BIOCHEMICAL AND PHYSIOLOGICAL FACTORS CONDUCIVE FOR THE DEVELOPMENT OF BACTERIAL BLIGHT OF COTTON AND ITS MANAGEMENT

BY

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I hereby declare that the contents of the thesis "Biochemical and physiological factors conducive for the development of bacterial blight of cotton and its management**" are product of my own research and no part has been copied from any published source (except the references, standard mathematical and genetic models/equations/formulas/protocols etc.). i further declare that the work has not been submitted for award of any other diploma/degree. the university may take action if the information provided is found inaccurate at any stage. in case of any default the scholar may be proceeded against as per hec plagiarism policy.**

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O! GOD OPEN OUR EYES,
TO SEE WHAT IS BEAUTIFUL,
OUR MINDS TO KNOW WHAT IS TRUE
OUR HEARTS TO LOVE WHAT IS GOOD

Dedicated to

HOLY PROFHET (PBUH)
THE GREATEST SOCIAL REFORMER

MY WORTHY PARENTS
THE TOIL AND SWEAT OF AFFECTIONATE
PARENTS AS MORAL SUPPORT ENSHRINED
AND GRAFTED IN ME UNTIRING ZEAL TO
GET ON THE HIGHER IDEALS OF LIFE

MY BROTHERS AND SISTERS
FOR THEIR LOVE, PATIENCE, ENCOURAGEMENT AND UNDERSTANDING THAT INSPIRED ME TO ACCOMPLISH THIS HUMBLE EFFORT

MY WIFE
WHOSE HANDS ALWAYS RAISED
IN PRAYERS FOR ME AND SHE
EXHIBITED A PROLONGED
PATIENCE FOR MY STUDY

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LIST OF USED SYMBOLS

Sr. #	Detail	Symbol
1	Adenosine triphosphate	ATP
2	Analysis of variance	ANOVA
3	Ayyub Agriculture Research Institute	AARI
4	*Azadirachta indica*	*A. indica*
5	*Bacillus Theoringensis*	Bt
6	Carbon dioxide	CO_2
7	Central Cotton Research Institute	NARC
8	Completely Randomized Design	CRD
9	Copper sulphate	$CuSO_4$
10	Cultivars	Cvs.
11	*Curcuma longa*	*C. longa*
12	*Datura alba*	*D. alba*
13	Deoxyribonucleic acid	DNA
14	Electron transport chain	ETC
15	Extracellular polysaccharides	EPS
16	Food and Agriculture Organization	FAO
17	Highly resistant	HR
18	Highly susceptible	HS
19	Hydrochoric acid	HCl
20	Hypersensitive response	HR
21	Immune	I

22	Integrated disease management	IDM
23	International Botanical Code for Nomenclature	IBCN
24	Least significant difference	LSD
25	Microgram	µg
26	Milligram	Mg
27	Milliliter	Ml
28	Moderately resistant	MR
29	Moderately susceptible	MS
30	*Moringa oleifera*	*M. oleifera*
31	*Nicotiana tabacum*	*N. tabacum*
32	Nicotinamide adenine dinucleotide	NAD
33	Nicotinamide adenine dinucleotide phosphate	NADP
34	Nitric acid	HNO_3
35	Nitrogen phosphorus potassium	NPK
36	Nutrient agar	NA
37	Parts per million	PPM
38	Percent	%
39	Plant to plant distance	P × P
40	Potassium chloride	KCl
41	Potassium sulphate	K_2SO_4
42	Probability	P
43	Reactive oxygen species	ROS

44	Resistant	R
45	Resistant gene	R gene
46	Ribonucleic acid	RNA
47	Row to row distance	R × R
48	Sodium hydroxide	NaOH
49	Standard	S
50	Superoxide dismutase	SOD
51	Susceptible	S
52	Systemic acquired resistance	SAR
53	Systemic induced resistance	SIR
54	Total soluble sugars	TSS
55	United States of America	USA
56	University of Agriculture Faisalabad	UAF
57	*Xanthomonas citri* pv. *malvacearum*	*Xcm*

ABSTRACT

Thirty varieties of cotton were screened against bacterial blight disease field conditions under completely randomized block design (RCBD) to find out their resistance status for two years i.e. 2013 and 2014. During both years eighteen varieties (Non Bt-PB-896 , Bt-CM 615, Bt-IR 901, Non Bt-BH 160, Bt-CRS 2007, Bt-VH 329, Bt-CM 616, Non Bt-Redacola, Bt- KZ 189, Non Bt-NIAB 111, Bt-IUB 222, Non Bt-Sindh 1, Bt-ASO 1, Non Bt-MNH 554, Bt-FH 183, Bt-MNH 886, Bt-53 and Bt-FH 177 expressed moderately resistant response. Six varieties (Bt-FH 143, Bt-Ali Akbar 802, Non Bt-CIM 573, BT-NS 131, Non Bt-CM 82 and Bt-NIBGI 2) exhibited moderately susceptible response. Four varieties (Bt-FH 142, Bt-FH 182, Bt-4243 and Bt-FH 169) showed susceptible response while Non Bt- Shahbaz and Non Bt- CRIS 134 expressed highly susceptible response against bacterial blight disease. Status of ionic contents and biochemical compound both in inoculated and un-inoculated cotton leaves were estimated under Nested Structured Design. Amount of all eight ionic contents decreased both in resistant and susceptible type of cotton plants after inoculation. Amount of N (2.78) and P (0.22) % while K (512.1), Ca (412.3), Mg (25.3), Cu (2.87), Zn (1.91) and Fe (1.96) ppm was observed in un-inoculated group of plants which decreased to 1.79 %, 0.14, 2.76 % and 191, 13.3, 1.85, 1.09, 0.99 ppm respectively. Level of protein decreased from 5.52 - 3.77 mg/g, total soluble phenols 3.73-1.83 mg/g, total soluble sugars 6.67-5.01 mg/g and chlorophyll contents from 1.56 - 0.68 mg/g respectively after inoculation. Amount of 2.17%, 0.16%, 378.8 ppm, 270.2ppm, 14.6ppm, 2.4ppm, 1.75ppm and 1.35 ppm of N, P, K, Ca, Mg, Cu, Zn and Fe was observed in susceptible type while in resistant type 2.40%, 0.19%, 408.3 ppm, 310.2 ppm, 21.1 ppm, 2.9 ppm, 1.83 ppm and 1.61 ppm respectively. Similarly amount of protein 4.37 mg/g, total soluble phenols 2.57 mg/g, total soluble sugars 5.64 mg/g and chlorophyll contents 0.83 mg/g was observed in susceptible type while 4.93, 2.98, 6.03 and 1.16 mg/g was estimated in resistant type of cotton plants. Level of proteins reduced from 5.52-3.77 mg/g, total soluble phenols 3.73-1.83 mg/g, total soluble sugars 6.67-5.01 mg/g and chlorophyll contents 1.56-0.68 mg/g respectively after inoculation. For management of bacterial blight of cotton five chemicals i.e., Flare, Plant protector, Mancozeb, Agrimycine, copper oxychloride and plant extracts (*N. tabacum*, *A. indica*, *M. oleifera*, *D. alba* and *C. longa*) at three concentrations were evaluated against *Xcm* under completely randomized design (CRD). Maximum inhibition was expressed by Flare whose main ingredient is streptomycin sulphate at all concentrations respectively while in case of plant extracts maximum inhibition of bacterial growth was expressed by *N. tabacum*. Impact of Flare and *N. tabacum* alone and in combination at different concentrations was also observed under greenhouse and field conditions. All treatments expressed significant results but maximum reduction in disease was expressed by combination of Flare + *N. tabacum* both under greenhouse and field conditions.

CHAPTER # 1 INTRODUCTION

Cotton (*Gossypium hirsutum*) is one of the most important fiber crop of Pakistan which belongs to Malvaceae family. It is grown in temperate and subtropical regions of the world including Pakistan (Smith, 1999). Worldwide area under cultivation of cotton is 33.1 million hectares with production of 116.7 million bales while in Pakistan it is cultivated on an area of 3.0 million hectares during 2013-14 with production of 9.5 million bales (Johnson *et al.,* 2014). Pakistan is the fourth largest producer of cotton after China, USA and India (Hanif and Jafri, 2008). It is a rich source of edible oil and fiber and it's by products are also used for livestock as food (Chaudhry and Guitchounts, 2003). Cotton seeds contribute 4% of vegetable oil production in Pakistan (Bruinsma, 2003). Several biotic and abiotic factors are responsible for its poor quality and yield. Many diseases attack on cotton crop but bacterial blight of cotton is a potential threat for its production which is caused by *Xanthomonas citri* pv. *malvacearum (Xcm)* (Saha *et al.,* 2001). This pathogen infects plant at any stage of the growth period by invading through stomata or wounds. Initially small, irregular and dark water soaked spots appears on lower epidermis of leaves that later becomes dark brown (Liberato *et al*., 2007).Water soaked abrasions on bolls, early stem and leaves senescence, stunted growth of infected plants are the characteristic symptoms of bacterial blight disease (Rungis *et al*., 2002). Bacterial blight of cotton causes 40% losses and when environmental conditions are favorable, losses exceed up to 50% (Verma, 1986).Several management strategies like cultural control, biological control and resistance varieties are used against this disease. Integrated management of bacterial blight of cotton is an environmentally sound and suitable strategy which minimize the use of chemicals by placing more reliance on resistant varieties, use of plant extracts and other non-chemical methods. Use of the resistant cultivars is the most efficient and eco-friendly method for the management of bacterial blight of cotton (Iglesias *et al.*, 2010; Jacobs *et al.*, 2010).

Moreover, resistant varieties also avoid the damage caused by other management strategies like acid delinting of seed and use of synthetic chemicals (Thaxton and El-Zik, 2001). In the absence of durable resistance in varieties, seed treatment with chemicals or acid delinting is recommended due to its quick action and readily availability (Singh *et al*., 2007). Although use of synthetic chemical is easy, direct and rapid means of controlling the disease but the continuous dependence on pesticides raise the problem of environmental pollution and

degradation. So, there is a dire need to find the alterantive of this method which is environment friendly and cost effective approach. Plant extracts can also use as an alternative of synthetic chemicals as these are non-phytotoxic, more systemic and easily biodegradable (Gottlieb *et al*., 2002). Plant extracts have minimal harmful effects on environment and human health as compared to the synthetic pesticides. Plant extract that based on therapeutics affect are easily available and cost effective (Kiran and Raveesha, 2006).

When *Xcm* attacks on cotton plant, it induces different biochemical and ionic changes in its metabolism. It interferes with its defense mechanism by disturbing the ionic content of the host plant which ultimately leads to disease development. Proper amount of nutrients also trigger the resistant mechanism of plants agaisnt pathogen while their deficiency makes them valunerable to disease by changing the physiology and biochemistry of host plant (Hajiboland, 2012). Nutrients reduces the disease severity by strenghthening the cell wall and promoting the growth of plant. (Huber and Graham, 1999). Minerals are the crucial part of plant nutrition, so their deficiency cause certain types of maladies either through disturbing metabolism or physiology of the plants by favoring plant pathogens or discouraging plant growth (Sahi *et al.,* 2010).

Nitrogen is responsible for production of enzymes, proteins and nucleic acid. It also interferes with disease resistance of infected host (Marschner, 1995). Phosphorus is second important element after nitrogen which inhibits the plant growth (Epstein and Bloom, 2005). It is a constituent of nucleic acids, phosphatides and coenzymes. Potassium is also an important element that is involved in biochemical and morpho-physiological processes that affects severity disease and incidence like N-metabolism, hormonal signaling, protein synthesis, osmoregulation, enzyme activation, photosynthesis (Epstein & Bloom, 2005; Taiz and Zeiger, 2010). Proper amount of potassium in a plant helps in hardening of plant structures, including cuticle, cell walls, outer wall of epidermis, sclerenchymatous tissues, and lignifications, as a result plant is protected from pathogens and insects (Datnoff *et al*., 2006). Magnesium and calcium is an important constituent of plant chlorophyll and cell wall respectively. It helps in translocation of starch and nutrient uptake. Any deviation in these nutrients from normal balance can badly affect pant metabolism and thus nutrients play a significant role in plant and pathogen interaction (Zafar *et al.,* 2010). *Xanthomonas citri* pv. *malvacearum* brings biochemical changes in an infected host. These changes cause increase or decrease of certain substances like phenols, proteins, enzymes, sugars and carbohydrates etc. Phenols and their oxidizing products produce

resistance in plant against pathogen infection (Rathi *et al.,* 1986). Protein contents are also affected by pathogen attack as they act as major structural constituent of plants (Palanisamy *et al.,* 2009). Chlorophyll is necessary plant pigment but pathogens interfere with chlorophyll contents of host plant (Khalil *et al*., 2014). It also helps in photosynthesis and prepares food for plants.

A healthy plant can compete to different challenges. The most suitable approach for the strengthening of plant health is to study mineral content of the healthy and diseased plants. Because, the pathogen of bacterial blight reduces the up-taking efficiency of plant and infected plants fail to optimize biochemical compounds like proteins, phenolics, chlorophyll contents, total soluble sugar and ionic contents i.e. N, P, K, Ca, Mg, Zn, Cu and Fe etc and ultimately become susceptible to disease. Application of nutrients through fertilization and alteration of soil environment for nutrient availability are important cultural controls that not only prevent the plant from diseases but also increase the agricultural production. The availability of nutrients through inorganic fertilizers provides the better means for reducing the severity of many diseases of crop plants (Savant *et al*., 1997). The ultimate objective of the integrated management of bacterial blight is to produce the optimum crop yield of high quality at minimum cost and preservation of the environment. The hypothesis of current studies was to characterize the physiological and biochemical changes in state of mutable disease intensity that may be helpful in the management of bacterial blight of cotton. In view of above observations, the present project was planned with the following objectives:

- To identify the source of resistance against bacterial blight of cotton.
- To observe biochemical changes in chlorophyll, phenolics, proteins, total soluble sugar and ionic contents i.e. N, P, K, Ca, Mg, Zn, Cu and Fe occurred in diseased leaves.
- Evaluation of different chemicals and plant extracts for the management of bacterial blight of cotton.

  In order to achieve the above mentioned objectives following line of work was adopted.

- Collection of germplasm from Department of Plant Breeding & Genetics, University of Agriculture Faisalabad, Ayub Agricultural Research Institute (AARI) Faisalabad and Central Cotton Research Institute (CCRI) Multan.

- Establishment of disease screening nursery in experimental area, Department of Plant Pathology, University of Agriculture Faisalabad for two years 2013 and 2014 under field conditions.
- Isolation, Purification and identification of bacteria from infected leaves.
- Pathogenicity test, establishment of plant under greenhouse conditions.
- Collection of inoculated and un-inoculated leaves of cotton from greenhouse for biochemical/physiological analysis.
- Different concentration of chemicals and plant extracts were evaluated against *Xanthomonas citri* pv. *malvacearum in-vitro*, green house and *in-vivo* conditions
- Data analysis.

CHAPTER # 2 REVIEW OF LITERATURE

2.1 **History, symptomology and transmission of bacterial blight of cotton**

Bacterial blight of cotton is one of the most destructive disease that causes considerable yield losses in rainy season (Delannoy *et al.*, 2005). *Xcm* is a pathogen of bacterial blight of cotton which requires high humidity for proliferation and spread (Voloudakis *et al.*, 2006). It was first time reported in 1892 in Alabama USA (Atkinson, 1892). Its occurrence was firstly observed in Tamil Nadu in 1918. In Pakistan, this disease was appeared in Burewala near Multan (Evans, 1965). During 2002-03 Harlapur *et al*., (2004) conducted a survey and observed the presence of bacterial blight in 27 locations of cotton growing areas of Marathwada. This disease was generally present in subtropical and temperate region of the world. The extensive dissemination of pathogen and high variability made its control more difficult (Kamal and Naim, 1983). The bacterium could survive in the field on debris of previously harvested crops and its initial inoculum was seed borne (Mohan, 1983). Viable propagules of *Xcm* can also be recovered from cotton seed even after the storage for more than two years at 5^{o}C. Studies reported that about 2% seed infection could lead to destructive epidemics within a field (Mehta *et al*., 2005). The bacterium attaches to the cotton leaf surface, enters leaves through open stomata or wounds and develops symptoms in susceptible plants. It also stimulates a hypersensitive reaction (HR) in resistant plants in which necrosis, desiccation and tissues collapse occur within two days (Delannoy *et al*., 2005). It also caused defoliation, stem blighting, black arm, girdling, weakened stem breaking and boll shedding. It can reduce the quality of cotton from lint staining, resulting in yield losses (Verma, 1986). Moreover damage of stem and boll tissues occurred which gradually produce brown necrotic, angular, waxy and water-soaked lesions on the leaf surface and these lesions were named as seedling blight, angular leaf spot, vein blight; black arm lesion and boll rot (Hillocks, 1992). The first symptom appeared as minute water soaked lesions (dull-green flaccid lesions) and then spread on the bottom of the young leaves (Verma, 1986). As the lesion size increased (about 4 mm in diameter in a susceptible cultivar), it turned into brown to black necrosis forming angular dead areas surrounded by chlorotic halos, or reddish or purplish ones (Innes, 1983). As the disease progresses, infected leaves defoliate prematurely (Ridgway *et al*., 1984) and disease spread along the veins of the host plant known as vein blight (Verma, 1986). Death of the growing point and the destruction of the terminal buds occurred in severe

cases (Innes, 1983). The bacterial ooze stained the cotton lint in infected bolls (Brown and Ware, 1958). Fruiting positions become vulnerable to black arm lesions due to the delicate nature of infected stems (Innes 1983; Akello and Hillocks, 2002). The bacterium over-wintered on infected lint, seed and plant residue remaining in the field after harvest and could also survive at least 22 months on the seed and 4 months on the seed lint (Kirkpatrick and Rothrock, 2001). Wind-driven rain, flowing water, and sprinkler irrigation were primary ways to spread this bacterium (Brown and Ware, 1958). Blowing sand was a general means to disseminate the pathogen. Dust and storms firstly produced wounds in the tissues of the plant which later cause infection in plants. Furthermore seeds, machines, insects and animals also responsible for transmission of this pathogen (Thaxton *et al*., 2001).

2.2 **Losses caused by bacterial blight of cotton**

Bacterial blight of cotton causes 26- 30% yield losses in different cotton growing areas of the world (Ramapandu *et al.,* 1979; Chidambaram and Kannan, 1989; Chattannavar *et al*., 2006). This disease was most severe in sub-humid and semi-arid areas which experienced wind, blowing rainfall ranges from 25.4 to 76.2 millimeters and dust events during growing season (Kirkpatrick and Rothrock, 2001). It was assumed that yield losses generally reached from 10 to 30% in Asian countries. In African countries they were recorded up to 50% (Bayles and Verhalen, 2007). About 37- 40% yield losses were observed in Faisalabad district (Bhutta and Bhatti 1983; Khan *et al.,* 1999). The disease infestation was more in the areas of high relative humidity that favored the growth and spread of the pathogen *Xcm* (Voloudakis *et al.,* 2006). Under natural environmental conditions, the infections of black arm of cotton damage 35% boll (Raj, 1988). In 2001 Mishra and Krishna conducted experiments and declared that losses at harvesting stage due to this disease ranges from 1 to 27% totally depending on variety and crop age. In Punjab it causes 50% losses where relative humidity and summer rain favored the disease development (Hussain and Ali, 1975). This disease affected plants at all growth stages which produced significant losses in terms of economy and yield of crop (Hillocks, 1992). Except exotic lines which were immune to all the races of *Xcm*, no one of the available commercial varieties was found to be resistant to the bacterial blight (Khan, 1996). In general, losses were less in leaves infection but in case of infected stem they increased up to 90% (Singh *at el*., 2007).

2.3 **Source of resistance against bacterial blight of cotton**

Search for source of resistance against bacterial blight disease of cotton was preliminary objective for management of this disease. As the development of resistant variety takes many years, so the screening of available germplasm of cotton to search out resistant cultivar of cotton was the short-term solution. Hussain, (1985) had done a screening test at the Central Cotton Research Institute (CCRI) Multan and observed that ten lines of exotic cotton introduced by the United States were immune to all the races of *Xcm* in Pakistan while none of the lines of local strains were immune against the pathogen. Mahmood and Hussain (1993) made a comparison of inoculating methods of *Xcm* in cotton cultivars for screening of cotton varieties in opposition to bacterial blight under glasshouse conditions. The result of experiment showed that scratching was less effective than hypodermic inoculation, band rubbing or spraying for screening of germplasm. Differentiation in the response range of infections and resurgence of microbial antagonists and plant extracts may be due to the fact that the pathogen multiplied at a high rate in susceptible varieties as compared to resistant or immune varieties. These responses were more important in B-284 (moderately resistant), CIM-109 (sensitive), CIM-1100, CIM-435 (moderately susceptible) as reported by Khan and Rashid (1999). In United States, as well as other countries, race 18 was the most frequently encountered race and no variety was immune against that race. Immunity, in the presence of the race 18 was only possible when more than one blight resistant genes present in the lines/varieties (Hussain & Brinkerhoff, 1978). In Texas, more than 75% of the cultivars planted in the last 5 years were susceptible to race 18 of *Xcm* (Thaxton *et al*., 2001). These cultivars had a source of stable immunity to all races of bacterial blight (Brinkerhoff *et al*., 1984), except a new race identified in the Upper Volta and Sudan (Bush, 1983). *Gossypium hirsustum* lines were found to be immune against bacterial blight (Bird 1960; Bird, 1962). However, a large number of commercial cultivars currently being grown were susceptible to infection (Thaxton *et al*., 2001; Sagaram *et al*., 2003; Nichols *et al*., 2007; Wheeler *et al*., 2007). Kharata & Chand, (1977) tested reaction of cotton lines against this disease and found Reba B50, B59, NG 8 and IAN 1327 immune to bacterial blight. Singh & Verma, (1971) tested genetic stock and found resistance response of 101-102B, Reba B50, BJA-592, HG-9 and PT 14 to all the races of *Xcm.* While Hussain & Brinkerhoff, (1978) evaluated and introduced 54 cotton breeding lines and they found 37 homozygous immunes, one homozygous resistant and 14 segregating with most of the plants immune or resistant.

Singh *et al.*, (2007) performed an experiment to find out the sources of resistance, 40 varieties were screened against *Xcm* under natural conditions none of those were found free from *Xcm*. Six varieties were resistant, ten moderately susceptible and twenty-four were found to be susceptible. Fifteen varieties of cotton were tested in research area of the Department of Plant Pathology University of Agriculture Faisalabad, to determine their genetic response against bacterial blight. Results showed that none of variety showed immune response while FH-114, Bt-121 and SLH-336 expressed moderately resistance response to bacterial blight with 15, 17 and 20% disease incidence respectively (Atiq *et al*., 2014).

2.4 **Characteristics of *Xanthomonas citri* pv. *malvacearum***

Genus *Xanthomonas* belongs to family *Pseudomonadaceae* (Schiegel, 1995). Colonies of this pathogen were smooth, yellow in color and viscid that expressed maximum growth at 25-30^{o}C (Bradbury, 1984). Certain species especially *Xanthomonas citri* (*Xc*) and *Xanthomonas fragriae* (*Xf*) produced a large amount of extracellular polysaccharides or *Xanthan* gums when grow in media comprising of functioning carbohydrates (Bradbury, 1984; Schiegel, 1995). Xanthomonadins (Yellow Pigments) were brominated aryl polyenes found in all species of *Xanthomonas* and was an important characteristic for identification. However, non- pigmented strains were also observed in this genus. Genetic variations in different isolates of several Xc pathovars were also observed by using different molecular techniques such as RFLP and rep-PCR (Norman *et al*., 1999; Restrepo *et al*., 2000).

Xcm can be cultured on nutrient glucose agar (NGA) media from infected leaves. After incubation of 2 to 3 days , opaque, yellow, convex, smooth with a rough edge round colonies were produced (Ridgway *et al*., 1984). Bacterium is gram negative and rod shaped with a polar flagellum. Size of the bacterial cells vary from 0.2-0.8×0.6-2.0µ (Bird 1981) to 1.2×0.9µ (Vasudeva *et al*., 1960). *Xcm* is an aerobic, gram-negative, and also has capsule which is a slime layer containing extracellular polysaccharides (EPS) and the slowly streaming out of the EPS from some pore-like structures. The cells might occured singly or in pairs, rarely in chains, non-spore formers, capsulated, facultative aerobe, oxidase negative. Requirements for normal growth included methionine, glutamic acid and nicotinic acid. While the growth was inhibited on 0.1 to 0.2 per cent triphenyl tetrazolium chloride nutrient agar, gelatin liquefied, starch and casein digested, acetone and indol (Schumann and Arcy, 2010).

2.5 **Impact of bacterial blight on ionic contents and biochemical compounds in leaves of cotton**

Growth and productivity of cotton significantly reduced by biotic and abiotic stresses. Biotic stresses or diseases lessened the uptake and utilization of nutrients by plants. So deficiency of plant mineral is compensated by nutrition management which changed plant responses to disease incidences (Walters & Bingham, 2007). In general, nutrients might cause changes in plant growth characteristics (anatomical and chemical composition) resulting in increase or decrease in tolerance to diseases (Zafar *et al*., 2010; Athar *et al*., 2011; Huber and Graham 2002). Application of mineral nutrients to mitigate the adverse effects of diseases on plants was suggested by a number of scientists (Graham, 1983; Huber & Graham, 1999, Athar *et al*., 2011; Huber and Jones, 2012). These nutrients also affect disease susceptibility through metabolic changes and enhancement of favorable environment for the development of disease. When a plant infected by a pathogen, it caused alterations in the physiology of plant particularly in translocation, assimilation and utilization of mineral uptake. Pathogens cause immobilization of nutrients in the soil and in infected tissues. They utilized nutrients, thus reducing their availability to the plant ultimately increased the plant's susceptibility to infection. Soil borne pathogens commonly infected plant roots and plant's vascular system, thus reducing the plant's ability to take up water and nutrients from soil and impair nutrient or water translocation (Spann *et al.,* 2010).

2.5.1 **Changes in ionic contents due to disease**

Huber, (1991) conducted an experiment and revealed that after the attack of pathogen in diseased plants, nitrogen contents decreased and large sized lesions were produced in field and greenhouse conditions due to utilization of nitrogen by pathogen. Nitrogen is an essential nutrient for all plants obtained from soil and decaying organic matter. A huge amount of N was required by the plant for formation of different types of enzymes, proteins and structures and its presence in balanced form was helpful for resistance towards bacterial blight of cotton. So plants express pronounced effect towards its application. Its excessive application errand some plant diseases while plants deficient in N contents favored some other types of symptoms. Decrease in N quantity favored the incidence of bacterial blight of cotton and an adequate application along with K, activated defense system and created resistance against pathogen attack (Chase, 1989; Vidhyasekaran, 1988; Agrios, 2005; Dordas, 2008). The susceptible black gram genotype

contained more nitrogen in both healthy and diseased plants than the resistant one (Iqbal *et al*., 2006). Randhawa (1994) reported that highest nitrogen contents in chickpea cultivars was responsible for resistance to Ascochyta blight. Plants grown under conditions of low N availability defended better against pathogens because there was an increase in the synthesis of defense-related compounds which were used as substrates for pathogens (Hoffland *et al*., 2000).

Phosphorus utilized by the plant for formation of essential molecules i.e. DNA, RNA, phospholipids, coenzyme NAD and NADP, ATP and other high energy compounds (Devlin and Witham, 1983) However, its role in resistance was variable. It has ability to decrease incidence of different plant diseases by promoting root growth. But there was some contradiction in results of different researchers. Decrease in Phosphorus quantity facilitated the growth and incidence of *Xcm*. Similar results were reported by Dordas (2008) who observed that low level of P favored the development of bacterial blight while its balanced application reduced the disease incidence (Huber and Graham, 1999; Kirkegaard *et al.,* 1999; Reuveni *et al.,* 2000). The content of Phosphorus was decreased significantly in infected susceptible genotype, whereas this decrease was remained non-significant in resistant genotype. The phosphorus content although higher in un-inoculated plants of susceptible lentil lines than the resistant ones, yet it decreased, as a result of inoculation with Ascochyta lentis while it increased in the resistant lentil lines and (Sahi *et al*., 2007; Awan and Strucmeyer 1957; Olanya *et al*., 2001) documented the decrease in phosphorus concentration. But Huber, (1991) reported that incidence of bacterial blight in cotton increased the phosphorus concentration because pathogen inhibited passage and utilization of that nutrients so its accumulation took place.Potassium role was crucial in metabolism of carbohydrates due to production of enzyme. Beside metabolism, it also played an important role in photosynthesis due to stomatal opening (Salisbury and Ross, 1992). It was involved in the activation of more than sixty enzymes which were necessary for different metabolic processes of plants. Mishra *et al.,* (2005) and Dordas, (2008) reported that decrease in K enhanced the severity of bacterial blight by favoring the growth of *Xcm*. Balanced amount of K in plants and soil boost up resistance in plants and also help plants to produce different types of physiological and biochemical resistance abilities against *Xcm* (Sharma and Duveiller, 2004; Mishra *et al.,* 2005). According to Zafar *et al*., (2010) disease occurred in susceptible variety S-12, whereas all the plants of resistant variety CIM-448 remained free of disease. The disease incidence on S-12 was more severe at lower

levels of K while the plants of this cultivar at the highest level of K (236 mg L-1) were almost free of disease.

Reduction in the accumulation of calcium was observed in diseased leaves of susceptible genotypes which showed severe symptoms like crinkling and puckering as compared to healthy leaves. While, reduction of calcium accumulation was also observed in leaves of inoculated plants of resistant genotype but it was less as compared to the susceptible genotype. Plants absorbed calcium as a Ca2+ cation. It played a significant role in stimulation of roots and leaf development, microbial activity and uptake of other nutrients. It prevented the penetration of pathogens and developed resistance in host plant which strengthened plant structure (Mishra *et al*., 2005). Calcium (Ca) is a major constituent of middle lamella of the cell wall which was present in the form of calcium pectate. It played a significant role in maintaining cell integrity and membrane permeability (Devlin and Withman, 1993). Reduction in Ca was observed in inoculated leaves of resistant and susceptible cultivars of cotton which enhanced the disease (Marschner, 1995; Mishra *et al.,* 2005). Similar results were reported by Dordas *et al.,* (2008) that there was decrease in calcium contents which increased the bacterial blight infection. Balanced amount of Ca provides protection against diseases by binding oxalic acid, strengthening cell wall and framework of cells by transformation of enzyme sensitive pectin and pectinic acid compounds of middle lamella which helps to resistance in host plant and prevent penetration of pathogen (Mishra *et al.,* 2005).Magnesium plays a significant role in synthesis of chlorophyll, photosynthesis and carbohydrate metabolism (Devlin and Withman, 1983). Mg was a vital element of structural tissues and takes part in different physiological and biochemical processes. It plays an important role in respiration, DNA and RNA formation, energy transfer reactions and also acts as a co-factor for many enzymes (Marschner, 2011). (Batson, 1971; Huber and Jones, 2012) observed reduction in Mg concentration in cotton leaves due to attack of *Xcm*. Reduction in Mg concentration caused hindrance in partitioning of dry matter roots and shoots which resulted in increased quantity of starch, amino acids and sugars in leaves, destruction of chlorophyll, reduction in electron transport chain (ETC) and production of reactive oxygen species (ROS) due to damage of CO_2 fixation in photosynthesis (Hermans *et al.*,2005; Cakmak and Kirby 2008). Zn plays a vital role in uptake and efficient use of water and worked as a catalyst in different metabolic and biochemical processes. It plays a significant role in starch and protein formation. Because Zn was an activator of SOD, so it was involved in protection of

membrane against reactive oxygen species (ROS) through detoxification of superoxide radicals (Cakmak, 2000) and damaged membrane due to free radicals which led to leakage of membrane which favored pathogenesis. (Marschner *et al*., 1991; Marschner, 1995; Dordas, 2008) suggested that decrease in concentration of Zn was due to disease. Low level of Zn increases severity of disease due to accumulation of amino acids and reducing sugars, which helped in disintegration of plasma membrane and increased pathogenesis (Grewal *et al.*, 1996; Mengel and Kirby, 2001). Iron the foremost component of chlorophyll and plays a vital role in nucleic acid metabolism. Reduction in iron content reduces chlorophyll contents of plants (Imran and Gurmani, 2011). It was also an important component of flavor-proteins and iron-porphyrn protein which included cytochromes, Peroxidases and Catalases. These proteins were responsible for increased catabolic activities in susceptible plants which were prone to bacterial blight infection (Delvlin and Withman, 1983). It was concluded that Fe concentration was decreased due to bacterial attack because plant pathogens generally had higher requirement of Fe and acted as virulence factor during the course of disease development because Fe activated the enzymes which were involved in the infection process of the host by the pathogen (Graham and Webb, 1991; Dordas, 2008). Copper is an imperative component of lignin and has a key role in protein and carbohydrate metabolism and acted as catalyst in different metabolic activities of the plant (Imran and Gurmani, 2011). When plant becomes infected, its defense system activated and starts secreting certain types of phenolics and flavonides both at the infection site and away from the site. Productions of these substances were controlled by nutrients of the plant. Therefore, shortage of elements like Cu, Fe, K and Zn were take place at the infection site and copper application reduce the intensity of bacterial diseases (Marschner, 1995).

2.5.2 **Changes in biochemical compounds due to disease**

Development stages of plants and their biological processes were influenced by total chlorophyll and the proportion of its components. Sain and Gour, (2008) observed decrease in chlorophyll contents with the increase in disease incidence. Stimulation of Chlorosis (loss of chlorophyll), necrosis and reduction in the green leaf area was due to lesion growth which was produced by infection but it had no effect on photosynthetic activity of the remaining green leaf tissue (Oijen, 1990). Sugars are important for the production of phenols, phytoalexins, lignin and cellulose, so they performed an imperative role in defense mechanism of plants. Gene regulation and host defense functions were accomplished by accumulation of soluble sugars. It was

observed that carbohydrate metabolism was influenced by pathogen attack. Starch accumulation, reduction in photosynthetic rate and reduction in total soluble sugar contents were characteristic properties of infected leaves (Goodman *et al.,* 1986; Sain and Gour, 2008). Chakrabarthy *et al*. (2002) reported that healthy leaves of the highly susceptible cotton possessed significantly higher amounts of total sugar than those of resistant and immune cotton cultivars/varieties. As a result of infection, concentration of total sugar declined more rapidly in susceptible plants than in the resistant plants. Bt cotton genotypes RCH-2 Bt, JKCH-1 Bt and JKCH-2 Bt exhibited more total sugar and reducing sugar content when compared with the non-Bt genotypes, Laxmi, Abhadita and DCH-32. It was also observed that there was decrease in the total sugar content in the non-Bt genotypes due to attack of *Xcm* (Govindappa, 2007). Total soluble phenol was present in more concentration in susceptible plants as compared to the resistant ones. Kumar *et al.,* (2007) reported that due to attack of bacterial blight disease on rice, there was an increase in total soluble phenols. Enhanced level of total soluble phenols advocated a preliminary effort by the host defense towards *Xcm*. Secondary phenolics compounds are produced by higher plants and their role in the metabolism of plants had not been adequately explained. Lignin and phenolics precursors play an important role in plant defense by increasing cell wall resistance to mechanical penetration, decreasing the susceptibility to cell wall degrading enzymes, restricting the entrance of enzymes and toxins produced by the pathogens (Ride, 1978). Borkar and Verma (1991) reported that the leaves of resistant cotton cv. 101-102 B to bacterial blight contained 69% more total phenol than the leaves of susceptible cotton cv. Acala-44. Kalappanavar and Hiremath, (2000) reported that resistant sorghum genotypes possessed higher content of phenols as compared to susceptible ones. The experiment conducted by Gallet *et al*., (2004) showed a substantive increase in phenolics and flavonoids in the leaves of highly resistant varieties. The induced accumulation of phenolics during infection protects the plant from further invasion and growth of the pathogens population. Decrease in protein contents indicated that bacterial infection increased with increase in protein contents of the plant. Sain and Gour, (2008) studied pathological, physiological and biochemical characterization of *Xanthomonas citri* and concluded that bacteria not only decrease protein contents of the host plant but also causes decrease in total nitrogen, chlorophyll and sugar contents. Boller, (1985) expressed that proteins are associated with the defense of plant against fungi and bacteria by their action on the cell walls of invading pathogens.

2.6 **Management of bacterial blight of cotton**

2.6.1 **Through Chemicals**

Beura *et al*., (1997) evaluated three sprays of Bordeaux mixture (1%) at 15 days interval and concluded that it was effective and reasonable under moderate disease incidence. (Khan *et al.,* 1999) compared the antibacterial activity of a variety of toxicants. Result indicated that Agromycine-100, Streptomycin sulphate and Vitavax showed more efficiency while Nemisopre, Penncozeb, Sandofan-M, Cuperosan and Beam were comparatively less effective against *Xcm.* Seed treatment with the 100 ppm streptomycin followed by 2 sprays. Streptomycin at the concentration 100ppm with 0.3% copper-oxy chloride was found most effective against bacterial blight of cotton (Pathak, *et al.,* 2006). Chattannvar *et al*., (2006) evaluated seven fungicides (copper oxychloride, wettable Sulphurpropineb, mancozeb, carbendazim, ziramand and tridmorph which were sprayed thrice at 15 days interval for the control of Red Acola. Carbendazim showed greatest disease control followed by Ziarm and triedmorph.

Hussain and Tahir, (1993) conducted field experiment to check the efficacy of different chemicals against *Xcm.* Agrimycin-100 (Streptomycin + oxytetracycline), Cobox, Trimiltox alone and in combination after inoculation was done. Agrimycine-100 with Trimiltox expressed lowest disease occurrence and highest yield followed by Agrmycine-100 and Cobox-Trimiltox when sprayed alone. While Agromycine-100 was least effective if applied as preventive measure of the disease. Among all tested fungicides, carbendazim (0.1%) expressed maximum disease control (55.71%) followed by triedmorph (0.1%), (30.88%). Singh *et al.,* (2007) evaluated twelve fungicides and two antibiotics against bacterial blight disease. Among all chemicals streptomycin sulphate expressed significant results both *in vivo* and *in vitro*. Similar results were also reported by Jagtap *et al.,* (2012). Efficacy of various chemicals i.e. streptomycin sulphate, Bordeaux mixture and copper oxychloride was evaluated against bacterial blight of cotton. These chemical were applied alone and with combination to each other. Result revealed that Copper oxychloride was efficient and gave significant results for the management of bacterial blight as compared to other chemicals (Sana *et al*., 2012).

2.6.2 **Through Plant Extracts**

Pesticides have made great contribution for quick and effective management of plant diseases and microbial contaminations in several agricultural commodities. Continuous and extensive use of these synthetic pesticides posing serious problem to the life supporting systems

due to their residual toxicity (Campos *et al*., 2005). Considering the deleterious effects of synthetic pesticides on life supporting system, there was an urgent need for alternative agents for the management of pathogenic microorganisms (Mahajan and Das, 2003). A green plant represents a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides (Cowan, 1999; Newman *et al*., 2000; Gibbons, 2005). Reports are available on the use of active agents from higher plants, in place of chemical fungicides, that are non-phytotoxic, more systemic and easily biodegradable (Gottlieb *et al*., 2002). These plant extracts were used against diseases because, these play an important role i.e. sustainable solutions in agriculture, Reduce crop losses, Eco-friendly, easily bio-degradable, Cheaper and are an important component in integrated diseases management.

Moringa oleifera is an antibacterial and its active ingredient was saponins which are flavonoids, they inactivate the activity of enzymes by rupturing the cell and damaging the intercellular components of the pathogen, when water (extraction of plant extracts in water) dips in to the cell of bacteria it causes to swell more and causes burst which leading to death. (Mustapha Hassan Bichi 2013). Neem (*Azadirachta indica*) is an antifeedant and antimicrobial which acts as a biopesticide and as antifeedant *i.e*. when a pathogen feed on the leaf which is treated with neem product, the presence of azadirachtin, salaninand melandriol there is production of an antiperistalitic wave in the alimentary canal and this produces something similar to vomiting sensation in the pest. Because due to this sensation pest does not feed on the neem treated surface and ability of pest to swallow is blocked which ultimately decrease activity of pathogen (Lokanadhan *et al.,* 2012). *Datura alba* is antibacterial and is rich in alkaloids which induce a stimulation of central nervous system and depression of the peripheral nerves. Antimicrobial substances affect the synthesis of peptidoglycan around the bacterial cell, and the cell dies by osmotic shock. (Maheshwari *et al*., 2013). *Curcuma longa*causes inactivation of the protein and its function and target on the microbial cell causing surface-exposed adhesions, degradation of cell wall polypeptides, and attack on membrane-bound enzymes. It also makes substrates unavailable to the microorganism. Turmeric causes inhibition of bacterial cell proliferation by blocking the assembly dynamics of FtsZ in the Z ring which ultimately reduced microbial activities. (M. M. Cowan, 1999). Tobacco (*Nicotiana* tabacum), is an alkaloid (active ingredient is nicotine) that is produced in the roots of tobacco plants and transported to leaves where it is stored in vacuoles. This toxic molecules disrupt pathogen metabolism and cellular

structure, bind to salivary proteins and digestive enzymes including trypsin and chymotrypsin resulting in protein inactivation of pathogen (Freeman and Beattie, 2008).

Khan *et al.,* (2000) managed bacterial blight of cotton by applying plant extracts through application of Neem seed oil (*A. indica*), leaf extracts of *Datura alba* and neem seed bitter at three different concentrations 1, 2 and 3% against the development of *Xcm* in lab and green house. *Datura alba* reduces the development of bacteria at 3% concentration as compared to others. Mukhtar and Chohan, (1999) evaluated different plant extracts and observed that plant extracts played a pivotal role for the enhancement of inhibition developed by *Xcm.* Among these plant extracts Datura (*Datura alba*) was rich in secondary metabolites and had antibacterial activity by different mechanisms. Antibacterial substances affected the synthesis of peptidoglycan around the bacterial cell and cell dies by osmotic shock. Neem (*A. indica*) had complex tetranortri-terpenoidlimonoid which was responsible for toxic effects pest repellent and pest reproduction controller. Tobacco (*Nicotiana tabacum*) contained nicotine which was an antibacterial compound (Saadabi *et al*., 2006). Moringa leaves act as a good source of natural antioxidant due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Anwar *et al.,* 2005). Sajid *et al.,* (2013) evaluated different plant extracts against growth of *Xcm* and found that maximum inhibition of bacterial growth was expressed by *N. tabacum.* Bambawale *et al*., (1995) conducted research on 14 diverse medicinal plant extracts against bacterial blight pathogen and showed that the extract of *Lawsonia inermis* and *A. indica* inhibited the colony growth of *Xcm*. Effect of Drava on angular leaf spot of cotton sowing resulted in deterioration of blight occurrence (Vinay *et al.,* 2007).Management of disease through plant extracts had been reported by different scientists in different crops because these are environment friendly. An experiment was conducted to check the efficacy of different concentration of various plants extracts namely *Moringa oleifera* (Sohangna), *Azadirachta indica* (Neem), *Mangifera indica* (Mango), *Mentha Piperita* (Mint), *Aloe vera* (Gavargandal), *Syzygium cumini* (Jaman) and *Citrus limon* (Lemon) against colony growth of *Xcm* though poisoned food technique. Three replications were made for each plant extract with 05, 25, 50 and 75ppm concentration. The results of study showed that *Mentha piperita*, *Aloe vera*, *Azadirachta indica* and *Syzygium cumini* exhibited the promising results against the infectivity of *Xcm* at all the tested concentrations under *in-vitro* conditions (Hasan *et al*., 2014).

2.6.3 **Induced Resistance**

A new research for the control of many plant diseases based on the motivation or stimulation of plants's own defence mechanism with the aid of artificial molecules having low molecular weight that incite systemic aquired resistance against vast range of disease causing pathogen in plants. The systemic aquired resistence (SAR) is the most important component of plant defence against distinct diseases where the initial infection offered systemic resistance to constant infection by a variety of microorganisms i.e. bacterial, viral and fungal infection causing agents (Gaffney *et al*., 1993). Many scientists have effectively used systemic aquired resistance (SAR) to manage many bacterial and viral diseases (Ghoshroy *et al*., 1998; Naylor *et al.*, 1998; Chivasa and Carr, 1998). Dong and Beer, (2000) and Ahn *et al.*, (2005) successfully used chemical salicylhydroxamic acid, cadmium, salicylic acid, riboflavin, antimycin A,2,6-dichloroisonicotinic acid and vitamin B1 to boost up the resistance of cotton plant against bacterial diseases (Naylor *et al*., 1998 and Chirkov *et al*., 2001). Using uniquely, the plant prospective to fight against pathogens, the induced resistance minimize the use of toxic synthetic chemicals for disease control, and thus suggested as an eco-friendly alternative, non-biocide and non conventional approach for protection of crop against disease and hence for sustainable agriculture (Soylu *et al*., 2003). Induced resistance is usually dependent on the stress by plant or establishment of a pathogen on the site of infection. Defence mechanisms include physical barriers, metabolites and production of essential protiens which limited the spread of disease causing microorganism that enter at that site. In moderen times a great number of chemicals (*viz.* Actigard, 2, 6-dichloroisonicotinic acid, salicylic acid, hydrogen peroxide, phosphates, calcium, jasmonic acid, ethylene, acetyl salicylic acid and methyl jasmonate, etc) had been reported which reduced the function of certain pathogens in the induction of systemic aquired resistance (Ryals *et al.*, 1996). Systemic induced resistance (SIR) produced by the application of nutrients is a substitute approach to decrease disease severity. In addition, there is a product that contain acibenzolar-S-methyl (commercial name actigard) is now commercially available which triggered the defence response against the enhancement of pathogen. The best SIR is a chemical that can reduced the adverse effect of the pathogens on the host having high level of effectiveness. NPK fertilizers used to reduce disease while other micro nutrients can also be used together with these macronutrients (Cristos, 2008).

CHAPTER # 3 MATERIALS AND METHODS

3.1 Evaluation of cotton germplasm for source of resistance against bacterial blight disease under field conditions for two years

Twenty Bt varieties, (Bt-NS 131, Bt-53, Bt Ali Akbar 802, Bt-KZ 189, Bt-FH 177, Bt-FH 182, Bt- ASO1, Bt-NIBGE 2, Bt-CM 615, Bt-CRS 2007, Bt-FH 142, Bt-IR 901, Bt-CM 616, Bt-FH 4243, Bt-FH169, Bt-VH 329, Bt-IUB 222, Bt-MNH 886, Bt-FH 183, Bt-FH 143) and 10 Non Bt, (MNH 554, CM 482, Sindh 1, NIAB 111, BH 107, Redacola, CRIS134, Shahbaz, PB 896 and CIM 573) of cotton were collected (on the basis of their good agronomic traits) from Central Cotton Research Institute Multan (CCRI), Department of Plant Breeding and Genetics, University of Agriculture Faisalabad and Ayub Agriculture Research Institute Faisalabad (AARI). Disease screening nursery was established in the experimental research Area 24.5×7 m, Department of Plant Pathology, University of Agriculture Faisalabad for two years 2013 and 2014 to find out source of resistance against bacterial blight disease of cotton. Seeds of above mentioned verities were neither treated with chemical nor given acid delinting to increase chances of primary infection of the disease. Sowing was done by using dibbler method (Islam *et al.,* 2001). Seeds of all varieties were sown with 30 cm plant to plant (P×P) and 75cm row to row (R×R) distance under randomized complete block design (RCBD) with three replications. No spreader was sown either around the field or between the lines. All the agronomic practices including recommended dose of fertilizers and irrigation schedule were followed to keep the crop in good condition. Data regarding disease incidence was collected on weekly basis by using (Brinkerhoff scale, 1977).

While disease incidence was calculated by using following formula.

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total No. of plants}} \times 100$$

3.2 Isolation, identification and purification of *X. citri* pv. *malvacearum*

Cotton leaves showing typical symptoms (water soaked lesions) of bacterial blight were collected from experimental area, Department of Plant Pathology, University of agriculture Faisalabad. For isolation of *Xcm*, diseased portion of leaves were cut into small pieces (1×1 cm), surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution and then washed with distilled water. Leaves were crushed with the help of pestle and mortar and distilled water was added in it

for preparation of bacterial suspension. Then bacterial suspension was transferred to nutrient agar media (NA) with inoculating loop (Nichrome, Qty/pk-12). All the plates were incubated (Drucker 614B) at 28^{o}C for 3 days. After 3 days, bacteria produced round colonies of yellow color on nutrient agar medium. Bacterial colony was examined under stereoscope (OLYMPUS, SZX-ILLB2-200) and was identified on the basis of morphological characteristics (size, shape, texture and colony color).

3.3 **Pathogenicity test**

To fulfill Koch's postulates, seeds of susceptible variety (Bt-FH 142) of cotton were acid-delinted with 0.2 % H_2SO_4 and washed thoroughly with distilled water and were sown in pots (12×9" dia.) which were filled with field soil (2kg/pot) and these pots were transferred to green house. Recommended agronomic practices were followed time to time and pots were watered once in a day. Plants at the age of 6-7 weeks were inoculated by using sterilized syringe (24 Gauge needle size) filled with 20 µl (local isolates) bacterial suspension, contained 10^{7}cfu ml^{-1}, which were measured by using colony counter (SUNTEX 560).Suspension was injected in midrib of leaves until the leaf became water soaked. In control treatment, only sterilized water was injected. After development of disease, bacterium was re-isolated from diseased leaves after one week of inoculation by using dilution plate technique (Harris and Sommers, 1968). Morphological characteristics (size, shape, texture and color of colony) of re-isolated bacterium were compared with bacterial culture that was used for inoculation. Re-isolated bacteria expressed exactly same colony characteristics as that of original culture.

3.4 **Determination of biochemical/physiological changes in leaves of diseased and healthy cotton plants**

After screening of cotton germplasm under field conditions, six varieties were selected for determination of biochemical/physiological changes and alterations in ionic status due to attack of bacterial blight disease in cotton leaves. For this purpose plants of cotton varieties were sown in pots (12×9") filled with loamy soil (2kg/pot) and transferred to greenhouse. Plant population was composed of two groups i.e. inoculated and un-inoculated and each group was consisted of two reaction types (Resistant and susceptible). Resistant reaction type contained Bt-MNH 886, Bt-FH 177 and Bt-ASO1while susceptible reaction type contained three varieties/lines viz. Bt-FH 142, Bt-FH 182 and Bt-FH 169. Each treatment was replicated five times. At the age of 6-7 weeks plants were inoculated by using syringe method (Richard Weindling 1948) and injected

20 µl (local isolates) bacterial suspensions (10^7cfu ml^{-1}) while un-inoculated plants served as control. In green house, humid condition was maintained by spraying water on daily basis. After 6 to 8 days of inoculation, typical symptoms (water soaked lesions, 72.70-79.20% disease incidence) of bacterial blight appeared on leaves, Samples of resistant and susceptible cultivars of inoculated and un-inoculated cotton plants were collected and washed in 0.2% detergent solution to remove dirt, followed by washing in 0.8% HCl (to remove metallic contaminants from them) and de-ionized water (to remove previous two solutions). Samples were air dried, placed in paper bags, oven (Heaes D650 Hanau) dried at 70°C for 72 hours to get constant weight. The dried samples were grind with mortar and pestle. Then grind samples (100 mg) were boiled in 10 ml of 1.4N HNO_3 on a hotplate (TH-550; Advantec, Tokyo, Japan) at 100 °C for 30 min. After cooling, suspension was diluted 250 times with distilled water, followed by analysis for the determination of N, P, K, Ca, Mg, Zn, Fe, and Cu following Bhargava and Raghupathi, (1995) method. Nitrogen and phosphorous contents were recorded on percent basis while contents of other elements were recorded as ppm (parts per million).

3.4.1 **Determination of ionic status from leaves of inoculated and un-inoculated cotton plants**

Un-inoculated and inoculated plant samples (comprising of leaves from both resistant and susceptible cultivars) were harvested and oven-dried (D6450 Hanus; Heraeus) for 48 hours at 70 °C. Then these samples were grinded in mortar with the help of pestle and dried samples (100 mg) were boiled in 10 ml of 1.4N HNO_3 on hot plate (TH-550; Advantec, Tokyo, Japan) at 100 °C for 30 min. After cooling, suspension was diluted 250 times with distilled water and was analyzed for the determination of N, P, K, Ca, Mg, Zn, Cu, and Fe by following Bhargava and Raghurpathi, (1995) while nitrogen was analyzed by Kjeldahl method (Kjeldahl, 1983).

3.4.1.1 **Determination of phosphorus from leaves of inoculated and un-inoculated cotton plants**

0.1 mL of sample solution, already prepared by wet digestion method, was taken in a volumetric flask (ASTM- E288). Then 8.6 ml of distilled water was added along with 1mL of ammonium molybdate reagent ($[NH4]_6Mo_7O_{24}.4H_2O$). After swirling the flask to mix solution, 0.4 mL of amino nephthol sulphonic acid ($C_{10}H_9NO_4S$) was added. Absorbency was measured using distilled water as reagent blank in place of sample solution at 720 nm on a spectrophotometer (Hitachi U-2001, model 121-003). Phosphorus concentration was determined by comparing the absorbency to a previously prepared standard curve (Fiske and Subbarow,

1925; Bolts and Mellon, 1948) by Atomic absorption spectrometer (Hitachi Polarized Zeeman).

3.4.1.2 **Determination of potassium contents from leaves of inoculated and un-inoculated cotton plants**

Potassium was measured by flame photometer (Janway, PFP-7). For the measurement of potassium, KCl was used as standard. Standard curves for K was prepared by using 10, 20, 30 and 40 ppm concentrations. Fresh working standards were prepared immediately before use.

3.4.1.3 **Determination of calcium, magnesium, copper, iron and zinc from leaves of inoculated and un-inoculated cotton plants**

Calcium (Ca), Magnesium (Mg), (Cu) Iron (Fe) and Zinc (Zn) were determined by using spectrophotometer (Hitachi U-2001, model 121-003). For the determination of these ions, Calcium chloride($CaCl_2$), Magnesium sulphate ($MgSO_4$), Copper sulphate ($CuSO_4$), Iron sulphate ($FeSO_4$) and Zinc oxide (ZnO) were used as standards respectively and their standard curves were obtained by using 10, 20, 40, 80, 100 ppm for Ca; 5, 10, 15 and 20 ppm for Mg; 2, 2.5, 3 and 3.5 ppm for Cu; 1,2,3 ppm for Fe and 0.2, 0.3, 0.5 and 2 ppm for Zn, respectively. These working standards were arranged as fresh just before exercise.

3.4.1.4 **Determination of total nitrogen from leaves of inoculated and un-inoculated cotton plants**

Total nitrogen in a sample was determined by following micro Kjeldahl method (46MC; Quickfit, England). (Kjeldahl, 1983). A known amount of oven (D6450 Hanus; Heraeus) dried sample (WI) was taken in a long neck Kjeldahl flask. Five gram of digestion mixture containing K_2SO_4 and $CuSO_4$ and 25 mL of concentrated sulphuric acid (H_2SO_4) were added. The sample was boiled in a digestion hood (KB8S Kjeldatherm), initially at low temperature and then at vigorous boiling till the contents became clear. After cooling, the contents of the flask were diluted with distilled water in a 250 mL volumetric flask (ASTM- E288). 10 mL of this solution was transferred to the micro Kjeldahl distillation apparatus (VAP20.Gerhardt) and was distilled in the presence of 10 mL of 40 percent NaOH solution. The ammonia (NH_3) so produced was collected in a beaker containing 10 mL of two percent of boric acid (H_3BO_3) solution having two drops of methyl red as an indicator. The distillate was titrated against standard 0.1 N sulphuric acid (H_2SO_4) to light pink point. The percentage of nitrogen was calculated according to the following formula:

$$\text{Nitrogen \%} = \frac{0.1\ \text{N}\ H_2SO_4 \times 0.0014 \times 250 \times 100}{\text{WI} \times 100}$$

3.4.2.1 **Determination of chlorophyll contents from leaves of inoculated and un-inoculated cotton plants**

Total chlorophyll contents were quantified from inoculated and un-inoculated leaves by following the method as described by Arnon (1949). Fresh leaves were taken and extracted overnight with 5 mL of 80% acetone (C_3H_6O). The extract was centrifuged (Dawlance, 9170 WB) at 14000 rpm for 5 minutes and absorbance of supernatant was measured at 645 nm and 663 nm by using spectrophotometer (Hitachi U-2001, model 121-003). The chlorophyll a and chlorophyll b were calculated by using following formulae (Inskeep and Bloom, 1985).

Total chlorophyll: mg/mL= 0.0202 A 645 + 0.00802 663

(Where A= Absorbance in 1.00 cm curve)

Chlorophyll contents in mg per gram fresh weight were calculated as

[mg. chlorophyll / mL × volume of extract (mL.)] ÷ Fresh weight (g)

3.4.2.2 **Determination of total soluble sugars from leaves of inoculated and un-inoculated cotton plants**

Total soluble sugars were quantified from leaves by the anthrone reagent (($C_{14}H_{10}O$) method (Yemm and Willis, 1954). Weighed sample (100 mg) was put into boiling tube and was hydrolyzed in a boiling water bath for 3 h with 5 mL of 2.5N HCl and was cooled to room temperature. It was neutralized with solid Sodium carbonate (Na_2CO_3) until the effervescence ceased. Volume was made up 100 mL and centrifuged (Dawlance, 9170 WB) Supernatant was collected and 0.5 and 1 mL aliquots were taken for analysis. The standards were prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mL of the working standards."0" served as blank. The volume was made up to 1 mL in all the tubes by adding distilled water and then 4 mL of anthrone reagent was added. It was heated in a boiling water bath. Cooling was done rapidly and the green to dark green color was observed at 630 nm. A standard graph was drawn by plotting concentration of the standard on the x-axis versus absorbance on the y-axisby using spectrophotometer (Hitachi U-2001, model 121-003). From the graph, the amount of carbohydrate (soluble sugar) present in the sample tube was calculated.

3.4.2.3 **Quantification of soluble protein contents from leaves of inoculated and un-inoculated cotton plants**

The soluble protein contents were determined by Bradford method (Bradford, 1976). 0.01, 0.02, 0.04, 0.06, 0.08, and 0.1 mL of standard protein solution was taken in a series of test tube. The volume in each test tube was made up to 1.0 mL with an appropriate buffer. 0.1 mL of

buffer alone served as the blank. 0.5 mL of protein reagent was added and mixed thoroughly by inversion. The absorbance was measured after 2 min and before 1 hour against a reagent blank at 525 nm. The amount of protein was calculated in the unknown sample from the standard curve.

3.4.2.4 **Determination of phenolic contents from leaf extracts of inoculated and un-inoculated leaves of cotton plants**

Phenolic contents were determined by following Julkenen-Titto procedure (1985). 0.1 gram of ground fresh material was extracted in 1ml 80 percent acetone at 50 °C and centrifuged (Dawlance, 9170 WB) for 5 minutes at 12000 rpm and the supernatant was taken in a microfuge tube and stored at -20 °C (Pol Eko Aparatura, ZLN-T 300 COMF) until used. An aliquot (100µl) was taken and diluted with milliq water to 1ml in 10 ml capacity test tubes. 0.5 ml of Folin-ciocalteu's phenol reagent was added and shaken vigorously. 2.5ml of Na_2CO_3 was added immediately and volume was made up to 5ml, vortexed vigorously for 5-10 seconds. It was waited for 20 minutes and absorbance was measured at 750 nm using a spectrophotometer (Hitachi U-2001, model 121-003). The spectrophotometer was set to zero against 80 percent acetone. The standard curve was prepared from 20, 40, 60, 80 and 100 µg of Tannic acid ($C_{76}H_{52}O_{46}$) (prepared from 100 µg/ml stock).

3.5.1 ***In- vitro*** **evaluation of different chemicals against *X. citri* pv. *malvacearum***

Five chemicals i.e Flare (T_1), Agrimycine (T_2), Copper oxychloride (T_3), plant protector (T_4) Mancozeb (T_5) were evaluated against colony growth of *Xcm* by using inhibition zone technique (Berry *et al*., 1979) under complete randomized design with three replications. Each chemical was tested at 0.25 (C_1), 0.30 (C_2) and 0.35% (C_3) concentrations. Bacterial culture was multiplied by adding freshly growing aqueous bacterial suspension to the Luke warm NA media in a flask (ASTM- E288). It was shaken well and poured in plates (60×15 mm).These plates were wrapped and incubated (Drucker, 614B) at 30°C. When bacterial colonies were formed, wells of 1cm were made by using sterilized cork borer at the center of plate. Chemical solutions of requisite concentrations were poured in petriplates with disposable sterile syringe (Glass and PTFE, 1050 model, 22 gauges) and were again wrapped and incubated (SANYO, 175 M) at 30°C. In control treatment (T_6), only sterile water was poured. Data regarding inhibition zone was recorded after five days.

3.5.2 *In vitro* evaluation of different plant extracts against *X. citri* pv. *malvacearum*

3.5.2.1 Preparation of plant extracts.

Anti-bacterial efficacy of five plant extracts (*Nicotiana tabacum*, *Azadirachta indica*, *Moringa oleifera*, *Datura alba*and *Curcuma longa*) were evaluated against bacterial colony growth. Fresh leaves of each plant were taken at flush stage, thoroughly washed with tap water and sun dried. When leaves giving brittle appearance, were grinded by using electric grinder (AG014, MAKUTE). Powder (25 g) of each plant leaves was taken, dissolved in 100 ml of acetone solvent and was mixed thoroughly by using electric stirrer (R30, UET Mixer) which then poured into plastic tubes and centrifuged (Dawlance, 9170 WB) at 6000 rpm for 5 minutes. After centrifugation, extract was taken out with the help of pipette (Nichiprt EXII, E13319791) and passed through filter paper (WhatmanNo.1). The extracts were arbitrarily considered as standard stored at -4^{o}C and used further in experiments. For Turmeric, 100g piece of turmeric bulb was taken and washed thoroughly in water, macerated well in mortar and pestle in 100ml of distilled water. Mixture was centrifuged (Dawlance, 9170 WB) at 9000 rpm and extract was separated.

3.5.2.2 *In-vitro* evaluation of plant extracts against *X. citri* pv. *malvacearum*

Five plant extracts i.e., *Nicotiana tabacum* (T_1), *Azadirachta indica* (T_2), *Moringa oleifera* (T_3), *Datura alba* (T_4) and *Curcuma longa* (T_5) were evaluated against *Xcm* by using inhibition zone technique (Berry *et al*., 1979) at 10, 15 and 20% concentration. Bacterial culture was multiplied by adding the freshly growing aqueous bacterial suspension to Luke warm NA media in a flask (Erlenmeyer, GW-11). It was shaken well and poured in petri plates (60 × 15 mm). These plates were wrapped and incubated (SANYO, 175 M) at 30°C.After solidification of culture media, wells of 1cm dia. were made by cork borer at the center of the plate. Extracts of each plant with three concentrations were poured in the wells with sterilized disposable syringe (Glass and PTFE, 1050 model). Overflowing was strictly avoided. In control treatment (T_6), only sterile water was poured. Petri plates were carefully wrapped with clingfilm and dispensed for 24 hours in refrigerator (Dawlance, 9170 WB) at (4°C). After dispensing, plates were incubated (SYNYO, 175M) at 30^{0}C. Experiment was conducted in Completely Randomized Design (CRD). Each treatment was replicated thrice. Data was recorded by measuring radius of Inhibition zones after 24, 48 and 72 hours. Treatments mean were compared by Fisher's Least Significant Difference Test (LSD).

3.5.3 **Evaluation of Flare and *N. tabacum* against *X. citri* pv. *malvacearum* under greenhouse conditions**

In lab. Flare and *N. tabacum* expressed the most effective results against *Xcm.* So these were also evaluated under greenhouse conditions to check their efficacy against bacterial blight of cotton. For this purpose seeds of susceptible variety (Bt-FH 142) with five replications, were sown in pots (2 plants/pot) which were partially filled with sterilized loamy soil (2kg/pot). These plants were artificially inoculated at the age of 6-7 weeks through syringe method by using20 µl (local isolates) bacterial suspension, contained10^7cells/ml. Flare and *N. tabacum* alone and in combination, were sprayed against disease, with five replication under complete randomized design (CRD) in greenhouse, Department of Plant Pathology, University of Agriculture, Faisalabad. Control plants were sprayed with distilled water only. Data regarding disease incidence was recorded after seven, fourteen and twenty one days of application

T_1= Flare (0.5, 0.55 and 0.6%)

T_2= *N.tabacum* (30, 35 and 40%)

T_3= Flare + *N.tabacum* (0.5, 0.55 and 0.6% + 30, 35 and 40%)

T_4= Control

3.5.4 ***In vivo* evaluation of Flare and *N. tabacum* against bacterial blight of cotton under field conditions**

To evaluate the efficacy of Flare and *N. tabacum* under field conditions, Seeds of susceptible variety (Bt-FH 142) were sown with 30cmplant to plant (P×P) and 75cm row to row (R×R) distance under randomized complete block design (RCBD) in research area, Department of Plant Pathology, University of Agriculture Faisalabad. After 6-7 weeks, when typical symptoms of blight appeared on the leaves, (water soaked lesions) treatments like Flare @0.75, 0.80 and 0.85% and *N. tabacum* @ 45, 50 and 55% alone and in combination were applied. Control plants were sprayed with distilled water only. Each treatment was applied with three replications with one control and each replication contained thirty plants. Data regarding disease incidence was recorded after seven, fourteen and twenty one days of application

T_1= Flare (0.75, 0.80 and 0.85%)

T_2= *N. tabacum* (45, 50 and 55%)

T_3= Flare + *N.tabacum* (0.75, 0.80, 0.85% + 45, 50, 55%)

T_4= Control

3.6 **Statistical analysis**

For biochemical and mineral analysis, plant population was comprised of two groups i.e. inoculated and un-inoculated. Each group consisted of two reaction types: resistant and susceptible. Three resistant: Bt-MNH 886, Bt-FH177 and Bt-ASO1, and three susceptible (Bt-FH 142, Bt-FH 182 and Bt-FH 169) varieties/lines were used for sample preparation. Standard analytical methods were used to estimate the minerals contents using Nested Design (Gomez and Gomez, 1984). For field experiment Randomized complete block design (RCBD) and for lab and greenhouse experiments, complete randomized design (CRD) was applied using Statistical Analysis System (SAS, 1990) and means were compared by using Least Significant Difference (LSD) test (Steel *et al*., 1997).

Table.1 **Brinkerhoff disease rating scale for recording bacterial blight of cotton**

Sr#	**Disease incidence** (%)	**Description**	**Response**
1	0	Immune	I
2	1-10	Highly Resistant	HR
3	11-20	Resistant	R
4	21-50	Moderately Resistant	MR
5	51-70	Moderately Susceptible	MS
6	71-80	Susceptible	S
7	<80%	Highly Susceptible	HS

Table.2 **Chemical with their active ingredients used against bacterial blight of cotton**

Sr#	**Common Name**	**Active ingredient**	**Concentration**	**Company**
1	Flare	Streptomycin sulphate	72% w/w	Kanzo Ag (Pak.)
2	Mancozeb	Ethylene Bisdithiocarbamate	88.23% w/w	Dow Agro Sciences (Pak.)
3	Copper oxychloride	Copper oxychloride	850g/kg	Agri Star (Pak.)
4	Agrimicin	Streptomycin sulphate	75ml/L	Nufarm (Pak.)
5	Plant protector	Benzoic acid	80% w/w	Top Farmers (Pak.)

Table.3 **Plants extracts used for their antibacterial potential against bacterial blight of cotton**

Sr. #	Common Name	Botanical Name	Active ingredients	Plant Part used	Authority
1	Sohanjna	*M. oleifera*	Saponins (Abalaka *et al.*, 2012)	Leaves	Lamarck
2	Neem	*A.indica*	Nimbine (Terpenoids) (Lokanadhan *et al.*,2012)	Leaves	A. Jussieu
3	Datura	*D. alba*	Scopolamine (Okwu and Igara, 2009)	Leaves	C. Linnaeus
4	Tobacco	*N.tabacum*	Nicotine (Bakht *et al.*,2012)	Leaves	C. Linnaeus
5	Turmeric	*C. longa*	Curcumin (Moghadamtousi *et al*., 2014)	Roots	C. Linnaeus

Table. 4 **Ingredients of Nutrient Agar Medium (Curran *et al*., 1937)**

Sr. #	**Name of Ingredients**	**Amount**
1	Beef extract	3.0 g
2	Glucose	2.5 g
3	Peptone	5.0 g
4	Agar agar	15 g
5	Distilled water	1 litre

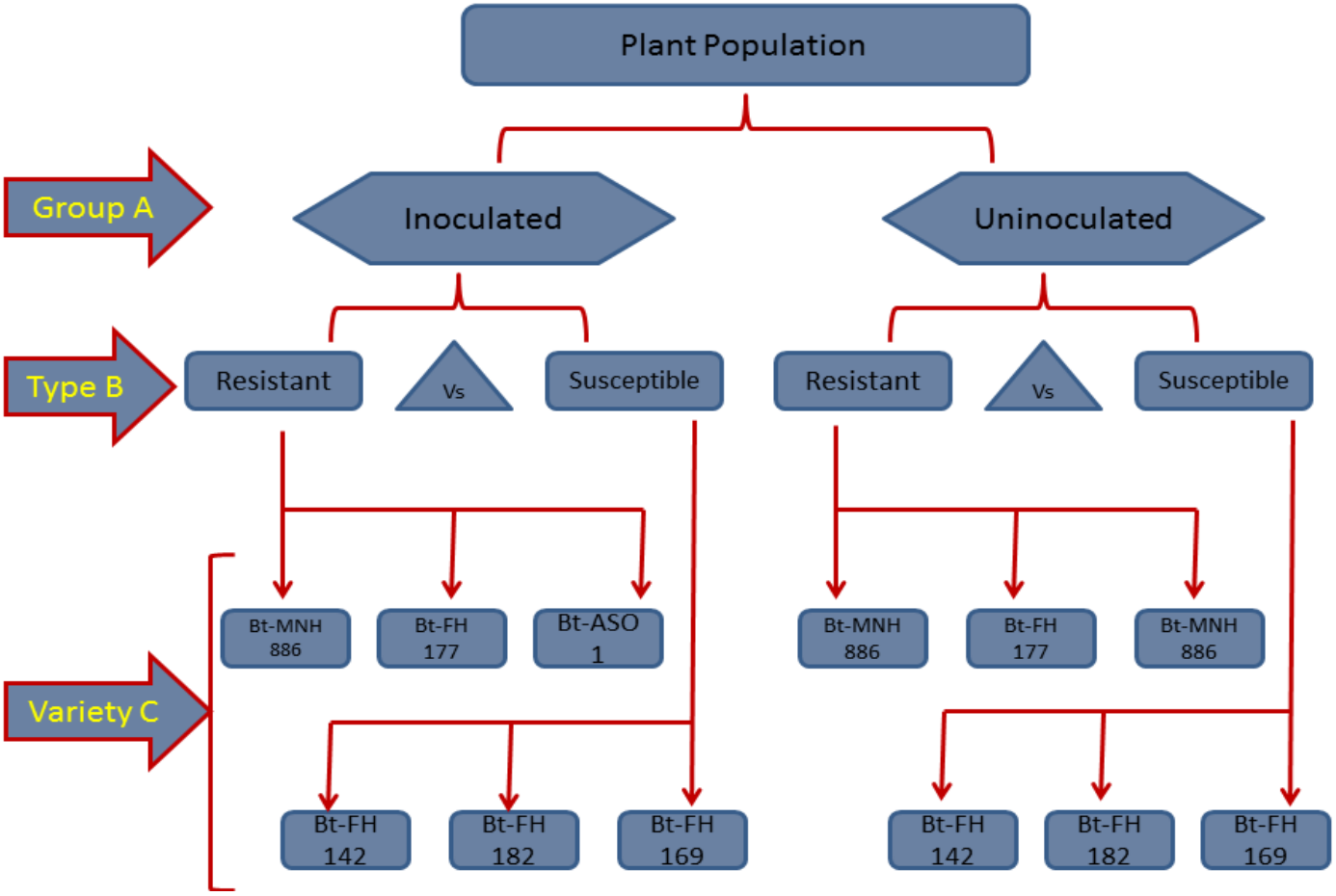

Fig.1 **Nested structured design for changes in ionic contents and biochemical compounds of cotton leaves due to bacterial blight**

CHAPTER # 4 RESULTS

4.1 Evaluation of cotton germplasm against bacterial blight under field conditions for two years

Thirty varieties were evaluated against bacterial blight of cotton for two years under field conditions. During, 2013 eighteen varieties expressed moderately resistant response i.e. Non Bt-PB-896 (22.30), Bt-CM 615(22.60), Bt-IR 901(23.50), Non Bt-BH 160(24.50), Bt-CRS 2007(25.30), Bt-VH 329(28.40), Bt-CM 616(30.40), Non Bt-Redacola (33.50), Bt- KZ 189 (35.00), Non Bt-NIAB 111(36.40), Bt-IUB 222 (39.30), Non Bt-Sindh 1(40.50), Bt-ASO 1(41.30), Non Bt-MNH554 (42.50), Bt-FH 183 (43.30), Bt-MNH 886 (43.40), Bt-53 (44.00) and Bt-FH 177 with 49% disease incidence (rating.4). Six varieties (Bt-FH 143, Bt-Ali Akbar 802, Non Bt-CIM 573, Bt-NS 131, Non Bt-CM 82 and Bt-NIBGI 2) exhibited moderately susceptible response with 52.50, 52.50, 55.60, 60.60, 62.40 and 66.30% disease incidence with rating 5. Four varieties (Bt-FH 142, Bt-FH 182, Bt-4243 and Bt-FH 169) showed susceptible response with 72.70, 75.60, 76.70 and 78.70% disease incidence respectively (rating 6) while Non Bt-Shahbaz and Non Bt- CRIS 134 expressed highly susceptible response with 81.60 and 83.60% disease incidence with rating 7 (Table.5 & fig.2).

During 2014, four varieties (Bt-4243, Bt-FH 169, Bt-FH 182and Bt-FH 142) showed susceptible response with 71.70, 75.70, 77.70 and 79.20 percent disease incidence (rating 6) while Bt-Ali Akbar 802 (58.50), Non Bt-CIM 573 (61.60), BT-NS 131(62.50), Bt-NIBGI 2 (63.50), Non Bt-CM 82 (67.60) and Bt-FH 143expressed moderately susceptible response with 68.70% disease incidence (rating 5) and Non Bt-Shahbaz and Non Bt-CRIS 134showed highly susceptible response with 82.40 and 85.60 percent disease incidence (rating 7). Eighteen varieties (Bt-MNH 886, Bt-FH 177, Non Bt-Sindh 1, Bt-FH 183,Bt-53, IUB 222, Bt-CM 616, Non Bt-MNH 554, Bt-ASO 1, Bt-CRS 2007, Non Bt-NIAB 111, Non Bt-BH 160, Non Bt-PB 896, Bt-KZ 189, Bt-CM 615, Bt-IR 901, Bt-VH 329and Non Bt-Redacola expressed moderately resistant response with 48.50, 46.00, 43.30, 42.30, 41.60, 40.60, 39.70, 37.70, 36.70, 34.70, 33.00, 30.70, 29.20, 28.20, 27.90, 26.80, 23.60 and 22.60% disease incidence with rating 4.(Table 6 & fig.3).

Table 5. **Response of cotton varieties against bacterial blight under field conditions during 2013.**

Sr.#	Varieties	Disease rating	Disease incidence (%)	Response
1	Non Bt-PB-896	4	22.30c	MR
2	BT-CM 615	4	22.60b	MR
3	BT-IR 901	4	23.50a	MR
4	Non Bt-BH 160	4	24.50Z	MR
5	Bt-CRS 2007	4	25.30Y	MR
6	Bt-VH 329	4	28.40X	MR
7	Bt-CM 616	4	30.40W	MR
8	Non Bt-Redacola	4	33.50V	MR
9	Bt- KZ 189	4	35.00U	MR
10	Non Bt-NIAB 111	4	36.40T	MR
11	Bt-IUB 222	4	39.30S	MR
12	Non Bt-Sindh 1	4	40.50R	MR
13	Bt-ASO 1	4	41.30Q	MR
14	Non Bt-MNH554	4	42.50P	MR
15	Bt-FH 183	4	43.30O	MR
16	Bt-MNH 886	4	43.40N	MR
17	Bt-53	4	44.00M	MR
18	Bt-FH 177	4	49.00L	MR
19	Bt-FH 143	5	52.50K	MS
20	Bt-Ali Akbar 802	5	52.50K	MS
21	Non Bt-CIM 573	5	55.60J	MS
22	Bt-NS 131	5	60.60I	MS
23	Non Bt-CM 82	5	62.40H	MS
24	Bt-NIBGI 2	5	66.30G	MS
25	Bt-FH 142	6	72.70F	S
26	Bt-FH 182	6	75.60E	S
27	Bt-4243	6	76.70D	S
28	Bt-FH 169	6	78.70C	S
29	Non Bt-Shahbaz	7	81.60B	HS
30	Non Bt-CRIS 134	7	83.60A	HS

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$)

MR = Moderately resistant MS = Moderately susceptible
S = Susceptible HS = Highly susceptible

Table.6 **Response of cotton varieties against bacterial blight under field conditions during 2014.**

Sr.#	Varieties/ Lines	Disease rating	Disease incidence (%)	Response
1	Non Bt-Redacola	4	22.60d	MR
2	Bt-VH 329	4	23.60c	MR
3	Bt-IR 901	4	26.80b	MR
4	Bt-CM 615	4	27.90a	MR
5	Bt- KZ 189	4	28.20Z	MR
6	Non Bt-PB-896	4	29.20Y	MR
7	Non Bt-BH 160	4	30.70X	MR
8	Non Bt-NIAB 111	4	33.00W	MR
9	Bt-CRS 2007	4	34.70V	MR
10	Bt-ASO 1	4	36.70U	MR
11	Non Bt-MNH 554	4	37.70T	MR
12	Bt-CM 616	4	39.70S	MR
13	Bt-IUB 222	4	40.60R	MR
14	Bt-53	4	41.60Q	MR
15	Bt-FH 183	4	42.30P	MR
16	Non Bt-Sindh 1	4	43.30O	MR
17	Bt-FH 177	4	46.00N	MR
18	Bt-MNH 886	4	48.50M	MR
19	Bt-Ali Akbar 802	5	58.50L	MS
20	Non Bt-CIM 573	5	61.60K	MS
21	Bt-NS 131	5	62.50J	MS
22	Bt-NIBGI 2	5	63.50I	MS
23	Non Bt-CM 82	5	67.60H	MS
24	Bt-FH 143	5	68.70G	MS
25	Bt-4243	6	71.70F	S
26	Bt-FH 169	6	75.70E	S
27	Bt-FH 182	6	77.70D	S
28	Bt-FH 142	6	79.20C	S
29	Non Bt-Shahbaz	7	82.40B	HS
30	Non Bt-CRIS 134	7	85.60A	HS

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$)

MR = Moderately resistant MS = Moderately susceptible
S = Susceptible HS = Highly susceptible

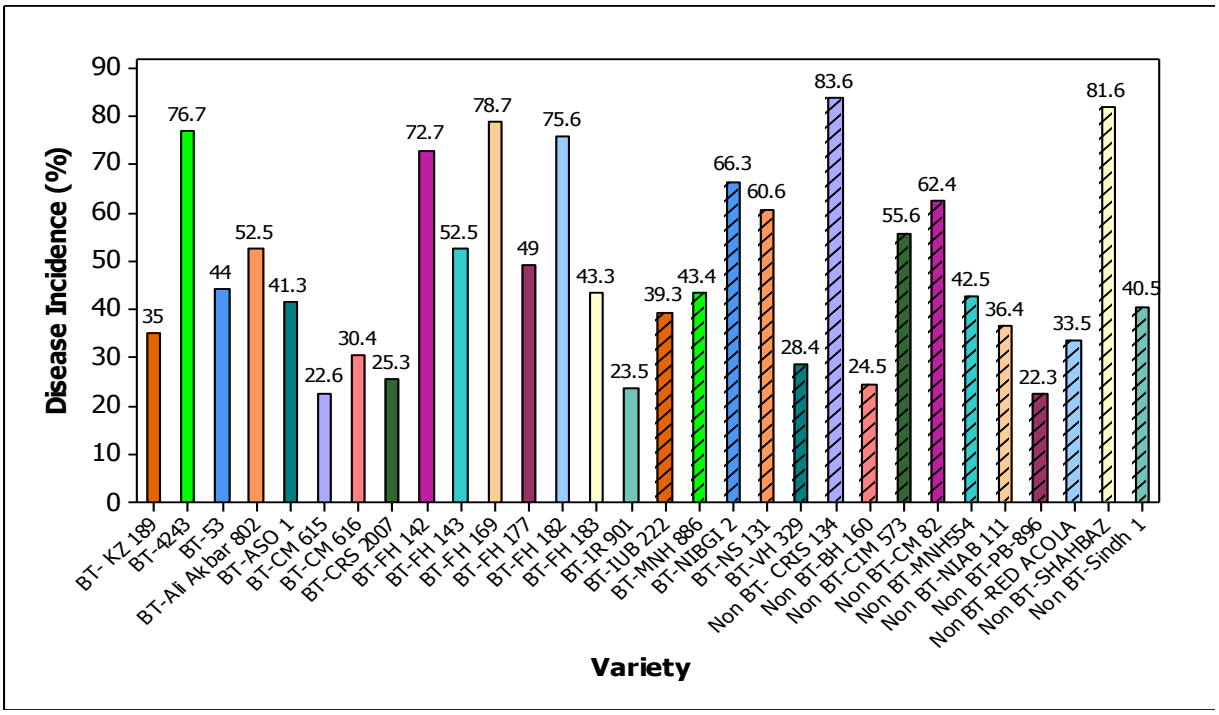

Fig.2 **Response of cotton germplasm against bacterial blight of cotton under field conditions during 2013**

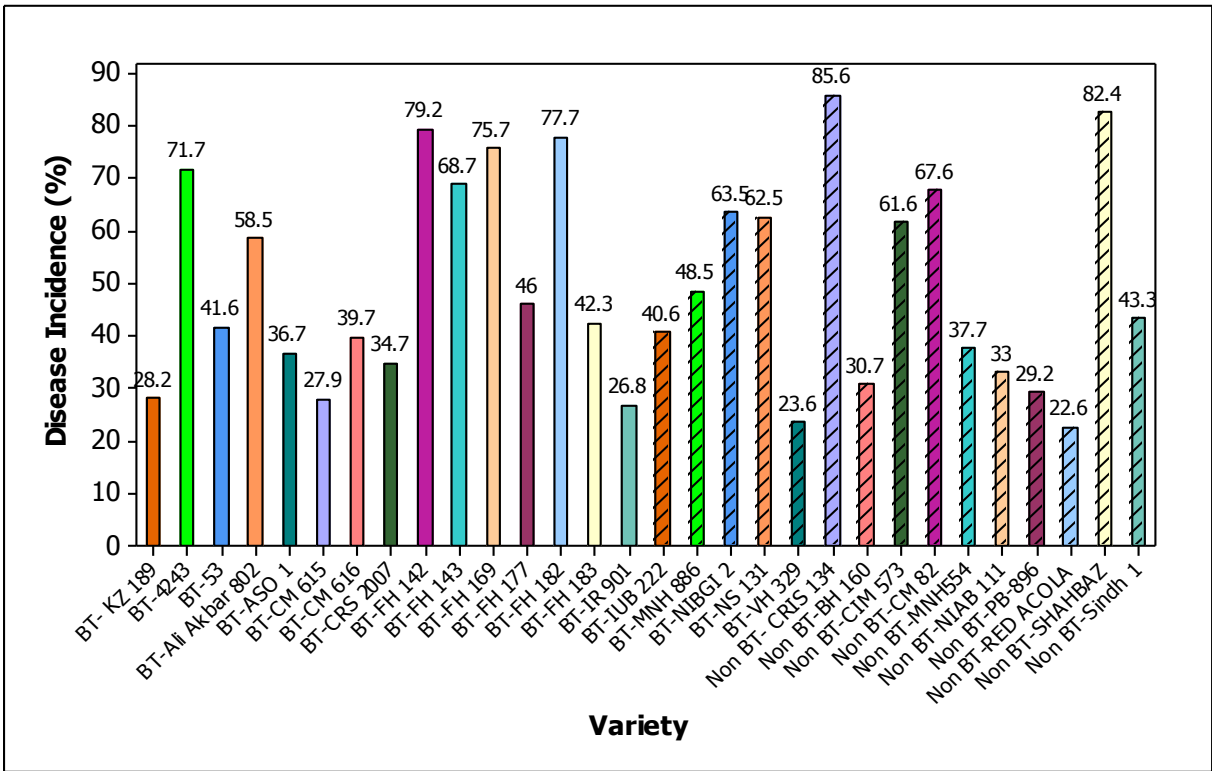

Fig.3 **Response of cotton germplasm against bacterial blight under field conditions during 2014**

4.2 **Determination of mineral and biochemical/physiological contents from leaves of inoculated and un-inoculated cotton plants**

Samples from inoculated and un-inoculated type from both moderately resistant and susceptible groups of six varieties were collected and dried with the help of oven, grind to fine powder and analyzed by following standard procedures for the determination of ionic contents of N, P, K, Ca, Mg, Fe, Zn, Cu and physiological and biochemical compounds like total soluble proteins, total soluble sugars, total chlorophyll contents and phenolic contents.

4.2.1 **Determination of nitrogen (percent dry weight) from leaves of inoculated and un-inoculated cotton plants**

Significant variation was observed between un-inoculated (averaging 2.78%) and inoculated group of plants (averaging 1.79%) indicating that N contents seem to affect metabolic activities as a result of disease stress as shown in Fig 4. This component counted for 94.30% of the total variance. Considerable variation was accounted for the plants marked as resistant and susceptible. The value of 2.40% was observed across the resistant and 2.17% across the susceptible type, significant at *P*>0.05 (Table 8). This component counted for 4.93% of the total variance. Varieties exhibited their natural tendencies with respect to N concentration explaining 0.50% of the total variance (Table 7). Maximum concentration was displayed by variety "Bt-ASO1" to the extent of 2.46% and minimum by Bt-FH 142 to the tune of 2.11% respectively.

Table.7 **Nested random effect's analysis of variance for nitrogen of inoculated and un-inoculated leaves of cotton plants**

SOV	DF	SS	MS	Pr>F	F value	Variance Component	% of total Variance
Group (A)	1	26.235	26.235	0.025*	37.906	0.473	94.30
Type (B)	2	1.384	0.692	0.022*	28.751	0.025	4.93
Variety (C)	8	0.192	0.024	0.000*	18.604	0.003	0.50
Error	96	0.124	0.001			0.001	0.26
Total	107	27.936				0.502	

* = Significant at *P*>0.05

Table.8 **Amount of nitrogen (%) in group (inoculated and un-inoculated), reaction type (resistant and susceptible), and varieties in cotton leaves**

Varieties (C)	**Bt-MNH 886**		**Bt-FH 177**		**Bt-ASO 1**		**Bt-FH 142**		**Bt-FH 182**		**Bt-FH 169**	
Type (B)	**Resistant**						**Susceptible**					
Group (A)	**Inoc.**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**
Amount of N in (C)	1.86	2.85	1.90	2.92	1.94	2.98	1.63	2.60	1.69	2.65	1.74	2.73
Av. amount of N in (C)	2.35		2.41		2.46		2.11		2.17		2.23	
Av. amount of N in (B)	**Resistant = 2.40**						**Susceptible = 2.17**					
Av. amount of N in (A)	**Inoculated = 1.79**						**Un-Inoculated = 2.78**					

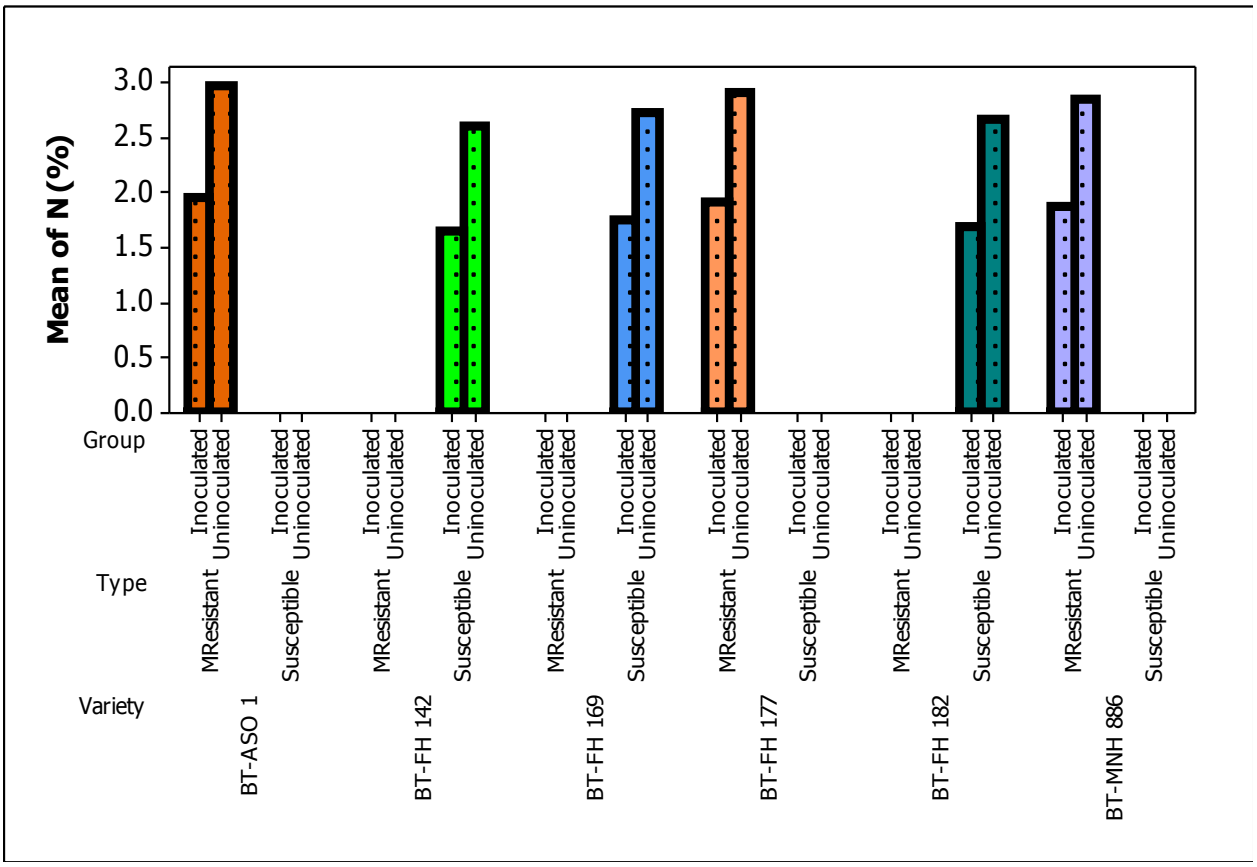

Fig.4 **Concentration of nitrogen (%) in resistant and susceptible cotton varieties of un- inoculated and inoculated plants**

4.2.2 **Determination of Phosphorus (percent dry weight) from leaves of inoculated and un-inoculated cotton plants**

Considerable variation was accounted for the plants marked as resistant and susceptible. The value of 0.19% was observed across the resistant type and 0.16% across the susceptible type, significant at *P*>0.05 (Table 10). This component counted for 8.51% of the total variance. Significant variation was observed between un-inoculated (0.22%) and inoculated group of plants (0.14%) indicating that P contents seem to affect metabolic activities (Fig. 5) This component counted for 90.48% of the total variance. Varieties exhibited their natural tendencies with respect to P concentration explaining 0.95% of the total variance (Table 9). Maximum concentration was displayed by variety named "Bt-ASO1" to the degree of 0.21% and minimum by Bt-FH 142 to the extent of 0.15%.

Table.9 **Nested random effect's analysis of variance for phosphorus of inoculated and un-inoculated leaves of cotton plants**

SOV	DF	SS	MS	Pr>F	F value	Variance Component	% of total Variance
Group (A)	1	0.353	0.353	0.044*	21.497	0.006	90.48
Type (B)	2	0.033	0.016	0.000*	27.632	0.001	8.51
Variety (C)	8	0.005	0.007	0.000*	137.250	0.000	0.95
Error	96	0.0004	0.000			0.000	0.06
Total	107	0.391				0.007	

* = Significant at *P*>0.05

Table. 10 **Amount of phosphorus (%) in group (inoculated and un-inoculated), reaction type (resistant and susceptible), and varieties in cotton leaves**

Varieties (C)	**Bt-MNH 886**		**Bt-FH 177**		**Bt-ASO 1**		**Bt-FH 142**		**Bt-FH 182**		**Bt-FH 169**	
Type (B)	**Resistant**						**Susceptible**					
Group (A)	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**
Amount of P in (C)	0.125	0.250	0.133	0.269	0.141	0.271	0.102	0.206	0.111	0.214	0.223	0.119
Av. amount of P in (C)	0.18		0.20		0.21		0.15		0.16		0.17	
Av. amount of P in (B)	**Resistant = 0.19**				**Susceptible = 0.16**							
Av. amount of P in (A)	**Inoculated = 0.14**				**Un-Inoculated = 0.22**							

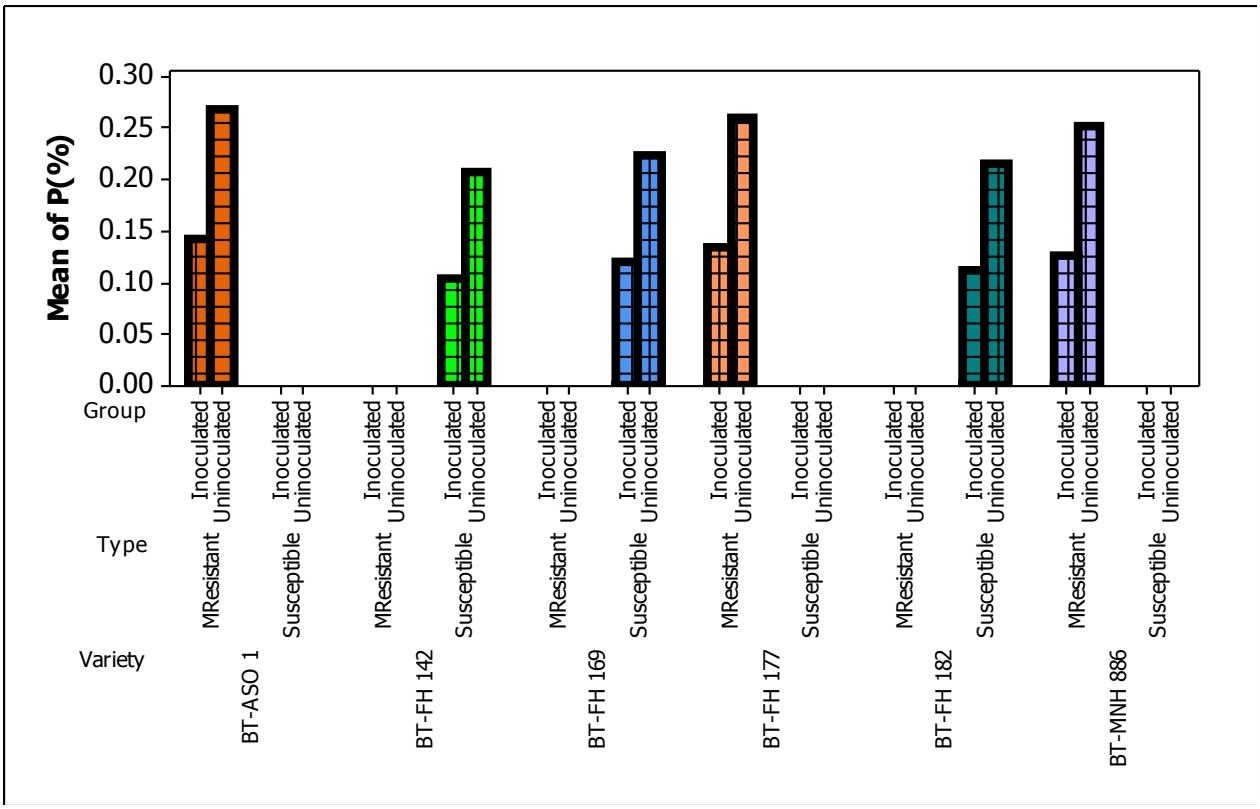

Fig.5 **Concentration of phosphorus (%) in resistant and susceptible cotton varieties of un-inoculated and inoculated plants**

4.2.3 **Determination of potassium (ppm) from leaves of inoculated and un-inoculated cotton plants**

Varieties exhibited their natural abilities with respect to K concentration explaining 0.11% of the total variance (Table 12). Maximum concentration was displayed by variety named "Bt-ASO 1" to the extent of 416 ppm and minimum by Bt-FH 142 to the tune of 372.5 ppm. Significant variation was observed between un-inoculated (averaging to 512.1 ppm) and inoculated plants group (averaging to 276.6 ppm) indicating that K content affect the development of thicker outer walls in epidermal cells and influenced plant metabolism as a result of disease pressure as shown in (Fig. 6). This component counted for 98.19% of the total variance. Significant variation was accounted for the plants marked as resistant and susceptible. The value of 408.3 ppm of K was observed in resistant type and 378.8 ppm in susceptible type, significant at $P>0.05$ (Table 11). This component counted for 1.63% of the total variance.

Table. 11 **Nested random effect's analysis of variance for potassium of inoculated and un-inoculated leaves of cotton plants**

SOV	DF	SS	MS	Pr>F	F value	Variance Component	% of total Variance
Group (A)	1	1.498	1.498	0.008*	118.859	27509.796	98.19
Type (B)	2	25208.666	12604.333	0.000*	41.394	455.549	1.63
Variety (C)	8	2436.000	304.500	0.008*	16.150	31.738	0.11
Error	96	1810.000	18.854			18.854	0.07
Total	107	1.527				28015.938	

* = Significant at *P*>0.05

Table.12 **Amount of potassium (ppm) in group (inoculated and un-inoculated), reaction type (resistant and susceptible), and varieties in cotton leaves**

Varieties (C)	**Bt-MNH 886**		**Bt-FH 177**		**Bt-ASO 1**		**Bt-FH 142**		**Bt-FH 182**		**Bt-FH 169**	
Type (B)	**Resistant**						**Susceptible**					
Group (A)	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**
Amount of K in (C)	286	521	292	527	298	534	256	491	262	497	266	503
Av. amount of K in (C)	403.5		409.5		416		372.5		379.5		384.5	
Av. amount of K in (B)	**Resistant = 408.3**						**Susceptible = 378.8**					
Av. amount of K in (A)	**Inoculated = 276.6**						**Un-Inoculated = 512.1**					

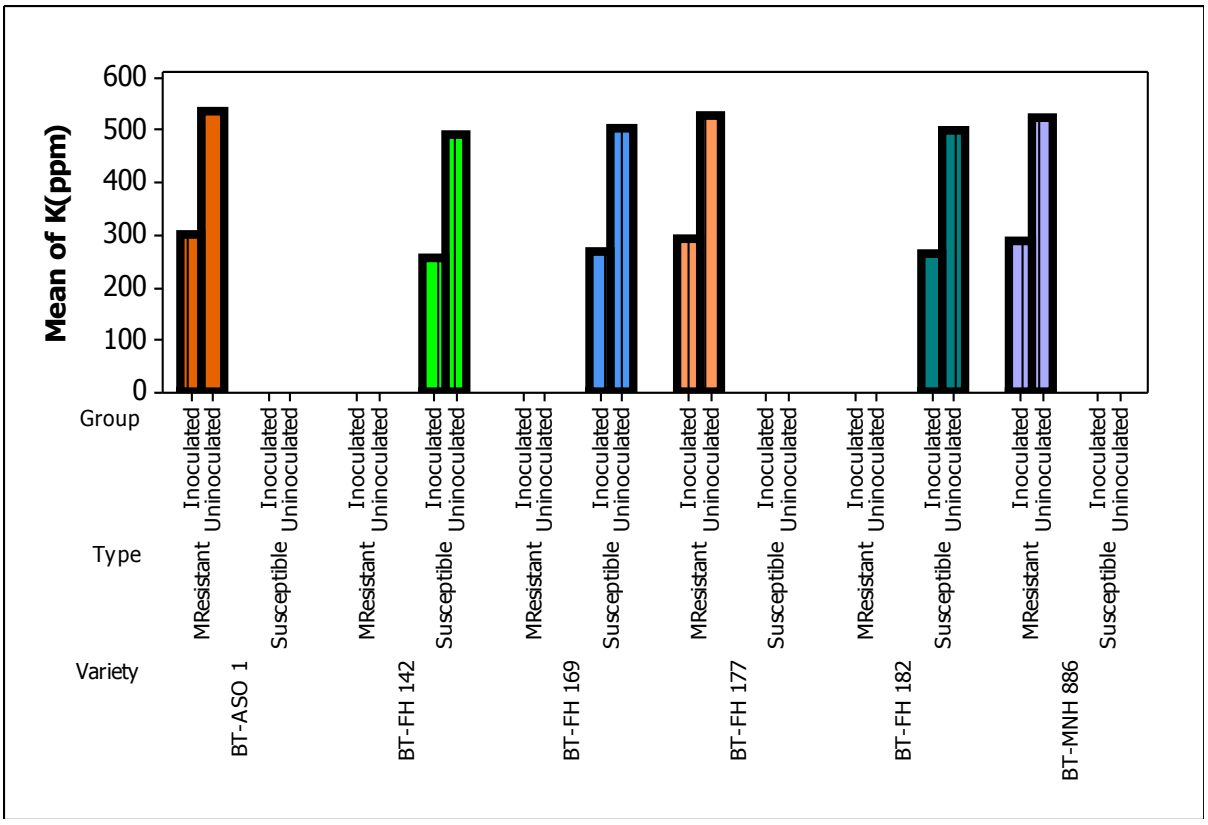

Fig.6 **Concentration of potassium (ppm) in resistant and susceptible cotton varieties of un-inoculated and inoculated plants**

4.2.4 **Determination of Calcium (ppm) from leaves of inoculated and un-inoculated cotton plants**

Varieties exhibited their natural abilities with respect to Ca concentration explaining 0.15% of the total variance (Table 14). Maximum concentration was displayed by variety named "Bt-ASO 1" to the extent of 325.5 ppm and minimum by "Bt-FH 142" to the tune of 265 ppm. Significant variation was observed between un-inoculated (averaging to 412.3 ppm) and inoculated plants group (averaging to 191 ppm) indicating that Ca content seems to affect the growth of new tissues as a result of disease pressure as shown in (Fig.7). This component counted for 95.62% of the total variance. Significant variation was accounted for the plants marked as resistant and susceptible. The value of 310.2 ppm was observed in resistant type and 270.2 ppm across the susceptible type and was significant at $P>0.05$ (Table 13). This counted for 4.14% of the total variance.

Table.13 **Nested random effect's analysis of variance for calcium of inoculated and un-inoculated leaves of cotton plants**

SOV	DF	SS	MS	Pr>F	F value	Variance Component	% of total Variance
Group (A)	1	1.1077	1.108	0.021*	46.544	20073.620	95.62
Type (B)	2	47601.666	23800.833	0.000*	77.317	870.111	4.14
Variety (C)	8	2462.667	307.833	0.000*	19.115	32.414	0.15
Error	96	1546.000	16.104			16.104	0.08
Total	107	1.159				20992.250	

* = Significant at *P*>0.05

Table.14 **Amount of calcium (ppm) in group (inoculated and un-inoculated), reaction type (resistant and susceptible), and varieties in cotton leaves**

Varieties (C)	**Bt-MNH 886**		**Bt-FH 177**		**Bt-ASO 1**		**Bt-FH 142**		**Bt-FH 182**		**Bt-FH 169**	
Type (B)	**Resistant**						**Susceptible**					
Group (A)	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**
Amount of Ca in (C)	205	410	210	416	217	534	165	365	171	371	178	378
Av. amount of Ca in (C)	307.5		313		325.5		265		271		274.5	
Av. amount of Ca in (B)	**Resistant = 310.2**						**Susceptible = 270.2**					
Av. amount of Ca in (A)	**Inoculated = 191**						**Un-Inoculated = 412.3**					

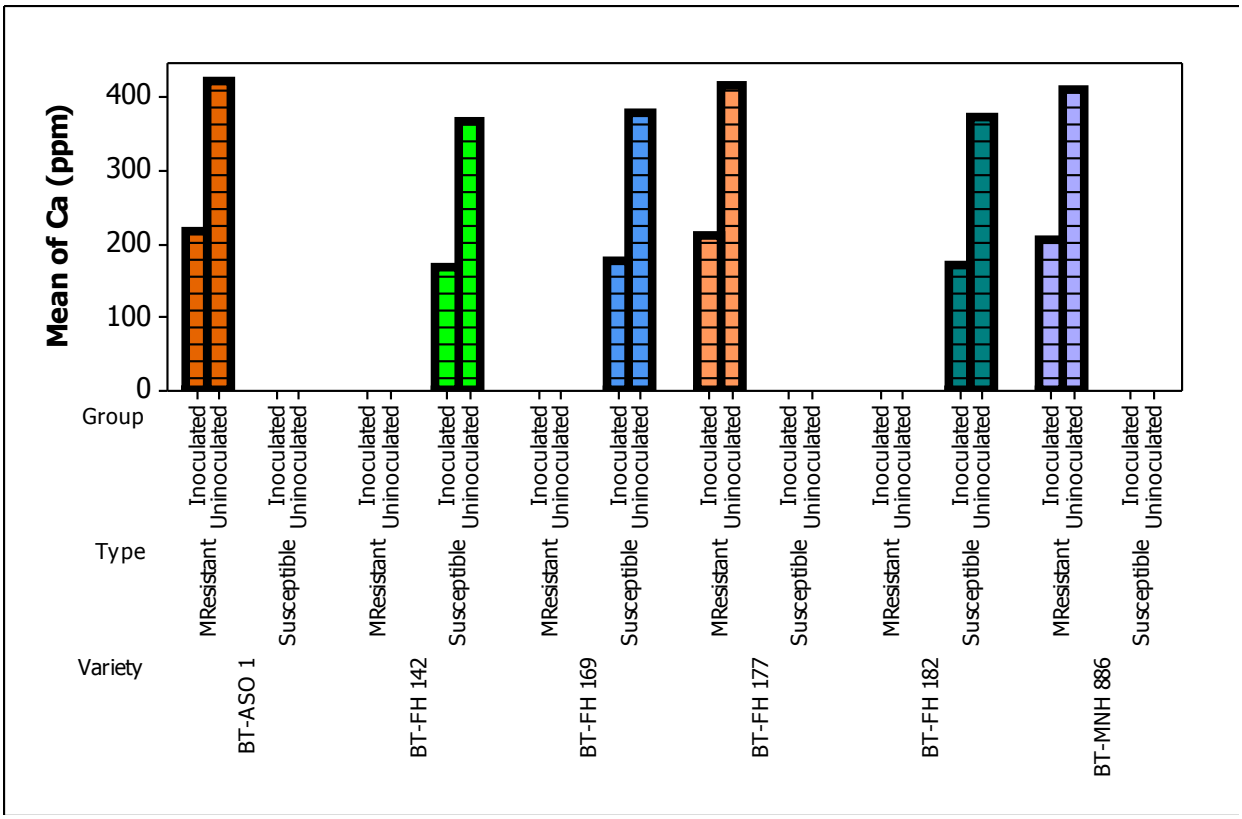

Fig .7 **Concentration of calcium (ppm) in resistant and susceptible cotton varieties of un-inoculated and inoculated plants**

4.2.5 **Determination of Magnesium (ppm) from leaves of inoculated and un-inoculated cotton plants**

In magnesium, significant variation was observed between un-inoculated (25.3 ppm) and inoculated plants group (13.3 ppm) representing that Mg content seems to affect the photosynthesis and other metabolic activities after disease appearance as shown in (Fig. 8). This component counted for 75.01% of the total variance. Substantial variation was accounted for the plants marked as resistant and susceptible. The value of 21.1 ppm was observed across the resistant type and 14.6 ppm across the susceptible type and was significant at $P>0.05$ (Table 16). This component counted for 23.55% of the total variance. Varieties reveal their natural ability with respect to Mg concentration explaining 0.65% of the total variance (Table 15). Variety "Bt-ASO1 exhibited maximum concentration to the extent of 23 ppm and minimum by Bt-FH 142 to the tune of 15.5 ppm.

Table.15 **Nested random effect's analysis of variance for magnesium of inoculated and un-inoculated leaves of cotton plants**

SOV	DF	SS	MS	Pr>F	F value	Variance Component	% of total Variance
Group (A)	1	43.141	5.393	0.000*	8.364	61.075	75.01
Type (B)	2	1046.054	523.027	0.000*	96.990	19.172	23.55
Variety (C)	8	3821.090	3821.090	0.114^{ns}	7.306	0.528	0.65
Error	96	61.897	0.645			0.645	0.79
Total	107	4972.183				81.419	

* = Significant at *P*>0.05

Table.16 Amount of magnesium (ppm) in group (inoculated and un-inoculated), reaction type (resistant and susceptible), and varieties in cotton leaves

Varieties (C)	**Bt-MNH 886**		**Bt-FH 177**		**Bt-ASO 1**		**Bt-FH 142**		**Bt-FH 182**		**Bt-FH 169**	
Type (B)	**Resistant**						**Susceptible**					
Group (A)	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**
Amount of Mg in (C)	14.97	28.16	15.54	29.07	16.02	30.02	10.46	20.63	11.25	21.60	11.81	22.53
Av. amount of Mg in (C)	21.5		22.3		23		15.5		16.4		17.2	
Av. amount of Mg in (B)	**Resistant = 21.1**				**Susceptible = 14.6**							
Av. amount of Mg in (A)	**Inoculated = 13.3**				**Un-Inoculated = 25.3**							

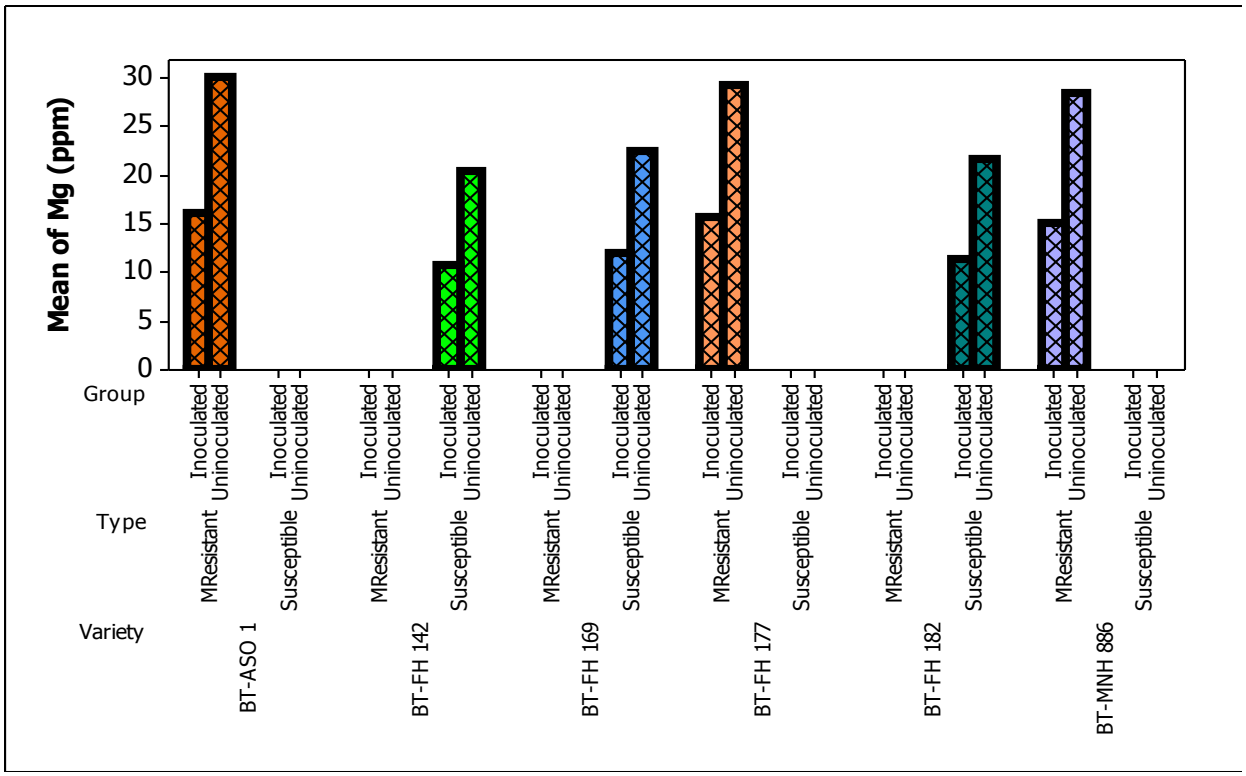

Fig.8 **Concentration of magnesium (ppm) in resistant and susceptible cotton varieties of un-inoculated and inoculated plants**

4.2.6 **Determination of copper (ppm) from leaves of inoculated and un-inoculated cotton plants**

Varieties exhibited their natural tendency with respect to Cu concentration explaining 0.56% of the total variance (Table 17). Maximum concentration was displayed by variety "Bt-ASO 1" to the level of 3 ppm and minimum by Bt-FH 142 to the amount of 2.3 ppm. Significant variation was observed between un-inoculated and inoculated plants group (Averaging 2.87 ppm to 1.85 ppm) indicating that Cu content seems to affect the chlorophyll formation and protein synthesis as a result of disease pressure as shown in (Fig. 9). This component accounted for 91.40% of the total variance. While considerable variation was accounted for the plants marked as resistant and susceptible. The value of 2.9 ppm was observed across the resistant type and 2.4 ppm across the susceptible type, significant at $P>0.05$ (Table.18). This component counted for 7.97% of the total variance.

Table.17 **Nested random effect's analysis of variance for copper of inoculated and un-inoculated leaves of cotton plants**

SOV	DF	SS	MS	Pr>F	F value	Variance Component	% of total Variance
Group (A)	1	70.374	70.374	0.040*	23.392	1.248	91.40
Type (B)	2	6.017	3.008	0.000*	43.318	0.109	7.97
Variety (C)	8	0.556	0.069	0.000*	69.595	0.008	0.56
Error	96	0.096	0.001			0.001	0.07
Total	107	77.042				1.365	

* = Significant at *P*>0.05

Table.18 **Amount of copper (ppm) in group (inoculated and un-inoculated), reaction type (resistant and susceptible) and varieties in cotton leaves**

Varieties (C)	**Bt-MNH 886**		**Bt-FH 177**		**Bt-ASO 1**		**Bt-FH 142**		**Bt-FH 182**		**Bt-FH 169**	
Type (B)	**Resistant**						**Susceptible**					
Group (A)	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**
Amount of Cu in (C)	1.98	3.63	2.08	3.73	2.17	3.81	1.53	3.14	1.63	3.21	1.73	3.29
Av. amount of Cu in (C)	2.8		2.9		3		2.3		2.4		2.5	
Av. amount of Cu in (B)	**Resistant = 2.9**				**Susceptible = 2.4**							
Av. amount of Cu in (A)	**Inoculated = 1.85**				**Un-Inoculated = 2.87**							

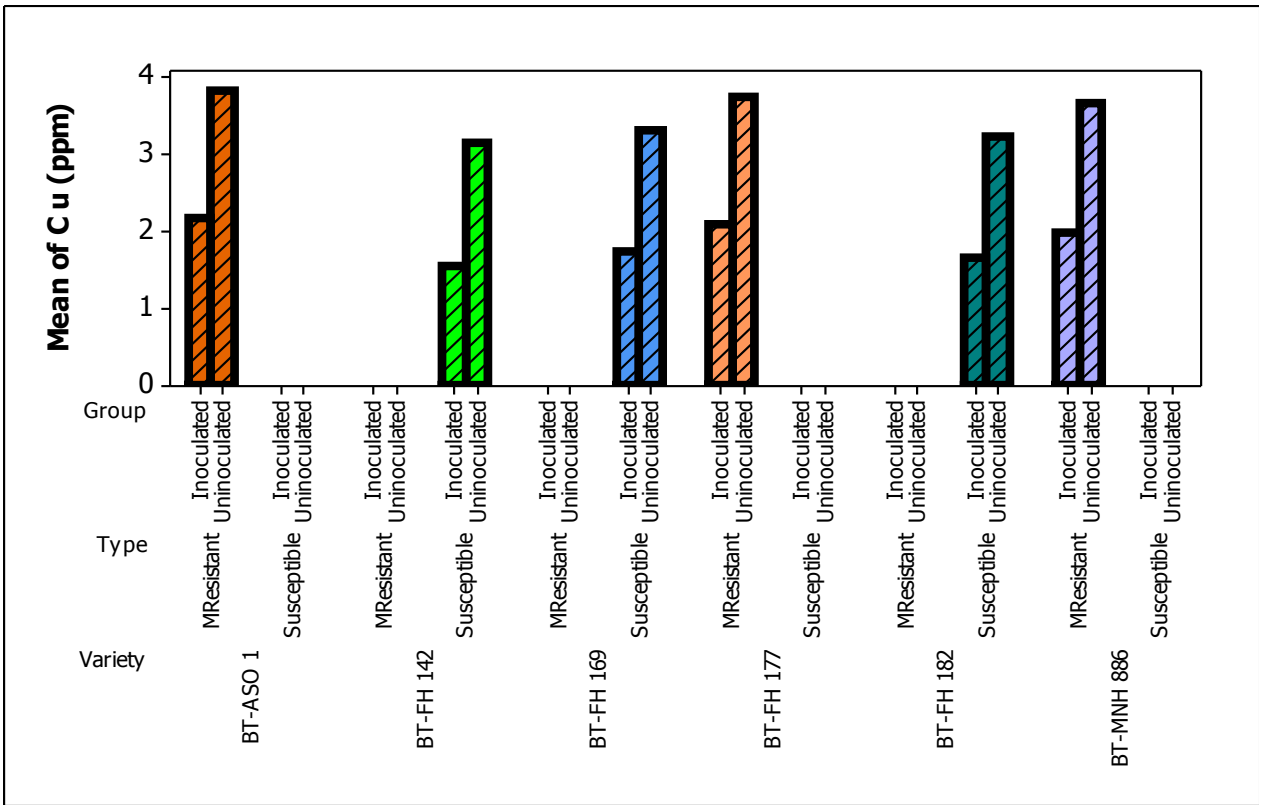

Fig.9 **Concentration of copper (ppm) in resistant and susceptible cotton varieties of un-inoculated and inoculated plants**

4.2.7 **Determination of zinc (ppm) from leaves of inoculated and un-inoculated cotton plants**

Significant variation was observed between un-inoculated (averaging to 1.91 ppm) and inoculated plants group (averaging to 1.09 ppm) indicating that Zn content seems to affect the enzyme activities as a result of disease as shown in (Fig. 10). This component counted for 67.58% of the total variance. Substantial variation was accounted for the plants marked as resistant and susceptible. The value of 1.83 ppm was observed across the resistant type and 1.75 ppm across the susceptible type and was significant at $P>0.05$ (Table 20) which counted for 30.70% of the total variance. Varieties exhibited their natural tendencies with respect to Zn concentration explaining 1.37% of the total variance (Table 19). Maximum concentration was displayed by variety named “Bt-ASO 1” to the extent of 1.80 ppm and minimum by Bt-FH 142 to the tune of 1.08 ppm respectively.

Table.19 **Nested random effect's analysis of variance for zinc of inoculated and un-inoculated leaves of cotton plants**

SOV	DF	SS	MS	Pr>F	F value	Variance Component	% of total Variance
Group (A)	1	0.519	0.065	0.000*	36.852	0.007	67.58
Type (B)	2	8.606	4.303	0.000*	66.327	0.157	30.70
Variety (C)	8	22.963	22.963	0.147^{ns}	5.337	0.346	1.37
Error	96	0.169	0.002			0.002	0.34
Total	107	32.257				0.511	

* = Significant at *P*>0.05

Table.20 **Amount of zinc (ppm) in group (inoculated and un-inoculated), reaction type (resistant and susceptible), and varieties in cotton leaves**

Varieties (C)	**Bt-MNH 886**		**Bt-FH 177**		**Bt-ASO 1**		**Bt-FH 142**		**Bt-FH 182**		**Bt-FH 169**	
Type (B)	**Resistant**						**Susceptible**					
Group (A)	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**
Amount of Zn in (C)	1.05	2.14	1.19	2.26	1.24	2.37	0.70	1.47	0.79	1.56	0.85	1.67
Av. amount of Zn in (C)	1.59		1.72		1.80		1.08		1.17		1.26	
Av. amount of Zn in (B)	**Resistant =1.83**				**Susceptible = 1.75**							
Av. amount of Zn in (A)	**Inoculated = 1.09**				**Un-Inoculated = 1.91**							

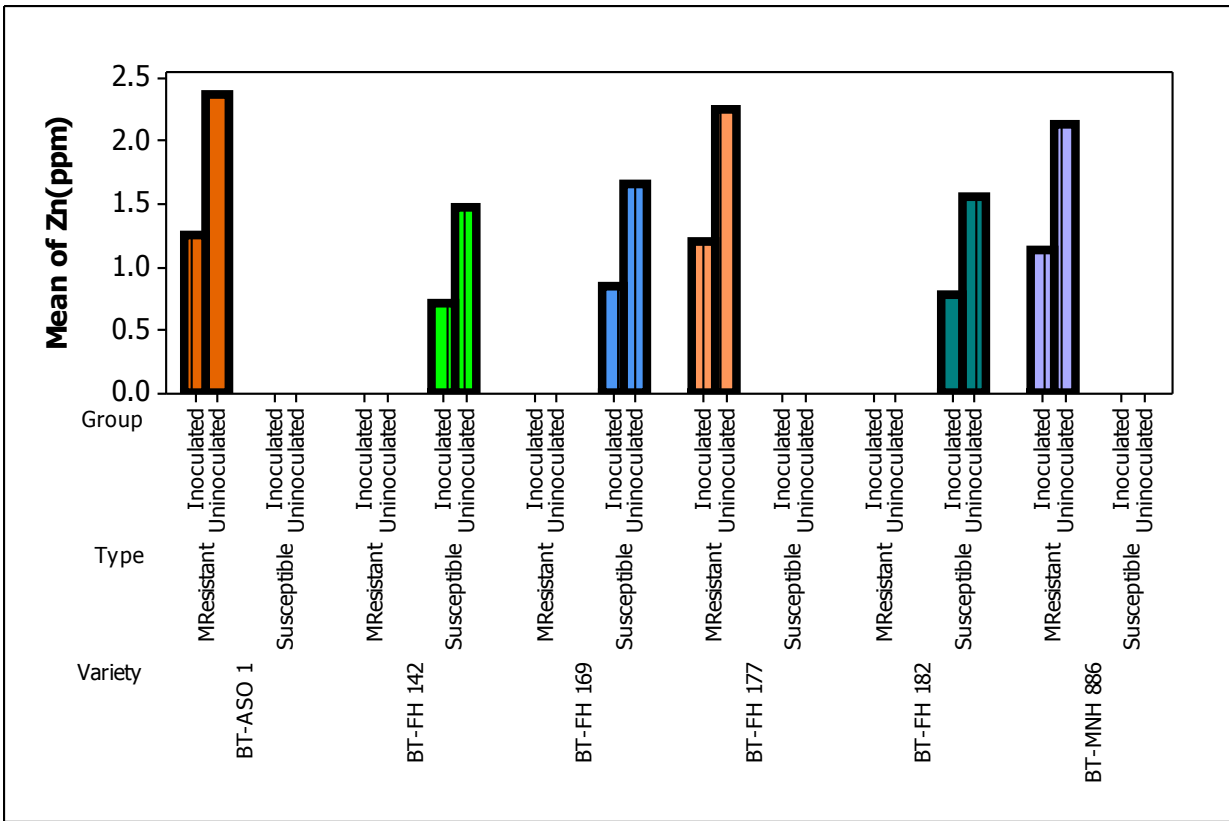

Fig.10 **Concentration of zinc (ppm) in resistant and susceptible cotton varieties of un-inoculated and inoculated plants**

4.2.8 **Determination of Iron (ppm) from leaves of inoculated and un-inoculated cotton plants**

Significant variation was observed for the plants marked as resistant and susceptible. The value of 1.61 ppm was observed across the resistant type and 1.35 ppm across the susceptible type and was significant at *P*>0.05 (Table 22). This component counted for 6.98% of the total variance. Varieties exhibited their natural tendencies with respect to Fe concentration explaining 0.37% of the total variance (Table 21). Maximum concentration was displayed by variety named "Bt-ASO 1" to the extent of 1.65 ppm and minimum by Bt-FH 142 to the tune of 1.30 ppm. Significant variation was also observed between un-inoculated (averaging to 1.96 ppm) and inoculated plants group (averaging to 0.99 ppm) indicating that Fe content seems to affect the chlorophyll synthesis activities as a result of disease stress as shown in (Fig. 11). This component counted for 92.56% of the total variance.

Table.21 **Nested random effect's analysis of variance for iron of inoculated and un-inoculated leaves of cotton plants**

SOV	DF	SS	MS	Pr>F	F value	Variance Component	% of total Variance
Group (A)	1	25.667	25.667	0.035*	27.064	0.458	92.56
Type (B)	2	1.897	0.948	0.000*	55.623	0.034	6.98
Variety (C)	8	0.136	0.017	0.000*	38.604	0.002	0.37
Error	96	0.042	0.0004			0.000	0.09
Total	107	27.742				0.495	

* = Significant at *P*>0.05

Table.22 **Amount of iron (ppm) in group (inoculated and un-inoculated), reaction type (resistant and susceptible), and varieties in cotton leaves**

Varieties (C)	**Bt-MNH 886**		**Bt-FH 177**		**Bt-ASO 1**		**Bt-FH 142**		**Bt-FH 182**		**Bt-FH 169**	
Type (B)	**Resistant**						**Susceptible**					
Group (A)	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**
Amount of Fe in (C)	1.09	2.05	1.13	2.09	1.15	2.15	0.81	1.8	0.86	1.84	0.90	1.88
Av. amount of Fe in (C)	1.57		1.61		1.65		1.30		1.35		1.39	
Av. amount of Fe in (B)	**Resistant = 1.61**				**Susceptible = 1.35**							
Av. amount of Fe in (A)	**Inoculated = 0.99**				**Un-Inoculated = 1.96**							

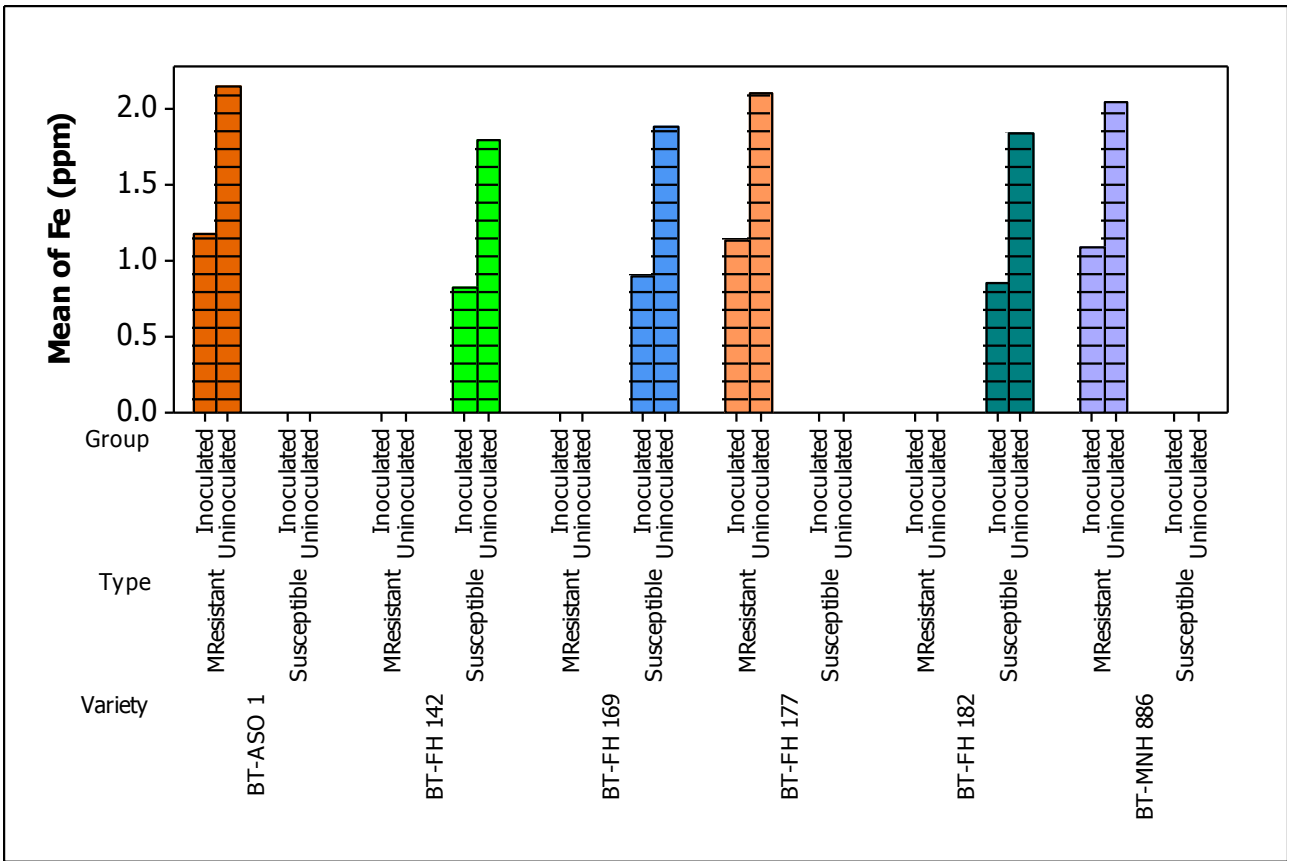

Fig.11 **Concentration of iron (ppm) in resistant and susceptible cotton varieties of un-inoculated and inoculated plants**

4.2.9 **Determination of protein (mg/g) from fresh leaves of inoculated and un-inoculated cotton plants**

Significant variation was observed between un-inoculated (5.52) and inoculated (3.77) mg/g plants group during disease stress which indicating that protein content seems to affect the enzyme activities (as shown in Fig 12) and accounted for 87.56 percent of total variance. Considerable variation was accounted for the plants marked as resistant and susceptible. The value of 4.93 was observed across the resistant type and 4.37 across the susceptible type and was significant at $P>0.05$ (Table 24). This component counted for 6.88% of the total variance. Varieties exhibited their natural tendencies with respect to protein concentration explaining 0.68% of the total variance (Table 23). Maximum concentration was displayed by variety named "Bt-ASO1" to the extent of 5.01 and minimum by Bt-FH 142 to the extent of 4.28 mg/g respectively.

Table.23 **Nested random effect's analysis of variance for protein of inoculated and un-inoculated leaves of cotton plants**

SOV	DF	SS	MS	Pr>F	F value	Variance Component	% of total Variance
Group (A)	1	87.642	87.642	0.038*	25.033	1.558	87.56
Type (B)	2	7.002	3.501	0.001*	17.882	0.122	6.88
Variety (C)	8	1.566	0.196	0.030*	2.253	0.012	0.68
Error	96	8.347	0.087			0.087	4.88
Total	107	104.552				1.780	

* = Significant at *P*>0.05

Table.24 **Amount of protein (mg/g) of fresh leaves in group (inoculated and un-inoculated), reaction type (resistant and susceptible), and varieties in cotton leaves**

Varieties (C)	**Bt-MNH 886**		**Bt-FH 177**		**Bt-ASO 1**		**Bt-FH 142**		**Bt-FH 182**		**Bt-FH 169**	
Type (B)	**Resistant**						**Susceptible**					
Group (A)	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**
Amount of protein in (C)	3.94	5.77	4.01	5.85	4.08	5.94	3.49	5.07	3.57	5.20	3.64	5.30
Av. amount of protein (C)	4.85		4.93		5.01		4.28		4.38		4.47	
Av. amount of protein (B)	**Resistant = 4.93**				**Susceptible = 4.37**							
Av. amount of protein (A)	**Inoculated = 3.77**				**Un-Inoculated = 5.52**							

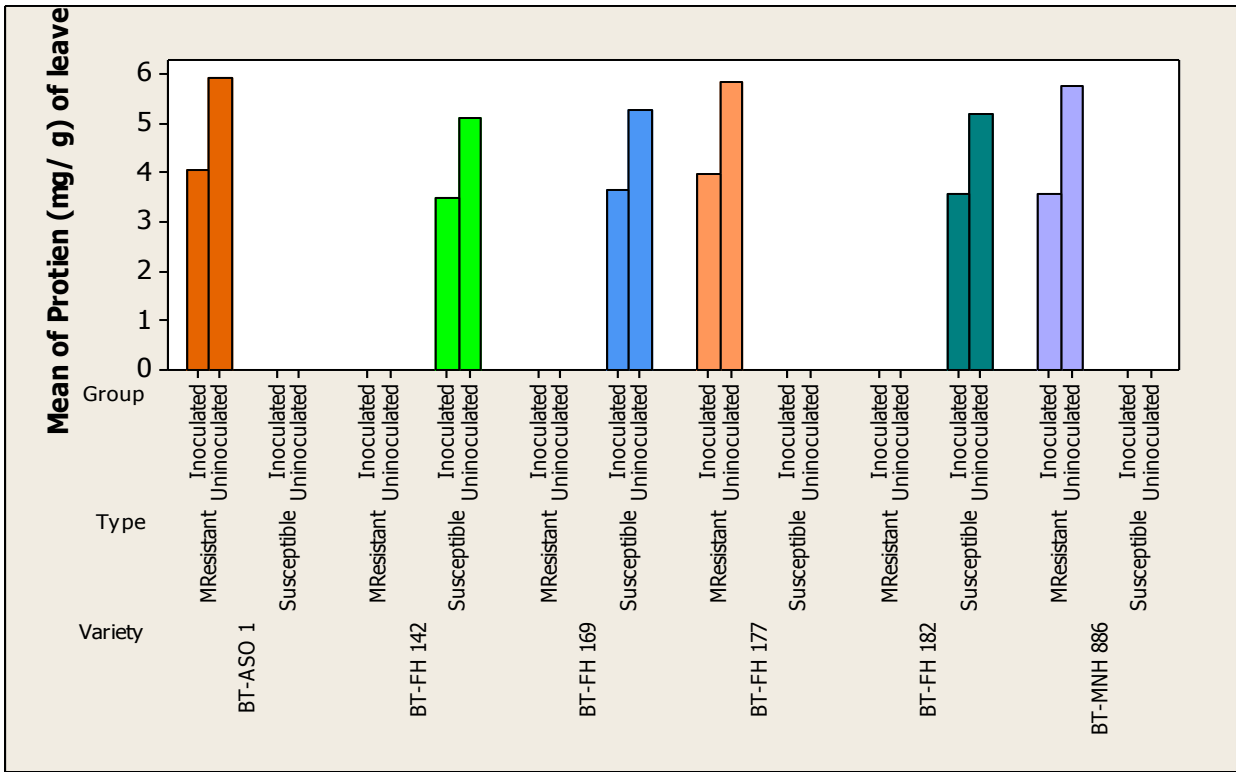

Fig.12 **Concentration of protein (mg/g) of fresh leaves in resistant and susceptible cotton varieties of un-inoculated and inoculated plants**

4.2.10 **Determination of Phenol (mg/g) from fresh leaves of inoculated and un-inoculated cotton plants**

Inoculated and un-inoculated plant group showed significant variation (1.83 and 3.73mg/g) respectively indicating that phenol content seem to affect the growth and defense activities as a result of disease appearance as shown in (Fig.13) and is counted for 95.25% of the total variance. Considerable variation was accounted for the plants marked as resistant and susceptible. The value of 2.98 mg/g was observed across the resistant type and 2.57 mg/g across the susceptible type and was significant at $P>0.05$ (Table 26). This component counted for 4.54% of the total variance. Varieties exhibit their natural tendencies with respect to phenol concentration explaining 0.18% of the total variance (Table 24). Maximum concentration was displayed by variety named "Bt-ASO 1" to the extent of 3.04 and minimum by Bt-FH 142 to 2.52 mg/g.

Table. 25 **Nested random effect's analysis of variance for phenol of inoculated and un-inoculated leaves of cotton plants**

SOV	DF	SS	MS	Pr>F	F value	Variance Component	% of total Variance
Group (A)	1	97.299	97.299	0.023*	42.356	1.759	95.25
Type (B)	2	4.594	2.297	0.000*	74.624	0.084	4.54
Variety (C)	8	0.246	0.039	0.000*	68.092	0.003	0.18
Error	96	0.043	0.0005			0.000	0.02
Total	107	102.183				1.847	

* = Significant at *P*>0.05

Table.26 **Amount of phenol (mg/g) in group (inoculated and un-inoculated), reaction type (resistant and susceptible), and varieties in cotton leaves**

Varieties (C)	**Bt-MNH 886**		**Bt-FH 177**		**Bt-ASO 1**		**Bt-FH 142**		**Bt-FH 182**		**Bt-FH 169**	
Type (B)	**Resistant**						**Susceptible**					
Group (A)	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**
Amount of phenol in (C)	1.94	3.91	2.00	3.97	2.05	4.04	1.61	3.43	1.67	3.49	1.72	3.55
Av. amount of phenol in (C)	2.92		2.98		3.04		2.52		2.58		2.63	
Av. amount of phenol in (B)	**Resistant = 2.98**				**Susceptible = 2.57**							
Av. amount of phenol in (A)	**Inoculated = 1.83**				**Un-Inoculated** = **3.73**							

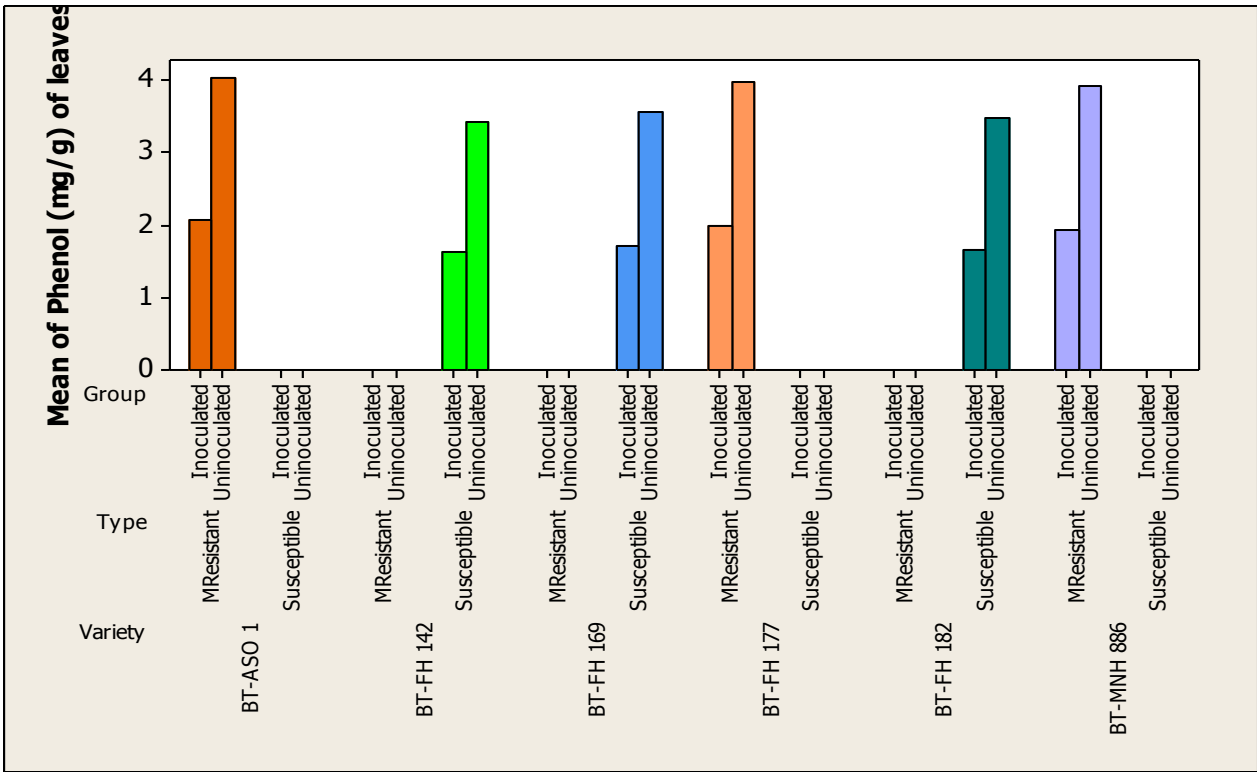

Fig.13 **Concentration of phenol (mg/g) of fresh leaves in resistant and susceptible cotton varieties of un-inoculated and inoculated plants**

4.2.11 Determination of total soluble sugar (mg/g) from leaves of inoculated and un-inoculated cotton plants

Total soluble sugar was observed in significant variation between un-inoculated (6.67) and inoculated plants group (5.01) mg/g respectively (Fig. 14) which accounted for 98.83 percent of the total variance. While resistant (6.03) and susceptible (5.64) type expressed significant variation with 1.12 percent of the total variance. The varieties, Bt-ASO 1 with 6.10 mg/g and Bt-FH 142 with 5.59 mg/g having the maximum and the minimum total soluble sugar concentration respectively with 0.05 percent of total variance (Table 27).

Table. 27 **Nested random effect's analysis of variance for total soluble sugar of inoculated and un-inoculated leaves of cotton plants**

SOV	DF	SS	MS	Pr>F	F value	Variance Component	% of total Variance
Group (A)	1	359.817	359.817	0.006*	174.659	6.625	98.83
Type (B)	2	4.120	2.060	0.000*	73.817	0.075	1.12
Variety (C)	8	0.223	0.028	0.000*	66.317	0.003	0.05
Error	96	0.040	0.0004			0.000	0.01
Total	107	364.201				6.704	

* = Significant at *P*>0.05

Table.28 **Amount of total soluble sugar (mg/g) of fresh leaves in group (inoculated and un-inoculated), reaction type (resistant and susceptible), and varieties in cotton leaves**

Varieties (C)	**Bt-MNH 886**		**Bt-FH 177**		**Bt-ASO 1**		**Bt-FH 142**		**Bt-FH 182**		**Bt-FH 169**	
Type (B)	**Resistant**						**Susceptible**					
Group (A)	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**
Amount of TTS in (C)	5.14	6.82	5.19	6.88	5.27	6.94	4.79	6.40	4.84	6.46	4.88	6.52
Av. amount of TTS in (C)	5.98		6.03		6.10		5.59		5.65		5.70	
Av. amount of TTS in (B)	**Resistant = 6.03**				**Susceptible = 5.64**							
Av. amount of TTS in (A)	**Inoculated = 5.01**				**Un-Inoculated = 6.67**							

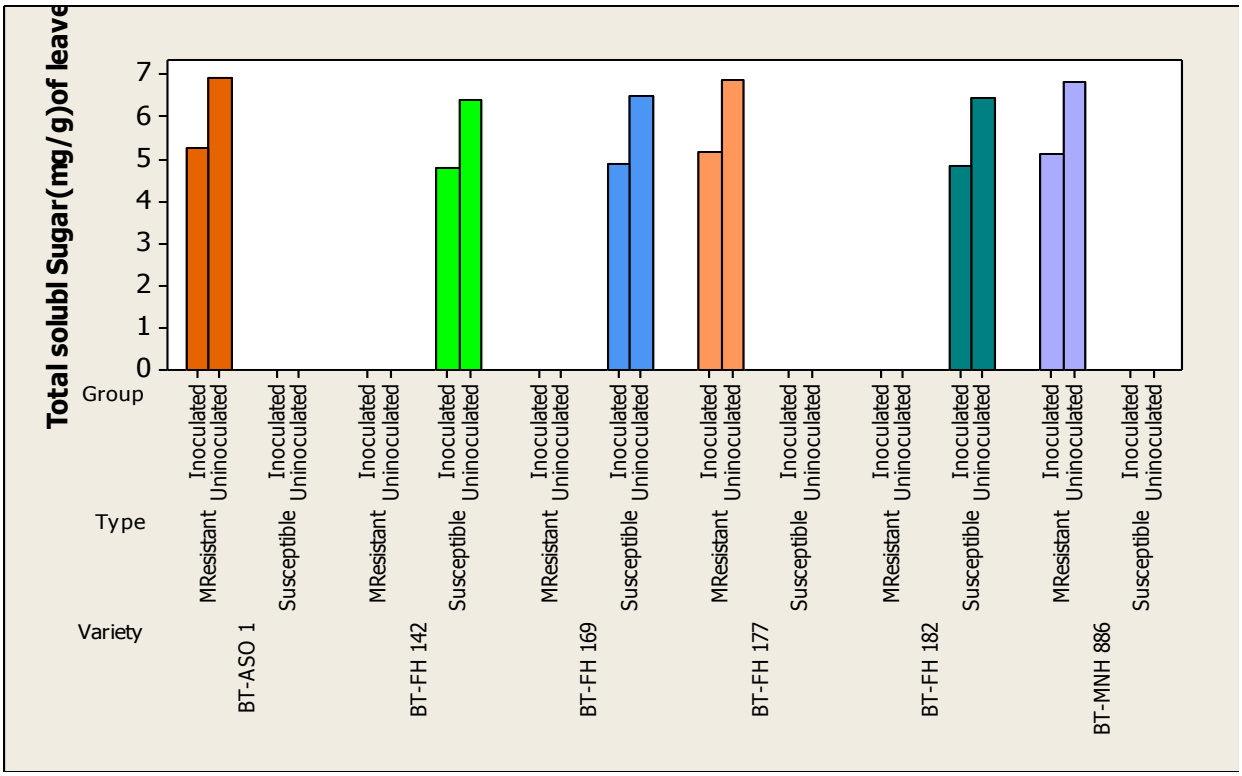

Fig.14 **Concentration of total soluble sugar (mg/g) in fresh leaves of resistant and susceptible cotton varieties of un-inoculated and inoculated plants.**

4.2.12 **Determination of chlorophyll contents (mg/g) from fresh leaves of inoculated and un-inoculated cotton plants**

Significant variation was observed between un-inoculated and inoculated group of plants (with 1.56 and 0.68 mg/g) indicating that total chlorophyll contents seem to affect metabolic activities as a result of disease stress (Fig. 15) and was counted for 74.85% of the total variance. Resistant and susceptible plants also showed significant variation (Table 30). The value of 1.16 was observed across the resistant type and 0.83 across the susceptible type and was significant at $P>0.05$ (Table 29) with 23.02% of the total variance. Varieties exhibited their natural tendencies with respect to total chlorophyll concentrations explaining 1.73% of the total variance. Maximum concentration was displayed by variety named “Bt-ASO 1” to the extent of 1.23 and minimum by variety “Bt-FH 142” 0.78 respectively (Table 30).

Table.29 **Nested random effect's analysis of variance for chlorophyll of inoculated and un-inoculated leaves of cotton plants**

SOV	DF	SS	MS	Pr>F	F value	Variance Component	% of total Variance
Group (A)	1	0.296	0.037	0.000*	40.584	0.174	74.85
Type (B)	2	2.956	1.478	0.000*	39.916	2.9564	23.02
Variety (C)	8	10.849	0.835	0.114^{ns}	7.339	0.004	1.73
Error	96	0.088	0.001			0.001	0.39
Total	107	14.189				0.232	

* = Significant at *P*>0.05

Table. 30 **Amount of chlorophyll (mg/g) fresh leaves in group (inoculated and un-inoculated), reaction type (resistant and susceptible), and varieties in cotton leaves**

Varieties (C)	**Bt-MNH 886**		**Bt-FH 177**		**Bt-ASO 1**		**Bt-FH 142**		**Bt-FH 182**		**Bt-FH 169**	
Group (A)	**Resistant**						**Susceptible**					
Type (B)	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**	**Inoc**	**Uninoc**
Amount of Chl. In (C)	0.77	1.43	0.81	1.51	0.87	1.59	0.49	1.07	0.55	1.13	0.60	1.18
Av. amount of Chl. in (C)	1.1		1.16		1.23		0.78		0.84		0.89	
Av. amount of Chl. in (B)	**Resistant = 1.16**				**Susceptible = 0.83**							
Av. amount of Chl. in (A)	**Inoculated = 0.68**				**Un-Inoculated** = **1.56**							

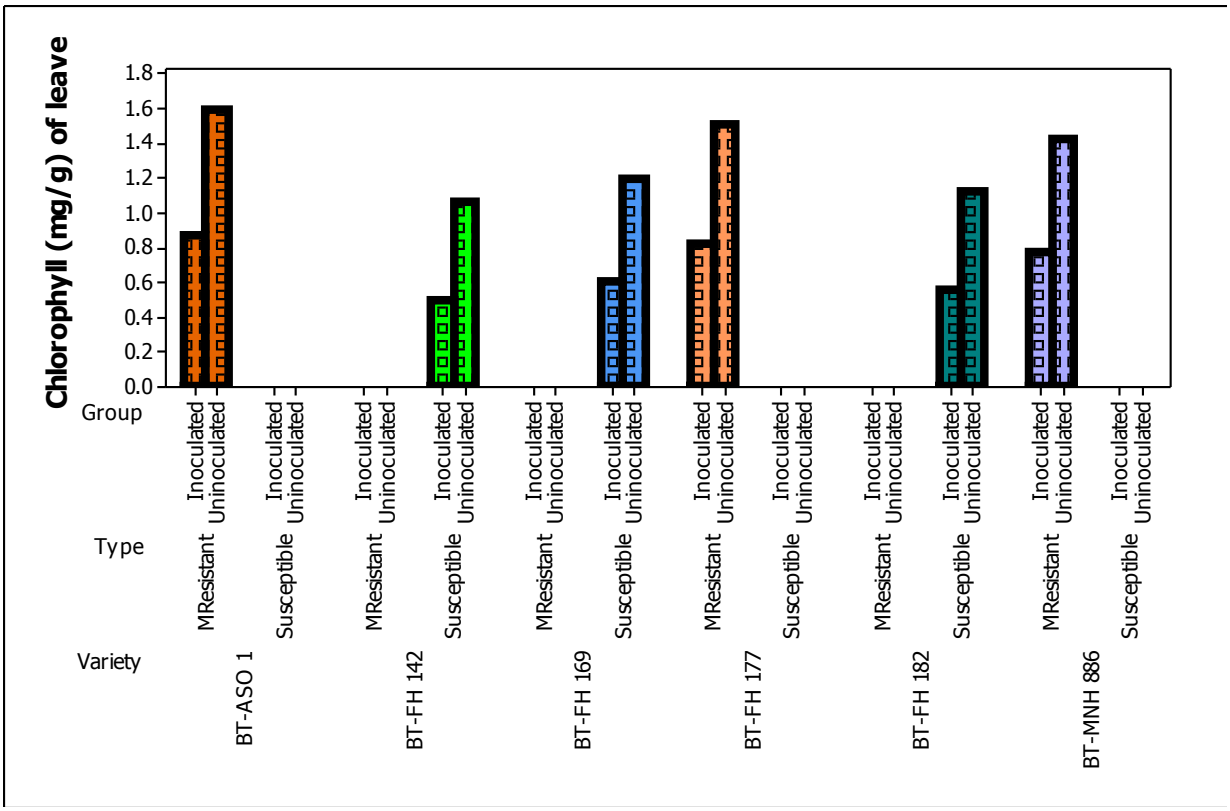

Fig.15 **Concentration of chlorophyll content (mg/g) fresh leaves in resistant and susceptible cotton varieties of un-inoculated and inoculated plants**

4.3.1 ***In-vitro*** **evaluation of chemicals against *X. citri* pv. *malvacearum***

All treatments (T), concentrations (C) and their interactions (T×C) expressed significant response against *Xcm* (Table 31). Maximum inhibition zone was expressed by Flare (1.69 cm) followed by Plant Protector (1.47cm), Mancozeb (1.29 cm), Agrimycine (1.15 cm) and Copper oxychloride (0.95 cm) respectively as compared to control (Table 32). In the interaction between treatments and concentrations (T×C) maximum inhibition zones (1.76 cm) was produced by Flare @ 0.35% followed by (1.69 cm) at 0.3% and (1.63 cm) at 0.25% concentration respectively while copper oxy chloride showed minimum inhibition zones (0.90 cm, 0.95 cm and 1.01cm) at (0.25, 0.3 and 0.35%) respectively, plant protector expressed 1.40, 1.47, 1.55, Mancozeb 1.24, 1.29, 1.34 and Agrimycine 1.10, 1.17 and 1.18 cm inhibition zone at 0.25,0.30 and 0.35% concentrations respectively as compared to control (Table. 33).

Table.31 **ANOVA for *in-vitro* evaluation of chemicals against *X. citri* pv. *malvacearum***

SOV	**DF**	**SS**	**MS**	**F**	**P**
Treatments (T)	5	15.851	3.170	53894.8	0.000*
Concentrations (C)	2	0.081	0.040	691.05	0.000*
T×C	10	0.022	0.002	38.25	0.000*
Error	34	0.002	0.00006		
Total	53	15.967			

* = Significant at P< 0.05

Table.32 ***In-vitro* evaluation of chemicals against *X. citri* pv. *malvacearum***

Sr #	**Treatments**	**Inhibition zones** (cm)
T_1	Flare	1.693a
T_2	Plant Protector	1.473b
T_3	Mancozeb	1.290c
T_4	Agrimycin	1.150d
T_5	Copper oxy chloride	0.953e
T_6	Control	0.000f
	LSD	**0.073**

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

Table. 33 **Impact of interaction between treatments and concentration** (T×C) **against *X. citri* pv. *malvacearum* under lab.conditions**

Sr#	**Treatments**	**Inhibition zones** (cm)		
		Concentrations (%)		
		0.25	**0.30**	**0.35**
T_1	Flare	1.63c	1.69b	1.76a
T_2	Plant Protector	1.40f	1.47e	1.55d
T_3	Mancozeb	1.24i	1.29h	1.34g
T_4	Agrimycin	1.10k	1.17j	1.18j
T_5	Copperoxy chloride	0.90n	0.95m	1.01i
T_6	Control	0.00 o	0.00 o	0.00 o
	LSD	**0.0127**		

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

4.3.2 ***In-vitro* evaluation of plant extracts against *X. citri* pv. *malvacearum***

All treatments (T) and concentrations (C) and their interactions (T×C) exhibited significant response against *Xcm* (Table 34). Maximum inhibition zone was produced by *Nicotiana tabacum* (0.65 cm) followed by *Azadirachta indica* (0.48cm), *Moringa oleifera* (0.35 cm), *Datura alba* (0.25 cm) and *Curcuma longa* (0.17 cm) as compared to control (Table 35). In the interaction between treatments and concentrations (T×C), maximum inhibition zone (0.70 cm) was produced by Tobacco at 20 % (0.65 cm) at 15% and (0.60 cm) at 10% respectively while *C. longa* exhibited minimum inhibition zones of (0.18cm, 0.17 cm and 0.15cm) while *A. indica* expressed 0.43, 0.49, 0.54, *M. oleifera* 0.32, 0.35, 0.38, *D. alba* 0.227, 0.260 and 0.283 cm inhibition zone at 10, 15 and 20% concentration respectively as compared to control (Table 36).

Table.34 **ANOVA for *in-vitro* evaluation of plant extracts against *X. citri* pv. *malvacearum* under lab. conditions**

SOV	**DF**	**SS**	**MS**	**F**	**P**
Treatments (T)	5	2.401	0.480	16208.9	0.000*
Concentrations (C)	2	0.033	0.016	557.69	0.000*
T×C	10	0.012	0.001	41.99	0.000*
Error	34	0.001	0.00003		
Total	53	2.450			

* = Significant at P< 0.05

Table.35 ***In-vitro* evaluation of plant extracts against *X. citri* pv. *malvacearum***

Sr #	**Treatments**	**Inhibition zones (cm)**
T_1	*N. tabacum* (Tobacco)	0.650a
T_2	*A. indica* (Neem)	0.486b
T_3	*M. oleifera* (Moringa)	0.350c
T_4	*D. alba* (Datura)	0.256d
T_5	*C. longa* (Turmeric)	0.168e
T_6	Control	0.000f
	LSD	**0.052**

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$

Table.36 **Impact of interaction between treatments and concentrations** (T×C) **against *X. citri* pv. *malvacearum* under lab. conditions**

Sr#	Treatments	**Inhibition zones** (cm)		
		Concentrations (%)		
		10	**15**	**20**
T_1	*N. tabacum*	0.600c	0.650b	0.700a
T_2	*A. indica*	0.430f	0.490e	0.540d
T_3	*M. oleifera*	0.320i	0.350h	0.380g
T_4	*D. alba*	0.227 l	0.260k	0.283j
T_5	*C. longa*	0.150 o	0.170n	0.186m
T_6	Control	0.000p	0.000p	0.000p
	LSD	**0.090**		

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$

4.3.3 **Evaluation of Flare and *Nicotiana tabacum* against bacterial blight of cotton under greenhouse conditions**

All treatments (T), concentrations (C), days (D) and their interaction (T×C), (T×D) and (T×C×D) expressed significant effect against bacterial blight of cotton (Table 37). Minimum disease incidence (32.27) % was observed when (Flare + *N. tabacum*) were in combination, followed by Flare (36.91) % and *N. tabacum* (41.60) % as compared to control (Table 38). Interaction between treatments and concentration (T×C) *N. tabacum* expressed 37.06, 41.83 and 45.90 @ 40, 35 and 30% concentration and Flare 32.50, 37.30 and 40.93 @ 0.6, 0.55 and 0.5 % concentration while minimum disease incidence was expressed by (Flare + *N. tabacum*) 27.86, 32.86 and 36.10% respectively at three concentrations as compared to control (Table 39). Interaction between treatments and days (T×D) exhibited that *N. tabacum* expressed (45.13, 42.16 and 37.50)% disease incidence while Flare (40.76, 37.00 and 32.96) and Flare + *N. tabacum* 36.50, 32.13 and 28.20% disease incidence after seven, fourteen and twenty one days respectively as compared to control (Table 39). In interaction between treatments, concentrations and days (T×C×D) maximum reduction in disease 39.40, 37.50 and 32.60 (after seven days), 35.40, 32.60, 28.40 after fourteen days, 33.50, 28.50 and 22.60% after twenty one days when (Flare + *N. tabacum*) was applied at three concentrations. Flare expressed (44.20,

41.60 and 36.50), (40.10, 37.40 and 33.50) and (38.50, 32.90 and 27.50)% disease incidence after seven, fourteen and twenty one days respectively @0.30, 0.35 and 0.40% concentration and minimum reduction in disease incidence was exhibited by *N. tabacum* (48.40, 45.20 and 41.80) after seven day, (45.80, 42.80 and 37.90) after fourteen days and (43.50, 37.50 and 31.50)% after twenty one days when applied @ 30, 35 and 40% as compared to control (Fig. 16).

Table.37 **ANOVA for evaluation of Flare and *Nicotiana tabacum* against bacterial blight of cotton under greenhouse conditions**

SOV	**DF**	**SS**	**MS**	**F**	**P**
Treatments (T)	3	30249.1	10083.0	206916	0.000*
Concentrations (C)	2	842.2	421.106	8641.59	0.000*
Days (D)	2	207.3	103.631	2126.63	0.000*
T×C	6	156.4	26.061	534.81	0.000*
T×D	6	1125.9	187.650	3850.79	0.000*
C×D	4	41.2	10.303	211.44	0.000*
T×C×D	12	18.9	1.573	32.30	0.000*
Error	70	3.4	0.048		
Total	107	32646.3			

* = Significant at $P< 0.05$

Table.38 **Evaluation of Flare and *Nicotiana tabacum* against bacterial blight of cotton under greenhouse conditions**

Sr#	**Treatments**	**Disease incidence (%)**
T_1	Flare +*N. tabacum*	32.27a
T_2	Flare	36.91b
T_3	*N. tabacum*	41.60c
T_4	Control	74.82d
	LSD	**0.1198**

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

Table.39 **Impact of interaction between treatments and concentrations** (T×C) **against bacterial blight of cotton under greenhouse conditions**

Sr #	**Treatments**	**Inhibition zones** (cm)		
		Concentrations (%)		
		I	**II**	**III**
T_1	Flare +*N. tabacum*	36.10i	32.86j	27.86 l
T_2	Flare	40.93f	37.30g	32.50k
T_3	*N. tabacum*	45.90d	41.83e	37.06h
T_4	Control	74.00c	74.66b	75.80a
	LSD	**0.2075**		

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

Table.40 **Impact of interaction between treatments and days** (T×D) **against bacterial blight of cotton under greenhouse conditions**

Sr#	**Treatments**	**Disease incidence (%)**		
		Days		
		D_7	**D_{14}**	**D_{21}**
T_1	Flare +*N. tabacum*	36.50i	32.13k	28.20 l
T_2	Flare	40.76f	37.00h	32.96j
T_3	*N. tabacum*	45.13d	42.16e	37.50g
T_4	Control	69.36c	75.43b	79.66a
	LSD	**0.2075**		

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

- I = 0.5% Flare + 30% *N. tabacum*
- II= 0.55 % Flare + 35% *N. tabacum*
- III= 0.6% Flare + 40% *N. tabacum*

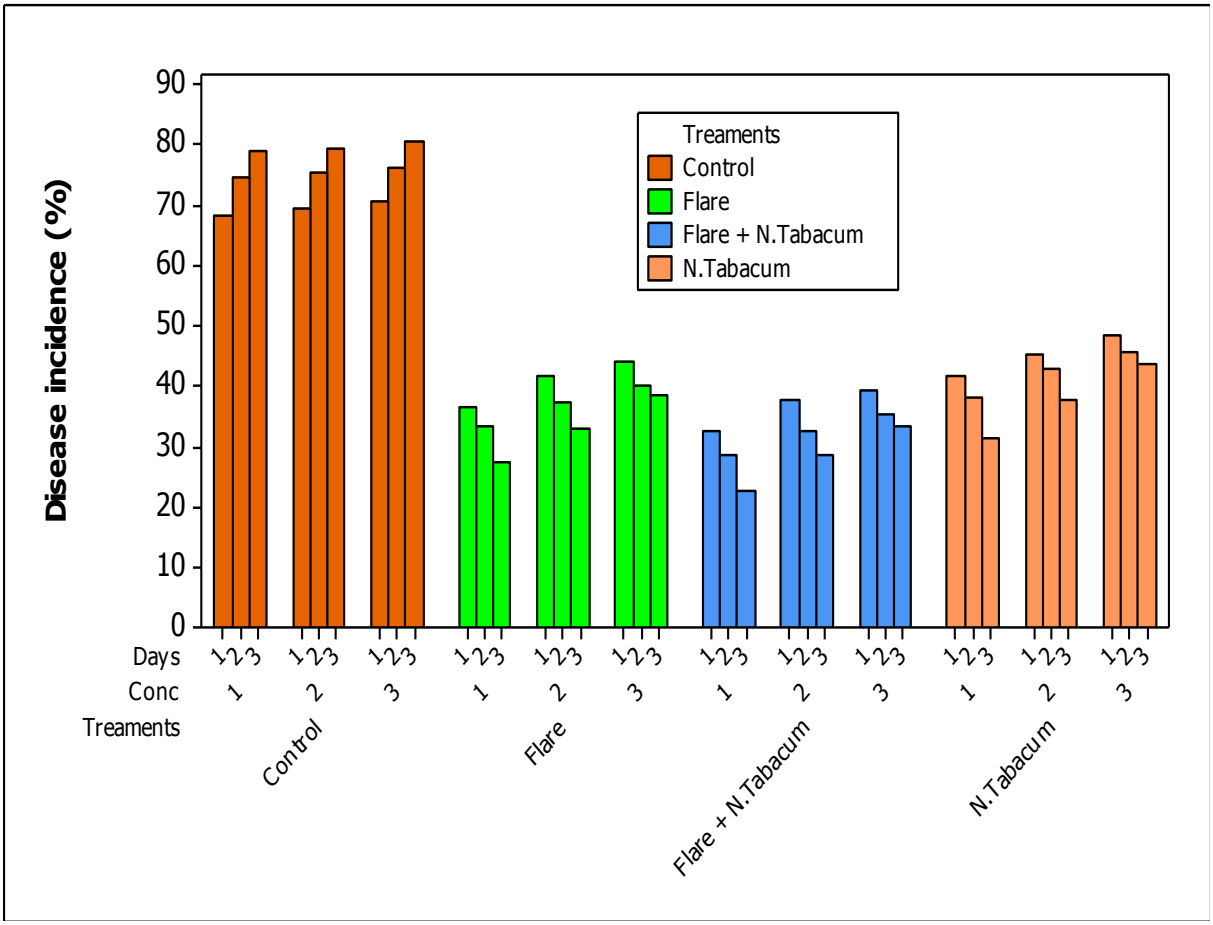

Fig.16 **Impact of interaction between treatments concentrations and Days** (T×C×D) **against *X. citri* pv. *malvacearum* under greenhouse conditions**

4.3.4 Evaluation of Flare and *Nicotiana tabacum* against bacterial blight of cotton under field conditions

ANOVA indicated that all treatments (T), concentrations (C), days (D) and their interaction (T×C), (T×D) and (T× C×D) expressed significant results (Table 41). Minimum disease incidence (40.41) % was observed when (Flare + *N. tabacum*) were applied in combination, followed by Flare (45.74) % and *N. tabacum* (50.41) % as compared to control (Table 42). Interaction between treatments and concentration (T×C), *N. tabacum* expressed 54.60, 50.26 and 46.36 @ 45, 50 and 55% concentration and Flare 49.70, 46.46 and 41.06 @ 0.75, 0.80 and 0.85% concentration while minimum disease incidence was expressed by (Flare

+ *N. tabacum*) 44.23, 41.93 and 35.06% respectively at three concentrations as compared to control (Table 43). Interaction between treatments and days (T×D) exhibited that *N. tabacum* expressed (53.90, 51.03 and 46.30) % disease incidence while Flare (49.26, 45.53 and 42.43) and (Flare + *N. tabacum*) 44.06, 40.80 and 36.36% after seven, fourteen and twenty one days respectively as compared to control (Table 44). In interaction between treatments, concentrations and days (T×C×D) maximum reduction in disease 46.50, 46.30, 39.40 (after seven days),44.30, 41.60 and 36.50 after fourteen days, 42.10, 37.30 and 29.30% after twenty one days when (Flare + *N. tabacum*) was applied at three concentrations. Flare expressed (52.50, 49.60 and 45.70), (48.90, 46.10 and 41.60) and (47.70, 43.70 and 35.90)% disease incidence after seven, fourteen and twenty one days respectively @ 0.75, 0.80 and 0.85% concentration and minimum reduction in disease incidence was exhibited by *N. tabacum* (56.70, 53.90 and 51.50) after seven day, (55.10, 50.30 and 47.70) after fourteen days and (52.00, 46.60 and 40.30)% after twenty one days when applied @ 45, 50 and 55% as compared to control (Fig. 17).

Table.41 **ANOVA for evaluation of chemical and plant extracts against *X. citri* pv. *malvacearum* under field conditions**

SOV	**DF**	**SS**	**MS**	**F**	**P**
Treatments (T)	3	29693.7	9897.91	320766	0.000*
Concentrations (C)	2	999.6	499.82	16197.8	0.000*
Days (D)	2	174.7	87.35	2830.87	0.000*
T×C	6	114.0	18.99	615.55	0.000*
T×D	6	1020.0	170.01	5509.45	0.000*
C×D	4	69.1	17.28	559.84	0.000*
T×C×D	12	33.5	2.79	90.42	0.000*
Error	70	2.2	0.03		
Total	107	32107.5			

* = Significant at $P< 0.05$

Table.42 **Impact of chemical and plant extracts against *X. citri* pv. *malvacearum* under field conditions**

Sr#	**Treatments**	**Disease incidence** (%)
T_1	Flare + *N. tabacum*	40.41d
T_2	Flare	45.74c
T_3	*N. tabacum*	50.41b
T_4	Control	82.93a
	LSD	**0.095**

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$)

Table.43 **Impact of various plant extracts and their concentrations on disease incidence caused by *X. citri* pv. *malvacearum* under field conditions**

Sr#	**Treatments**	**Disease incidence (%)**		
		Concentrations (%)		
		I	**II**	**III**
T_1	Flare + *N.tabacum*	44.23h	41.93i	35.06k
T_2	Flare	49.70f	46.46g	41.06j
T_3	*N.tabacum*	54.60d	50.26e	46.36g
T_4	Control	80.96c	83.43b	84.40a
	LSD	**0.165**		

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

Table.44 **Impact of various plant extracts and days on disease incidence caused by *X. citri* pv. *malvacearum* under field conditions**

Treatments	Disease incidence (%)		
	D_7	D_{14}	D_{21}
Flare + *N. tabacum*	44.06i	40.80k	36.36 l
Flare	49.26f	45.53h	42.43j
N. tabacum	53.90d	51.03e	46.30g
Control	77.63c	83.60b	87.56a
LSD	**0.165**		

Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test ($P \leq 0.05$).

- **I** = 0.75 Flare + 45% *N. tabacum*
- **II** = 0.80 Flare + 50% *N. tabacum*
- **III** = 0.85 Flare + 55% *N. tabacum*

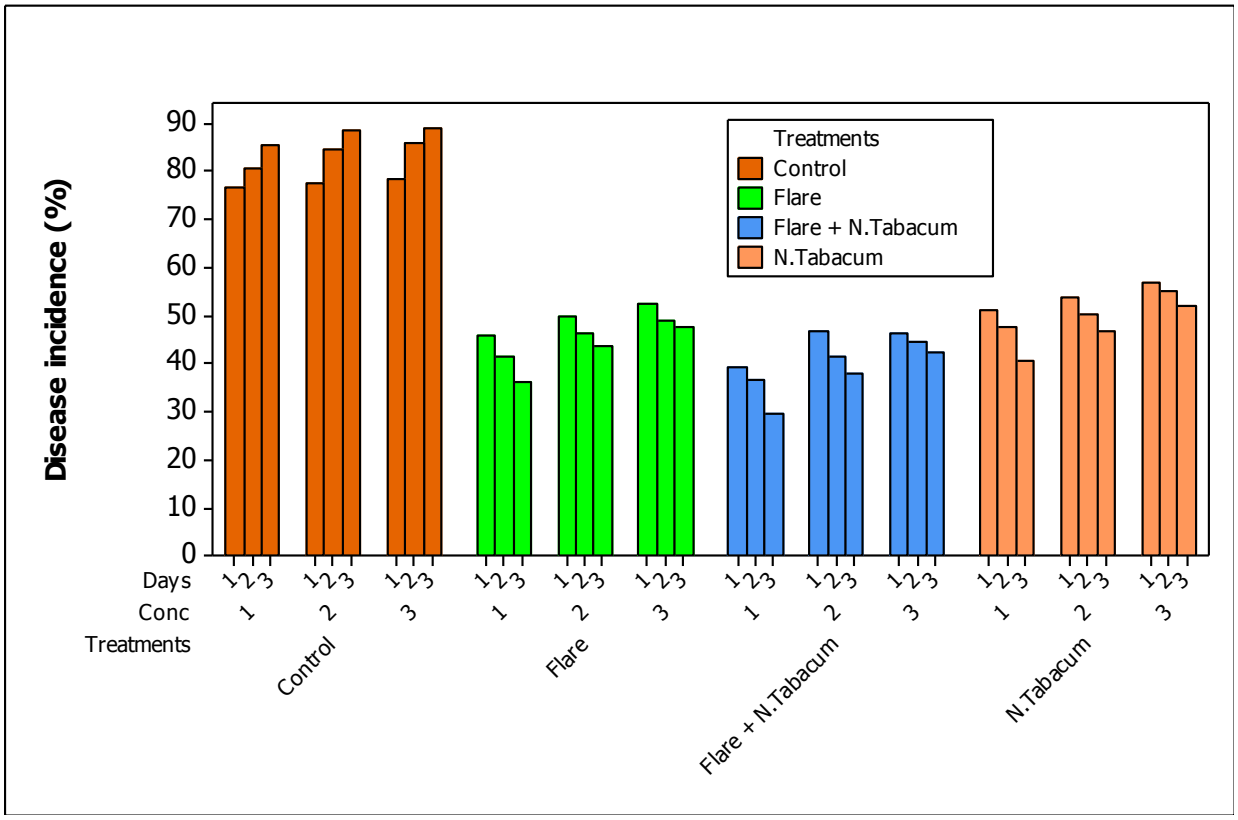

Fig.17 **Impact of interaction between treatments concentrations and Days** (T×C×D) **against *X. citri* pv. *malvacearum* under field conditions**

CHAPTER # 5 DISCUSSION

1. Evaluation of cotton germplasm for source of resistance against bacterial blight

The Symptomology of a disease plays an imperative role in the understanding of altered physiological changes and the establishment of pathogen with in host but there are many factors which distresses the symptoms uttered by pathogenic bacteria like infection time, type of infection, plant age, strain of bacteria, genetic makeup of the host plant and environmental conditions. In Pakistan the production of cotton crop is threatened by bacterial blight caused by the *Xcm.* The early infection of the pathogen causes heavy losses in yield. It has been reported that the bacterial blight of cotton cause 40-50 percent losses depending upon the infection time and the genotype of the host (Verma, 1986; Khan *et al.,* 1999; Bayles *et al.,* 2007). As the infection of pathogen affects the vegetative portion of plant and the components of yield, therefore *Xcm.* is responsible for low yield of cotton. Susceptible germplasm of cotton, conducive environmental conditions and presence of virulent pathogen play a major role in the development and spread of epidemics of the bacterial blight of cotton. Under such conditions the use of resistant varieties is only an effective and durable way to protect crop from *Xcm.* The development of resistant variety by presenting resistant genes into cotton cultivars is only a single possible solution but it takes long time. So, the screening of available germplasm should be done as it short term and easy method to get resistant varieties. That is why in present study, a lot of thirty varieties was evaluated against bacterial blight of cotton under field conditions for two years (2013 and 2014). During both years eighteen varieties expressed moderately resistant response (Non Bt-PB-896, Bt-CM 615, Bt-IR 901, Non Bt-BH 160, Bt-CRS 2007, Bt-VH 329, Bt-CM 616, Non Bt-Redacola, Bt- KZ 189, Non Bt-NIAB 111, Bt-IUB 222, Non Bt-Sindh 1, Bt-ASO 1, Non Bt-MNH554, Bt-FH 183, Bt-MNH 886, Bt-53 and Bt-FH 177 respectively with rating 4, while six varieties Bt-FH 143, Bt-Ali Akbar 802, Non Bt-CIM 573, Bt-NS 131, Non Bt-CM 82and Bt-NIBGI 2 exhibited moderately susceptible response with rating 5. Bt-FH 142, Bt-FH 182, Bt-4243, Bt-FH 169 showed susceptible while Non Bt-Shahbaz and Non Bt- CRIS 134 expressed highly susceptible response. Outcomes of the present study are supported by the work of Atiq *et al*., (2014) who tested fifteen varieties of cotton to determine their genetic response against bacterial blight. He observed that none of variety showed immune response while FH-114, Bt-121 and SLH-336 expressed moderately resistance While Kirn, SLH Bt-6, Bt-666, CIM-595, FH-113 and Bt-MK2 showed moderately susceptible and SG-1, Bt-222, Bt-457, Bt-7, SLH-

317 and Bt-986 showed highly susceptible response against the bacterial blight disease of cotton. Similar work was also performed (Singh *et al.,* 2007; Nichols et al., 2007; Wheeler *et al.,* 2007) and concluded that no variety expressed immune response towards bacterial blight of cotton and the best management strategy to manage disease is use of resistant germplasm. Drishak *et al.,* (2014) screened cotton varieties against bacterial blight by using different inoculation techniques and found that no variety expressed highly resistant response. As the result of present screening, the selected resistant varieties can be further used in breeding program as source of resistance against bacterial blight. If these resistant varieties possess all desirable agronomic characteristics then these can also be introduced at commercial level.

2. **Changes in ionic contents and biochemical compounds in cotton leaves due to bacterial blight**

In plants deficiency/excessive amount of nutrients cause different maladies which are affected by amount of elements, form of elements, type of disease and environmental conditions affect the appearance of disease. Elements like carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), Sulphur (S), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B), molybdenum (Mo) and chlorine (Cl) are necessary for the growth of plants and completion of their life cycle. Some essential nutrients have also been produced in some plants i.e. sodium (Na), cobalt (Co), vanadium (V) and silicon (Si). These four nutrients are almost never deficient in soil. Because nutrients expressed a variety of effects on disease progress in different plant species that simplification/generalization becomes difficult. Different plants and pathogen species come in contact with each other under different climatic conditions but the consequence of their interaction cannot be varied. Plants obtained all nutrients from soil and they generally supply most of the nutrients for the growth and activities of pathogens. So it is possible that different types of nutrients may affect the resistance status of the host as well as virulence of the pathogen. Plants obtaining well balanced nourishment, with all necessary elements easily available in proper amount undergo a smaller amount of disease and get protection from upshots of fresh infection and expressed pronounced growth, development and yield (Mishra *et al*., 2005). A makeable effect of bacterial blight disease was observed on the nutritional status of leaves in cotton.

Nitrogen is an essential nutrient for all plants obtained from soil and decaying organic matter. A huge amount of N is required by the plant because it is essential for formation of

different types of enzymes, proteins and structures and its balanced quantity is essential for resistance towards bacterial blight of cotton. That is why plant expressed pronounced effect towards its application. Its excessive application errand some plant diseases while plants deficient in N contents favor some other types of symptoms. In present study decrease in N level was observed and decrease in nitrogen quantity favor the incidence of bacterial blight of cotton and adequate application along with K activate defense system and create resistance against pathogen attack (Chase, 1989; Vidhyasekaran 1988; Agrios, 2005; Dordas, 2008). Phosphorus is also utilized by the plant for formation of essential molecules i.e. DNA, RNA, phospholipids, coenzyme NAD and NADP, ATP and other high energy compounds (Devlin and Witham, 1983). However it role in resistance is variable. It can decrease incidence of different plant diseases by promoting root growth but in present study decrease in P quantity was observed which facilitate the growth of *Xcm* and enhanced the incidence of bacterial blight of cotton. Similar results were reported by Dordas (2008) who also observed that low level of P favor the development of bacterial blight and balanced application of P reduced the incidence of bacterial blight (Huber and Graham, 1999; Kirkegaard *et al.*,1999; Reuveni *et al.,* 2000).Potassium role is crucial in metabolism of carbohydrate due to production of enzyme. Beside metabolism they also play an important role in photosynthesis due to stomatal opening (Salisbury and Ross, 1992). It is involved in the activation of more than sixty enzymes which are involved in different metabolic processes of plants. In contemporary study, decrease in K amount was noted due to bacterial blight disease. Outcome the present study is supported by the work of Mishra *et al.,* (2005) and Dordas (2008) who decrease in K enhanced the severity of bacterial blight by favoring the growth of *Xcm* (Sharma *et al.,* 2005). Its balanced amount is plant and soil boost up resistance in plants and plant started to produce different types of physiological and biochemical enhance resistance ability of the plant by creating different types of barriers against *Xcm* (Sharma and Duveiller, 2004; Mishra *et al.,* 2005).Plants absorb calcium as a Ca2+ cation. It helps in stimulation of root and leaf development, microbial activity and uptake of other nutrients. It prevents the penetration of pathogens and develops resistance in host plant which strengthens the plant structure (Mishra *et al*., 2005). Calcium is a major constituent of middle lamella of the cell wall which is present in the form of calcium pectate. It plays a significant role in maintaining cell integrity and membrane permeability (Devlin & Withman, 1993). In infected leaves of cotton there was a reduction in calcium concentration which was observed both in resistant and

susceptible cultivars which enhanced disease (Marschner, 1995; Mishra *et al.*, 2005). Similar results were reported by Dordas, (2008) that there was decrease in calcium contents which enhanced the bacterial blight infection. A balanced amount of Ca provide protection against diseases by binding oxalic acid or strengthening cell wall and framework of cells by transformation of enzyme sensitive pectin and pectinic acid compounds of middle lamella to rather enzyme tolerant calcium pectate and develop resistance in host plant and prevent penetration of pathogen (Mishra *et al.*, 2005). Magnesium plays a significant role in synthesis of chlorophyll, photosynthesis and carbohydrate metabolism (Devlin & Withman, 1983). Since Mg is a vital element of structural tissues and take part in different physiological and biochemical processes. It is necessary for integrity and safeguarding of ribosome and is concomitant with growth, mitosis, protein level, metabolism of carbohydrate and oxidative phosphorylation. It also takes part in respiration, DNA and RNA formation, energy transfer reactions and also acts as a co-factor for many enzymes (Marschner, 2011). It current study level of Mg was decreased in cotton leaves due to attack of bacterial blight disease. Outcome of present study is supported by the work of Batson (1971) and Huber and Jones, (2012) who also observed decrease in Mg amount in cotton leaves due to attack of *Xcm*. Reduction in Mg concentration cause hindrance in partitioning of dry matter root and shoots which result in increased quantity of starch, amino acids and sugars in leaves, destruction of chlorophyll, reduction in electron transport chain (ETC) and production of reactive oxygen species (ROS) due to damage of CO_2 fixation in photosynthesis (Hermans *et al.*, 2005; Cakmak and Kirby, 2008).Zn is an important micronutrient in cotton plant because it plays a vital role in uptake and efficient use of water and work as a catalyst in different metabolic and biochemical processes. It play a significant role in starch and protein formation. As a Zn/Cu activator of SOD, it is involved in protection of membrane against reactive oxygen species (ROS) through detoxification of superoxide radicals (Cakmak, 2000) and damaged membrane due to free radicals leads to leakage of membrane which favor pathogenesis. In present study Zn concentration was decreased in cotton leaves due to attack of bacterial blight disease. Upshot of the present study is supported by the work of Marschner (1991); Marschner (1995); Dordas (2008) who also reported decrease in concentration of Zn due to disease. Low level of Zn increase severity of disease due to accumulation of amino acids and reducing sugars, which help in disintegration of plasma membrane and increase pathogenesis (Grewal *et al.*, 1996; Mengel and Kirby., 2001).

Application of Zn to the soil can decrease the incidence of bacterial blight of cotton. Iron is the foremost component of chlorophyll and has vital role in nucleic acid metabolism and its deficiency can reduce chlorophyll contents of plants (Imran and Gurmani, 2011). It is vital components of flavoproteins and iron-porphyrn protein which includes cytochromes, Peroxidases and Catalases. These proteins are responsible for increased catabolic activities in bacterial blight susceptible plants (Delvlin & Withman, 1983). In present study it was concluded that Fe concentration was decreased due to bacterial attack. It may due the fact that plant pathogens generally have higher requirement of Fe and act as virulence factor during the course of disease development because Fe activate enzymes which are involved in the infection process of the host by the pathogen (Graham and Webb, 1991; Dordas, 2008). Copper is an important component of lignin and have a key role in protein and carbohydrate metabolism and acts as catalyst in different metabolic activities of the plant (Imran and Gurmani, 2011). In contemporary study decrease in Cu concentration was observed. Outcome of the present study is supported by the fact that when plant became infected, its defense system activated and starts secreting certain types of phenolics and flavonides both at the infection site and away from the site. Production of these substances is controlled by nutrients of the plant. Therefore shortage of elements like Cu, Fe, K, Mn and Zn takes place at the infection site and copper application reduce the intensity of bacterial diseases (Marschner, 1995).

There was a decrease in the total chlorophyll contents of both resistant and susceptible cultivars upon inoculation in present study. Development stages of plants and their biological processes were influenced by total chlorophyll and the proportion of its components. Outcome of the current study is supported by the work of Sain and Gour, (2008) who observed decrease in chlorophyll contents with the increase in disease incidence. Stimulation of Chlorosis (loss of chlorophyll), necrosis, and reduction in the green leaf area is due to lesion growth which is produced by this infection. But it has no effect on photosynthetic activity of the remaining green leaf tissue (Oijen, 1990). Sugars are antecedent for production of phenols, phytoalexins, lignin and cellulose, so they perform an imperative role in defense mechanism of plants. Gene regulation and host defense functions were accomplished by accumulation of soluble sugars. Decrease in total soluble sugar contents was noted during present study both in resistant and susceptible cultivars upon infection. Numerous reports indicated that carbohydrate metabolism is influenced by pathogen attack. Starch accumulation, reduction in photosynthetic rate and

reduction in total soluble sugar contents are characteristic properties of infected leaves (Goodman *et al.*, 1986; Sain and Gour, 2008). In susceptible cotton plant leaves, the total soluble phenol is present in less concentration than the resistant ones as observed in the present study. This result is in agreement with the findings of Borthakuar and Addy, (1988) in case of rice against sheath blight, Borkar and Verma (1991) in case of cotton against bacterial blight. While enhanced level of total soluble phenols advocated a preliminary effort by the host defense towards pathogens. Secondary phenolic compounds are produced by higher plants and their role in the metabolism of plants had not been adequately explained. Lignin and phenolic precursors play an important role in plant defenses by increasing cell wall resistance to mechanical penetration, decreasing the susceptibility to cell wall degrading enzymes, restricting the entrance of enzymes and toxins produced by the pathogens, preventing nutrient flow toward the pathogen, and as toxic products active against the pathogen (Ride, 1978).Results of the present study indicated that protein contents in cotton leaves decreased due to attack of *Xcm*. Decrease in protein contents indicated that bacterial infection increased with decrease in protein contents of the plant. Outcomes of the extant study are supported by the work of Sain and Gour (2008) who studied pathological, physiological and biochemical characterization of *Xanthomona scitri* and concluded that bacteria not only decrease protein contents of the host plant but also decrease total nitrogen, chlorophyll and sugar contents.

3. **Management of bacterial blight of cotton**

Most suitable, economical, safe, reliable and practicable management of bacterial blight of cotton is, is the use of resistant varieties but if resistant varieties are not available and disease appears in the field suddenly and at a very rapid rate in the field, the farmers have only one option to spray crop with some effective chemical. So in present study five chemicals (Flare, Plant protector, Mancozeb, Agrimycine and copper oxychloride at three concentrations were evaluated against *Xcm*. Maximum inhibition was expressed by Flare whose main ingredient is streptomycin sulphate. Plant extracts were selected because, these play an important role i.e. sustainable solutions in agriculture, Reduce crop losses, Eco-friendly, easily bio-degradable, Cheaper and are an important component in integrated diseases management. In current research above plant extracts used on the basis of their easily availability in local area. Similarly five plants extract (*N. tabacum, A. indica, M. oleifera, D. alba*and *C. longa*) were also evaluated against growth of *Xcm* under lab. Conditions by using inhibition zone technique. Among plant

extracts *N. tabacum* expressed maximum inhibition zone. Then Flare and *N. tabacum* were evaluated under greenhouse and filed conditions against bacterial blight of cotton. Both Flare and *N. tabacum* expressed significant results but maximum reduction in disease was expressed by combination of Flare + *N .tabacum* both under greenhouse and field conditions. Fallouts of the present study are reinforced by the work of Singh *et al.,* (2007) who evaluated twelve fungicides and two antibiotics against bacterial blight disease. Among all chemicals streptomycin sulphate expressed significant results both *in vivo* and *in vitro*. Similar results were also reported by Jagtap *et al.,* (2012). A great potential of antibacterial activity is present in a number of plant (Cao *et al.,* 2001). So in present study different plant extracts were used against bacterial blight disease and outcomes of the present study are supported by the work of Sajid *et al.,* (2013) who evaluated three chemicals (plant protector, agrimycine and copper oxy chloride) and three plant extracts (*N. tabacum, C. colocynthis and C. longa*) against *Xcm* at different concentrations and observed that *N. tabacum* expressed pronounced results.

New room for management of bacterial blight of cotton is given by the current study. Plant extracts especially *N. tabacum* and streptomycin sulphate gave us a casement for future biochemical mount of work for isolation, purification and concentration of antibacterial compounds. Selection of suitable formulation and method of application could be the future aspects of plant product especially *N. tabacum* related research. By addition of synergistic additives and by applying other methods, commercialization of environmental friendly plant products especially *N. tabacum* can further be improved.

CHAPTER # 6 SUMMARY

Cotton is the utmost economical and sensitive fiber crop of Pakistan. This crop not only strengthens economy of Pakistan but also boost up livelihood of farmers. It is grown in tropical and sub-tropical areas of the world. It is unique because it provides food, edible oil and fiber. It is prone to attack by a number of fungal, bacterial and viral diseases but the most destructive one is the blight of cotton caused by *Xanthomonas citri* pv. *malvacearum* which reduce yield and produce inferior quality fiber. Under conducive environmental conditions, it cause up to 50 % losses in yield. *Xcm* is gram negative aerobic bacterium with single polar flagellum which enters host plant through stomata and wounds and initiate infection process. Small, irregular water soaked spots on lower epidermis were observed on lower side of epidermis which later became dark brown. These characteristic symptoms expressed true picture of disease development.

Epitome of research attempt undertaken was to evaluate available germplasm of cotton for source of resistance against bacterial blight disease under field conditions for two years (2013 & 2014).During both years eighteen varieties (Non Bt-PB 896, Bt-CM 615, Bt-IR 901, Non Bt-BH 160, Bt-CRS 2007, Bt-VH 329, Bt-CM 616, Non Bt-Redacola, Bt- KZ 189, Non Bt-NIAB 111, Bt-IUB 222, Non Bt-Sindh 1, Bt-ASO 1, Non Bt-MNH554, Bt-FH 183, Bt-MNH 886, Bt-53 and Bt-FH 177 with rating.4) expressed moderately resistant response. Six varieties (Bt-FH 143, Bt-Ali Akbar 802, Non Bt-CIM 573, BT-NS 131, Non Bt-CM 82and Bt-NIBGI 2) exhibited moderately susceptible response with rating 5. Four varieties (Bt-FH 142, Bt-FH 182, Bt-4243 and Bt-FH 169) showed susceptible response while Non Bt-Shahbaz and Non Bt- CRIS 134 expressed high susceptible response with rating 7.

Nutrients not only strengthen health of plants but also activate defense mechanism of the host plants and enable them to withstand different biotic and a biotic stresses. In present study amount of all eight minerals decrease both in resistant and susceptible type of cotton plants upon inoculation. Amount of N (2.78) and P (0.22) % while K (512.1), Ca (412.3), Mg (25.3), Cu (2.87ppm), Zn (1.91) and Fe (1.96) ppm was observed in un-inoculated group of plants which decrease to 1.79, 0.14, 2.76, 191, 13.3, 1.85, 1.09, 0.99 respectively. Level of protein decrease from 5.52–3.77 mg, Phenol 3.73–1.83 mg, total soluble sugars 6.67–5.01 mg and chlorophyll contents from 1.56–0.68 mg respectively upon inoculation. Amount of 2.17, 0.16, 378.8, 270.2, 14.6, 2.4, 1.75 and 1.35 of N, P, K, Ca, Mg, Cu, Zn and Fe was observed in susceptible type while in resistant type 2.40, 0.19, 408.3, 310.2, 21.1, 2.9, 1.83 and 1.61 respectively. Similarly

amount of protein 4.37, total soluble phenols 2.57, total soluble sugars 5.64 and chlorophyll contents 0.83 was observed in susceptible type while 4.93, 2.98, 6.03 and 1.16 was estimated in resistant type of cotton plants. Level of protein reduced from 5.52- 3.77, total soluble phenols 3.73-1.83, total soluble sugars 6.67-5.01 and chlorophyll contents 1.56-0.68 respectively after inoculation.

For management of bacterial blight of cotton five chemicals i.e., Flare, Plant protector, Mancozeb, Agrimycine , copper oxychloride and plant extracts ((*N.tabacum, A. indica, M. oleifera, D. alba* and *C. longa*) at three concentrations were evaluated against *Xcm*. Maximum inhibition was expressed by Flare whose main ingredient is streptomycin sulphate at all concentrations followed by Plant protector, Mancozeb, Agrimycine and copper oxy chloride respectively while in case of plant extracts maximum inhibition of bacterial growth was expressed by *N.tabacum* followed by *A. indica, M. oleifera, D. alba* and *C. longa*. Impact of Flare and *N. tabacum* alone and in combination at different concentrations was also observed under greenhouse and field conditions. All treatments expressed significant results but maximum reduction in disease was expressed by combination of Flare + *N. tabacum* both under greenhouse and field conditions at all concentrations, followed by Flare and *N. tabacum*.

CONCLUSIONS AND RECOMMENDATIONS

- No variety expressed resistant to highly resistant response while few varieties like Non Bt-PB-896, Bt-CM 615, Bt-IR 901, Non Bt-BH 160, Bt-CRS 2007 etc. exhibited moderately resistant response towards bacterial blight of cotton. So it is the need of hour to identify resistant genes in cotton cultivars against bacterial blight of cotton and should be introduced in high yielding varieties.
- Moderately resistant cotton varieties should be recommended for farmers with exploitation of their high yielding potential and selection of planting dates is very important. So planting at recommended date of sowing should be encouraged because it is better option to save farmers from heavy losses due to bacterial blight of cotton.
- Nitrogen, phosphorus, potassium, calcium, magnesium, zinc, copper, iron, total soluble sugars, total soluble proteins, chlorophyll contents and total phenols decreased in cotton leaves due to attack of bacterial blight disease. So it is necessary to conduct research for management of blight disease in cotton through application nutrition in future.
- Measurement of plant nutrients may be used as biochemical markers to identify source of resistance against bacterial blight of cotton which may be helpful in management of bacterial blight of cotton through nutrients and is supportive for researchers to develop such type of nutritional product which may be effective against *Xanthononas citri* pv. *malvacearum*.
- Flare and *N. tabacum* expressed minimum disease incidence under greenhouse and field conditions. Because use of chemicals pollute our environment continuously, so it is essential to exploit antibacterial potential different plant extracts. It is also necessary to search out specific operative compound (s) or element (s) present in various plant extracts which should be employed for proper management of bacterial blight of cotton in future.

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