DISTRIBUTION OF BLACK CHAFF DISEASE OF WHEAT CAUSED BY *XANTHOMONAS CAMPESTRIS* PV. *TRANSLUCENS* IN DIFFERENT ECOLOGICAL ZONES OF PUNJAB AND ITS MANAGEMENT

Dr Abdul Rashid, Muhammad Shahjahan (Plant Pathology) **,Prof. Dr. Iftikhar Waris,** Dr. Ijaz Pervaiz and Dr. Khalid Miraj(W.S.Homeopathic research Centre LHR.)

Abstract:

Wheat is a commonly cultivated as staple food crop in Pakistan. It is heavily attacked by a number of diseases like rusts, smuts, bunts and black chaff, a bacterial diseased caused by *Xanthomonas campestris* pv. *translucens* due to the prevalence of diverse environmental conditions in different ecological zones of Punjab. Maximum disease was recoded in semiarid and central districts of Punjab as up to 25%, 23% and 18% in Layyah, Bhakkar and Okara respectively. Disease was recoded minimum in arid areas like Attock and Okara as 8% and 6%. four plant extracts, bulb of *Allium sativum, Allium cepa*, chilies (*Capsicum annum* L.), *Terminalia chebula* and bio-product Biosal and Vampire were tested for their efficacy in vitro against Xanthomonas *campestris* pv. *translucens.* Biosal and Vampire showed efficient results to reduce the pathogen growth on Nutrient Glucose Agar (NGA) medium.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most essential cereal of the world which belongs to poaceae family. Globally it is most cultivated cereal crop after maize. Among the bacterial disease of wheat bacterial leaf streak (black chaff) caused by *Xanthomonas campestris* pv. *tranlucens* is most important (Scharen *et al.,* 1976).Seed borne infection of bacterial pathogens are important not only for its association with the seeds which cause germination failure and/or causing disease to the newly emerged seedlings or growing plants, but also contaminate the soil by establishing its inoculums permanently. It was therefore necessary to search for control measures that are cheap, ecologically sound and environmentally safe to eliminate or reduce the incidence of these economic important pathogens, so as to increase seed germination and to obtain healthy and vigorous plant as well as better yield of wheat. In last decade much concentration has been given to non-chemical substances for the management of pathogen. Chemical fungicides can control the plant diseases, but it has bad effects on human health, plants, fishes and other animals etc which is harmful to our environment. Approximately, three million people are the victims of pesticide poisoning and 200,000 die each year around the world. A majority of affected people belongs to the developing countries (FAO, 2002). It is also assumed that in the developing countries like Pakistan, the occurrence of pesticide poisoning may even be greater than estimated. This may be due to under-reporting, lack of data and misdiagnosis (Tariq, 2005). It has been described that 37,000 cancer cases are linked with pesticide consumption in developing countries each year (WHO, 1990).

On the other hand, several higher plants and their constituents have shown success in plant disease control and are proved to be harmless and non-phytotoxic unlike chemical fungicides[7-12]. Plant extracts have played significant role in reducing

the incidence of seed-borne pathogens and in the improvement of seed quality and emergence of plant seeds in the field.

MATERIAL AND METHODS

Field survey

Survey was conducted at different ecological zones of Punjab to collect the disease samples showing typical disease symptoms. Randomly 10 plots will be selected from each district. One district from each zone data regarding disease incidence on leaf and spikes will be recorded on visual basis on the basis of symptomology. The samples were taken randomly from the fields by measuring the one square meter and by counting the number of healthy plants and infected plants in this square meter and record diseases incidence by given formula (Gnanamanickam *et al*.,1999).

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

Samples were preserved in plastic container at 28°C for three months.

Preparation of plant extracts and bio-products.

Plant extract of Bulb *of Allium sativum* and *Allium cepa, Terminalia chebula* and Chilies (*Capsicum annum* L.) were extracted with alcohol and water following the method described by Mahadevan and SridhM in 1982. Five gram tissues were cut into pieces and immediately plunged in ethyl alcohol/water in a beaker and allowed to boil for 5 to 10 min. Used 5 to 10 mL of alcohol or water for every gram tissue. The extraction was done on top of a steam bath. The extracts were cooled in a pan of cold water. The tissues were crushed thoroughly in a mortar with a pestle and then passed through two layers of chess cloth. The ground tissues were re-extracted in ethyl alcohol distilled water of the plant materials. Both the extracts were cooled and filtered through Whatman's No. 1 filter paper. The volume of the extract was evaporated on a steam bath to dryness and adequate amount of distilled water was added for 5 g of tissues to adjust the ratio of the plant extracts at 2%, 3% and 5% concentration and used for poisoned food technique for *Xanthomonas campestris* pv. *translucens* on Nutrient Glucose Agar medium.

Vampire and Biosal were liquid product and their concentration were set as 2%, 3% and 5%.

Preparation of Nutrient Glucose Agar

Nutrient Glucose Agar	(NGA)	
Ingredients	g/l	
Beef extract	3.0g	
Peptone	5g	
Glucose	2.5g	
Agar	15g	(Wilson *et al*., 1967)

First of all the ingredients were weighed of above mention medium, then mixed all the ingredient except glucose in 500 ml of water slowly and made up the volume up to one liter. Boiled the contents, add agar slowly into the medium while heating, and stir the content thoroughly so that these may not stick to the bottom of the pan. After removing from the heat, glucose was added into the solution. Filtered the medium through the cheese cloth. Poured the media into the test tubes and conical flasks and pluged it with cotton.

Isolation of the organism associated with bacterial leaf streak (Black chaff) disease of wheat from leaves.

While conducting survey of the wheat fields, the leaves showing typical symptoms of disease like bacterial lesion and wheat spike were collected in polythene bag and were brought to the laboratory for isolation of the bacterium by using Riker and Riker method (Riker and Riker., 1936). Infected tissue of the leaf was obtained with the help of sterilized cork borer (diametr 1cm). The disc thus obtained were disinfected with 0.1% mercuric chloride (HgCL2) and given three washing with sterilized water to reduce the injurious effect of Hgcl2.the disc were ground in the sterilized pestle and mortar and total volume of mixture was adjusted to 10ml by the addition of sterilized water, followed by the tenfold dilution by the mixture. One milliliter of each dilution was pipetted into a Petri dish and warm at 45 ^{o}C. Nutrient agar was poured in it. Then this Petri dish was gently shaken. The Petri dishes containing different solution of the bacterial suspension

were incubated at 30 ºC. Yellow and round colonies appeared 96 hours after incubation. Isolated colonies of the bacterium were picked and single colony transferred to each agar slant to prepare pure culture. The bacterium was identified by morphological and biochemical characteristics. The stock culture of the bacterium was maintained on nutrient agar in culture tubes at 4 ºC in refrigerator.

Isolation of the organism associated with bacterial leaf streak (Black chaff) disease of wheat from seeds.

One hundreds wheat seeds of showing typical symptoms of disease like black chaff were soaked over night in distilled water at 25 ºC ± 2 than 0.2ml was transferred by micropipette on nutrient agar medium, and spread on plate with the help of bended glass rod i.e Agar plate Technique (Bhutta, 1992). After 72-hours bacterial colonies were recorded. Culture was purified on yeast extract purified dextrose purified media. The bacterium thus obtained was identified following morphological and biochemical test (Buchanan and Dowson, 1957). The stock culture of the bacterium was maintained on nutrient agar in culture tube at 30 ºC.

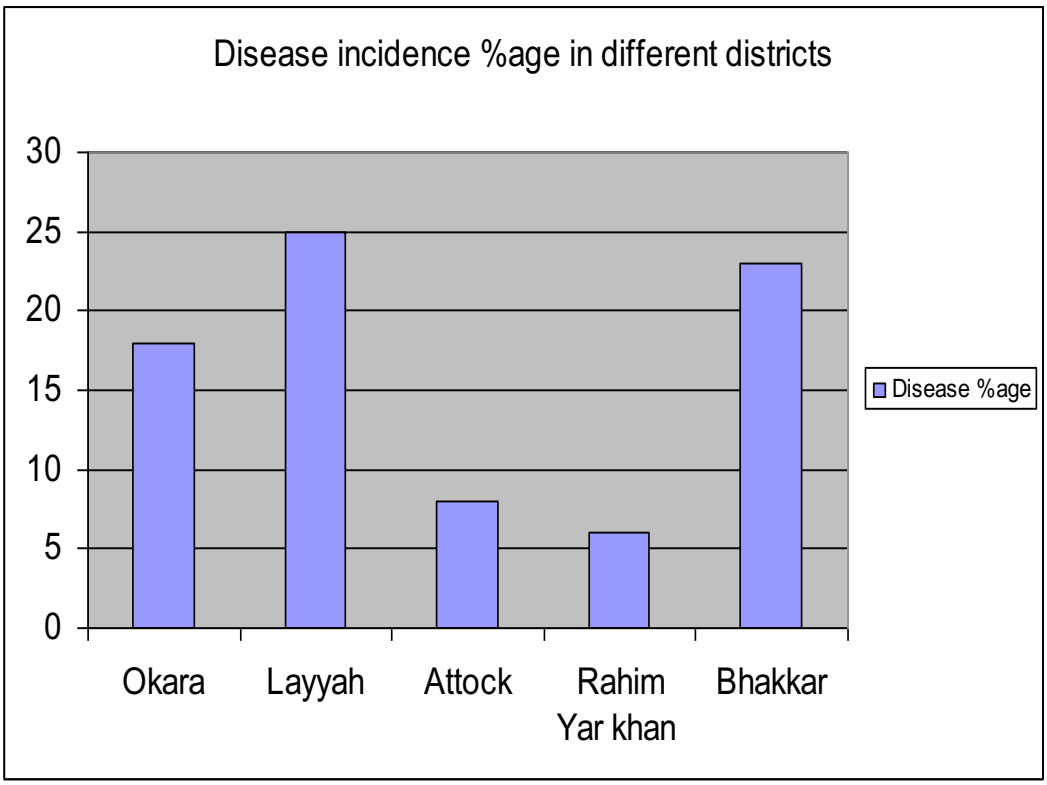
Statistical Analysis

All the data on percent plant infection. Disease severity in the field conditions was subjected to statistical analysis. Analysis of variance (ANOVA) was used to determine the effect of plant extract and bio-pesticides, and treatment means when compared by LSD or DMR test. (Steel *et al.,* 1997)

RESULTS AND DISCUSSION

Incidence of Black chaff disease of wheat in different districts of **Punjab.**

The recorded disease incidence of the different selected districts of Punjab from different type of soil structure and climatic conditions varied from one another and location to location.



Black chaff disease incidence in different districts of Punjab.

Analysis of Variance for Data of different districts

SOV	DF	SS	MS	F-ratio
Replications	2	12.13	6.07	1.83^{NS}

Locations	4	876.27	219.07	66.05**
Errors	8	26.53	3.32	
Total	14	914.93		

NS: non- significant. *: Significant **: Highly significant

Analysis of variance shows that district to district results are highly significant but from same location replications are non significant

Effect of different plant extracts on growth of Xanthomonas *campestris* pv. *translucens* after 4 days.

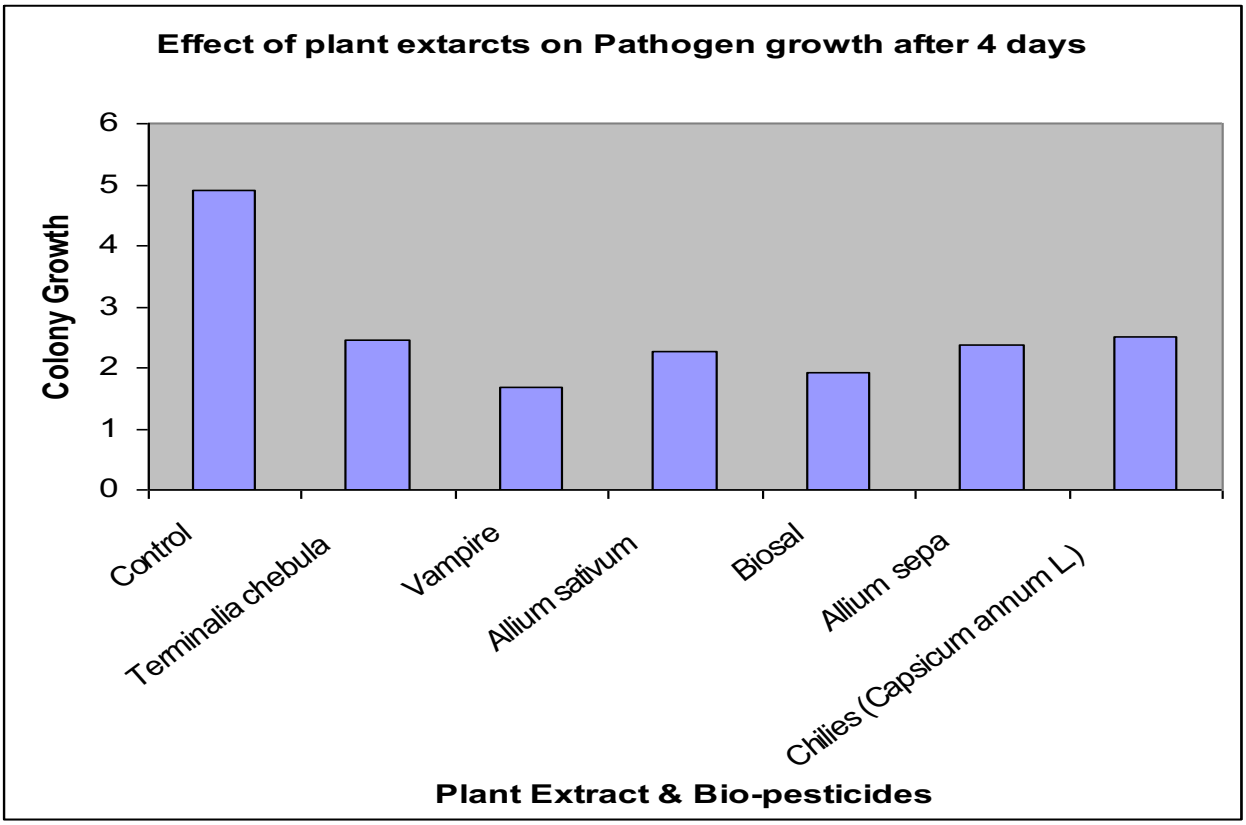
Six plant extracts and bio- product having antibiotic actions were tested against *Xanthomonas campestris* pv. *translucens* . Least colony growth was observed in case of Vampire at 4%, 2% and 3% concentration with 1.5, 1.8, 1.7 cm growth respectively. *Allium sepa* at 2% , Biosal at 2% , *Allium sativum* at 4% showed 2.6, 2.1 and 2.1 cm growth of pathogen respectively.2.1, 1.9 cm and 1.8 cm average growth of *Xanthomonas campestris* pv. *translucens* were observed in case of Biosal at2%, 3% and 4% concentration. *Allium sepa* at 3% and 4% show 2.3 and 2.2cm respectively. *Terminalia chebula* at 3% concentration showed 2.5 cm Chilies (*Capsicum annum* L.) at 3% showed growth 0f 2.6cm *Terminalia chebula* at 3% showed 2.5 cm, 67.10mm and 69.6mm of growth respectively. 72.1mm growth of pathogen was observed in *Allium sativum* at 3% and chilies (*Capsicum annum* L.) in at 2%. While Chilies (Capsicum annum L.) showed 2.7 cm at 2% showed maximum colony growth of 76mm respectively.

Colony growth of bacterium under lab conditions after 4 days

Treatments	**Average colony growth (cm) at different concentrations**									
	2 %			**3 %**			**4%**			**Mean**
	R1	R2	R3	R1	R2	R3	R1	R2	R3	
Terminalia chebula	2.2	2.6	2.7	2.4	2.6	2.5	2.4	2.5	2.3	2.45 c

Vampire	1.5	1.9	2	1.5	1.9	1.7	1.5	1.3	1.7	1.67 g
Allium sativum	2.12	2.8	2.28	2.12	2.35	2.43	2.2	2.1	2	2.27 e
Biosal	2.3	1.9	2.1	1.7	1.9	2.1	1.7	1.9	1.8	1.93 f
Allium sepa	2.4	2.8	2.6	2.4	2.2	2.3	2.3	2.2	2.1	2.37 d
Chilies (*Capsicum annum* L.)	2.6	2.5	3	2.6	2.5	2.7	2.2	2.5	2.5	2.50 b

Control= 4.9cm a



Colony growth in cm of pathogen in response to the treatments after four days

Analysis of Variance for Data of treatments

SOV	DF	SS	MS	F-ratio
Replications	2	131.4	65.7	2.52*
Treatments	5	2395.6	479.1	18.39**
Errors	10	260.6	26.1	

Total	17	2787.6		

NS: non- significant. *: Significant **: Highly significant

Results: treatments showed highly significant results while Dose Concentration is significant at 5 % but Non-significant at 1%

Analysis of variance given in Table indicated significant F values for treatment doses, doses and their interaction. From fungicides means, it appeared that response of each fungicide is different. Best response was in Vampire followed by Biosal, *Allium sepa* , *Allium sativum*, *Terminalia chebula* and Chilies (*Capsicum annum* L.).

Effect of different plant extracts on growth of *Xanthomonas campestris* pv. *translucens* after 8 days.

Maximum inhibition was observed in Biosal at 2%, 3% and 4% concentration as growth of pathogen was measured as 3.5, 3.2 and 3.0 cm. *Allium sepa* at 2%, Vampire at 2%, *Allium sativum* at 4% showed 5.0, 3.7, 5.1cm respectively. 3.1, 4.7 and 4.5 percent of inhibitions were observed in case of Vampire at 4%, *Allium sepa* at 3% and 4% respectively. While Vampire showed 3.1cm colony growth at 3% concentration, and *Terminalia chebula* at 4% showed 5.1 cm colony growth. *Terminalia chebula* at 2% concentration, Chilies (*Capsicum annum* L.) at 4%, *Terminalia chebula* at 3% concentration showed 5.6, 4.1 and 5.4 colony growth respectively. 5.2 and 5.3cm colony growth was observed in *Allium sativum* at 3% and in Chilies (*Capsicum annum* L.) at 2%. While chilies (*Capsicum annum* L.) showed 4.3cm at 3% concentration. Biosal and Vampire show excellent responce to inhibit the bacterial growth and followed by chilies (Capsicum annum L.), *Allium sepa, Allium sativum, Terminalia chebula* respectively.

Colony growth of pathogen in poisoned food technique after 8 days

Treatments	**Average colony growth (cm) at different concentrations**									
	2 %			**3 %**			**4%**			**Mean**
	R1	R2	R3	R1	R2	R3	R1	R2	R3	

Terminalia chebula	5.5	5.7	5.6	5.5	5.4	5.3	4.9	5.3	5.1	5.37 b
Vampire	3.5	3.7	3.9	2.9	3.3	3.1	2.9	3.3	3.1	3.30 f
Allium sativum	5.5	6.1	5.2	5.2	5.1	5.3	5.2	5.1	5	5.30 c
Biosal	3.15	3.25	4.1	3.15	3.25	3.2	3.1	2.9	3	3.23 g
Allium sepa	5.2	4.7	5.1	4.5	4.7	4.9	4.5	4.7	4.3	4.70 d
Chilies (*Capsicum annum* L.)	5.5	5.3	5.1	4.5	4.1	4.3	3.9	4.1	4.3	4.67 e

Control = 6.2cm a

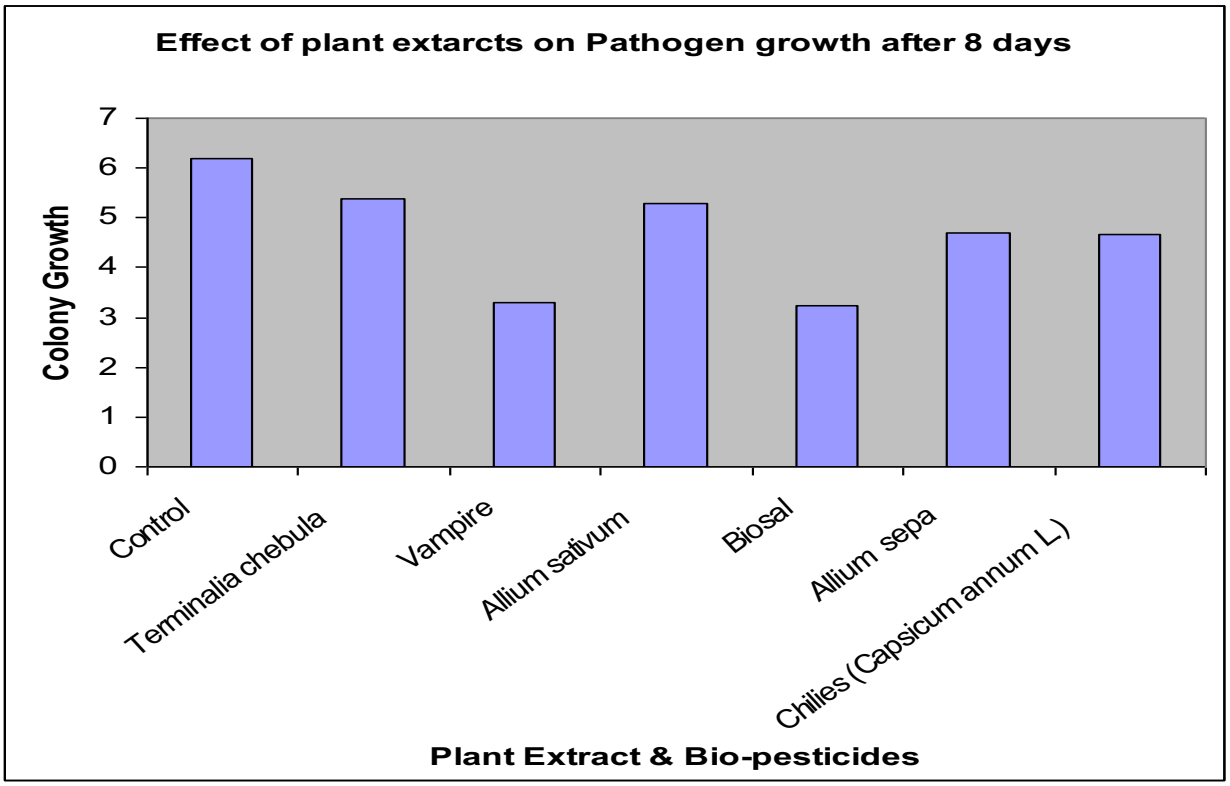


Figure 4.13: colony growth of pathogen in response to the treatments after eight days

Analysis of Variance for data of treatments after 8 days

SOV	DF	SS	MS	F-ratio
Replications	2	1.29	0.64	22.05*
Treatments	5	13.35	2.26	91.08**

Errors	10	0.2933	0.02933	
Total	17	149450		

NS: non- significant. *: Significant **: Highly significant

Treatments as well as Dose Concentration show highly significant results

Discussion

Bacterial leaf streak (BLS) disease of wheat is a common, important and destructive for wheat in Pakistan as well as in other wheat growing countries. High disease incidence is encountered every year, which are attributing to the combination of factors as insect pest vector and favourable environmental conditions.

A survey was carried out in selected districts of different ecological zones of Punjab to assess the incidence of bacterial leaf streak disease of wheat. BLS disease of wheat was prevalent in all the visited locations. The disease was severe in some locations and was mild in others. The disease was present on all varieties sown in these locations Bhutta and Ahmad (1995) conducted an extensive survey to assess the occurrence, distribution and importance of bacterial leaf streak (black chaff) disease of wheat in Pakistan. Satya *et al*. (2005) described the initial symptoms in case of black chaff disease of wheat were leaf curling which appeared after 3 days, in case of Chakwal-97 and Inqulab-91, as these varieties are highly or moderately susceptible to *Xanthomonas translucens* pv. *undulosa(Xtu)*.

Use of disease resistant varieties is advocated as an effective solution of controlling BLS disease of wheat, efforts were directed towards controlling of the disease bacterium *Xanthomonas campestris* pv. *translucens* through plant extracts, bio-products under lab conditions.

The efficacy of the plant extracts was examined by antibacterial action and was used as seed treatment to know the enhancement of seed germination and seedling vigor. Besides premising results, plant extracts were evaluated under greenhouse experiments to test the efficacy in controlling bacterial blight disease incidence. Leaf extract of *Datua alba*, seed oil of neem (*Azadirachta indica*), neem seed bitter and nimbokil 60 EC were evaluated at 1, 2 and 3% concentration on the growth of *Xanthomonas campestris* pv. *malvacearum,* in-vitro and on the green house grown cotton varieties/lines. At 3%

concentration *Datura alba* significantly retarded the growth of bacterium followed by nimbokil, neem seed bitter and neem seed oil respectively. None of the plant extract showed effectiveness at 1% concentration. There was significantly lees number of leaf shedding. Less number of bare nodes and more number of bolls, increase boll weight and yield of seed cotton of varieties sprayed with standard concentrations of *Datura alba* and nimbokil 60 EC as compared to untreated control. Disease severity was less in treated varieties as compared to untreated varieties (Khan *et al*., 2000)

From above discussion it may also be suggested that resistant varieties must be introduced against this pathogen. Still no variety has resistance against this pathogen, disease was found in almost all locations of different ecological zones of Punjab.. Secondly perform in-vivo experiment to check the antibacterial efficacy of different homeopathic (bio-pesticides) products like Vampire having no health hazards effects on our staple food.

Summary

In Pakistan wheat is considered to be an important agronomic crop. Bacterial leaf streak disease of wheat cause considerable reduction in the yield of wheat up-to 40-50 percent. The plant extracts and bio-pesticides have been used for the management of bacterial leaf streak (black chaff) disease of wheat. Epidemiological factors play an important role in development of disease.

Keeping in view all these factors present study was planned to asses the incidence of bacterial leaf streak disease of wheat in selected district from different ecological zones of Punjab and to determine the most effective bio-pesticides for the enhancing of yield of this crop.

For this purpose an experiment was conducted in bacteriological lab of Department of Plant Pathology, University of Agriculture Faisalabad, in Complete Randomized Design (CRD). There were six treatments out of untreated control, having

All the six treatments showed good results but Biosal and Vampire were most effective to inhibit the pathogen growth of *Xanthomonas campestris* pv. *translucens*.

From this study we can concluded that bacterial leaf streak disease of wheat have its economic importance and it may cause maximum yield losses in future if favorable conditions for the development of pathogen will be occur. So we must be developing resistant variety and its management practices through bio-pesticides.

LITERATURE CITED

Spencer, D.M., J.N. Topps and R.L. Wain, 1957. Fungistatic properties of tissue. An antifungal substance from the tissue of *Vicia faba.* Nature, 179: 651-662.

Singh, H.N.P., M.M. Prasad and K.K. Shinha, 1993. Efficacy of leaf extracts of some medicinal plants against disease development in banana. Lett. Microbiol., 17: 269-271.

Mahadevan, A. and R. Sridhar, 1982. Methods in Physiological Plant Pathology. 2nd Edn., Sivakami Publications. Madraj, pp: 316.

Montana and losses from them. Plant reporter, 60: 686-690.

FAO (Food and Agriculture Organization). 2002. FAO/WHO global forum of food safety regulators. Food and Agriculture Organization of the United Nations, Marrakech, Morocco.

GOP (Government of Pakistan). 2006. Pre-feasibility study for pesticide industry. Employment and Research Section, Planning and Development Division, Govt. of Pakistan, Islamabad, Pakistan.

Tariq, M.I. 2005.Leaching and degradation of cotton pesticides on different soil series of cotton growing areas of Punjab, Pakistan in Lysimeters. Ph.D. Thesis, Univ. Punjab, Lahore, Pakistan.

WHO (World Health Organization). 1990. Public health impact of pesticides used in agriculture. World Health Organization, Geneva, Switzerland.

Gnanamanickam, S.S., V.B. Priyadarisini, N.N. Narayanan, P. Vasudeven and S. Kavitha, 1999. An overview of bacterial blight disease in rice. Current Sci., 77(11): 1435-1444.

Wilson, E.E., F.M. Zeitoun and D.L. Fredrickson, 1967. Bacterial phloem canker, a new disease of persian walnut trees. Phytopathology, 57(5): 618-621.

Riker, A.J. and A.S. Riker, 1936. Introduction to researches on plant diseases. Swift an Chigago, 16(4): 4-5.

Bhutta, A.R., 1992. Comparative studies for detection of *Xanthomonas campestris* pv. *malvacearum* from cotton seed in Pakistan. Pak. J. Agric. Res., 13(3): 277-281.

Buchanan, P.E. and N.E. Gibbons, 1974. Burgey's Manual of Determinative Bacteriology, 8th Edit. Williams Wilkinson Baltmore, Co., pp. 244.

Dowson, W.J., 1957. Plant disease due to bacteria. Cambridge University Press. 2nd Ed. pp. 39-48.

Steel, R.G.A. and J. H. Torrie, 1960. Principles and Procedures of Statistics. MeGraw Hill Book co. Ince., New York, U.S.A.

Khan, M.A., A. Rashid and R.A. Chohan, 2000. Biological control of bacterial blight of cotton using some plant extracts. Pak. J. Agric. Sci., 34(4): 105-108.

Satya, P., V.P. Singh and A.K. Singh, 2005. Genetic analysis of pyramided genes for resistance to bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) and development of resistant lines of basmati rice (*Oryza sativa*). Indian J. Agric. Sci., 275(7): 428-431.

Bhutta, A.R. and S.I. Ahmad, 1995. Detection of *Xanthomonas campestris* pv. *translucens* in wheat seed lots in Pakistan. Pak. J. P. Pathal., Vol. 7(2): 114-117.