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Success of Bio Products against Bacterial Leaf Blight Disease of Rice caused by *Xanthomonas oryzae pv. oryzae*

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Abstract
Bacterial leaf blight of rice (BLB) is a devastating disease in rice growing areas of Pakistan. Due to absence of appropriate antibiotic or bactericides in market, disease destroys the whole crop whenever hits to its epidemics in the region. Therefore, present work was conducted to evaluate some newly available bio products in the market to manage the disease efficiently. Five Bio products namely Biosal, Protector, Vega Plus, Rigorous and Vampire were evaluated by in-vivo and in-vitro experiments. In-vitro evaluation was carried out against colony growth of *Xanthomonas oryzae* pv*. oryzae* by using inhibition zone technique with different dozes (100 ppm, 200 ppm and 300 ppm) in a CRD experiment with 3 replications. In-vivo evaluation was performed by foliar sprayed bio products on moderately resistant to moderately susceptible varieties (KSK-133 and IRRI-6) in an RCBD Experiment in triplicate. Data were collected at an interval of 7 days. In-vitro experiments revealed that Biosal was most effective while Protector and Vega Plus proved approximately equal in performance for minimizing the pathogen colony. Similarly, In-Vivo evaluation recorded Biosal as most significant while Protector and Rigorous proven comparative for disease reduction under natural field Conditions.
Keywords: Rice, Bacterial Blight, Bio Products, Effectiveness, Benefits.

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INTRODUCTION

Rice (*Oryzae sativa* L.) is abundantly grown in the tropical and sub-tropical zones across the world (Ezuka and Kaku, 2000). As Asian countries use Rice as staple food so Asia region holds top position in Rice cultivation bearing 90% of the total rice cultivated globally (Smith and Bruce, 2000; Zafar, 2004). GDP of Pakistan is highly depended upon rice export and the country is ranked 2nd worldwide for rice cultivation (Zahid, 2005). Total rice production area in Pakistan is 2850,000 hectares with annual production 10351 Metric Tonns (USDA, 2015).

This highly valuable crop is pressurized by diverse fungal and bacterial attacks (Khan, 2009). Among them, Bacterial Leaf Blight (BLB) caused by *Xanthomonas oryzae pv. oryzae* is one of the most destructive diseases resulting in considerable losses (Swings, 1990). It is observed in all continents of the world but particularly destructive in Asian Regions causing growth and yield losses (Narayanan, 2002; Amna, 2008). In highly susceptible varieties, up to 80% yield loss is common (Lee and Khush, 2000). It affect crop at all stages and show either “Kresek” or leaf blight symptoms (Akhtar, 2008). Diseased plants usually produce sterile panicles or with immature grains leading to complete loss of the crop.

Management strategies against the BLB may involve use of resistant Varieties, Use of Chemicals or use of Eco friendly bio products. But currently In Punjab, all the commercial basmati rice cultivars are categorized susceptible to the disease (Akhtar, 2008; Ali, 2009). Regarding Chemical management, Several Chemicals such as Copper-oxychloride, Bleaching Powder, acetylene compounds and streptomycin solutions have been declared as inhibiting agents against the bacterium growth (Khan, 2012). But the chemicals are expensive; their handling is dangerous; they have residual effects showing detrimental aspects upon human beings and environment (Damalas, 2011). So finding the alternatives to these chemicals is the best solution. Coping this, the direction goes towards the use of bio products or plant extracts. Recent studies have reported the effectiveness of some plant extracts against the BLB disease (Gurjar *et al.,* 2012; Jabeen, 2011, 2013; Meena, 2013; Shivalingaiah, 2013; Yugander, 2015). Jabeen (2011) investigated the

antibacterial potential of twenty five indigenous medicinal plants for management of bacterial leaf blight (BLB) of rice and documented about the success of *Anethum graveolens*, *Ferula assfoetida* and *Terminalia chebula* decoctions for disease reduction. Keeping in view the potential of plant based Bio products and dearth of data, the experiment was executed to test some available bio products. For this purpose Five Bio products that are available in the market named as Biosal, Protector, Vega Plus, Rigorous and Vampire were evaluated in two different In-vivo and In-vitro experiments for their effectiveness against the disease and its causal pathogen.

MATERIALS AND METHODS

Sampling, Identification and Purification of the Bacteria

The disease samples were collected from Sheikhupura, Hafiz Abad, Gujranwala and Sialkot region of Punjab Province in 2014-15. Upper three leaves of each plant were collected. The leaves were cut into small pieces (5mm), placed in ethanol (75%) for 12 seconds, rinsed twice in distilled water leading to the dipping into 300 *ul* distilled water for 15-20 minutes in centrifuge tubes. A loopful of bacterium containing water was streaked on Potato starch agar (peptone 1.2%, sucrose 1.2%, agar agar 2%) plates and placed in incubator at 28^0C for 72 hours. The bacterial colonies were picked up with the sterilized wire loop and placed upon fresh ager plates for purification. Identification of bacterium was done by Morphological tests (Dowson, 1957). Moreover Gram's Staining and pathogenicity test were performed for confirmation of Koch's postulates upon susceptible varieties i.e. Basmati 2000, Basmati Super. Purified bacterium culture was put into test tube containing fresh ager medium and kept refrigerated at 4°C.

In vitro* evaluation of different Bio products against *Xanthomonas oryzae* pv. *oryzae

Five Bio products namely Biosal (*Azadirachtine 70%)*, Protector (*Neem Oil* + *Garlic Oil* + *Emulsifier* + *Balsamodendron myrrha Extract)*, Vega Plus (*Avena sativa* + *Carbo vegitabilis* + *Bryonia alba* + *Turnera* + *Alfalfa* + *Ginkgo bilboa*), Rigorous (*Mother tincture of Calendula* + *Mother tincture of Ginkgo biloba* + *Bellis perennis* + *Stellaria media* + *Stimatamaydis* + *Baptisia* + *Astraglus mollissimus*) and Vampire (*Thuja Occid* + *Silicea* + *Natrum arsinicicum* + *Gelsemium* + *Berberis vulgaris* + *Hydrastis*) were evaluated against colony growth of *Xanthomonas oryzae* pv*. oryzae* by using inhibition zone technique at three different concentrations (100 ppm, 200 ppm and 300 ppm) and one Sterile distilled water treatment as control. Experiment was laid out in Completely randomized design in triplicate. Multiplication of bacterial culture was performed by adding the freshly grown bacterial aqueous suspension in a flask containing luke warm NGA media. After shaking well, suspension was poured in plates, wrapped and incubated at 30°C. After colony appearance, 1cm wells were created by cork borer at the center of the plate. Bio products solutions of mentioned concentrations were added in plates with the help of sterile disposable syringe, wrapped and incubated. Data regarding inhibition zones and all the treatments was recorded after 24, 48 and 72 hours. All the data were subjected to analysis of variance (ANOVA) along with application of LSD test at 5% significant level (Steel, 1997).

Evaluation of Bio products against Bacterial Leaf Blight of Rice under field conditions

Five bio products (Protector, Biosal, Vampire, Rigorous and Vega Plus) were experimented against the Bacterial Leaf Blight of Rice upon KSK-133 and IRRI-6 varieties. These varieties are considered moderately resistant and moderately susceptible respectively. For the experimental layout, RCBD was used along with three replications. From the multiplied bacterial culture, dilution plate technique was adopted to generate aqueous suspension (1 $x10^8$ CFU/ml) of the bacterium (Clifton, 1958). At maximum tillering stage of the crop, artificial inoculation was performed by clipping technique. After appearance of the disease symptoms, The bio products were foliarly sprayed at recommended doses (Biosal = 500ml/acre, Protector = 500ml/acre , Vega Plus = 1000ml/acre ,Rigorous = 1500 ml/acre, Vampire = 1000ml/acre) after an interval of seven days while spraying Sterilized water as control check.

Statistical Analysis

The collected data regarding disease severity was recorded (Chaudhary, 1996), and were subjected to Analysis of variance (Steel *et al.,* 1980).

RESULTS

In-vitro evaluation of bio products against *Xanthomonas oryzae* pv. *oryzae*

Statistical results explained that Biosal was more significant as compared to Protector Vega plus, Rigorous and Vampire. In different time durations after 24 hours, treatments showed variation among different concentration levels (100, 200 and 300 ppm) for all the bio products.

After 24 hours

After 24 hours 100 ppm concentration of all the bio products made 0.0cm inhibition zone while at 200 ppm inhibition zone (IZ) by Biosal was recorded as 0.90 cm as compared to Protector and Vega plus which shown results as 0.60 and 0.63 cm simultaneously. At 300 ppm the IZ by Biosal was enhanced as 1.85 cm comparing with Protector and Vega Plus i.e 1.40 cm and 0.70 cm. But interestingly, the increased concentration of Vega Plus did not increased its effect upon the Pathogen growth but Increased Concentration of Rigorous and Vampire improved their effect as results were recorded as 1.35cm and 1.16 cm as compared to their effect at 200 ppm i.e. 0.46 cm and 0.20 cm. Overall IZ recorded for control treatment was 0.00 cm (Figure 1).

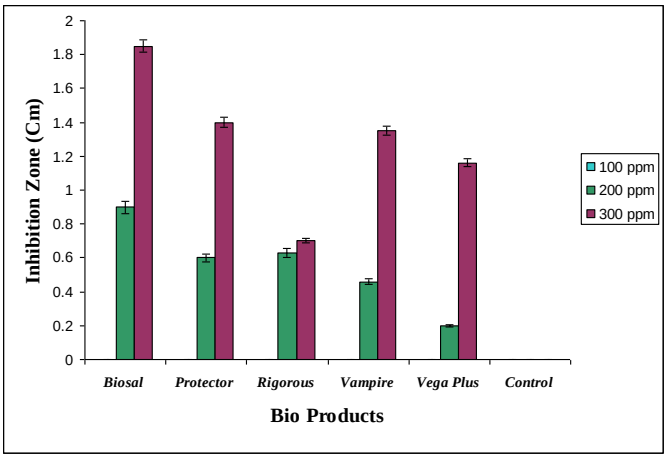

Fig. 1. Comparison of mean for evaluation of various Bioproducts against *Xanthomonas oryzae* pv*. oryzae* after 24 hours with 100, 200 and 300 ppm concentration.

After 48 hours

Increase in the time interval shown directly proportional results towards the size of Inhibition Zone. The IZ diameter at 100 ppm for Biosal was 0.53 cm comparing to Protector and Vega Plus which were closely nearing each other i.e. 0.43cm and 0.40 cm. The IZ size by all others was minimum than the above mentioned products. Similarly increase in the IZ size was recorded at 200 ppm concentration of all the products. 300 ppm concentration results were comparative regarding all the concentration in spite of Biosal which taken the lead and shown IZ as 2.13 cm but Protector, Vega Plus, Rigorous and Vampire shown IZ 1.70 cm, 1.00 cm, 1.63 cm, 1.43 cm respectively while control IZ was 0.00 cm (Figure 2).

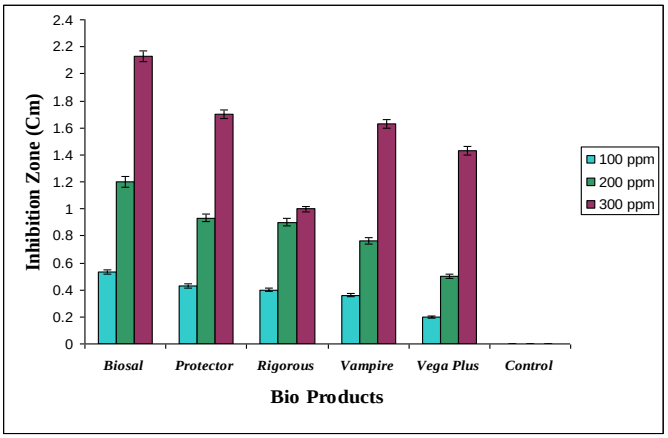

Fig. 2. Comparison of mean for evaluation of various Bio products against *Xanthomonas oryzae* pv*. oryzae* after 48 hours with 100, 200 and 300 ppm concentration.

After 72 hours

After 72 hours, of interval the results showed dissimilarity at 100 and 200 ppm. Some Bio products that were thought to be under power were showing good competition with others. Seemingly at 100 ppm of all the bio products showed some comparative IZ after 72 hours. 200 ppm concentration of Biosal, Protector and Vega Plus (IZ:- 1.50 cm, 1.23 cm and 1.20 cm) proven better as compared to Rigorous, Vampire and control (IZ:- 1.06 cm, 0.80 cm and 0.00cm). But consequently, the Results at 300 ppm become better and IZ was increased with the increase in concentration. Here also Biosal was commendable comparing to others (Figure 3).

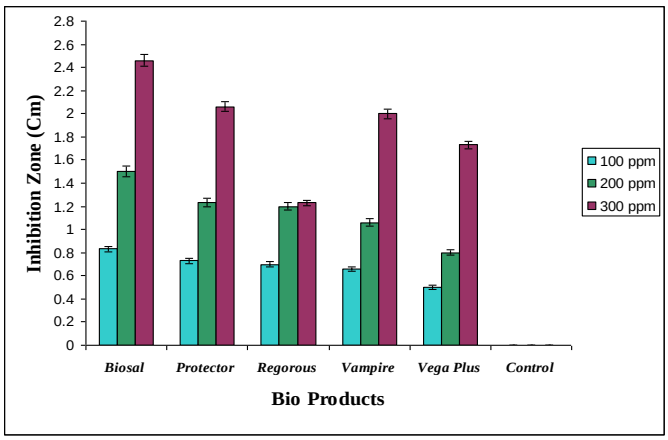

Fig. 3. Comparison of mean for evaluation of various Bio products against *Xanthomonas oryzae* pv*. oryzae* after 72 hours with 100, 200 and 300 ppm concentration.

In-Vivo Evaluation of bio products against BLB disease under natural Field Conditions

Field Evaluation of bio products against the bacterial Leaf Blight disease was conducted in 2014-15. The application of bio products to manage BLB disease revealed that in first week Biosal, Protector and Rigorous proved highly significant and average disease severity was recorded as 18 %, 28 %, 36% respectively whereas., Vampire and Vega plus were less effective in disease reduction and the mean percentage recorded was 44 %, 54 %, respectively comparing with the control (65.00%) (Figure 4).

After the second spray Biosal, Protector and Rigorous remained successful against BLB with following mean percent disease reductions 14 %, 23 %, 30 %. While vampire and Vega plus remained least effective by recording reduction in mean percentage disease severity recorded was 38 %, 49 % respectively comparing the control treatment (64.66%) (Figure 5).

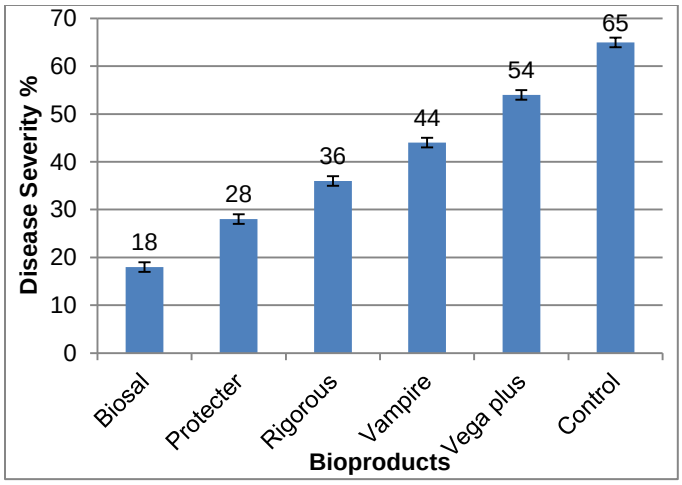

Fig. 4. Comparison of mean for evaluation of bio products against Bacterial leaf blight of rice after 7 days of artificial inoculation under field conditions.

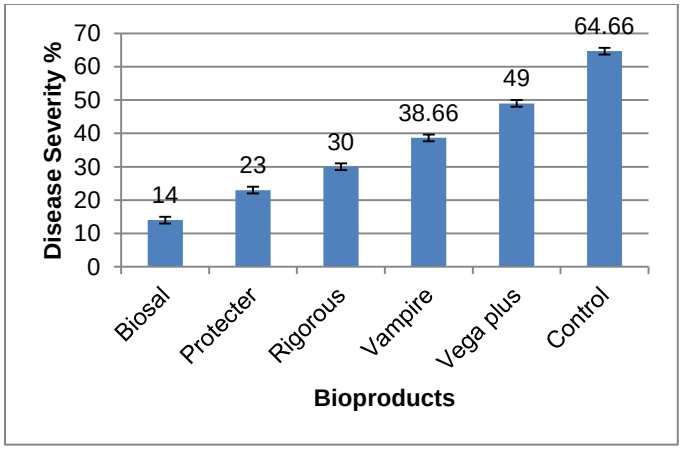

Fig. 5. Comparison of mean for evaluation of bio products against Bacterial leaf blight of rice after 14 days of artificial inoculation under field conditions.

DISCUSSION

BLB of rice has become a formidable disease. This causes destruction of the crop growth and yield. Management of the diseases is mostly done by usage of chemicals but those have resulted adverse effects upon environment as well as human health (Damalas, 2011). So the trend is shifting towards finding eco friendly organic products for controlling disease causing agents. Many of the botanical extracts have been recognized having antibacterial potential in recent years (Jabeen, 2013; Meena, 2013). Keeping an eye of the positivity of previous results, we attempted to evaluate some commercial bio products against the target disease.

Our results assured, that bio products are successful in reducing the pathogen viability in laboratory conditions and also minimize the disease severity in the field as well. Our finding have been proved connecting with the findings of other researchers. Jabeen (2011, 2013) who investigated the antibacterial potential of twenty five and eight different indigenous medicinal plants in the laboratory conditions for management of bacterial leaf blight (BLB) of rice respectively and documented about the success of *Anethum graveolens*, *Ferula assa-foetida*, *Terminalia chebula* and *Curcuma longa* for their significant activity in disease reduction. We found that Biosal and Protector exhibited excellent results by showing larger Inhibition zones at different concentrations and exposure time period as compared to Vega Plus, Rigorous and Vampire. The Bio Products performed better when they are used at higher concentrations to cope against the said disease. For example, Biosal and Protector applied at 300 ppm recorded the inhibition zone 2.46 cm and 2.06 cm respectively as compared to Vega Plus(1.23 cm), Rigorous (2.00 cm) and vampire (1.73 cm). Previous reports also strengthen our results that *Cocculus hirsutus* extract inhibited the *Xanthomonas oryzae* pv. *oryzae*, when used at a relatively higher concentration i.e 300 ppm (Shivalingaiah, 2013). We further explore that Bioproducts responded well when the pathogen was exposed to them for longer period of time at higher dozes. Such as Biosal exhibited the IZ=2.46 cm at a doze of 300 ppm when exposure period was maximum i.e 72 hours. In a similar context to our findings was Yougander (2015) who recognized the potential of Plant extracts at higher concentrations with longer exposure time to minimize the disease severity as he tested 30 different plant extracts and reported six of them (*Allium sativum, Curcuma domestica, Syzygium aromaticum, Eucalyptus globules, Datura metel, Ocimum sanctum*) successful against the bacteria when exposed against the extracts for more than 6 days. Biosal (*Azadirectine* 70%) exhibited inhibition zone size as 1.85 cm at 300 ppm concentration with an exposure time of 24 hours. Correspondingly, the results of Jabeen (2013) match with our results who evaluated 24 organic extracts of different indigenous plants and mentioned about the minimization of pathogen by *Azadirechtin-D* by recording IZ as 1.83 cm.

It is equally important to test the bio products in field conditions after recording their success in the laboratory against the causative pathogen because in natural field conditions many other factors such as insect pests, other pathogens, fertilizer and water stress and environmental variations play an important role in varying the performance of the test products. Average disease severity recorded was 18% after the first spray of Biosal at recommended doze which performed the best comparing with others (Protector, Rigorous, Vampire, Vega Plus) which exhibited higher disease severity i.e 28%, 36%, 44%, 54%. Our findings coordinate with Suresh (2014) who evaluated streptomycin, Copper Compounds and Azadirectin at different dozes and reported that these compounds and

Azadirectin minimized the disease severity. The disease severity reduced up to certain levels after the second spray of the bioproducts. Biosal reduced the average disease severity up to 14% while others recorded disease severity as 23%, 30%, 38% and 49% respectively. Results of Singh (2015) cope with our efforts as various plant extracts were proved successful against *Xanthomonas oryzae* pv. *oryzae.* He recorded the disease reduction by 0.3% Neem oil and 0.3% grass oil up to 33% and 34.5% respectively.

CONCLUSION

Regarding In-vitro management, the concentration of the bio products along with the time interval shown their equal importance and direct proportion with the results. Using the increased concentration of bio products proven better after 72 hours of time interval. Under Natural Filed Conditions, pathogen performs differently and disease progression is minimized by almost all the bio products up to some extent that shows there is potential in Bio products to manage the BLB disease. Previously, limited studies have been carried out to inquire about the bio products so the need of time is to turn the direction towards the exploration of more botanicals to manage the disease.

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CONFLICT OF INTEREST AND FUNDING

The author has carried out the research to explore the potential of available bio products against the most threatening disease of rice crop and has received no financial support. The authors further declare that they have no interest of competition with anyone.

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