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Distribution of black chaff disease of wheat caused by *Xanthomonas campestris* PV. *translucens* in different ecological zones of Pakistan and its management through plant extracts and bio-products

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ABSTRACT

Wheat is an important cereal crop chiefly attacked by black chaff disease. Survey of five districts was conducted, maximum disease incidence was noticed in Layyah district. Bacterium was isolated from leaves, spikes and seed tissues, yellowish colonies appeared after 48 hours of incubation. Four plant extracts (Allium sativum, Allium cepa, Terminalia chebula and Capsicum annum) were evaluated at 2, 3 and 5% concentrations against Xanthomonas campestris pv. translucens. Capsicum annum significantly retarded bacterial growth at 5% dose after 8 days. Two bio-products (Vampire and Biosal) were evaluated at 2, 3 and 5% doses against bacterial colony growth. Maximum inhibition was noticed by vampire at 5% after 8 days.

Keywords: Wheat; Black chaff; Bacterial leaf streak of wheat; Plant extracts; Bio-products; *Xanthomonas campestris* pv. *translucens*,

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important cereal crop of world, belongs to family “Poaceae”[1]. It is a staple food of Pakistan, cultivated under the area of 8666 thousand hectares with 23517 thousand tons production[2].Black chaff disease of wheat is an important bacterial disease of wheat caused by *Xanthomonas campestris* pv. *translucens.* Symptoms appear after 10-14 days. Narrow water soaked necrotic lesions form at the center with rusted margins on leaves[3]. Bacterial slime oozes and dries on leaf surface gives flaked off appearance. Disease symptoms hardly appear on seedlings. Black chaff with purple-black discoloration appear on glumes and seeds surface. *Xanthomonas campestris* pv. *translucens* is seed born pathogen. Transmission rate is quite slow but can be sporadic under favourable conditions. Bacterium is disseminated primarily through infected seeds. Rains splashes and aphids are secondary means for dispersing of inoculum[4]. Bacterium may survive up to 63 months on seeds[5]. Infection cycle completes in 10 days [6].

Fungicides are fatal for human health, plants, fisheries and animals. Approximately, three million people are the victims of pesticide poisoning each year around the world [7].

Plants are not only a food source but also have unique medicinal properties. In many remedies, these are vitally used as basic ingredients for curing diseases in men and animals. Plant extracts are eco-friendly and cheap source for plant disease management. Plants having different compounds in its different parts are antibacterial and antifungal, gaining popularity as an alternate to agro chemicals day by day.

The purpose of this study was to observe disease incidence on different areas of the country. In vitro evaluation of plant extracts and bio-products at significant concentration will be an effective tool for disease management.

MATERIALS AND METHODS

A survey of five districts (Attock, Layyah, Okara, Rahim Yar Khan and Bhakkar) of Pakistan was conducted to assess disease incidence of "black chaff disease of wheat". Three wheat plots from each district were selected randomly and incidence was measured by using following formula;

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

Diseased samples (leaves, spikes and seeds) were preserved at 4 ^{o}C in refrigerator.

Isolation from Infected leaves and spikes:
Diseased tissues (leaves and spikes) were excised into small pieces, surface sterilized with 0.1% $HgCl_2$ and rinsed twice with tap water. After drying one gram infected tissues were macerated in small amount of distilled water. Bacterium was isolated by using dilution plate technique[8]. Nutrient Glucose Agar (beef extract= 3g, peptone = 5g, glucose = 2.5g, agar = 15g and water = 1L) medium was used for bacterial colony isolation. Plates were wrapped and incubated at 30^{o}C for 48 hours.

Isolation from diseased seeds:
Infected seeds with typical disease symptoms were surface sterilized (0.1% $HgCl_2$) and soaked in distilled water for overnight. 100 µl aliquot was spread on the surface of nutrient glucose ager medium. Plates were wrapped and incubated at 30^{o}C for 48 hours. Colonies were purified by streaking plate method and preserved.

***In vitro* evaluation of different plant extracts and bio-products:**
Four plant extracts (*Allium sativum, Allium cepa,Terminalia chebula* and *Capsicum annum*) and three bio-products (Biosal& Vampire) were evaluated at 2%, 3% and 5% concentrations by using food poisoning technique[9] against *Xanthomonas campestris* pv. *translucens.* In control, sterile water was used. Each treatment was replicated thrice and arranged in Completely Randomized Design (CRD). Data was recorded after 4 and 8 days of interval by measuring bacterial colony diameter in centimeter scale.

Preparation of plant extracts:
Twenty five grams bulbs of *Allium sativum Allium cepa,Terminalia chebula* and *Capsicum annum* were crushed into small pieces and grinded with 75 ml of distilled water. Resultant was filtered through "Whatman's No. 1" filter paper and considered as standard. Three doses 2%, 3% and 5% were made by adding the requisite amount of distilled water [10].

Statistical Analysis:
Disease incidence recorded at different districts was compared by Analysis of Variance (ANOVA). Treatments means were comparedby Fisher's Least Significant Difference (LSD) test[11]. Data was statistically analyzed by using SAS software [12] and graphs were made in Microsoft Excel [13].

RESULTS AND DISCUSSION

Significant difference in disease incidence of "black chaff disease of wheat"was noted in five districts of Pakistan (Table 1). Maxmim disease incidence was oberved in Layyah district (23%) followed by Bhakkar (17.5%), mimimum incidence was noted in Rahim Yar Khan among surveyed districts (Fig 1).

Wheat (*Triticum aestivum*) is a staple food of Pakistan, extesively cultivated in arid, semi arid and irrigated zones. Layyah is a semi arid area having totally different climate compared to Rahim Yar Khan district.Change in disesae incidence might be due to the variation of climatic condition. As a little information is known about the disease [14] biology in Pakistan it needs further research on epidemiological aspects.Bhutta and Ahmad [15] surveyed different districts of Pakistan, highest percentage of infected samples was found from Hyderabad (38.46 %) followed by those from D.I. Khan (28.57 %). None of the samples from R.Y. Khan and Sukkur was found infected.

Isolation from leaves and seeds:
Yellowinsh round muccoid bacterail colonies apperared after 48 hours of incubation. Colonies were purified and identified as non-sporing, aerobic, motile, Gram-negative rod, occurring singly or in pairs with a single polar flagellum.

In 1986, Sands *et al.*, isolated bacterium using Wilbrink's medium and charcterized as areobic, non spore forming, Gram-negative rod having mucoid yellowish colonies.

***In vitro* evaluation of different plant extracts:**
After 4 days of treatment, *Allium sativum* significantly controlled *Xanthomonas campestris pv.. translucens* colony growth as compared to the others at 2% concentration. No significant difference was observed by *Terminalia chebulaand* and *Allium sativum* at 2 and 3% doses. At 3% concentration, no statistically significant difference was noted among *Terminaliachebula*, *Allium sativum* and *Allium sepa* except *Capsicum annum*. Maximum bacterial colony inhibition after 4 days was observed by *Allium sativum* plant extract at 5% dose. After 8 days; no statistical difference was seen between *Terminalia chebula* and *Allium sativum*, significant colony inhibition was noticed by *Allium sepa* at 2% dose. At 3% and 5%after 8 days, *Capsicum annum*, significantly suppressed *Xanthomonas campestris pv.. translucens* colony (Table 2; Fig 2)

***In vitro* evaluation of bio-products:**
After4 days, significant results were noted by both products (Vampire & Biosal). Vampire was significantly more effective as compared to Biosal at 2% dose. Significant difference was noted by Vampire & Biosal at 3 and 5% doses. Vampire significantly inhibited 1.33 cm bacterial colony as compared to Biosal at 5%. After 8 days, Vampire was significantly more effective at 5%, suppressed 2.73 cm *Xanthomonas campestris pv.. translucens* colony (Table 3; Fig 3).

Plant disease management through biological means is eco-friendly and has gained much popularity since last decades. Plant extracts are a cheap and alternative to chemicals having broad spectrum activity against bacterial and fungal pathogens. *Capsicum annum* belongs to family “Solanaceae” containing 16% Oleoresin compound [17] having antibacterial property.Oleoresin acts on the bacterial cell wall, removes proteins and the S-layer, and interferes with the cell division process[18].

Khan, Rashid and Riaz [10], evaluated leaf extract of *Daturaalba*, seed oil of neem (*Azadirachta indica*), and nimbokil 60 EC at 1, 2 and 3% concentration against growth of *Xanthomonas campestris pv.. malvacearum*. At 3% concentration *Datura alba* significantly retarded the bacterium growth followed by nimbokil. None of the plant extract showed effectiveness at 1% concentration.Sajid, Rashid, Ehetisham-ul-Haq, Javed, Jamil, Mudassir, Farooq, Ahmad, Latif, Chohan, Ahmad and Kamran [19] evaluated three plant extracts *Citrullus colocynthis, Nicotiana tobaccum* and *Curcuma lunga* at 5, 10 and 15% concentrations. Tobacco’s extract (*Nicotiana tobaccum*) significantly controlled *Xanthomonas campestris pv.. malvacearum* at 10% concentration.Jabeen [20], twenty five different plant species were evaluated against bacterial leaf blight of rice by disc plate diffusion method. Out of twenty five plants, significant activity was noted by *Azadirachta indica*, *Thuja orientalis*, *Terminalia bellirica*, and *Anethum graveolens*.

Table 1: Analysis of Variance (ANOVA) for disease incidence of five districts of Pakistan

SOV	DF	SS	MS	F-ratio	P
Replications	2	72.9	36.45		
Districts	4	859.5	214.875	48.29	0.0000**
Total	8	968	4.45		
Error	14	35.6			

*** = Highly significant, CV = 13.18, α = 0.05*

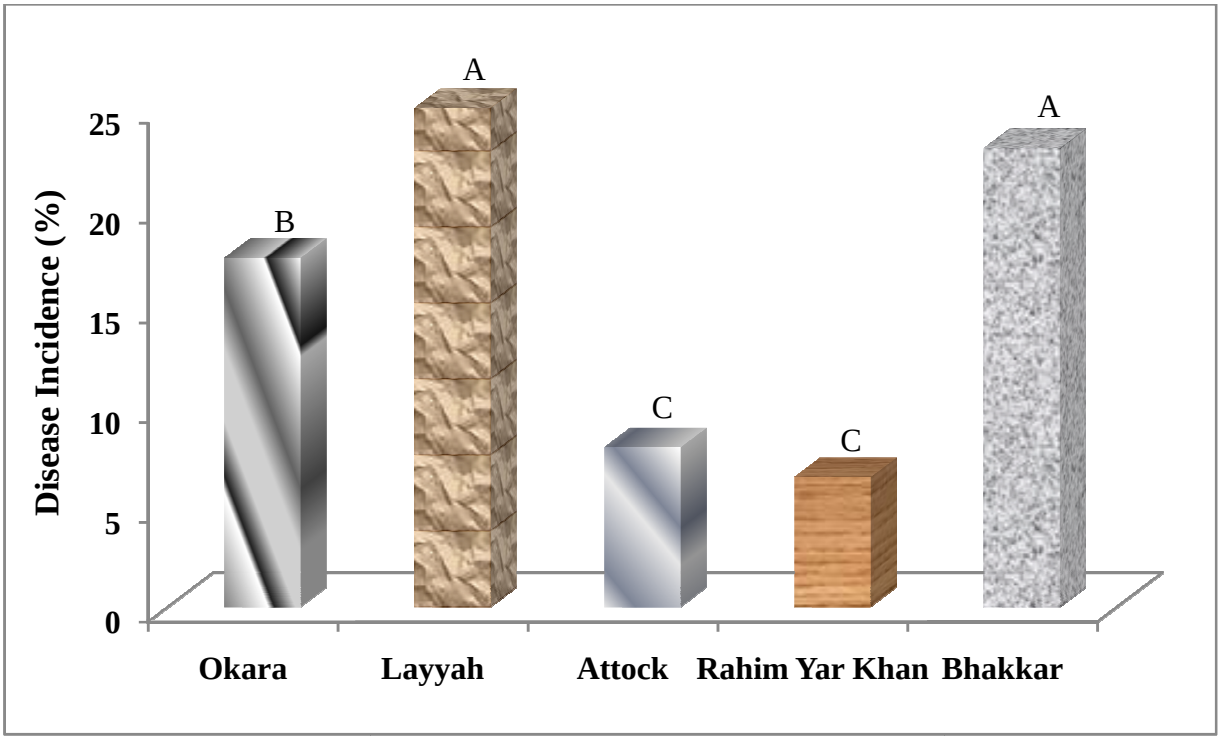

Fig 1: Comparison of disease incidence among different districts of Pakistan (LSD = 3.971)

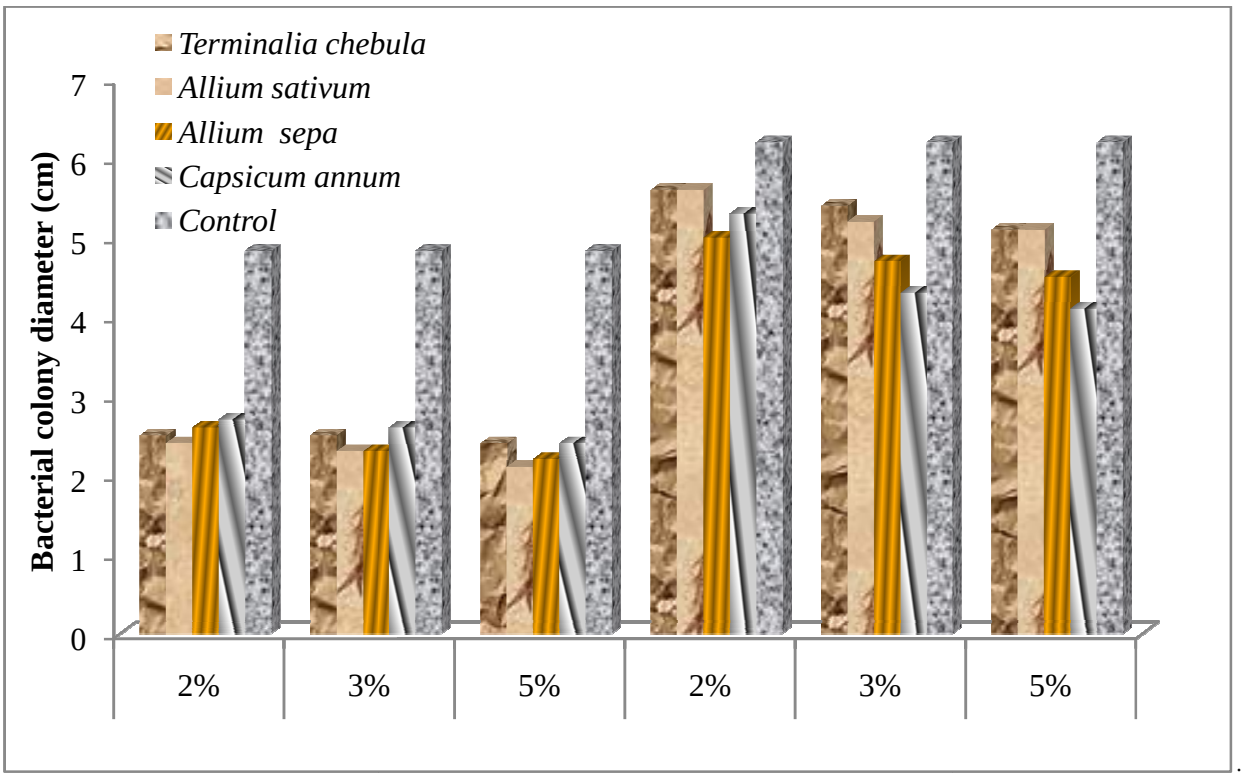

Fig 2: Response of plant extracts at various doses against bacterial colony diameter

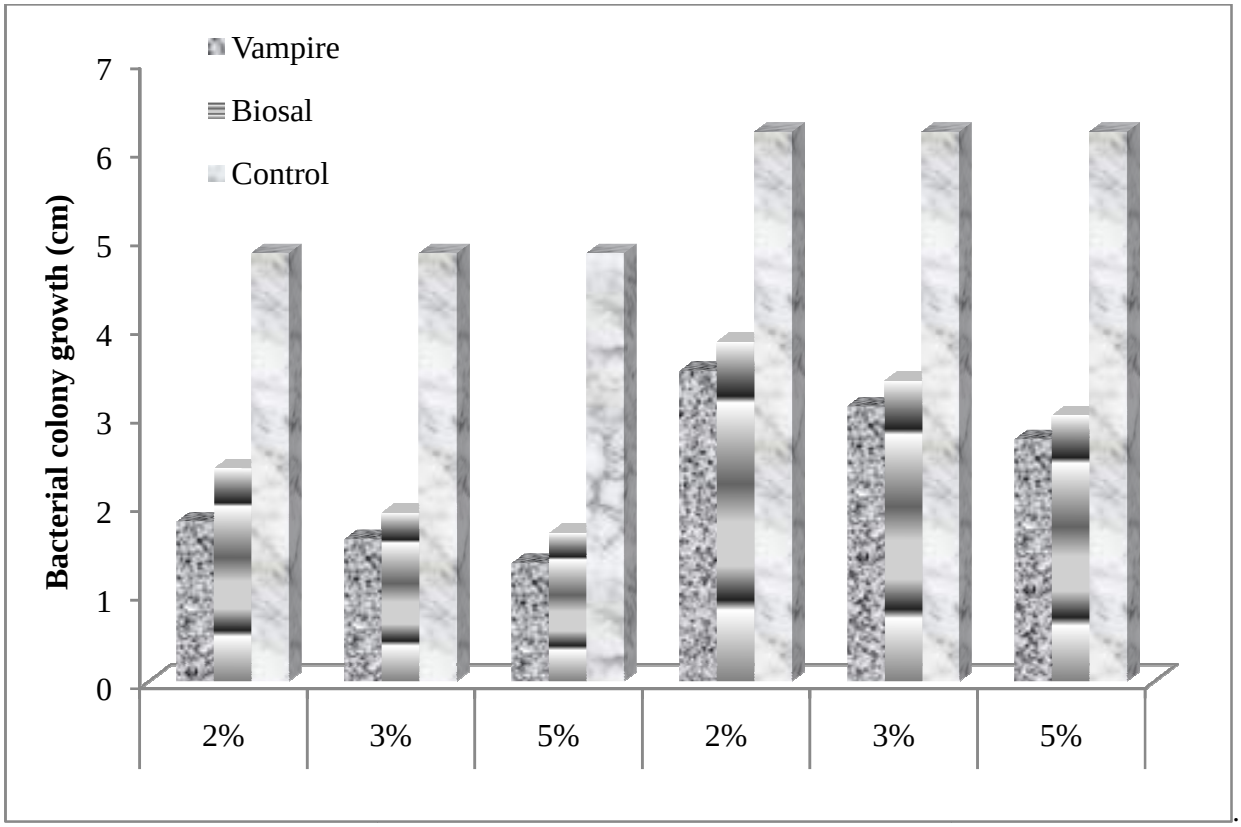

Fig 3: Representation of bio-products impact against bacterial colony diameter

Table 2: Means comparisons of plant extracts at three doses against colony with respect to different time intervals

		After 4 Days			After 8 Days		
	Time	**Doses**			**Doses**		
	Doses	**2%**	**3%**	**5%**	**2%**	**3%**	**5%**
Treatments	*Terminaliachebula*	2.50 KLM	2.50 KLM	2.40 LMN	5.60 B	5.40 BC	5.10 DEF
	Allium sativum	2.40 LMN	2.30 MNO	2.10 O	5.60 B	5.20 CDE	5.10 DEF
	Allium sepa	2.60 KL	2.30 MNO	2.20 NO	5.00 EF	4.70 GH	4.50 HI
	Capsicum annum	2.70 K	2.60 KL	2.40 LMN	5.30 CD	4.30 IJ	4.10 J
	Control	4.83 FG	4.83 FG	4.83 FG	6.20 A	6.20 A	6.20 A
					LSD = 0.2696		**α = 0.1**

Mean values sharing simmilar letters do not differ significantly

Table 3: Mean comparisons of different bio-products at various dosesagainst colony with respect to different time intervals

		After 4 Days			After 8 Days		
	Doses	**2%**	**3%**	**5%**	**2%**	**3%**	**5%**
Treatments	Vampire	1.80 HI	1.60 IJ	1.33 J	3.50 D	3.10 E	2.73 F
	Biosal	2.40 G	1.90 H	1.67 HI	3.83 C	3.39 D	3.00 EF
	Control	4.83 B	4.83 B	4.83 B	6.20 A	6.20 A	6.20 A
					LSD = 0.2690		**α = 0.1**

Mean values sharing simmilar letters do not differ significantly

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