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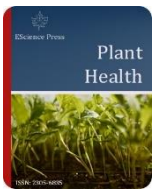

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Screening of Various Wheat Lines Against Puccinia Triticina and its Management through Synthetic Fungicides

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ABSTRACT

Wheat is an important grain crop in the world and in Pakistan. Leaf rust is one of the detrimental disease and widespread in the world due to its distribution and higher adaptive capabilities. For screening of wheat germplasm against leaf and stripe rust, 150 varieties/lines were evaluated each entry was planted as single row and Morocco (susceptible) was included to every 10 entry in the nursery Moreover the use of fungicide on wheat crop is prohibited in Pakistan as it is being consumed as staple food, because of the widespread losses being caused by the leaf rust fungicides are being used to minimized the losses being caused by the disease so the potential of different chemicals for controlling leaf rust caused by *Puccinia triticina* was evaluated under this study. Therefore, three foliar different fungicides/chemicals were selected for evaluation named as Mycoguard (Azoxysterobin), Score (Diaphenaconazole) and Tilt (Propioconazol), with doses of 125ml/10L, 125ml/10L and 200ml/10L respectively were tested under field conditions and T4 was kept as control these chemicals, when applied at booting and leaf emergence stages. Screening result showed, out of 150 varieties/advanced lines screened against leaf rust of wheat. 26 were moderately resistant, 28 lines showed moderately susceptible response, 51 were resistant and remaining lines were susceptible. Among above captioned fungicides Mycoguard significantly reduced the disease upto 56%. The second best treatment was score which induced reduction upto 50%. All the data was statistically analyzed. Therefore, it is suggested that Mycoguard can be effectively employed to control leaf rust disease in wheat crop.

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INTRODUCTION

Wheat (*Triticum aestivum*.L) is an important grain crop in the world. In Pakistan, it is consumed as staple food. Wheat provides carbohydrates, proteins and certain amino acids including glutamine and proline, alanine, asparagine and glycine (Cornell, 2003). Agriculture sector is prevailing as the backbone of Pakistan's economy at this time. It is accounted for 19.5 percent of GDP of the

country and 42.5 percent of employment as well as employment opportunity in the financial year 2016–2017 (GOP, 2019). Wheat at this time stands as the second largest crop in the country and it is alone responsible for 1.9 percent in the GDP of Pakistan, and 3.4 percent in the employment as well as employment opportunity during the said year. Considering the mentioned facts wheat is being considered as backbone of national economy and its domestic consumption continues to increase steadily year after year, therefore its exports are expected to be affected significantly in upcoming years; therefore it is alarming and will be a major setback in coming years for country's economy. Wheat production forecasting for many years to come is one way of lightening this predicament which is to minimize the diseases by various methods as the losses caused by diseases are alarming certain measures may be (Akram *et al*., 2017). Leaf rust caused by (*Puccinia triticina*) is the most violent and widespread disease due to its distribution and higher adaptive capabilities (Agrios, 2005). This disease has a complex etiology that is difficult to understand. The responsible fungus overwinters in the early drilled crop. It has alternate hosts including species of *Thailctrum*, *Isopyrum* and *Clematis*. Environmental factors (temperature, rainfall, leaf wetness and relative humidity) play key role in establishing the disease in epidemic form while affecting various developmental stages of the pathogen. During spring, the disease manifests itself at temperatures between 15°C to 22 °C under higher relative humid conditions, afterwards symptoms can be observed on larger scale at the start of the summer season when days are windy and dry, and nights are cooler allowing for the disease establishment. This climatic change favors the early development of the disease (Agrios, 2005). If understood clearly, the epidemiological studies allow developing effective disease control measures thereby incorporating and optimizing the use of better performing resistant germplasm, chemicals, culture practices and fertilizer applications for advocating a proper wheat growing plan, as well as for disease outbreak prediction and forecasting (Agrios, 2005).

MATERIALS AND METHODS

Establishment of screening nursery: For screening of wheat germplasm against leaf and stripe rust, 150 varieties/lines were sown on mid of November in wheat experimental area of Ayub Agriculture research institute (AARI). Each entry was planted as single row and Morocco was included to every 10 entry in the nursery. The line length was one meter and line to line distance was 30cm with 1m path. Two lines of Morocco were sown to trap air borne infection and spread it.

Inoculation: In order to ensure maximum disease pressure, artificial inoculation was done by transplanting rusted plants, rubbing, spraying with rust inoculums and dusting with talcum powder and rust inoculums during the crop season (Stubbs *et al*., 1986). To maintain the rust inoculums pressure nursery was inoculated twice in a week at booting stage. A suspension of artificial urediniospores was prepared in a lab and sprayed on wheat germplasm. After every 10th line/variety a line of highly susceptible wheat cultivar i.e. Morocco, was sown to act as rust spreader row (highly susceptible to all the prevalent rust races and provides a substrate for rapid multiplication and distribution of rust inoculums).

Recording of leaf rust data: Modified Cobb's scale was used to record the Leaf rust severity and disease response data described by Peterson *et al*. (1948). The data was collected with an interval of fifteen days. The final data was collected when the disease severity reached upto 80-100% in spreader morocco.

Table 1. Rust reaction, code for field response and response value.

Reaction	Code	Field response	Value
No disease	0	No visible infection.	0.0
Resistant	R	Necrotic areas with or without minute uredia.	0.2
Moderately Resistant	MR	Small uredia present surrounded by necrotic Area.	0.4
Moderately resistant, Moderately Susceptible	MRMS	Small uredia present surrounded by necrotic areas as well as medium uredia with no necrosis but possible some distinct chlorosis.	0.6
Moderately Susceptible	MS	Medium uredia with no necrosis but possible some distinct chlorosis.	0.8
Moderately Susceptible-susceptible	MSS	Medium uredia with no necrosis but possible some distinct chlorosis as well as large uredia with little or chlorosis present.	0.9
Susceptible	S	Large uredia and little or no chlorosis present.	1.0

Cobb's scale (Peterson *et al*., 1948) was used only to record the rust severity data.

Collection of Environmental data: Environmental data consisting of higher and lower air temperature, relative humidity, rainfall and wind speed was recorded by conventional instruments installed in an observatory in the field of Ayub Agricultural Research Institute, Faisalabad, about 1.5 kilometers away from wheat experimental area.

Area Under Disease Progress Curve (AUDPC): In order to screen out resistant varieties against leaf rust 120 lines were sown. The inoculation was done with the mixture of virulent races and the data was recorded with a fifteen days interval. Area Under Disease Progress Curve (AUDPC) was calculated by using following formula developed by CIMMYT (Shaner and Finney, 1980)

Chemical Control: Different chemicals were applied to evaluate the efficiency of chemicals in controlling the leaf rust disease. Susceptible variety MORROCO was selected for this purpose. Trial was conducted at experimental area of Ayub Agricultural Research Institute (AARI), Faisalabad. Fungicides were applied between leaf emergence and booting stage. Three chemicals, Tilt, Mycoguard and Score were evaluated at 200mL/ 10 L, 125ml/10 L and 125mL/10 L the recommended dose concentration respectively. Trial was conducted in Randomize Complete Block Design (RCBD), with three replications.

Statistical Analysis: A simple correlation was done between the different environmental factors such as maximum & minimum temperature, relative humidity, rainfall and wind speed and leaf rust response was observed through modified Cobb's scale described by Peterson *et al*., (1948) for wheat varieties/lines. All the rust severities and environmental data were subjected to correlation and regression analysis to determine the relation of epidemiological factors with wheat rusts. Data was analyzed statistically to determine the effectiveness of fungicides at 5% least significant difference using appropriate statistical tools.

RESULTS

Screening of wheat varieties/lines against leaf rust: Out of 150 varieties/advanced lines screened against leaf rust of wheat. 26 were moderately resistant, 28 lines showed moderately susceptible response, 51 were resistant and remaining lines were susceptible.

Table 2. Response of different lines/varieties against leaf rust on the basis of AUDPC.

Sr.	Lines/ Varieties	Assessment 1	Assessment 2	Assessment 3	Mean Severity	AUDPC	Disease Reaction	Sr.	Lines/ Varieties	Assessment 1	Assessment 2	Assessment 3	Mean Severity	AUDPC	Disease Reaction
1	V-1	5	15	20	13.33	263	R	76	V-76	0	5	20	8.33	175	MS
2	V-2	0	0	0	0	0	R	77	V-77	0	5	20	8.33	175	S
3	V-3	5	20	30	18.33	368	MS	78	V-78	10	20	40	23.33	455	S
4	V-4	0	0	0	0	0	R	79	V-79	0	0	5	1.66	35	MS
5	V-5	0	0	0	0	0	R	80	Morocco	40	60	80	60	1,120	S
6	V-6	10	25	30	21.66	420	MR	81	V-81	0	5	10	5	105	MS
7	V-7	5	20	30	18.33	368	R	82	V-82	0	0	0	0	0	R
8	V-8	0	0	0	0	0	R	83	V-83	0	0	0	0	0	R
9	V-9	10	30	40	26.66	525	MS	84	V-84	0	5	10	5	105	MS
10	Morocco	40	60	80	60	1,120	S	85	V-85	0	0	0	0	0	R
11	V-11	0	0	0	0	0	R	86	V-86	0	5	10	5	105	MS
12	V-12	0	0	0	0	0	R	87	V-87	0	0	5	1.66	35	MR
13	V-13	10	15	20	15	280	MR	88	V-88	5	15	20	13.33	263	S
14	V-14	5	10	20	11.66	228	MS	89	V-89	0	10	20	10	210	MS
15	V-15	5	30	30	21.66	438	MS	90	Morocco	40	60	80	60	1,120	S
16	V-16	10	30	30	23.33	455	MS	91	V-91	10	20	40	23.33	455	MR
17	V-17	0	0	0	0	0	R	92	V-92	40	60	80	60	1,120	S
18	V-18	0	0	0	0	0	R	93	V-93	20	40	60	40	770	S
19	V-19	0	20	30	16.66	350	MS	94	V-94	20	40	60	40	770	S
20	Morocco	40	60	80	60	1,120	S	95	V-95	10	20	40	23.33	455	MR
21	V-21	0	0	0	0	0	R	96	V-96	0	0	0	0	0	R
22	V-22	40	60	80	60	1,120	S	97	V-97	20	40	60	40	770	S
23	V-23	0	0	0	0	0	R	98	V-98	0	0	0	0	0	R

24	V-24	0	0	0	0	0	R	99	V-99	40	60	80	60	1,120	S
25	V-25	20	50	70	46.66	910	S	100	Morocco	40	60	80	60	1,120	S
26	V-26	0	0	0	0	0	R	101	V-101	20	40	60	40	770	MR
27	V-27	20	50	60	40	840	S	102	V-102	10	20	30	20	385	MR
28	V-28	20	40	60	40	770	MS	103	V-103	10	20	30	20	385	MR
29	V-29	30	40	50	40	735	MS	104	V-104	20	40	60	40	770	S
30	Morocco	40	60	80	60	1,120	S	105	V-105	10	20	30	20	385	S
31	V-31	30	40	60	43.33	805	MSS	106	V-106	20	40	60	40	770	MS
32	V-32	20	40	50	36.33	700	MS	107	V-107	0	0	0	0	0	R
33	V-33	10	20	40	23.33	455	MR	108	V-108	0	0	0	0	0	R
34	V-34	5	20	40	21.66	438	MR	109	V-109	0	0	0	0	0	R
35	V-35	0	0	0	0	0	R	110	Morocco	40	60	80	60	1,120	S
36	V-36	0	0	0	0	0	R	111	V-111	0	0	0	0	0	R
37	V-37	0	0	0	0	0	R	112	V-112	0	0	0	0	0	R
38	V-38	30	40	60	43.33	805	S	113	V-113	20	30	40	30	560	MR
39	V-39	0	0	0	0	0	R	114	V-114	30	40	60	63.33	805	S
40	Morocco	40	80	60	60	1,120	R	115	V-115	0	0	0	0	0	R
41	V-41	40	50	70	53.33	980	S	116	V-116	0	0	0	0	0	R
42	V-42	0	0	0	0	0	R	117	V-117	30	40	60	63.33	805	S
43	V-43	40	60	80	60	1,120	S	118	V-118	0	0	0	0	0	R
44	V-44	30	40	60	43.33	805	S	119	V-119	0	0	0	0	0	R
45	V-45	20	40	60	40	770	S	120	Morocco	40	60	80	60	1,120	S
46	V-46	20	40	70	43.33	840	MS	121	V-121	0	0	0	0	0	R
47	V-47	10	30	50	30	595	S	122	V-122	0	0	0	0	0	R
48	V-48	0	0	0	0	0	R	123	V-123	0	0	0	0	0	R
49	V-49	20	40	40	33.33	630	S	124	V-124	20	30	40	30	560	MR
50	Morocco	40	60	80	60	1,120	S	125	V-125	30	40	60	63.33	805	S
51	V-51	10	20	40	23.33	455	MS	126	V-126	5	10	20	11.66	228	MR
52	V-52	15	30	40	28.33	543	S	127	V-127	10	20	40	23.33	455	MR
53	V-53	20	30	40	30	560	MS	128	V-128	0	0	0	0	0	R
54	V-54	5	20	30	18.33	368	S	129	V-129	5	10	20	11.66	228	MR
55	V-55	0	0	0	0	0	R	130	Morocco	40	60	80	60	1,120	S
56	V-56	0	0	0	0	0	R	131	V-131	20	40	60	40	770	S
57	V-57	0	10	30	13.33	280	S	132	V-132	10	20	30	20	385	MR
58	V-58	10	40	80	43.33	875	MSS	133	V-133	0	0	0	0	0	R
59	V-59	10	60	80	50	1,015	S	134	V-134	0	0	0	0	0	R
60	Morocco	40	60	80	60	1,120	S	135	V-135	10	30	50	30	595	MR
61	V-61	20	50	80	50	980	S	136	V-136	10	20	40	23.33	455	S
62	V-62	10	30	60	33.33	665	S	137	V-137	20	30	40	30	560	MR
63	V-63	0	5	20	8.33	175	S	138	V-138	0	0	0	0	0	R
64	V-64	10	30	60	33.33	665	MS	139	V-139	0	0	0	0	0	R
65	V-65	0	0	0	0	0	R	140	Morocco	40	60	80	60	1,120	S
66	V-66	20	40	100	53.33	1,050	S	141	V-141	30	40	50	40	735	MR
67	V-67	0	10	30	13.33	280	S	142	V-142	0	0	0	0	0	R
68	V-68	20	30	40	30	560	MS	143	V-143	0	10	20	10	210	MR
69	V-69	0	0	10	3.33	70	MS	144	V-144	0	5	10	5	105	MS
70	Morocco	40	60	80	60	1,120	S	145	V-145	0	5	10	5	105	MS
71	V-71	0	0	0	0	0	R	146	V-146	0	10	20	10	210	MR
72	V-72	0	0	0	0	0	R	147	V-147	0	10	20	10	210	MR
73	V-73	10	20	60	30	595	MR	148	V-148	0	30	40	23.33	490	MS
74	V-74	0	20	40	20	420	R	149	V-149	5	10	20	11.66	228	MS
75	V-75	0	40	0	0	280	R	150	Morocco	40	60	80	60	1,120	S

HR; Highly resistant, **R**; Resistant, **MR**; Moderately resistant, **MS**; Moderately susceptible, **S**; Susceptible, **HS**; Highly susceptible.

Environmental factors were favoring the leaf rust development

Different epidemiological factors were favoring the disease development such as Temperature (lower, high), relative humidity, wind velocity and rainfall. Temperature favors the disease development with the increase of maximum (20-27°C) and minimum temperature (9-15°C) disease severity increased.

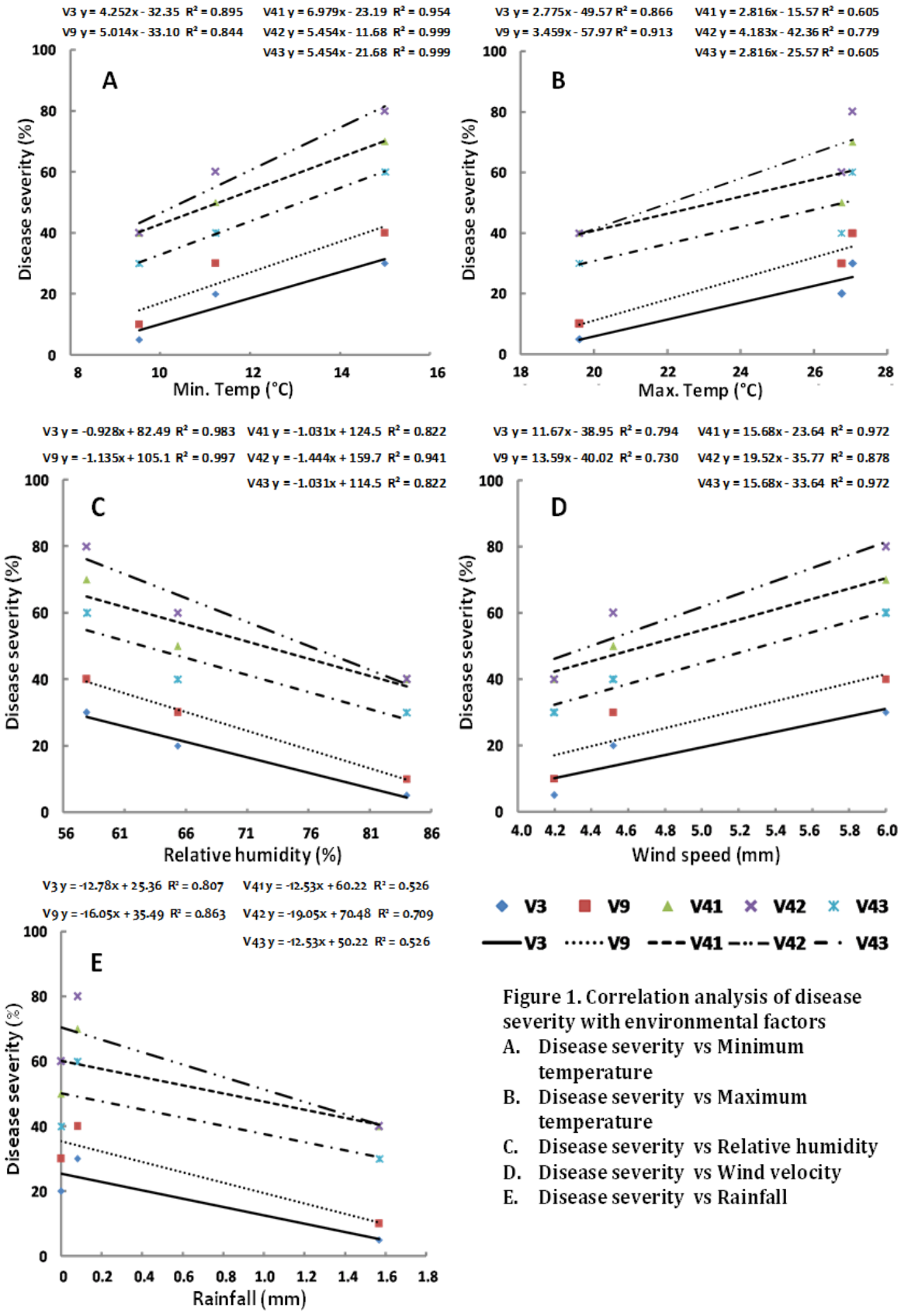

Figure 1. Correlation analysis of disease severity with environmental factors

A. Disease severity vs Minimum temperature
B. Disease severity vs Maximum temperature
C. Disease severity vs Relative humidity
D. Disease severity vs Wind velocity
E. Disease severity vs Rainfall

It showed there was a significant relationship between temperature and disease severity. As compared to temperature with the increase of relative humidity (56-86) and rainfall (0-1.6) disease severity decreased so, there was a non-significant relationship between disease severity and rainfall and relative humidity. Wind speed (4.2-6) was also conducive for the disease development as with the increase of wind speed disease severity increased.

Environmental factors were favoring the leaf rust development: Different epidemiological factors were favoring the disease development such as Temperature (lower, high), relative humidity, wind velocity and rainfall. Temperature favors the disease development with the increase of maximum (20-27°C) and minimum temperature (9-15°C) disease severity increased. It showed there was a significant relationship between temperature and disease severity. As compared to temperature with the increase of relative humidity (56-86) and rainfall (0-1.6) disease severity decreased so, there was a non-significant relationship between disease severity and rainfall and relative humidity. Wind speed (4.2-6) was also conducive for the disease development as with the increase of wind speed disease severity increased.

Evaluation of fungicides: Three different chemicals were selected for evaluation named as Mycoguard, Score and Tilt with dose of 125ml/10L, 125ml/10L and 200ml/10L respectively. Chemicals were applied 30, 45 and 40 days before the harvesting of the crop. The data was collected after 3, 7 and 10 days of application and it was found that Mycoguard significantly reduced the disease upto 56%. The second best treatment was score which induced reduction upto 50%.

Reduction in mean disease severity: Mean disease was also calculated and there was significant reduction in mean disease severity was seen. After 3, 7 and 10 days the mean disease severity of Mycoguard was 23.33, 23.33 and 26.33 respectively as shown in the table 3. The mean disease severity of score after 3, 7 and 10 days interval was 26.6, 30 and 30. Tilt also showed significant reduction in mean disease severity upto 26.66, 30 and 40 on 3 different time intervals. In a nut shell, Mycoguard is the best treatment which significantly reduced the mean disease severity.

Table. 3. Fungicides treatments results after 3, 7 and 10 days of application

Treatments	Trade name	Replication	Before application	After 3 days of application	After 7 days of application	After 10 days of application
T1	Control	R-1	40S	40S	50S	60S
		R-2	40S	40S	50S	60S
		R-3	40S	40S	50S	60S
T2	Tilt	R-1	40S	30S	30S	40S
		R-2	40S	30S	30S	40S
		R-3	40S	20S	30S	40S
T3	Mycoguard	R-1	40S	20S	20S	30S
		R-2	40S	30S	30S	20S
		R-3	40S	20S	20S	30S
T4	Score	R-1	40S	30S	30S	30S
		R-2	40S	20S	30S	30S
		R-3	40 S	30S	30S	30S

DISCUSSION

In Pakistan, per hectare average production of wheat is much lesser, as compared to the contemporary leading wheat producing countries like China, Australia and USA. However, the maximum potential of Pakistani wheat varieties (7000 to 8000 kg/hac) is achievable provided the prevalent diseases are handled properly according to the best agriculture practices (Asif and Kamran, 2011; Ahmed *et al*., 2012). Important diseases include Bunts, smuts, and viral diseases. Among these, the three main fungal diseases of wheat are Stripe rust (Puccinia striiformis), Leaf rust (Puccinia triticina) and Stem rust (Puccinia graminis) that cause huge losses in terms of lower quality and low production of wheat (Singh *et al*., 2004). The improper use of chemicals has called for developing alternative eco-friendly phyto-sanitary measures, in addition to determining the most suitable chemical pesticides while developing resistant varieties, bio control practices and integrated disease management strategies safer for the stakeholders (Atlas and Bartha,

1998). Nevertheless, the chemical control remains an effective method for controlling plant diseases in the meantime, that cannot be left ignored. Azoxystrobin, the synthetic compounds called β-methoxyacrylates are derived from naturally occurring compounds called strobilurins (Inoue *et al.*, 2012). Biochemically, Azoxystrobin interferes with ATP synthesis by binding to the Qo site of complex III within the mitochondrion and hence disrupts electron transport chain (Hewitt, 1998).. This compound has been effectively used against four groups of fungi viz. Ascomycota, Deuteromycota, Basidiomycota and the Oomycota. In addition it is preventive against powdery mildew, downy mildew, rust and rice blast diseases (Anand *et al.*, 2010). Propiconazole is derivative of triazole, a demethylated inhibitor interferes with ergosterol biosynthesis by suppressing the oxidative removal of 14 α -methyl group from 24-methylenedihydrolanosterol in fungi (Sisler *et al.*, 1984). The deficiency of ergosterols results in instability of plasma membrane that leads to failure of fungus nutrition and ultimately death (Baldwin and Wiggins, 1984). Difenoconazole is the first sterol inhibitor compound. Biochemically, inhibitors are divided into two groups based on their action. Firstly, it inhibit the sterol C-14 demethylation which interferes with the P-450 enzyme and secondly, interferes with the C-14 reductas (Dahmen and Staub, 1992).

CONCLUSION

It was concluded that leaf rust is one of the damaging wheat disease and Mycoguard significantly reduced the disease upto 56%. The second best treatment was score which induced reduction upto 50% Therefore, it is suggested that Mycoguard can be effectively employed to control leaf rust disease in wheat crop.

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