) content in leaf of YMV infected Blackgram

TREATMENTS	Carotenoids (mg g ⁻¹)		
	15 DAE	30 DAE	45 DAE
T ₁ : Control	0.259	0.222	0.079
T ₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	0.316	0.242	0.084
T ₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	0.296	0.226	0.083
T ₄ : 0.25% Boron spray	0.275	0.304	0.267
T ₅ : 50 ppm salicylic acid spray	0.303	0.303	0.104
T ₆ : Complete nutrient solution spray	0.421	0.248	0.308
T ₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	0.346	0.227	0.237
T ₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	0.292	0.223	0.184
T ₉ : 0.25% Boron + 50ppm SA spray	0.389	0.284	0.172
T ₁₀ : Complete nutrient solution + 50ppm SA spray	0.435	0.317	0.212
SEm ±	0.025	0.0045	0.011
CD (P ≤ 0.05)	0.053	0.0095	0.024
CV (%)	10.39	2.13	8.11

Table 4.4. Effect of Calcium, Boron and Salicylic acid application on leaf dry matter (g plant⁻¹) in YMV infected Blackgram

TREATMENTS	Leaf dry matter (g plant ⁻¹)		
	30 DAE	60 DAE	AT HARVEST
T₁ : Control	0.56	1.73	2.05
T₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	1.17	3.02	4.14
T₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	0.67	2.15	3.44
T₄ : 0.25% Boron spray	0.64	2.48	3.47
T₅ : 50 ppm salicylic acid spray	0.76	1.74	3.54
T₆ : Complete nutrient solution spray	0.86	1.50	3.46
T₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	1.21	3.15	4.34
T₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	0.95	1.74	2.71
T₉ : 0.25% Boron + 50ppm SA spray	1.31	2.26	3.00
T₁₀ : Complete nutrient solution + 50ppm SA spray	0.83	1.84	2.95
SEm ±	0.10	0.26	0.28
CD (P ≤ 0.05)	0.22	0.54	0.59
CV (%)	14.89	14.70	10.40

Table 4.5. Effect of Calcium, Boron and Salicylic acid application on stem dry matter (g plant⁻¹) in YMV infected Blackgram

TREATMENTS	STEM DRY MATTER (g plant ⁻¹)		
	30 DAE	60 DAE	AT HARVEST
T₁ : Control	0.11	0.20	0.69
T₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	0.20	0.33	1.06
T₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	0.17	0.23	0.74
T₄ : 0.25% Boron spray	0.15	0.25	0.84
T₅ : 50 ppm salicylic acid spray	0.16	0.27	1.00
T₆ : Complete nutrient solution spray	0.16	0.28	1.00
T₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	0.18	0.32	1.20
T₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	0.14	0.21	0.81
T₉ : 0.25% Boron + 50ppm SA spray	0.16	0.23	0.99
T₁₀ : Complete nutrient solution + 50ppm SA spray	0.18	0.25	1.02
SEm ±	0.01	0.02	0.05
CD (P ≤ 0.05)	0.041	0.04	0.11
CV (%)	14.6	10.04	7.15

Table 4.6. Effect of Calcium, Boron and Salicylic acid application on total dry matter (g plant⁻¹) in YMV infected Blackgram

TREATMENTS	TOTAL DRY MATTER (g plant ⁻¹)		
	30 DAE	60 DAE	AT HARVEST
T₁ : Control	0.55	2.92	8.70
T₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	1.52	5.14	12.22
T₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	0.86	3.14	10.20
T₄ : 0.25% Boron spray	1.03	4.15	9.70
T₅ : 50 ppm salicylic acid spray	0.82	4.89	8.76
T₆ : Complete nutrient solution spray	1.14	4.50	11.30
T₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	1.49	5.12	12.35
T₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	1.11	5.09	9.60
T₉ : 0.25% Boron + 50ppm SA spray	0.88	3.35	10.30
T₁₀ : Complete nutrient solution + 50ppm SA spray	0.63	3.20	11.17
SEm ±	0.12	0.24	0.65
CD (P ≤ 0.05)	0.26	0.50	1.37
CV (%)	15.49	7.08	7.69

Table 4.7. Effect of Calcium, Boron and Salicylic acid application on total phenols content (mg/100mg) in leaf of YMV infected Blackgram

TREATMENTS	Total phenols (mg/100mg)				
	15 DAE	30 DAE		45 DAE	
		HEALTHY	DISEASE	HEALTHY	DISEASE
T₁ : Control	0.50	0.473	0.71	0.62	0.83
T₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	0.81	0.93	0.98	1.12	1.24
T₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	0.65	0.74	0.81	0.76	0.87
T₄ : 0.25% Boron spray	0.64	0.65	0.76	0.86	0.96
T₅ : 50 ppm salicylic acid spray	0.76	0.82	0.89	0.90	0.99
T₆ : Complete nutrient solution spray	0.70	0.76	0.84	0.86	1.05
T₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	0.80	0.99	0.91	1.13	1.26
T₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	0.72	0.75	0.80	0.80	0.94
T₉ : 0.25% Boron + 50ppm SA spray	0.64	0.74	0.82	0.89	1.14
T₁₀ : Complete nutrient solution + 50ppm SA spray	0.72	0.81	0.88	0.96	1.08
SEm ±	0.02	0.01	0.03	0.02	0.03
CD (P ≤ 0.05)	0.04	0.03	0.067	0.043	0.064
CV (%)	3.67	2.46	4.68	2.84	3.60

Table 4.8. Effect of Calcium, Boron and Salicylic acid application on peroxidase activity (Δ Abs/min/g) in leaf of YMV infected Blackgram

TREATMENTS	Peroxidase activity(Δ Abs/min/g)				
	15 DAE	30 DAE		45 DAE	
		HEALTHY	DISEASE	HEALTHY	DISEASE
T ₁ : Control	0.16	0.19	0.37	0.27	0.47
T ₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	0.27	0.33	0.49	0.40	0.61
T ₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	0.22	0.27	0.38	0.32	0.50
T ₄ : 0.25% Boron spray	0.22	0.24	0.41	0.28	0.54
T ₅ : 50 ppm salicylic acid spray	0.32	0.32	0.48	0.37	0.53
T ₆ : Complete nutrient solution spray	0.21	0.26	0.37	0.31	0.56
T ₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	0.31	0.35	0.53	0.36	0.64
T ₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	0.27	0.29	0.43	0.36	0.52
T ₉ : 0.25% Boron + 50ppm SA spray	0.25	0.29	0.42	0.33	0.59
T ₁₀ : Complete nutrient solution + 50ppm SA spray	0.21	0.26	0.40	0.32	0.49
SEm \pm	0.01	0.01	0.01	0.01	0.02
CD (P \leq 0.05)	0.02	0.03	0.03	0.03	0.04
CV (%)	6.17	7.96	4.15	6.69	4.65

Table 4.9. Effect of Calcium, Boron and Salicylic acid application on phenylalanine ammonia lyase (η moles of transcinnamic acid/min/g) in leaf of YMV infected Blackgram

TREATMENTS	PAL activity (η moles of transcinnamic acid/min/g)				
	15 DAE	30 DAE		45 DAE	
		HEALTHY	DISEASE	HEALTHY	DISEASE
T₁ : Control	0.54	0.61	0.77	0.726	1.46
T₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	0.86	0.91	1.01	1.42	1.75
T₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	0.75	0.83	0.95	0.99	1.56
T₄ : 0.25% Boron spray	0.69	0.78	0.87	0.86	1.72
T₅ : 50 ppm salicylic acid spray	0.81	0.95	1.06	1.15	1.6
T₆ : Complete nutrient solution spray	0.79	0.84	1.02	1.02	1.5
T₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	0.89	1.00	1.14	1.31	1.69
T₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	0.81	0.90	1.02	1.08	1.52
T₉ : 0.25% Boron + 50ppm SA spray	0.73	0.85	0.95	1.00	1.51
T₁₀ : Complete nutrient solution + 50ppm SA spray	0.69	0.81	0.94	0.93	1.60
SEm ±	0.01	0.01	0.02	0.03	0.03
CD (P ≤ 0.05)	0.02	0.02	0.06	0.07	0.05
CV (%)	2.16	3.01	3.6	4.3	2.69

Table 4.10. Effect of Calcium, Boron and Salicylic acid application on membrane injury index (%) of YMV infected Blackgram

TREATMENTS	Membrane injury index (%)		
	15 DAE	30 DAE	45 DAE
T₁ : Control	18.08	21.79	25.17
T₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	10.19	13.32	15.13
T₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	12.25	13.77	14.4
T₄ : 0.25% Boron spray	14.20	15.29	16.52
T₅ : 50 ppm salicylic acid spray	15.30	16.12	15.35
T₆ : Complete nutrient solution spray	11.18	13.45	16.21
T₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	12.36	14.23	16.72
T₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	13.22	15.38	17.99
T₉ : 0.25% Boron + 50ppm SA spray	14.22	15.60	20.13
T₁₀ : Complete nutrient solution + 50ppm SA spray	15.34	16.19	20.06
SEm ±	0.23	0.43	0.29
CD (P ≤ 0.05)	0.49	0.92	0.62
CV (%)	2.12	3.46	2.05

Table 4.15. Effect of Calcium, Boron and Salicylic acid application on potassium content (%) in leaf of YMV infected Blackgram

TREATMENTS	Potassium (%)				
	15 DAE	30 DAE		45 DAE	
		HEALTHY	DISEASE	HEALTHY	DISEASE
T₁ : Control	1.07	1.48	1.23	1.743	1.46
T₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	1.82	2.98	3.92	2.84	3.97
T₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	1.79	2.45	3.46	2.64	3.64
T₄ : 0.25% Boron spray	1.14	1.86	2.18	2.01	2.8
T₅ : 50 ppm salicylic acid spray	1.23	1.97	2.77	1.79	3.03
T₆ : Complete nutrient solution spray	1.24	1.36	2.06	2.05	2.48
T₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	1.44	1.55	1.89	2.10	2.39
T₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	1.57	1.86	2.14	2.13	3.45
T₉ : 0.25% Boron + 50ppm SA spray	1.38	1.75	1.63	2.1	2.87
T₁₀ : Complete nutrient solution + 50ppm SA spray	1.49	2.02	2.28	2.25	2.64
SEm ±	0.07	0.05	0.04	0.04	0.05
CD (P ≤ 0.05)	0.15	0.10	0.102	0.100	0.11
CV (%)	6.55	3.17	2.52	2.70	2.38

Table 4.14. Effect of Calcium, Boron and Salicylic acid application on calcium content (mg g⁻¹) in leaf of YMV infected Blackgram

TREATMENTS	Calcium content (mg g ⁻¹)				
	15 DAE	30 DAE		45 DAE	
		HEALTHY	DISEASE	HEALTHY	DISEASE
T ₁ : Control	13.33	14.01	16.56	15.58	17.30
T ₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	20.08	19.26	22.26	20.39	22.22
T ₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	19.47	18.68	20.14	19.74	21.14
T ₄ : 0.25% Boron spray	16.07	16.33	17.79	16.57	18.25
T ₅ : 50 ppm salicylic acid spray	16.71	17.33	17.36	18.89	19.4
T ₆ : Complete nutrient solution spray	16.09	16.57	18.00	17.58	19.52
T ₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	19.84	16.38	17.85	18.07	19.45
T ₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	18.44	18.41	19.01	19.58	20.95
T ₉ : 0.25% Boron + 50ppm SA spray	16.31	15.54	17.58	17.46	18.47
T ₁₀ : Complete nutrient solution + 50ppm SA spray	17.11	15.68	18.04	17.67	18.88
SEm ±	0.74	0.44	0.51	0.50	0.36
CD (P ≤ 0.05)	1.56	0.94	1.08	1.06	0.76
CV (%)	5.24	3.27	3.42	3.40	2.26

Table 4.16. Effect of Calcium, Boron and Salicylic acid application on boron content (mg kg⁻¹) in leaf of YMV infected Blackgram

TREATMENTS	Boron content (mg kg ⁻¹)				
	15 DAE	30 DAE		45 DAE	
		HEALTHY	DISEASE	HEALTHY	DISEASE
T₁ : Control	7.79	9.55	8.12	17.90	19.84
T₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	11.43	13.67	18.76	26.20	38.25
T₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	10.69	12.86	17.33	24.90	37.77
T₄ : 0.25% Boron spray	11.43	14.03	18.40	23.24	35.62
T₅ : 50 ppm salicylic acid spray	8.95	10.23	11.35	21.92	30.67
T₆ : Complete nutrient solution spray	9.44	11.49	13.44	26.13	30.13
T₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	11.25	12.48	15.50	25.74	32.44
T₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	9.88	10.91	16.63	26.05	33.37
T₉ : 0.25% Boron + 50ppm SA spray	11.02	13.24	12.40	25.80	34.17
T₁₀ : Complete nutrient solution + 50ppm SA spray	9.44	12.10	12.33	24.15	30.27
SEm ±	0.41	0.41	0.16	0.783	1.02
CD (P ≤ 0.05)	0.86	0.87	0.49	1.64	2.16
CV (%)	5.00	4.21	2.00	3.96	3.90

Table 4.17. Effect of Calcium, Boron and Salicylic acid application on yield components of YMV infected Blackgram

TREATMENTS	Yield components		
	Number of pods plant-1	Number of seeds pod ⁻¹	Pod length (cm)
T₁ : Control	5.7	5.2	3.2
T₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	6.2	6.3	3.9
T₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	6.1	5.8	3.6
T₄ : 0.25% Boron spray	5.9	5.3	3.8
T₅ : 50 ppm salicylic acid spray	5.9	5.4	4.1
T₆ : Complete nutrient solution spray	5.8	5.3	3.6
T₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	6.0	5.8	3.5
T₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	6.0	5.9	3.9
T₉ : 0.25% Boron + 50ppm SA spray	5.7	5.8	4.0
T₁₀ : Complete nutrient solution + 50ppm SA spray	6.4	5.9	3.5
SEm ±	0.7	0.3	0.2
CD (P ≤ 0.05)	1.5	0.6	0.5
CV (%)	15.1	6.7	8.4

Table 4.18. Effect of Calcium, Boron and Salicylic acid application on yield and yield components on YMV infected Blackgram

TREATMENTS	Yield components		
	100 Seed weight (g)	Pod weight (g)	Seed Yield plant ⁻¹
T ₁ : Control	3.26	5.46	1.62
T ₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	3.93	6.83	2.23
T ₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	3.93	6.53	2.16
T ₄ : 0.25% Boron spray	3.80	6.37	1.79
T ₅ : 50 ppm salicylic acid spray	3.80	6.30	1.64
T ₆ : Complete nutrient solution spray	3.73	6.80	1.89
T ₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	3.93	6.96	2.18
T ₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	3.53	6.96	1.98
T ₉ : 0.25% Boron + 50ppm SA spray	4.00	7.00	1.92
T ₁₀ : Complete nutrient solution + 50ppm SA spray	4.26	6.26	2.28
SEm ±	0.28	0.53	0.19
CD (P ≤ 0.05)	0.60	1.11	0.40
CV (%)	9.24	9.94	11.82

Table 4.19. Effect of calcium, boron and salicylic acid application on yield (kg ha⁻¹) of YMV infected blackgram

TREATMENTS	Yield kg ha⁻¹
T₁ : Control	472.5
T₂ : Gypsum 300Kg + boron 1 kg ha ⁻¹	650.4
T₃ : 0.3% Calcium (CaNO ₃ .4H ₂ O) spray	640.0
T₄ : 0.25% Boron spray	522.0
T₅ : 50 ppm salicylic acid spray	478.3
T₆ : Complete nutrient solution spray	551.2
T₇ : Gypsum 300Kg + boron 1 kg ha ⁻¹ + 50ppm SA spray	635.8
T₈ : 0.3% Calcium (CaNO ₃ .4H ₂ O) + 50ppm SA spray	577.5
T₉ : 0.25% Boron + 50ppm SA spray	560.0
T₁₀ : Complete nutrient solution + 50ppm SA spray	665.0
SEm ±	29.69
CD (P ≤ 0.05)	62.37
CV (%)	6.32

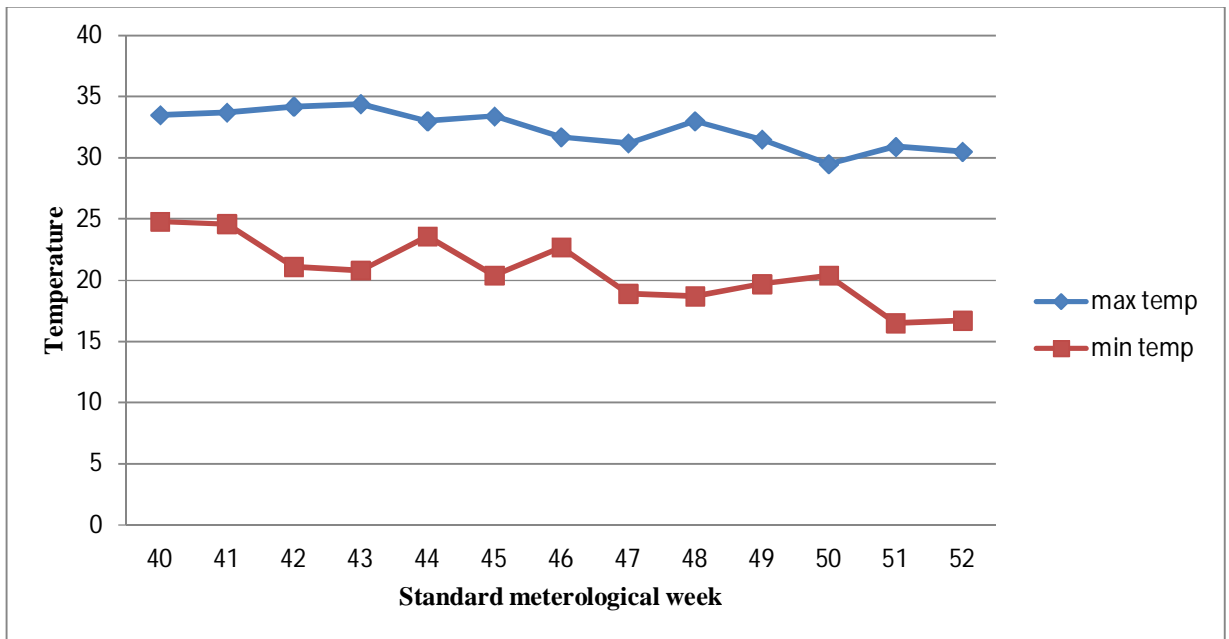


Fig. 3.1. Weekly temperature during crop growth period

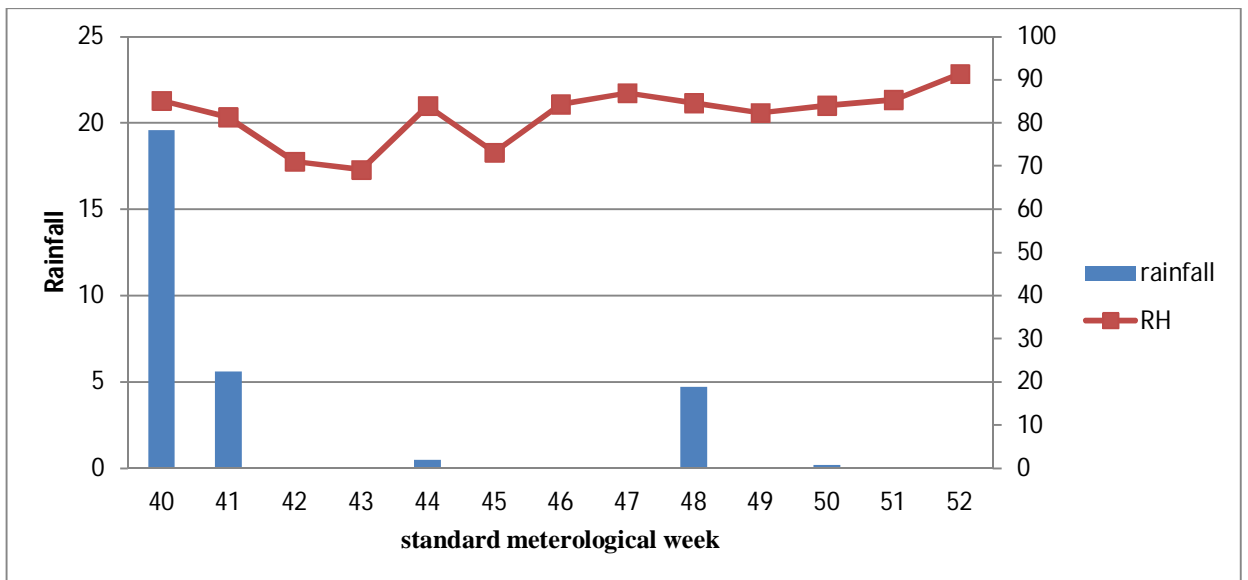


Fig. 3.2. Weekly rainfall and mean relative humidity during crop growth period