Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

EVALUATION OF PLANT EXTRACTS AS BIOCONTROL AGENTS AGAINST *XANTHOMONAS AXONOPODIS* PV CITRI THE CAUSE OF CITRUS CANKER

Hafiz A.S. Tahir, Shahbaz T. Sahi, Amer Habib*, Imran U. Haq, Arbab Ahmad, Waqas Ashraf
Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan.

A B S T R A C T

Ten plant extracts; *Allium sativum L.*, *Allium cepa L.*, *Azadirachta indica*, *Capsicum Annum*, *Calotropisgi gantea* , *Dalbrgia sissoo* , *Eucalyptus camelduensis*, *Gardenia florida Melia azedarach* and *Zingiber officinalis* were tested *in vitro* at Standard dose S and S/2 against *Xanthomonas axonopodis pv. citri*. All the plant extracts at both the doses reduced the multiplication of *Xac* except Zingiber *officinalis* and *Capsicum Annum. Allium sativum*and *Azadirachta indica*at doses (S, S/2) exhibited statistically significant inhibition zones as compared to other treatments, followed by *Dalbergia sisso*. *Gardenia florida* and *Melia azedarach* which produced similar effect and raked 4th. *Calotropisgi gantea* ranked 5th while Eucalyptus *camelduensis* (Sufeda) and Allium *cepa L.* inhibited the growth of *Xac*at a minimum level. *Capsicum Annum* and *Zingiber officinalis* produced no inhibition zone and showed similar results as a control. All the plant extracts at standard doses reduced the multiplication of *Xac* maximumas compared to S/2 except Allium *cepa L., Dalbrgia sissoo*, *Eucalyptus camelduensis* and *Melia azedarach* which showed similar effects at both doses. The most effective treatments proved *in vitro* then tested after combining with streptomycin sulphate *in vitro* and found that Streptomycin sulphate 1 % reduced the multiplication of *Xac* significantly, followed by Streptomycin sulphate in combination with Allium *sativum* L. and Streptomycin sulphate in combination with *Azadirachta indica* at standard dose. These treatments then tested in greenhouse the results confirmed the findings of *invitro* trials. There is a need to promote research in the field of plant extracts usage as biocontrol agents and find out plants having toxicity against plant pathogens, as the plant extracts are environment friendly and cheaper.

Keywords: Citrus canker, plant extract, Xanthomonas *axonopodis pv. citri*, Streptomycin sulphate.

INTRODUCTION

Citrus is the largest fruit crop worldwide that is widely consumed both as fresh fruit and juice with high vitamin C content and antioxidental potential (Gorinstein *et al*., 2001). The area under citrus cultivation lies predominantly within tropical and subtropical regions covering around 137 countries (Ismail and Zhang, 2004). South-East Asia is believed to be the origin of citrus at least 4000 years BC by most researchers but exact center of its origin is not clear (Davies and Albrigo, 1994). Citrus fruits have been reported to prevent liver, lungs, and skin diseases, birth defects and contribute to a balance and healthy life style (Swarmura, 2000). Moreover, several studies have shown that citrus fruits and their components are protective against a variety of human cancers (Tian *et al*., 2001; Manthey and Guthrie, 2002; Rafter, 2002; Kim *et al*., 2005). Citrus is the leading fruit crop in Pakistan and its export is a good source of foreign exchange. Citrus industry in Pakistan has a wide distribution covering an area of 193977 thousand hectares with annual production of about 2147340 tones (GOP, 2012). In Punjab Province Citrus ranks second after Guava occupying an area of 183568 hactares with annual production of 2076831 tones (GOP, 2012).

Citrus plant is attacked by number of fungal, bacterial, viral diseases like citrus canker, gummosis, citrus decline, Citrus tristiza virus, and greening etc, but citrus canker caused by the bacterium *Xanthomonas axonopodis* pv. *citri*. (Hasse) Dows, is probably the worst enemy to the citrus plantations (Sahi *et al.,* 2007). It is a common and widely distributed disease of Indo-Pak sub-continent (Arif *et al.*, 1962) and most commonly occurs

* Corresponding Author:
Email: amer.habib@uaf.edu.pk

in citrus growing regions of the Punjab on most of the commercially important citrus cultivars (Hafiz & Sattar, 1952, Atiq *el al.,* 2007.). The disease is present in Pakistan, India, China, Japan, South-East Asia and the islands of Indian Ocean (Das, 2003).

Symptoms are usually developed on all above ground parts, leaves, twigs and fruits in the form of conspicuous raised necrotic lesions on diseased plants. These corky lesions can be felt by drawing the fingers over the surface of infected tissues. Circular lesions become raised and blister-like, on leaves, stems, thorns and fruit, which later on turn into white or yellow spongy pustules. Pustules may coalesce to split the epidermis on stem along the stem length, and girdling of young stems may occur. Older lesions on leaves and fruit have developed more elevated margins surrounded by a yellow chlorotic halo and a sunken center which are especially noticeable on fruits. As the lesions do not penetrate far into the rind therefore internal quality of fruit is not affected. Defoliation, die-back, deformation of fruit and premature fruit drop is resulted in case of severe infection (Rossetti, 1977; Civerolo, 1981; Chand and Pal, 1982; Stall and Seymour, 1983).

The use of chemicals is the best strategy for preventing pre and post-harvest crop losses caused by insect pests and diseases, but due to their residual toxicity, excessive use of pesticides are causing very serious health hazardous effects on human being, animal life and the whole environment. (Ferrer and Cabral, 1991; Gassner *et al*., 1997; Andrea *et al*., 2000; Harris *et al*., 2001; Campos *et al*., 2005). Due to extensive and repeated use of pesticides resistance has been developed against synthetic pesticides in many phytopathogenic bacteria (Sundin *et al*., 1994; Clarke *et al*., 1997; Williams and Heymann, 1998; White *et al*., 2002).

To avoid or reduce the deleterious effects of synthetic pesticides on ecosystem or the environment, it is very necessary to find out alternative approaches for the management of plant pathogenic microorganisms (Hostettmann and Wolfender, 1997). Green plants as an alternate to synthetic pesticides are proved to be effective chemotherapeutants and can be used as valuable source of natural pesticides (Balandrin *et al*., 1985; Mahajan and Das, 2003). The use of several plant by-products, which posses antimicrobial properties, on several pathogenic bacteria and fungi has been reported by many Researchers (Dorman and Deans, 2000; Parameswari and Latha, 2001; Rath *et al*., 2001; Britto and Senthilkumar, 2001; Bylka *et al*., 2004; Shimpi and Bendre, 2005; Kilani, 2006). The purpose of this study is to find out best suitable plant extracts and their combination with toxicants against *Xanthomonas axonopodis* pv. *citri*.

MATERIALS AND METHODS

Isolation of *Xanthomonas axonopodis*: From the citrus orchards, the leaves showing typical symptoms of disease were collected in polythene bags and brought to the Bacteriology lab of department of Plant Pathology, University of Agriculture Faisalabad, for isolation of the bacterium by using dilution plate technique (Kiraly *et al.,* 1974) and petri dishes with different dilutions were kept at 30 °C. The bacterium was identified by morphological and biochemical characteristics (Breed *et al*., 1957). The stock culture of the bacterium was maintained on nutrient agar in culture tubes at 4oC in refrigerator.

Pathogencity test: The isolated bacterium was examined for its pathogenicity on healthy citrus. One year old citrus plants were obtained from nursery of Department of Horticulture University of Agriculture Faisalabad. These plants were transplanted in pots containing field soil previously sterilized with Formalin (1:320). The bacterium from stock culture was multiplied on nutrient agar by incubating it for 48 hours at 30°C. Aqueous suspension of the bacterium having a concentration of approximately 10^8 cells/ml was prepared by plate count method.

Just before the inoculation, plants were irrigated, and covered with polythene bags for 2 hours to allow the stomata to open to the maximum (Weindling, 1948, Gunn, 1962). The abaxial surface of the leaves was inoculated by spraying machine at pressure of 1.1 kg/cm^2 until the tissue showed water soaking. In control the plants were sprayed only with sterilized water. The inoculated and control consisted of 3 replications. The plants were kept under observation for two weeks in green house and symptoms, if any were recorded. Isolation of bacterium from diseased tissue was carried out in the same way as mentioned above and the morphological characteristics (Breed *et al*, 1989) of the isolates were compared with the culture of bacterium inoculated.

Preparation of plant extracts: Fresh (leaves and Rhizome /Bulb/Branches) plant material of 10 plants were collected. For preparation of aqueous extracts of above motioned plant, 75 g fresh material was macerated separately with 25 ml sterilized distilled

water in a blender. The macerates were first filtered through four layered sterilized muslin cloth and then filtered through whatman filter paper no. 4. The extract obtained through this procedure were considered standard (S) arbitrary (Ilyas *et al*.,1997) and were stored in deep freezer for cold sterilization and for further studies in the laboratory.

Antibacterial activity assay of plant extracts: Bacterial suspension containing concentration of 10^8 cfu/ml of *Xac*from 48 hours old culture was mixed with the Luke warm nutrient agar @ 1ml /25ml of media and poured into sterilized petri dishes of 9 cm diameter. These petri dishes were gently shaken to mix the bacteria uniformly in the nutrient agar and allowed to solidify.

Wells (1cm) were made in the centre of petri dishes with the help of sterilized cork borer (1cm) and plant extracts at standard doses (S) and S/2 were poured into these wells with the help of sterilized syringes. All these petri dishes were placed in refrigerator at 4°C for 24 hours and transferred to an incubator at 28±2°C for 48 hours and inhibition zones if any were recorded. The experiment was conducted in completely randomized design with 3 replications in each treatment. Control was similarly carried out, except, sterilized water instead of plant extract.

Evaluation of effective toxicants and plant extracts against *Xac.* in Green House: One year old healthy rough lemon (Khatti) plants which are highly susceptible to *Xanthomonas axonopodis pv citri* were taken from the research area, Department of Horticulture, University of Agriculture Faisalabad. These plants were transplanted to pots (one plant/pot) containing sterilized soil. Providing the conventional agronomic practices for 15 days, the most effective plant extracts and toxicants alone and in different combinations were sprayed on the abaxial surface of the citrus plants. There were three replications for each treatment. The plants were irrigated and covered with polythene bags for 2 to 2 ½ hours to provide artificial condition of humidity. Then aqueous suspension of the bacterium prepared from 48 hours old actively growing culture of *Xac* was inoculated with the help of a spraying gun which could produce a pressure of 1.1 Kg/cm^2. The plants inoculated with pathogen only served as a control. Data regarding the disease intensity were recorded starting from 10th day to 45 days at 5 days interval according to scale used by Sahi *et al.,* 2007 adopted from Horsfall and Heuberger, (1942).

0 = Free from infection
1 = Traces to 25% leaf area killed
2 = 26-50% leaf area killed
3 = 51-75% leaf area killed
4 = 76-100% leaf area killed

Table 1: Different plant parts used to prepare plant extracts.

No	Name of Plant	Plant Part
1	*Allium cepa.* L. *(*Onion)	Bulb
2	*Allium sativum* L. (Garlic)	Rhizome
3	*Azadirachtaindica* L. (Neem)	Leaves/ Twigs
4	*Calotropisgigantea* L.(Aak)	Leaves/ Twigs
5	*Capsicum annum* L. (Chilli)	Fruit
6	*Dalbergiasissoo*Roxb. (Shishum)	Leaves/ Twigs
7	*Eucalyptus camelduensis*Dehnh. (Sufeda)	Leaves/ Twigs
8	*Gardenia florida*J.Elis. (Gardenia)	Leaves/ Twigs
9	*Melia azedarach* L. (Bakayn)	Leaves/ Twigs
10	*Zingiberofficinaeles* Roscoe. (Ginger)	Rhizome

RESULTS

In vitro evaluation of plant extracts against Xanthomonas axonopodis pv. citri.All the plant extracts at both concentrations viz., standard dose S and S/2 reduced the multiplication of Xac significantly as compared to control except *Zingiber officinalis* and *Capsicum annum*. *Allium sativum* and *Azadirachta indica* at both doses exhibited statistically significant inhibition zones as compared to other plant extracts.

The ten test plant extracts when compared on the basis of mean inhibition zone at both concentrations, *Allium sativum* produced max inhibition zone (2.44 cm) followed by *Azadirachta indica* and *Dalbergia sisso* (2.33 cm. and 1.99 cm). *Gardenia florida* and *Meliaazedarach* showed similar results statistically (1.79 and 1.80 cm respectively) followed by *Calotropisgigantea* (1.60 cm). *Allium sativum* and *Eucalyptuscamelduensis* produced least inhibition zone (0.33 cm and 0.63 cm). *Zingiber officinalis* and *Capsicum annum* produced no inhibition zone (Table 2).

Table 2. Evaluation of plant extracts as biocontrol agents against Xac after 48 hours.

No	Plant Extract	Inhibition zone at S (cm)	Inhibition zone at S/2 (cm)
1	*Allium sativum* L. (Garlic)	2.53a	2.35b
2	*Allium cepa L.* (Onion)	0.33k	0.32k
3	*Azadirachtaindica*(Neem)	2.27bc	2.20c
4	*Capsicum Annum (Chilli)*	0.10l	0.00m
5	*Calotropisgigantea* (Aak)	1.67h	1.53i
6	*Dalbrgiasissoo* (Shishum)	2.03d	1.95de
7	*Eucalyptus camelduensis* (Sufeda)	0.67j	0.60j
8	*Gardenia florida* (Gardenia)	1.87ef	1.72gh
9	*Melia azedarach* (Bakayn)	1.80fg	1.80fg
10	*Zingiber officinalis* (Ginger)	0.00m	0.00 m
11	Control	0.00m	0.00m
	LSD		0.0429

*Mean values sharing similar letters do not differ significantly as determined by LSD test at 5 % level of probability.

In vitro* evaluation of different combinations of effective plant extracts and antibiotics against *Xac. Streptomycin sulphate 1 % in combination with *Azadirachta indica* at standard dosereduced the multiplication of *Xanthomonas axonopodis* pv. *Citri* significantly by 3.62 cm inhibition zone as compared to untreated control followed by Streptomycin sulphate alone by 3.51 cm inhibition zone and Streptomycin sulphate in combination with *Allium sativum* L. with 2.55 cm inhibition zone statistically (Table 3). *Allium sativum* L. and *Azadirachta indica*alone at standard doses produced minimum inhibition zones of 2.55 cm and 2.28 cm respectively against *Xanthomonas axonopodis* pv. *citri*as compared to in combination with Streptomycin sulphate at 1 % concentration.

Table 3. In vitro Evaluation of different combination of effective plant extracts and antibiotics against Xac.

No	Treatments	Inhibition zones (cm)
1	*Allium sativum* L.	2.55d
2	*Azadirachtaindica*	2.28e
3	Streptomycin sulphate	3.51b
4	Streptomycin sulphate + *Allium sativum* L.	3.42c
5	Streptomycin sulphate + *Azadirachtaindica*	3.62a
6	Control	0.00f
	LSD	0.0354

Mean values sharing the same letter do not differ significantly at 5 % level of Confidence

Evaluation of the effective treatments against *Xac* under greenhouse conditions: Analysis of variance treatments applied to greenhouse grown citrus plants to control citrus canker disease revealed the significant interaction between treatments applied and the number of days. This indicated that there was significant difference in citrus canker disease development after 45 days when treatments were applied to citrus plants. One way interaction i.e treatment and days was also found statistically significant.

Streptomycin sulphate at 1 % concentration and its combination with *Allium sativum* L. and *Azadirachta indica* extracts at standard doses statistically produced different results but found effective to control citrus canker disease up to 59.43, 50.51 and 44.90 % respectively as compared to control after 45 days (Table 4).

Disease development and no of days are positively correlated i.e. as no of days increasing disease development maximum. There is highly significance among no of days at all treatment as R^2 = > 0.9 in all treatments (Figure1). Rate of disease development per day of Streptomycin sulphate, Streptomycin sulphate + *Allium sativum* L. and Streptomycin sulphate + *Azadirachtaindica*is minimum as value of X are .0248, .0219 and .0277 respectively while rate of disease development per day of *Allium sativum* L and *Azadirachta indica*is maximum as value of X are .0795, .0756 and .0756 respectively (Figure 2).

Table 4. Evaluation of the effective treatments against Xac. on greenhouse grown citrus plantsfor the control of canker disease.

No	Treatments	Days after inoculation						
		15	20	25	30	35	40	45
1	*Allium sativum*	2.75m	2.77m	3.12k	3.57i	4.10fg	4.62c	4.72c
2	*Azadirachtaindica*	2.87lm	2.92l	3.25jk	3.72h	4.12f	4.62c	4.75bc
3	Streptomycin sulphate	1.23z	1.33yz	1.45xy	1.58ux	1.68vwx	1.87stu	1.95rs
4	Streptomycinsulphate + *Allium sativum*	1.63u	1.72vu	1.82tuv	1.93rst	2.02qr	2.15q	2.30p
5	Streptomycinsulphate + *Azadirachtaindica*	1.78uv	1.87stu	1.97rs	2.15q	2.32op	2.45no	2.57n
6	Control	2.88lm	2.97l	3.35j	3.97g	4.30e	4.45d	5.15a
	LSD				0.0697			

Table 5. Mean values showing the effect of different plant extracts against Xac. under green house conditions.

No	Treatments	Disease incidence	Percent decrease over control
1	*Allium sativum* L.	3.65c	6.88
2	*Azadirachtaindica*	3.74b	4.59
3	Streptomycin sulphate	1.59f	59.43
4	Streptomycin sulphate + *Allium sativum* L.	1.94e	50.51
5	Streptomycin sulphate +*Azadirachtaindica*	2.16d	44.90
6	Control	3.93a	

LSD 0.0205

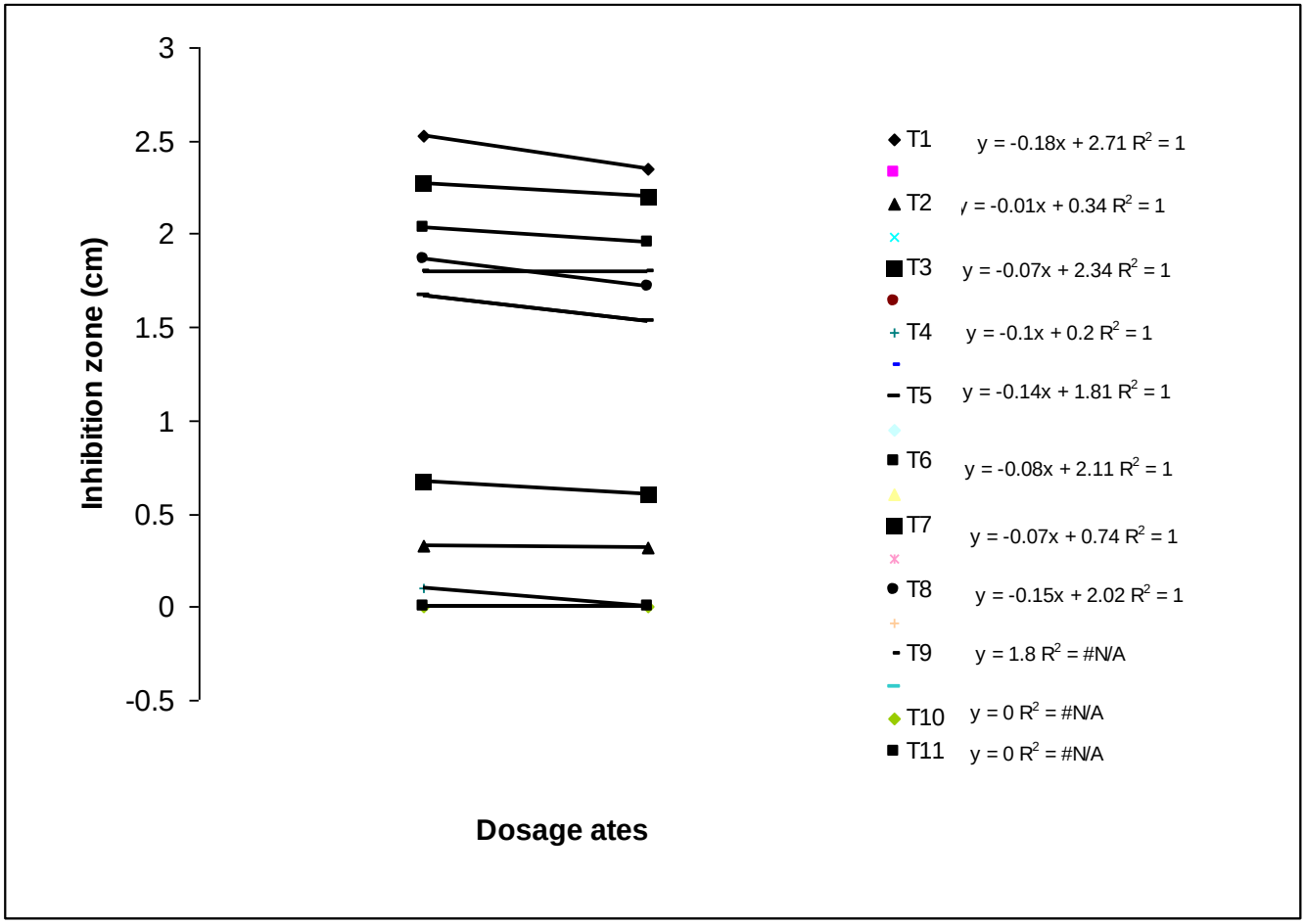

Figure 1. Effect of plant extracts at different doses against *Xac*

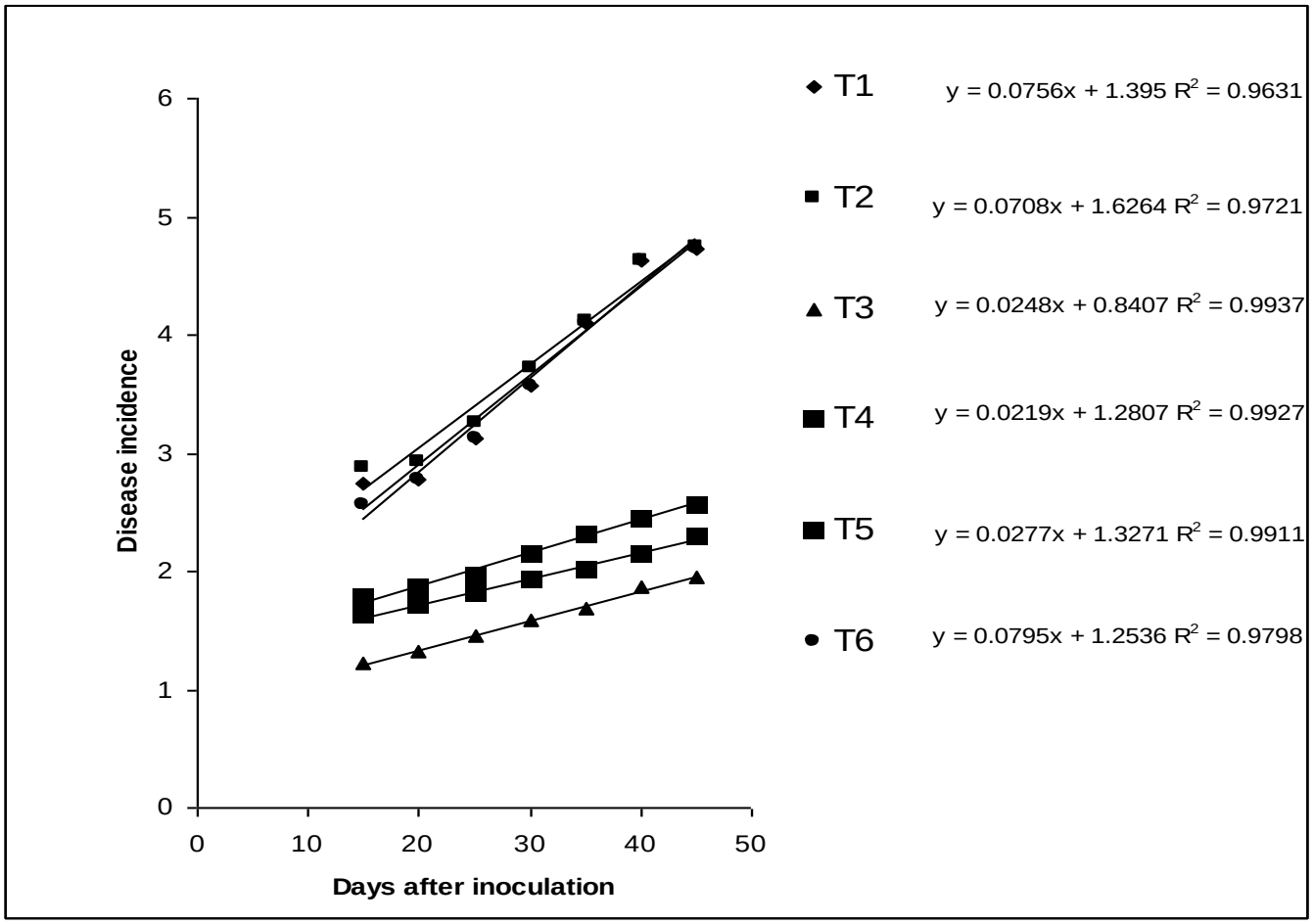

Figure 2 Effect of different treatments on greenhouse grown citrus plants for the control of canker disease.

DISCUSSION

Biopesticides can be used as an effective substitute for chemicals. The use of several plant by-products, which posses antimicrobial properties, on several pathogenic bacteria and fungi has been reported by many Researchers (Dorman and Deans, 2000; Parameswari and Latha, 2001; Rath *et al*., 2001; Britto and Senthilkumar, 2001; Bylka *et al*., 2004; Shimpi and Bendre, 2005; Kilani, 2006). The purpose of this study is to find out best suitable plant extracts and their combination with toxicants against *Xanthomonas axonopodis pv. citri.*

All the plant extracts at both the doses standard dose S and S/2 reduced the multiplication of *Xanthomonas axonopodis pv. citri.* Except Zingiber *officinalis* and *Capsicum annum* significantly as compared to control. *Allium sativum* and *Azadirachta indica*at both doses exhibited statistically significant inhibition zones as compared to other plant extracts. Dalbergia sisso inhibited the growth of *X. axonopodis* pv. *ctri* after *Allium sativum* and *Azadiracachta indica* followed by *Gardenia florida* and *Melia azedarach* which produced similar effect .*Calotropisgigantea* inhibited the growth of *X. axonopodis* pv. *citri* after *Gardenia florida* and *Melia azedarach* while *Eucalyptus camelduensis* (Sufeda) and *Allium cepa L.* inhibited the growth of *X. axonopodis* pv. *ctri* minimum . *Capsicum Annum* and *Zingiber officinalis* produced no inhibition zone and showed similar result as control. All the plant extracts at standard doses reduced the multiplication of *Xanthomonas axonopodis* pv. *Citri* maximum as compared to S/2 except *Allium cepa L., Dalbrgia sissoo*, *Eucalyptus camelduensis* and *Melia azedarach* which showed similar effects statistically at both doses. These results agreed to Moses and Chandramohan (1993) who tested 16 crude and centrifuged plant extracts in vitro against *X. campestris pv. citri*and reported that extracts of Neem cake, *Allium sativum* (garlic) showed best results.

Streptomycin sulphate at 1 % concentration and its combination with *Allium sativum* L. and *Azadirachta*

indica extracts at standard doses statistically produced different results but found effective to control citrus canker disease up to 59.43, 50.51 and 44.90 % respectively as compared to control after 45 days. *Allium sativum* L. and *Azadirachta indica* did not control canker disease on greenhouse grown citrus plants as in case of in vitro evaluation. This produced statistically different results than streptomycin sulphate alone and in combinations. These results agreed to Khan *et al*., (2003) who tested Streptomycine sulphate and garlic (Allium sativum) at different concentrations against the multiplication of *Xanthomonas campestris* pv. *citri* on nutrient agar using dual culture assays and found effective results, Garlic extract proved effective in *vitro* but was not so effective on the inoculated plants grown in green house. Ateeq, (2008) reported that under field conditions, combination of Streptomycin sulphate at 1 % and *A. indica* at 7.5 % , Streptomycin sulphate at 1 % proved to the most effective to control disease after 15 days of spray.

Plant extracts can be used as biocontrol agents in controlling plant diseases. There is a need to promote research in the field of plant extracts usage as biocontrol agents and find out plants having toxicity against plant pathogens, as the plant extracts are environment friendly and cheaper.

REFERENCES

Andrea, M.M., T.B. Peres, L.C. Luchini, and J. A. Pettinelli. 2000. Impact of long-term pesticide applications on some soil biological parameters. J. Env. Sci& Health 35: 297-307.

Arif, A.G., M. Akhtar and M. Ibrahim. 1964. Citrus diseases and their control. Punjab Fruit J., 26/27: 369-70.

Atiq, M., M.A. Khan and S.T. Sahi. 2007. Screening of citrus germplasm for the sources of resistance against citrus canker disease caused by *Xanthomonasaxonopodis*pv. *citri.* Pak. J. Phytopathol., 19(2): 222-226.

Awan, M.Z., M. Ishfaq, I.A. Hafiz, M. Ijaz and C.M. Ayyub. 1992. Incidence of citrus canker in Barani area at Chakwal. *In.* Proceed. 1st Inter. sem. citriculture in Pakistan.Dec. 2- 5.Uni. Agri. Faisalabad.

Balandrin, M.F., J. A. Klocke, E.S. Wurtele, and W.H. Bollinger, 1985. Natural plant chemicals: Sources of Industrial and medicinal materials. Science 228: 1154-1160.

Britto, S.J. and S.Senthilkumar, 2001.Antibacterial activity of *Solanum incanum*L. leaf extracts.Asian J. Microbl.Biotechnology &Env. Sci. 3: 65-66

Bylka, W., M. Szaufer-Hajdrych, I. Matalawska, and O. Goslinka, 2004. Antimicrobial activity of isocytisoside and extracts of Aquilegia vulgaris L. Letters in Applied Microbiology 39: 93-97.

Bylka, W., M. Szaufer-Hajdrych, I. Matalawska, and O. Goslinka, 2004. Antimicrobial activity of isocytisoside and extracts of *Aquilegia vulgaris*L. Letters in Applied Microbiology 39: 93-97.

Campos, A., C.M. Lino, S.M. Cardoso, and M.I.N. Silveira. 2005. Organochlorine pesticide residues in European sardine, horse mackerel and Atlantic mackerel from Portugal. Food Additives and Contaminants 22: 642-646.

Chand, J.N. and V. Pal, 1982.Citrus canker in India and its management. In: Problems of citrus diseases in India (S.P. Raychaudhuri and Y.S. Ahlawat, Eds.). Surabhi Printers and Publishers, New Delhi. pp. 21-26.

Chandramohan.1993. Potential of neem and other plant extracts for management of crop diseases. In: Neem for the management of crop diseases. (V. Maria pan ed) Associated pub. Co., New Delhi. Pp: 91-97 Citric, 1: 402-405.

Civerolo, E.L. 1981. Citrus bacterial canker disease: An overview. Proc.Intn. Soc. Citric., 1: 390-394.

Clarke, J.H., W.S. Clark and M. Hancock. 1997. Strategies for the prevention of development of pesticide resistance in the UK– Lessons for and from the use of herbicides, fungicides and insecticides. Pesti. Sci. 51: 391-397.326.

Das, A.K. 2003. Citrus canker – A review J. Appl. Hort. 5(1):52-60.

Davies, F.S., and L.G. Albrigo. 1994. Citrus. (Wallingford: CAB International).

Dorman, H.J.D. and S.G. Deans, 2000.Antimicrobial agents from plants antibacterial activity of plant volatile oils.Applied Microbiology 88: 308-316.

Dorman, H.J.D. and S.G. Deans, 2000.Antimicrobial agents from plants antibacterial activity of plant volatile oils.Applied Microbiology 88: 308-316.

Ferrer, A. and R. Cabral. 1991. Toxic epidemics caused by alimentary exposure to pesticides: a review. Food Additives and contaminants 8: 755-776.

Gassner, B., Wuthrich, A., J. Lis, G. Scholtysik, and M. Solioz. 1997. Topical application of synthetic Pyrethroids to cattle as a source of persistent

environmental contamination. Envl. Sci. & Health 32: 729-739.

Gorinstein, S., O. Martin-Belloso, Y. Park, R.Haruenkit, A. Lojek, I.Milan, Caspi, A. I. Libman, S. Trakhtenberg, 2001.Comparison of some biochemical characteristics of different citrus fruits. Food Chem. 74 (3), 309-315.

Govt. of Pak. 2012.Fruit, vegetables and condiments statistics of Pakistan 2011-12.Ministry of National Food Security & Research (Economic Wing), Islamabad, Pakistan: 6-9.

Gunn. R. E. 1962. Bacterial blight of cotton.A seedling inoculation technique. Emp. Cott. Gr. Rev., 39: 188-190.

Hafiz, A. and A. Sattar. 1952. Canker of citrus. Research on plant diseases of the Punjab. pp: 142-5.

Harris, C.A., Renfrew, M.J. and Woolridge, M.W. 2001.Assessing the risks of pesticides residues to consumers: recent and future developments. Food Additives and Contaminant 18: 1124-1129.

Horsfall, J.G. and J.W. Heuberger. 1942. Measuring magnitude of a defoliation disease in tomatoes. Phytopath., 32: 226-232.

Hostettmann, K. and J. Wolfender. 1997. The search for biological active secondary metabolites. Pesticides Science 51: 471-482.

Ilyas M. B., K. Iftikhar and M. Anwar-ul- Haq. 1997. Effect of different neem products on the vegetative growth and sclerotial production of *Macrophominaphaseolina*. Pak. J. Phytopathol., 9 (1): 77-79.

Ismail, M., J. Zhang, 2004.Postharvest citrus diseases and their control.Outlook Pest Manag. 1(10): 29-35.

Kapoor, A. 2001. Neem: The wonder plant. Pesticides Information 27: 33-34.

Khan, M.A., F. Iqram and A. Rashid. 2003. Comparative efficacy of garlic extract and streptomycin sulphate against *Xanthomonascampestris*pv. *citri*(Hasse) dye *in vitro*, and on the control of citrus canker in green house. Pak J. of Botany 35(5): 987-991.

Kilani, A.M. 2006. Antibacterial assessment of whole stem bark of *Vitexdoniana*against some Enterobactriaceae.African J. Biotech. 5: 958-959.

Kim, M. J., H. J. Park, M.S. Hong, H. J. Park, M.S. Kim, K. H. Leem, J. B. Kim, Y. J. Kim, and H. K. Kim. 2005. Citrus reticulatablanco induces apoptosis in human gastric cancer cells SNU-668. Nutrition and Cancer 51: 78-82.

Mahajan, A. and S. Das, 2003.Plants and microbes-Potential source of pesticide for futureuse. Pesticides Information 28(4): 33-38.

Manthey, J.A., and Guthrie, N. 2002.Antiproliferative activities of citrus flavonoids against six human cancer cell lines. J. Agric. & Food Chemistry 50: 5837-5843.

Mehrotra, R.S. 1980. Bacteria and Bacterial Diseases.636-8. Plant Pathology.Tata McGraw–Hill pub. Co. Ltd. New Delhi.

Parameshwari, C.S. and V. Latha, 2001. Antibacterial activity of *Ricinuscommunis*leaf extracts. Indian drugs. 38: 587-588.

Rafter, J.J. 2002. Scientific basis of biomarkers and benefits of functional foods for reduction of disease risk: Cancer. British J. Nutrn., 88, 219-224.

Rath, C.C., S.K. Dash, R.K. Mishra, 2001. *In-vitro* susceptibility of Japanese mint (*Menthaarvensis*L.) essential oil against five human pathogen. Ind. Perfum. 45: 57-61.

Rossetti, V. 1977. Citrus canker in Latin America: A review. Proc. Int.

Sahi S.T., M. U. Ghazanfar, M. Afzal, A. Rashed and A. Habib, 2007. Incidence of citrus canker disease caused by *Xanthomonascampestris*pv. *citri* (Hasse) Dows on Kinnow (*Citrusreticulata*) and its chemotherapy Pak. J. Bot., 39(4): 1319-1327.

Shimpi, S.R. and R.S. Bendre, 2005. Stability and antibacterial activity of aqueous extracts of *Ocimumcanum*leaves. Indian Perfumer 49(2): 225-229.

Stall, R.E. and C.P. Seymour, 1983. Canker, a threat to citrus in the Gulf- Coast States.Plant Dis., 67: 581-585.

Sundin, G.W., Demezas, D. H. and Bender, C. L. 1994.Genetic plasmid diversity within natural populations of *Pseudomonas syringae*with various exposures to copper and Streptomycin bactericides. Applied and Environmental Microbiology 60: 4421-4431.

Sawarmura, M., 2000. Aroma and functional properties of Japanese yuzu (citrus junolanka) essential oil. Aroma Res.1 (1):14-19.

Tian, Q., E.G. Miller, H. Ahmad, L. Tang, and B. S. Patil, 2001.Differential inhibition of human cancer cell proliferation by citrus limonoids. Nutrition and Cancer 40, 180-184.

Verma, J. and N.K.Dubey, 1999.Prospectives of botanical and microbial products aspesticides of Tomorrow. Current Science 76: 172-179.

Weindling, R. 1948. Bacterial blight of cotton under conditions of artificial inoculation. USDA Washington, D. C. Tech. Bull. No. 956-204.

White, D.G., S. Zhao, S. Simjee, D. D. Wagner and P. F. McDermott, 2002.Antimicrobial resistance of foodborne pathogens. Microbes and Infection 4: 405-412.

Williams, R.J. and Heymann, D.L. 1998. Containment of antibiotic resistance. Science 279: 1153-1154.