Impact of chemicals, plant extracts and their combination on bacterial blight of cotton

Muhammad Sajid [Corresp., 1] , **Shahabaz Talib Sahi** [2] , **Muhammad Atiq** [2] , **Muhammad Abid** [Corresp., 1] , **Rashida Perveen** [1] , **Muhammad Rizwan Bashir** [3] , **Hafiz Arslan Anwaar** [2]

[1] Department of Plant Pathology, Faculty of Agricultural Sciences and Techology, Bahauddin Zakariya University, Multan, Pakistan

[2] Deparment of Plant Pathology, University of Agriculture Faisalabad, Pakistan

[3] Oilseeds Research Institute, Ayub Agriculture Research Institute Faisalabad, Pakistan

Corresponding Authors: Muhammad Sajid, Muhammad Abid
Email address: sajid1694@gmail.com, muhammad.abid@bzu.edu.pk

Five chemicals, incuding Flare, Plant Protector, Mancozeb, Agrimycine, and Copper oxychloride, and five plant extracts including *N. tabacum*, *A. indica, M. oleifera, D. alba* and *C. longa* were evaluated against bacterial blight of cotton caused by *Xanthomonas citri* pv*. malvacearum* (a bacterium). The impact of chemicals and plant extracts on bacterial development was tested in laboratory while on disease reduction was tested in green house and field experiments. Laboratory experiments showed that maximum inhibition zone of bacterial growth was expressed by Flare (1.693cm) at all concentrations followed by Plant Protector (1.473 cm), Mancozeb (1.290 cm), Agrimycine (1.150 cm) and copper oxy-chloride (0.953) cm respectively while in case of plant extracts maximum inhibition was expressed by *N. tabacum* (0.650 cm) followed by *A. indica* (0.486)*, M. oleifera* (0.350)*, D. alba* (0.256 cm) and *C. longa* (0.168 cm). Green house experiment revealed that the best result was produced by the combination of Flare and *N. tabacum* by indicating lowest disease incidence (32.27%) at all the tested concentration. Same results were obtained in field experiment, where the lowest disease incidence (40.41%) was recorded when the ,Flare and *N. tabacum* were applied in combination although it was higher then green house. This study concludes that *N. tabacum* and Flare are better option against bacterial disease development and even their combination is more significant lowering the bacterial blight disease incidence on cotton. Selection of suitable formulation and method of application could be the future aspects of plant product especially *N. tabacum* related research.

Title: Impact of chemicals, plant extracts and their combination on bacterial blight of cotton

Short Title: Management of Bacterial blight of cotton

Muhammad Sajid[1,*], Shahbaz Talib Sahi[2], Muhammad Atiq[2], Muhammad Abid[1], Rashida Parveen[1], Muhammad Rizwan Bashir[3], Hafiz Arslan Anwaar[2]

[1]Department of Plant Pathology, Bahauddin Zakariya University Multan, Pakistan

[2]Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan

[3]Oilseeds Research Institute, Ayub Agriculture Research Institute Faisalabad, Pakistan

[*]**Corresponding author:**: Muhmmad Sajid**,** Email: sajid1694@gmail.com

ABSTRACT

Five chemicals, incuding Flare, Plant Protector, Mancozeb, Agrimycine, and Copper oxychloride, and five plant extracts including *N. tabacum*, *A. indica, M. oleifera, D. alba* and *C. longa* were evaluated against bacterial blight of cotton caused by *Xanthomonas citri* pv. *malvacearum* (a bacterium). The impact of chemicals and plant extracts on bacterial development was tested in laboratory while on disease reduction was tested in green house and field experiments. Laboratory experiments showed that maximum inhibition zone of bacterial growth was expressed by Flare (1.693cm) at all concentrations followed by Plant Protector (1.473 cm), Mancozeb (1.290 cm), Agrimycine (1.150 cm) and copper oxy-chloride (0.953) cm respectively while in case of plant extracts maximum inhibition was expressed by *N. tabacum* (0.650 cm) followed by *A. indica* (0.486)*, M. oleifera* (0.350)*, D. alba* (0.256 cm) and *C. longa* (0.168 cm). Green house experiment revealed that the best result was produced by the combination of Flare and *N. tabacum* by indicating lowest disease incidence (32.27%) at all the tested concentration. Same results were obtained in field experiment, where the lowest disease incidence (40.41%) was recorded when the ,Flare and *N. tabacum* were applied in combination although it was higher then green house. This study concludes that *N. tabacum* and Flare are better option against bacterial disease development and even their combination is more significant lowering the bacterial blight disease incidence on cotton. Selection of suitable formulation and method of application could be the future aspects of plant product especially *N. tabacum* related research.

Key words: Bacterial blight, Cotton, *Xanthomonas citri* pv. *malvacearum,*Chemicals, Plant extracts, Flare, Nicotiana tabacum

1- INTRODUCTION

Cotton (*Gossypium hirsutum*) is the most important fiber crop of Pakistan which plays a significant role in the economy of country. It is grown in temperate and subtropical regions of the world including Pakistan (Smith, 1999). It is cultivated on an area of 33.1 million hectares in the world while on 3.0 million hectares in Pakistan during 2013-14 with the production of 116.7 and 9.5 million bales respectively (Johnson *et al.,* 2014). Its present area, production and yield in

the world depicted that Pakistan is the fourth largest producer of cotton after China, USA and India (Hanif and Jafri, 2008). Cotton is unique among agricultural crops because it provides food, edible oil, fiber and other byproducts for livestock food (Chaudhry and Guitchounts, 2003). Bacterial blight of cotton caused by *Xanthomonas axonopodis* pv. *malvacearum (Xam)* is one of the serious diseases of cotton (Saha *et al.,* 2001) 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). About 37- 40% yield losses were observed in Pakistan, Faisalabad district (Bhutta and Bhatti 1983; Khan *et al.,* 1999). This bacterium enters in healthy plants through stomata or wounds, initiates infection process during any stage of the growth period and producing typical symptoms including small, irregular and dark water socked spots on lower epidermis of leaves that later becomes dark brown (Liberato *et al*., 2007).

Use of resistant varieties is genuine method for the management of disease because it makes possible to avoid other management strategies like acid-delinting of seed, use of chemicals and the destruction of diseased plant residues followed by tillage operations (Thaxton and El-Zik, 2001; Turkkan and Dolar, 2009). Many other stratigies that are used for the management of bacterial blight of cotton are being applied as an alternate source of chemicals for the management of disease but absence of durable resistance in varieties, treatment with chemicals is recommended quick action and readily availability (Singh *et al*., 2007). Numerous reports on the use of active ingredeints from plants in place of chemicals are available owing to there non-phytotoxic nature, more systemic and easily biodegradable behavior (Gottlieb *et al*., 2002). This led to screen out a large number of plants for antibacterial activity against important seed borne phytopathogenic *Xanthomonas* pathovars under *in vitro* and *in vivo* conditions, with an ultimate aim of developing plant based formulations for disease management (Kiran and Raveesha, 2006). Although chemicals are easily available, direct and rapid in action which consiquently reduce the losses caused by this disease but the continuous dependence on pesticides has proven increasingly unsuitable by causing environmental pollution and degradation due to their judicious use. Therefore, it is dire need to conduct *in-vitro and in-vivo* and research for an apt management of bacterial lignt of cotton through chemicals and plant extracts. The current research was conducted with an ultimate objective of integrated management of this disease, to produce the optimum crop yield of high quality at minimum cost as well as to preserve the environment.

2-MATERIALS AND METHODS

2.1- Isolation 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 contained 10^7cfu ml^{-1} 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°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).

2.2- Pathogenicity test

To fulfill Koch's postulates, seeds of susceptible variety (Bt-FH 142) of cotton were sown in pots (12×9 cm dia.) which were filled with sterilize soil (2kg/pot) and these pots were transferred to green house by adopting CRD Design. Recommended agronomic practices were followed time to time and pots were watered once in a day. Plants at the age of 5-6 weeks were inoculated by using sterilized syringe (24 Gauge needle size) filled with 20 µl (local isolates) bacterial suspension, contained 107-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. 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.

2.3- Laboratory evaluation of different chemicals against *X. citri* pv. *malvacearum*

Five chemicals i.e Flare, Agrimycine, Copper oxychloride, plant protector and Mancozeb were used in the research Table-1.These chemicals were evaluated against the 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%, 0.30% and 0.35% concentrations. Bacterial culture was multiplied by adding freshly growing aqueous bacterial suspension (1×10^7 cfu/ml) to the Luke warm NA media in a flask (ASTM- E288). It was shaken well and poured in plates (90×15 mm). These plates were wrapped with clingfilm and incubated at 30°C. When bacterial colonies were formed, wells of 6 mm 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 with clingfilm and incubated at 30°C. The plates with no chemicals and only having 20 µl sterile water were considered as control against treatments. Data regarding inhibition zone was recorded after three days of interval.

Table 1 Chemical with their active ingredients used against bacterial blight of cotton

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

2.4- Laboratory evaluation of different plant extracts against *X. citri* pv. *malvacearum*

Preparation of plant extracts

Five plants extracts i.e. *Nicotiana tabacum*, *Azadirachta indica*, *Moringa oleifera*, *Datura alba* and *Curcuma longa*) were used in this experiment Table-2. Anti-bacterial efficacy of these five plants extracts was evaluated against bacterial colony growth. Fresh leaves of each plant were taken at flush stage, thoroughly washed with tap water and sun dried for two days. 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, supernatant 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°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. Five plant extracts i.e., *N. tabacum*, *A. indica*, *M. oleifera*, *D. alba* and *C. longa* 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 (90 × 15 mm). These plates were wrapped with clingfilm and incubated (SANYO, 175 M) at 30°C. After solidification of culture media, wells of 6 mm 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, 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.

Table 2 Plants extracts used for their antibacterial potential against bacterial blight of cotton

Common Name	Botanical Name	Active ingredients	Plant Part used	Authority
Sohanjna	*Moring oleifera*	Saponins (Abalaka *et al.*, 2012)	Leaves	Lamarck
Neem	*Azadirachta indica*	Nimbine (Terpenoids) (Lokanadhan *et al.*,2012)	Leaves	A. Jussieu
Datura	*Datura alba*	Scopolamine (Okwu and Igara, 2009)	Leaves	C. Linnaeus
Tobacco	*Nicotiana tabacum*	Nicotine (Bakht *et al.*,2012)	Leaves	C. Linnaeus
Turmeric	*Curcumba longa*	Curcumin (Moghadamtousi *et al*., 2014)	Roots	C. Linnaeus

2.5- Evaluation of Flare and *N. tabacum* against bacterial blight of cotton in greenhouse

In labarotary, Flare and *N. tabacum* expressed the most effective results against *Xcm*. So these were also evaluated in 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, contained 10^7cells/ml. Flare and *N. tabacum* alone and in combination, were sprayed against disease, with three 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

2.6- Evaluation of Flare and *N. tabacum* against bacterial blight of cotton in field

To evaluate the efficacy of Flare and *N. tabacum* in field conditions, Seeds of susceptible variety (Bt-FH 142) were sown with 30 cm plant 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 5-6 weeks, when typical symptoms of blight appeared on the leaves, (water soaked lesions) treatments i.e. Flare at the rate of 0.75%, 0.80% and 0.85% and *N. tabacum* at the rate of 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

2.7- Statistical analysis

The statistical analysis was performed by using SAS/STAT statistical software version 6 (SAS Institute, 1990). Data was analyzed statistically and means were compared by using Least Significant Difference (LSD) test (Steel *et al*., 1997).

3- RESULTS

3.1- Impact of chemicals on *X. citri* pv. *malvacearum* growth

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 3). In the interaction between treatments and concentrations maximum inhibition zones 1.76 cm was produced by Flare at 0.35% followed by 1.69 cm at 0.30% and 1.63 cm at 0.25% concentration respectively. Copper oxy chloride showed minimum inhibition zones 0.90 cm, 0.95 cm and 1.01cm at 0.25%, 0.30% and 0.35% respectively, plant

protector expressed 1.40 cm, 1.47 cm, 1.55 cm, Mancozeb 1.24 cm, 1.29 cm, 1.34 cm. Agrimycine 1.10 cm, 1.17 cm and 1.18 cm inhibition zone at 25 %, 30 % and 35 % concentrations respectively as compared to control (Table.3).

Table 3. Impact of different chemicals in three different concentrtions on the growth of *X. citri* pv. *malvacearum*.

Treatments	**Inhibition zones** (cm)			**Mean Inhibition zones** (cm)
	0.25%	**0.30%**	**0.35%**	
Flare	1.63c	1.69b	1.76a	1.693a
Plant Protector	1.40f	1.47e	1.55d	1.473b
Mancozeb	1.24i	1.29h	1.34g	1.290c
Agrimycin	1.10k	1.17j	1.18j	1.150d
Copper oxy chloride	0.90n	0.95m	1.01i	0.953e
Control	0.00 o	0.00 o	0.00 o	0.000f

Means in the column and rows with the dirrefent letters are significantly different at ($p<0.05$)

3.2- Impact of plant extracts on *X. citri* pv. *malvacearum*

Maximum inhibition zone was produced by *Nicotiana tabacum* 0.65 cm followed by *Azadirachta indica* 0.48 cm, *Moringa oleifera* 0.35 cm, *Datura alba* 0.25 cm and *Curcuma longa* 0.17 cm as compared to control (Table 4). In the interaction between treatments and concentrations, maximum inhibition zone 0.70 cm was produced by Tobacco at 20 % 0.65 cm at 15 % and 0.60 cm at 10 % respectively. *C. longa* exhibited minimum inhibition zones of 0.18 cm, 0.17 cm and 0.15 cm, *A. indica* expressed 0.43 cm, 0.49 cm, 0.54 cm, *M. oleifera* 0.32 cm, 0.35 cm, 0.38 cm, *D. alba* 0.22 cm, 0.26 cm and 0.28 cm inhibition zone at 10 %, 15 % and 20 % concentration respectively as compared to control (Table 4).

Table 4 Impact of different plant extracts in three different concentrtions on the growth of *X. citri* pv. *malvacearum*

Treatments		**Inhibition zones** (cm)			**Mean inhibition zones** (cm)
		10%	**15%**	**20%**	
N. tabacum	(Tobacco)	0.600c	0.650b	0.700a	0.650a
A. indica	(Neem)	0.430f	0.490e	0.540d	0.486b
M. oleifera	(Moringa)	0.320i	0.350h	0.380g	0.350c
D. alba	(Datura)	0.227 l	0.260k	0.283j	0.256d
C. longa	(Turmeric)	0.150 o	0.170n	0.186m	0.168e

Control	0.000p	0.000p	0.000p	0.000f

Means in the column and rows with the dirrefent letters are significantly different at ($p<0.05$)

3.3- Impact of Flare and *Nicotiana tabacum* against bacterial blight of cotton in greenhouse

Minimum disease incidence 32.27 % was observed when Flare and *N. tabacum* were applied in combination, followed by Flare 36.91 % and *N. tabacum* 41.60 % as compared to control (Table 5). In interaction between treatments and concentration *N. tabacum* expressed 37.06 %, 41.83 % and 45.90 % disease incidence at 40 %, 35 % and 30 % concentration and Flare showed 32.50 %, 37.30 % and 40.93 % disease incidence at 0.60 %, 0.55 % and 0.50 % concentration. 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 5). In interaction between treatments, concentrations and days, maximum reduction in disease was observed (39.40 %, 37.50 % and 32.60 %) after seven days, 35.40 %, 32.60 %, 28.40 % after fourteen days, and 33.50 %, 28.50 % and 22.60 % after twenty one days when Flare and *N. tabacum* was applied in combination 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 at 0.30 %, 0.35 % and 0.40 % concentration. 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 at 30 %, 35 % and 40 % as compared to control (Table 6).

Table 5 Impact of Flare and *Nicotiana tabacum* and their mixture in three different concertations on bacterial blight of cotton in greenhouse

Treatments	**Disease incidence (%)**			**Mean Disease incidence (%)**
	C1	**C2**	**C3**	
*Flare +*N. tabacum*	36.10i	32.86j	27.86 l	32.27a
**Flare	40.93f	37.30g	32.50k	36.91b
****N. tabacum*	45.90d	41.83e	37.06h	41.60c
Control	74.00c	74.66b	75.80a	74.82d

* C1=0.50% Flare + 30% *N. tabacum,* C2= 0.55% Flare + 35% *N. tabacum,* C3= 0.60% Flare + 40% *N. tabacum;* ** C1= 0.50%, C2= 0.55%, C3= 0.60%; *** C1= 30%, C2= 35%, C3= 40%. Means in the column and rows with the dirrefent letters are significantly different at ($p<0.05$)

Table.6 Impact of Flare and *Nicotiana tabacum* and their mixture in three different concertations on three cosective weeks on bacterial blight of cotton in green house

Treatments	Disease incidence (%)								
	Week 1			Week 2			Week 3		
	C1	C2	C3	C1	C2	C3	C1	C2	C3
*Flare+*N.tabacum*	32.60	37.50	39.40	28.40	32.60	35.40	22.60	28.50	33.50
**Flare	36.50	41.60	44.20	33.50	37.40	40.10	27.50	32.90	38.50
****N.tabacum*	41.80	45.20	48.40	37.90	42.80	45.80	31.50	37.50	43.50
Control	68.40	69.30	70.40	74.60	75.40	76.30	79.00	79.30	80.70

* C1=0.50% Flare + 35% *N. tabacum,* C2= 0.55% Flare + 35% *N. tabacum,* C3= 0.60% Flare + 40% *N. tabacum;* ** C1= 0.50%, C2= 0.55%, C3= 0.60%; *** C1= 30%, C2= 35%, C3= 40%.

3.4- Impact of Flare and *Nicotiana tabacum* against bacterial blight of cotton in field

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 7). In interaction between treatments and concentration, *N. tabacum* expressed (54.60%), (50.26%) and (46.36%) disease incidence at 45 %, 50 % and 55 % concentration, Flare showed (49.70%, 46.46% and 41.06%) disease incidence at 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 7). In interaction between treatments, concentrations and days, maximum reduction in disease (46.50%, 46.30%, 39.40%) was observed after seven days, (44.30%, 41.60% and 36.50%), fourteen days, and (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 at 0.75%, 0.80% and 0.85% concentration. 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 at 45%, 50% and 55% concentration as compared to control (Table 8).

Table 7 Impact of Flare and *Nicotiana tabacum* and their mixture in three different concertations on bacteraial blight of cotton in field experiment

Treatments	Disease incidence (%)			Mean Disease incidence (%)
	C1	C2	C3	
*Flare +*N. tabacum*	44.23h	41.93i	35.06k	40.41d
**Flare	49.70f	46.46g	41.06j	45.74c
****N. tabacum*	54.60d	50.26e	46.36g	50.41b
Control	80.96c	83.43b	84.40a	82.93a

* C1= 0.75% Flare + 45% *N. tabacum,* C2= 0.80% Flare + 50% *N. tabacum,* C3= 0.85% Flare + 55% *N. tabacum;* ** C1= 0.75%, C2= 0.80%, C3= 0.85%; *** C1= 45%, C2=50%, C3=55%. Means in the column and rows with the dirrefent letters are significantly different at ($p<0.05$)

Table.8 Impact of Flare and *Nicotiana tabacum* and their mixture in three different concertations on three cosective weeks on bacteraial blight of cotton in filed

Treatments	Disease incidence (%)								
	Week 1			Week 2			Week 3		
	C1	C2	C3	C1	C2	C3	C1	C2	C3
*Flare + *N.tabacum*	39.40	46.50	46.30	36.50	41.60	29.30	44.30	37.70	42.10
** Flare	45.70	49.60	52.50	41.60	46.10	35.90	48.90	43.70	47.70
****N. tabacum*	51.10	53.90	56.70	47.70	50.30	40.30	55.10	46.60	52.00
Control	76.70	77.60	78.60	80.70	84.40	85.50	85.70	88.30	88.90

* C1= 0.75% Flare + 45% *N. tabacum,* C2= 0.80% Flare + 50% *N. tabacum,* C3= 0.85% Flare + 55% *N. tabacum;* ** C1= 0.75%, C2= 0.80%, C3= 0.85%; *** C1= 45%, C2=50%, C3=55%.

4- DISCUSSION

The most suitable, economical, safe, reliable and practicable management of bacterial blight of cotton, is the use of resistant varieties. 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. 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, they 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 present esearch above plant

extracts were 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* in lab.conditions by using inhibition zone technique. Among plant extracts *N. tabacum* expressed maximum inhibition zone, (0.650cm). Then Flare and *N. tabacum* were evaluated in 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 in 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.

The management of bacterial blight of cotton is achievable by plant extracts, which we selected based on their well-established antibacterial activity as documented by various researchers *Moringa oleifera* (saponins) Abalaka *et al.,* 2012, *Azadirachta indica* (Nimbine) Lokanadhan *et al.,*2012, *Datura alba* (scopolamine) Okwu and Igara, 2009, *Nicotiana tabacum* (Nicotine) Bakht *et al.,* 2012 and *Curcuma longa* (Curcumin) Moghadamtousi *et al.,* 2014 as antibacterial agent gave a casement for future and selection of suitable formulation and method of application of plant product *in vitro,* green house and *in vivo* especially *N. tabacum* related research.

5- CONCLUSIONS

Flare and *N. tabacum* expressed maximum inhibition zone in lab and minimum disease incidence under greenhouse and field conditions. The use of mixture of Flare and *N. tabacum* showed the best results in disease reduction. Our results urges the need of the effective use of mixture of botanicals with chemicals which can reduce the dose of pesticide. The use of chemicals pollute our environment continuously, so it is essential to exploit antibacterial potential different plant extracts.

LITERATURE CITED

Abalaka, M. E., S. Y. Daniyan, S. B. Oyeleke, S. O. Adeyemo. 2012. The Antibacterial Evaluation of *Moringa oleifera* Leaf Extracts on Selected Bacterial Pathogens. J. Microb. Res. 2(2) 1-4

Allen, S. J. and K. L. D. West. 1991. Predominance of race 18 of Xanthomonas campestrispv. malvacearumon cotton in Australia. Plant Dis. 75: 43-44.

Anwar, F., M. Ashraf and M. I. Bhanger. 2005. Inter provenance variation in the composition of Moringa oleifera oilseeds from Pakistan. JAOCS. 82(1): 45-51.

Bakht, J., Azraand M. Shafi. 2012. Antimicrobial activity of *Nicitiana tabacum* using different solvents extracts. Pak. J. Bot. 44(1): 459-463.

Barry, L. A., M. B. Coyle, C. Thornsberry, E. H. Geriach and R. W. Hawkinsons. 1979. Methods of Measuring Zones of Inhibition with the Bauer-Kirby Disk Susceptibility Test. J. Clin. Microb. 10(6): 885-889.

Bruinsma, J. 2003. World Agriculture towards 2015/2030. An FAO Perspective. Earth Scans Publications Limited. London.

Cao, K. Q., H. C. Ariena and D. V. Bruggen. 2001. Inhibitory efficacy of several plant extracts and plant products on Phytophthora infestans. J. Agri. Uni. Hebei. 24(2): 90-96.

Chattannvar, S. N., A. S. Sharmila and B. M. Khadi. 2006. Field evaluation of fungicides against angular leaf spot of cotton. J. Cotton Res. Dev. 20: 145-146.

Chaudhry, M. R. and A. Guitchounts. 2003. Cotton Facts. Technical paper No. 25 of the common fund for commodities. International cotton advisory committe. pp. 158.

Chidambaram, P. and A. Kannan. 1989. Grey mildew of cotton. Tech. Bull. Central Inst. cotton Res. regional station coimbatore India. Eur. J. Plant Pathol. 103: 687-693.

Gottlieb, O. R., M. R. Borin and N. R. Brito. 2002. Integration of ethnobotany and phytochemistry dream or reality. Phytochemistry. 60: 145-152.

Hanif, M. N. and S. K. Jafri. 2008. Financial Development and Textile Sector Competitiveness. A case study of pakistan. SBP Working Paper Series No. 20.

Harris, R. and L. Sommers. 1968. Plate-dilution frequency technique for assay of microbial ecology. Applied Microbiology 16: 330-334.

340 Jagtap G. P., A. M. Jangam and U. Deya. 2012. Management of bacterial blight of cotton caused
341 by Xanthomonas axonopodis pv. malvacearum. Sci. J. Microbiol. 1(1): 10-18.
342 Johnson, J., S. M. Donald, L. Meyer, B. Norrington and C. Skelly. 2014. The world and United
343 States cotton outlook. Agric. Outlook Forum. USDA. pp. 1-16.
344 Kiran, B. and K. A. Raveesha, 2006. Antifungal activity of seed extract of Psoralea corylifolia L.
345 Pl. Dis. Res. 20(2): 213-215.
346 Liberato, J. R., S. J. Allen, N. D Suassuna, Y. R Mehta, S. R koenning and R. G. Shivas.
347 2007. Bacterial blight of cotton Xanthomonas axonopodis pv. malvacearum. Pest and
348 diseases image library- PaDIL. Victoria, dez. 18. pp.
349 Lokanadhan, S., P. Muthukrishnan and S.Jeyaraman 2012. Neem products and their agricultural
350 applications. J. Biopest, 5: 72-76.
351 Moghadamtousi, S. Z., H. A. Kadir, P. Hassandarvish, H. Tajik, S. Abubakar and K. Zandi.
352 2014. A Review on Antibacterial, Antiviral, and Antifungal Activity of Curcumin.
353 BioMed. Res. Int. Volume 2014, Article ID 186864, 12 pages
354 http://dx.doi.org/10.1155/2014/186864
355 Okwu, D.E. and E.Igara. 2009. Isolation, characterization and antibacterial activity of alkaloid
356 from *Datura* metel Linn leaves. Afr. J. Pharm. Pharmacol. 3(5) pp. 277-281.
357 Okwu. D. E and E. C. Igara. 2014. Isolation, characterization and antibacterial activity of
358 alkaloid from Datura metel Linn leaves. Afri. J. Pharm. Pharmacology. 3(5): 277-281.
359 Ramapandu, S., K. Sitaramaiah, K. Subbarao. and M. P. Prasadarao. 1979. Screening of cotton
360 germplasm against bacterial blight caused by Xanthomonas citri pv.malvacearum. Indian
361 Phytopathol. 32: 486-487.
362 Saha, S., R. P. Singh, J. P. Verma and J. Jayaraman. 2001. Population dynamics of cotton
363 phylloplane bacteria antagonistic towards Xanthomonas axonopodis pv. malvacearum.
364 Indian Phytopathol. 54: 409-413.
365 Sajid, M., A. Rashid, M. Ehetisham-ul-haq, M. T. Javed, H. Jamil, M. Mudassir, M. Farooq, F.
366 Ahmad, M. Latif, M. A. Chohan, M. Ahmad. A. Kamran. 2013. In vitro evaluation of
367 chemicals and plant extracts against colony growth of Xanthomonas axonopodis pv.
368 malvacearum causing bacterial blight of cotton. Eur. J. Exp. Biol. 2013. 3(1): 617-621.
369 SAS Institute Inc. 1990. SAS/STAT User's guide, version 6.

370 Singh, A., S. S. L. Srivatava and M. Akram. 2007. Studies on bacterial leaf blight of cotton
371 (Gossypium spp.). Int. J. Sustain. Crop Prod. 2: 25-29.
372 Smith, W. C. 1999. Production statistics. Cotton Origin, history, Technology and production.
373 John Wiley and Sons. Inc. 435-449. pp.
374 Steel, R. G. D., J. H. Torrie and D. A. Dickey. 1997. Principles and procedures of statistics. a
375 biometrical approach. 3rd edi. McGraw Hill Pub. New York.
376 Thaxton, P. M., T. D. Brooks and K. M. E. Zik. 2001. Race identification and severity of
377 bacterial blight from natural infestations across the cotton belt. In: Proceeding Beltwide
378 cotton conference. Memphis. TN. pp 137-138.
379 Turkkan, M. and F. S. Dolar. 2009. Determination of pathogenic variability of Didymella rabiei
380 the agent of ascochyta blight of chickpea in Turkey. Turk. J. Agric. For. 33: 685-591.