RESEARCH ARTICLE

EFFECT OF TREATMENT IMPOSED ON TOTAL SOLUBLE PROTEIN CONTENT IN WHEAT LEAVES INFECTED BY BROWN RUST (*PUCCINIA RECODITA F*.SP. TRITICI ROB. EX. DESM.) AT KANPUR AND IARI REGIONAL STATION WELLINGTON (T.N.).

Akash Tomar*, Ved Ratan , Javed Bahar Khan, Dushiyant Kumar, Devesh Nagar and Sonika Pandey

Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture & Technology Kanpur 208002 (U.P.) India
Email: atakshay343@gmail.com

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Abstract: In India, wheat (*Triticum aestivum* L.) is a staple food. Rust caused by. *Puccinia Recondita* f. sp. *tritici* Rob. ex. Desm. (Brown rust) is the most destructive and one of the most common diseases of wheat worldwide. It probably results in higher total annual losses worldwide because of its more frequent and widely distributed diseases of wheat in India and elsewhere that affects its yield potential. Although, chemical control of these diseases is known but is not economic and environmental friendly to be used on a large scale. . The chemical changes in leaves due to infection of brown rust protein quantification were done by Lowry method. The soluble protein contents in treatment T_{16} (Soil treatment with Mycorrhiza (VAM) @ 5 gm / plot + Soil treatment with *Trichoderma harzianum* @ 5 gm / plot + Three spray with Propiconazole @ 25 EC 0.1 %) treated leaves were 0.37 mg/ml, followed by T1 (0.32 mg/ml) and T3 (0.28 mg/ml) which is the highest among all the treatments.

Keywords: Soluble protein, Treatment, Brown rust, Wheat

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important food crops and is a staple food for over one-third of the world's population. In Pre-historic times, it was grown in ancient Persia, Egypt, Greece, and Europe as early as 10,000 to 15,000 B.C. and in China about 3000 B.C. From all possible records, it seems that its center of origin in South-Western Asia. It is believed that Aryans brought wheat grains to India, and since then it has been cultivated in India.

The pieces of evidence from the ancient sites of Jarmo in Eastern Iraq and the excavations of Mohenjo-Daro in the Indian subcontinent indicate that wheat was cultivated in India more than 5,000 years ago. Specific references are made to wheat in "Atharva Veda" which is believed to have been written around 15000 to 5,000 B.C. More of the earth's surface is covered by wheat than with any other food crop. Wheat is the third most-produced cereal after maize and rice, but in terms of dietary intake, it is currently second to rice as the main food crop, given the more extensive use of maize as an animal feed. As a hardy crop, which can grow in a wide range of environmental conditions and that permits large-scale cultivation as well as long-term storage of food, wheat has been key to the emergence of city-based societies for millennia. India is the second-largest producer of the wheat in the world and is outranked only by China. But in terms of productivity, India ranks 38 in the world. In India, Wheat is the second most important cereal crop occupying 52.8 percent of the total Rabi food grains, and it ranks second in production and area after rice. It covered about 30.50 million hectares area during 2016-17 with a record production of 98.38 million tones with the productivity of 3216 kg/hectare.

MATERIALS AND METHODS

Total Soluble Protein Extraction

Total Soluble protein in wheat leaves infected by brown rust was extracted by using method developed by Goggin *et al*., (2011). Leaves from treated wheat plants (approx. 500mg) were frozen by liquid nitrogen, grinding to a fine powder using mortar and pestle then transferred to a fresh centrifuge tube. Two ml of extraction buffer (Tris-HCl 1M, pH 8, EDTA, 0.25), SDS, 10%, glycerol, 50%) was added and mixed well. The content of the tubes were centrifuge at 12000 rpm for 20 min at 4°C. After centrifugation process supernatant was discarded. Mixed the pellets with 1ml of sample buffer (80% Acetone, 0.07% β-mercaptoethanol and 2mM EDTA) and centrifuged at 12000 rpm for 5 minutes. The process was repeated until all chlorophyll removed. Mixed clear pellet with milli Q water and stored at -20°C. Protein concentration of all the samples was determined using Lowry *et al*., (1951) and Yurganova *et al*., (1989).

Protein Quantification

For quantification of protein content 1mg/ml of BSA standard was used. Different dilutions of the standard were made. To each tube of standard and sample 2ml of complex forming reagent was added and kept for 10 minutes at room temperature. After 10 minutes of incubation period, 0.2ml of Folin-Ciocalteu reagent

*Corresponding Author

solution was added to each tube and incubated for 20-30 minutes at room temperature in dark. After incubation period sample absorbance was taken at 660nm by using spectrophotometer (Bio-Rad). Calibration curve was constructed by plotting absorbance reading on Y axis against standard protein concentration (mg/ml) on X axis. Sample concentration was calculated using standard graph as a reference.

Observations on total protein content in wheat plants treated with different concentrations was revealed that treatment was found best field conditions at Kanpur and IARI Regional Station Wellington (T.N.), yielded highest protein respectively.

Value of concentration and Absorbance with slandered graph.

concentration	Absorbance
0	0
0.1	0.17
0.2	0.3
0.4	0.64
0.6	0.89
0.8	1.24

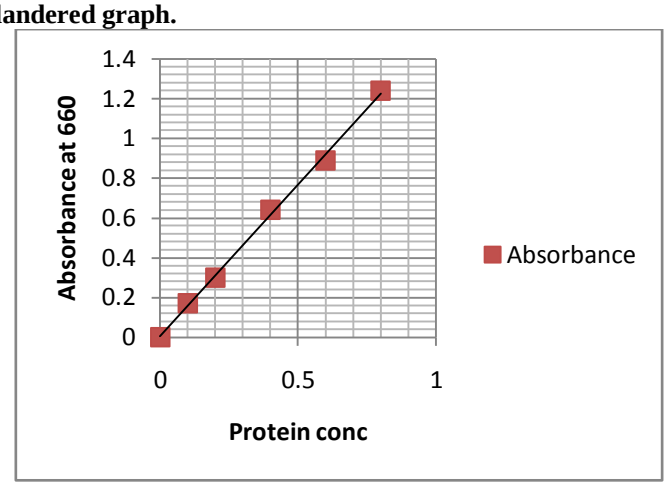

Table 1. Total treatment with different combination

T_1	Seed treatment with carbendazim @ 2 gm/ kg seed
T_2	Seed treatment with Carbendazim @ 2 gm/ kg seed + Soil treatment with *Trichoderma harzianum* @ 5 gm / plot
T_3	Seed treatment with Carbendazim @ 2 gm/ kg seed + Soil treatment with Mycorrhiza (VAM) @ 5 gm / plot
T_4	Seed treatment with Carbendazim @ 2 gm/ kg seed + Three sprey with Propiconazole @ 25 EC 0.1 %
T_5	Seed treatment with Carbendazim @ 2 gm/ kg seed + Three sprey with Triadimefon @ 25 EC 0.1 %
T_6	Seed treatment with Carbendazim @ 2 gm/ kg seed + Three sprey with Hexaconazole @ 25 EC 0.1 %
T_7	Soil treatment with *Trichoderma harzianum* @ 5 gm / plot
T_8	Soil treatment with *Trichoderma harzianum* @ 5 gm / plot + Three sprey with Propiconazole @ 25 EC 0.1 %
T_9	Soil treatment with *Trichoderma harzianum* @ 5 gm / plot + Three sprey with Triadimefon @ 25 EC 0.1 %
T_{10}	Soil treatment with *Trichoderma harzianum* @ 5 gm / plot Three sprey + with Hexaconazole @ 25 EC 0.1 %
T_{11}	Soil treatment with Mycorrhiza (VAM) @ 5 gm / plot
T_{12}	Soil treatment with Mycorrhiza (VAM) @ 5 gm / plot + Three sprey with Propiconazole @ 25 EC 0.1 %
T_{13}	Soil treatment with Mycorrhiza (VAM) @ 5 gm / plot + Three sprey with Triadimefon @ 25 EC 0.1 %

T_{14}	Soil treatment with Mycorrhiza (VAM) @ 5 gm / plot + Three sprey with Hexaconazole @ 25 EC 0.1 %
T_{15}	Soil treatment with Mycorrhiza (VAM) @ 5 gm / plot + Soil treatment with *Trichoderma harzianum* @ 5 gm / plot
T_{16}	Soil treatment with Mycorrhiza (VAM) @ 5 gm / plot + Soil treatment with *Trichoderma harzianum* @ 5 gm / plot + Three sprey with Propiconazole @ 25 EC 0.1 %
T_{17}	Soil treatment with Mycorrhiza (VAM) @ 5 gm / plot + Soil treatment with *Trichoderma harzianum* @ 5 gm / plot + Three sprey with Triadimefon @ 25 EC 0.1 %
T_{18}	Soil treatment with Mycorrhiza (VAM) @ 5 gm / plot+ Soil treatment with *Trichoderma harzianum* @ 5 gm/ plot + Three sprey with Hexaconazole @ 25 EC 0.1 %
T_{19}	Three sprey with Propiconazole @ 25 EC 0.1 %
T_{20}	Three sprey with Triadimefon @ 25 EC 0.1 %
T_{21}	Three sprey with Hexaconazole @ 25 EC 0.1 %
T_{22}	Three sprey with Propiconazole @ 25 EC 0.1 % + (F.Sp.) with Triadimefon @ 25 EC 0.1 % + (F.Sp.) with Hexaconazole @ 25 EC 0.1 %
T_0	Control

RESULTS

Soluble Average protein concentration

The data presented in Table showed that the soluble protein contents in treatment T_{16} treated leaves were 0.37 mg/ml, followed by T1 (0.32 mg/ml) and T3 (0.28 mg/ml), T2 and T5 (0.25 mg/ml) which is the highest among all the treatments. The soluble average protein contents of control T_0 was 0.06 mg/ml. The decrease protein in infected leaves with comparison to treatment imposed may be due to utilization of some protein by the pathogen.

Table 2. Effect of Treatment Impose on total soluble protein content in Wheat leaves after Eighth week of disease observation of Brown Rust in Kanpur and IARI regional station Wellington.

S.No.	Treatments	Protein concentration mg/ml		Average
		Kanpur	Wellington	
1.	**T_1**	**0.30**	**0.34**	0.32
2.	**T_2**	0.25	0.26	0.25
3.	**T_3**	0.30	0.27	0.28
4.	**T_4**	0.18	0.26	0.22
5.	**T_5**	0.21	0.29	0.25
6.	**T_6**	0.12	0.18	0.15
7.	**T_7**	0.11	0.32	0.21
8.	**T_8**	0.23	0.19	0.21
9.	**T_9**	0.16	0.20	0.18
10.	**T_{10}**	0.14	0.18	0.16
11.	**T_{11}**	0.20	0.23	0.21
12.	**T_{12}**	0.19	0.25	0.22

13.	**T_{13}**	0.12	0.29	0.20
14.	**T_{14}**	0.15	0.17	0.16
15.	**T_{15}**	0.26	0.21	0.23
16.	**T_{16}**	**0.30**	**0.44**	0.37
17.	**T_{17}**	0.09	0.16	0.12
18.	**T_{18}**	0.10	0.16	0.13
19	**T_{19}**	0.15	0.20	0.17
20	**T_{20}**	0.14	0.20	0.17
21	**T_{21}**	0.16	0.23	0.19
22	**T_{22}**	0.17	0.26	0.21
23	**$T_{0\ control}$**	0.07	0.05	0.06
CD at 5%				**0.026**

DISCUSSION

The data presented in Table showed that the soluble protein contents in treatment T_{16} treated leaves were 0.37 mg/ml, followed by T1 (0.32 mg/ml) and T3 (0.28 mg/ml), T2 and T5 (0.25 mg/ml) which is the highest among all the treatments. The soluble average protein contents of control T_0 was 0.06 mg/ml. The decrease protein in infected leaves with comparison to treatment imposed may be due to utilization of some proteins. The reduced disease incidence indicates that some protein must be associated with induction of resistance against the pathogen. Antoniew *et al.* (1980) considered that pathogen related proteins (PR protein) are involved in plant defense response to pathogens. Boller (1985) was also of the opinion that proteins are associated with defense of plants against fungi and bacteria by their action on cell walls of invading pathogen. Most of antifungal proteins are in the form of chitinase, PR-1, peroxides, β-glycosidase etc. In the presence of defense response, synthesis of protein related enzymes are enhanced and accumulation of these antifungal elements causes lysis of the cell wall of pathogens. Such results are in agreement with Vannacci, G. *et. al*.(1987), Vidhyasekaran, P. (1974).

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