INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2003/05–1–83–85
http://www.ijab.org

Effect of Bio-Control Agents on Leaf Rust of Wheat and Influence of Different Temperature and Humidity Levels on Their Colony Growth

SHEROZE, A., A. RASHID†, A.S. SHAKIR AND S.M. KHAN
Department of Plant Pathology, University of Agriculture, Faisalabad–38040, Pakistan
†Directorate of Pest Warning and Quality Control of Pesticides, Chiniot–Pakistan

ABSTRACT

Out of five bio-control agents viz., *Verticillium lecanii, Paecilomyces fumosoroseus Beauveria bassiana, Cladosporium cladosporiodes and Metarrhizium anisopliae,* the best combination for the reduction of leaf result pustule development was *Verticillium sp.* and *Paecilomyces sp.* and *Verticillium* sp. with *Beauveria* sp. Overall 80% relative humidity and 30°C favoured the maximum growth of the bio-control agents. No growth was observed at 45°C. Although there was an increase in colony diameter after 2nd week but the pattern of growth was the same as after one week of incubation of bio-control agents application. The growth of bio-control agents did not differ among themselves.

Key Words: Wheat Leaf rust bio-control agents; Temperature; Humidity

INTRODUCTION

Wheat is principal staple food for Pakistan and leaf rust or brown rust caused by *Puccinia recondita* Roberge ex Desmaz f. sp. *Tritici* (Eriks. & Henn.) D.M. Henderson, appears on wheat crop in the months of March-April in the form of orange colour pustules scattered on leaf and leaf sheath. It is the most destructive and devastating disease due to its time of appearance, nature of attack, regular occurrence and prolonged season prevalent for the disease development in the wheat growing areas of Pakistan. During the rust epidemic of 1978, a loss of 10% in yield was primarily attributed to rust attack that compelled the Government to import large quantities of wheat (Hassan, 1979). The epidemics of 1976 had 50-80% severity on most of the common cultivars and 30% losses in yield were recorded in Punjab (Khan, 1985). In 1973, leaf rust intensity ranged from 40-50% with 100% infection on susceptible varieties. However crop escaped severe losses due to short duration of the favourable season of rust (Hassan, 1973). Sowing of resistant variety is a valid option in any disease management strategy then is chemical control.

Use of rusticide is avoided in Pakistan due to high cost of chemicals, low market price of wheat, risks of health hazard and lack of proper disease forecasting system to apply fungicide economically. Some researchers have tried to find out bio-control of leaf rust (Sheroze *et al*., 2002) but their work needs more attention for the economic use of bio-control agents at proper time for safer agro-ecosystem. The present task's aim is to study some of the environmental factors and their impact on the effective use of bio-control agents against leaf rust of wheat.

MATERIALS AND METHODS

Glass house studies. The efficacy of different biological control agents namely *Verticillium lecanii, Paecilomyces fumosoroseus, Beauveria bassiana, Cladosporium cladosporioides and Metarrhizium anisopliae* was tested against leaf rust on a susceptible wheat cultivar Morocco. These bio-control agents were used alone and also in combination. During experimentation at CDRI, Murree in the glasshouse, temperature of 28°C and 80% humidity was maintained. As the plants attained two leaf stage, these were thinned to four per pot. The plants were artificially inoculated with uredospores suspension by syringe method. One ml of spore suspension was injected into the stem when the upper leaves were still rolled up. The next day these plants were sprayed with uredospores suspension in water and covered with a polythene sheet to produce an artificial moist chamber. In check, plants were sprayed with distilled water. The data were recorded for number of pustules per leaf. Just with the initiation of pustules, the plants were sprayed with the spore suspension of these bio-control agents separately after an interval of one week. For this purpose a loopful of the inoculum was thoroughly mixed in 100 ml of distilled sterilized water before application. The number of rust pustules were counted on randomly selected leaves of each pot. Modified Cobb's scale (Peterson *et al.*, 1948) was used. Data were analysed statistically (Steel & Torrie, 1984) for interpretation of the results.

To study the effect of temperature and humidity levels on colony growth, the five biological control agents namely *Verticillium lecanii, Paecilomyces fumosoroseus, Beauveria bssiana, Cladosporium cladosporioides* and *Metarrhizium anisopliae* were used against leaf rust of wheat in December 2000 at University of Agriculture, Faisalabad. These were

multiplied on Saboraud dextrose agar for further studies. Saturated solutions of ammonium chloride, sodium chloride and ammonium sulphate were used for maintaining different humidity levels.

RESULTS AND DISCUSSION

During laboratory studies the effect of different levels of relative humidity on colony growth of five bio-control agents indicated that growth of Beauveria bassiana was the maximum. Overall, 80% relative humidity favoured the growth. According to the treatment means, growth of all the five bio-control agents however, differed significantly except T_1 and T_5 (Table I). Data on the effect of temperature on different bio-control agents showed that out of four temperatures namely 20, 25, 30 and 45°C, the growth was maximum at 30°C in all the five bio-control agents but no growth was observed at 45°C. Although there was an increase in colony diameter after 2nd week, but the pattern of growth was the same as after one week of incubation. According to the treatment means, the growth of bio-control agents (T_4 and T_5) and (T_1 and T_2) did not differ among themselves (Table II).

Glass house studies. Bio-control agents when applied alone

Table I. Comparative effect of different levels of relative humidities (percent) on colony growth of bio-control agents at 30°C

Period or Time Temperatures (0°C)		Average Colony growth (mm) of bio-control agents				
		V. Lecanii	***P. fumosoroseus***	***B. bassiana***	***C. cladosporioides***	*M. **Anisopliae***
		T_1	**T_2**	**T_3**	**T_4**	**T_5**
After one week	75	20.00	16.00	23.00	14.00	17.00
	80	23.00	20.00	18.67	20.00	18.67
	90	19.67	16.67	23.67	16.33	21.33
After two weeks	75	35.00	32.00	36.30	28.00	32.00
	80	37.33	33.00	38.00	31.00	36.67
	90	36.00	34.00	37.33	28.33	35.00
Mean		**28.50 ab**	**22.28 c**	**30.44 a**	**22.94 d**	**27.89 b**

Table II. Comparative effect of different temperatures on colony growth (mm) of bio-control agents

Period or Time Temperatures (0°C)		Average Colony growth (mm) of bio-control agents				
		V. lecanii	***P. fumosoroseus***	***B. bassiana***	***C. cladosporioides***	*M. **anisopliae***
		T_1	**T_2**	**T_3**	**T_4**	**T_5**
After one week	20	10.33	10.00	9.00	10.67	11.00
	25	12.33	12.00	13.33	11.66	12.66
	30	17.00	13.33	16.00	13.00	13.33
After two weeks	20	16.33	17.67	27.00	18.67	20.67
	25	20.33	20.00	25.33	23.00	22.23
	30	23.33	23.00	27.67	25.00	25.67
Mean		**16.56 b**	**15.83 b**	**19.17 a**	**17.00 ab**	**17.89 ab**

Table III. Comparative effect (in percent) of bio-control agents (alone) on pustule development after 3 applications in glass house on a susceptible variety Morocco

	Rust development (percent) on Morocco					
	Verticillium (T_1)	**Paecilomyees (T_2)**	**Beauveria (T_3)**	**Cladosporium (T_4)**	**Metarrhizium (T_5)**	**Control (T_6)**
First Application	4.50	6.00	3.75	10.25	9.50	14.50
2nd Application	6.25	8.75	6.50	10.50	9.50	16.75
3rd Application	8.00	9.75	7.25	10.75	10.00	14.50
Mean	6.25 c	8.167 bc	5.833 c	10.50 b	9.58 b	15.25 a

Table IV. Comparative effect (in percent) of bio-control agents (in combination) on pustule development after 3 applications in glass house on a susceptible variety Morocco

	Rust development (percent) on Morocco					
	Verticillium + Paecilomyces	**Paecilomyees + Metarrhizium**	**Beauveria +Verticillium**	**Cladosporium + Beauveria**	**Metarrhizium + Cladosporium**	**Control**
	T_1	T_2	T_3	T_4	T_5	T_6
First Application	2.25	3.75	2.00	3.75	4.75	6.50
2nd Application	3.50	5.50	5.25	5.50	6.25	12.75
3rd Application	5.75	8.25	6.25	8.00	9.00	14.00
Mean	3.83 b	5.50 b	4.50 b	5.75 b	6.83 b	11.08 a

showed a differential response as regards pustule development. As compared with control, though there was a decrease in rust development in all the cases but no pronounced difference was observed. Among the five bio-control agents, application of Beauveria bassiana proved comparatively better in reducing the rust Pustule development (Table III). Microscopic examination of rust uredospores after two weeks of application of the bio-control agents revealed that there was coagulation and disintegration effect on the cytoplasm.

The bio-control agents when used in combination gave a different response. When *Verticilliuim lecanii* was combined with *Paecilomyces fumosoroseus* and *Beauveria bassiana* there was less rust development as compared to other combinations. However, all the treatments differed from that of check (Table IV). Out of the treatments T_1 and T_3 proved better in checking the rust development as compared to check.

Data revealed that when *Verticillium lecanii* was combined with *Paecilomyces* and *Beauveria*, there was less (3.83 and 4.5%) rust development as compared to control (11.08 (Table IV). The efficacy of *Verticillium lecanii* is well documented against cereal rusts (Alien, 1982; Srivastava 1985; Whipps, 1993) due to parasitization of uredospores. Coagulation and disintegration of the cytoplasm, rapidly utilizing of spore contents and parasitization of spores and fruiting structures of the rust fungi in these studies there is synergistic effect of *Verticillium lecanii* with other bio-control agents. Hall (1981) reported that *Verticillium lecanii* frequently occurred as parasite of rust and it colonized the sori of Puccinia. In these studies, *Verticillium lecanii* also showed excellent results alongwith combinations with *Paecilomyces fumosoroseus* and *Beauveria bassiana*. Moreover hyperparasitism of *Verticillium lecanii* and *Cladosporium cladosporiodes* were compared in soybean rust, it was evident that when uredospores were sprayed with conidial suspension of these bio-control agents, the hyphae of these fungi grew over the uredinia and penetrated directly (Sultana *et al.,* 2000). The less attack of leaf rust was may be due to this hyperperasitism. The best suited temperature for the growth of bio-control agents was 30°C as compared to 20°C, and 25°C (Table II). Khan *et al.* (1998) reported that infection of *Puccinia recondita* f.sp.*Tritici* occurred over a. wide range of temperature (5-25°C) and bio-control agents continue to grow even at 30°C. Similarly overall 80% relative humidity favoured the growth of the bio-control agents and maximum colony growth (38.00 mm) was observed in case of *Beauveria bassiana* (Table I). The findings of these studies may become the foundation stone for the use or bio-control agents against leaf rust of wheat as safer and least hazards for the humanity.

REFERENCES

Alien, D.J., 1982. *Verticillitim lecanii* on the bean rust fungus, *Uromyces appendiculatus*. *Trans. Brit. Myco. Soc.*, 55: 173.

Hall, R.A., 1981. The fungus *Verticillium lecanii* as a microbial insecticide Against Aphids and Scales. *In: Microbial Control of Pests and Plant Diseases.* pp: 483–98. Academic Press.

Hassan, S.F., 1973. Wheat diseases and their relevance to improvement and production in Pakistan. *Proc. Fourth FAO\Rockfeller Foundation Wheat Sem.*, pp: 84–6. Islamabad.

Hassan, S.F., 1979. Wheat disease situation in Pakistan. *Paper presented at Nat. Sem. on Wheat Res. Prod.*, Islamabad, pp: 6–9.

Khan, M.A., 1985. Wheat breeding rust epidemiology in Pakistan. *Proc. Nat. Sem. on Epidemiology and Forecasting of Plant Diseases*. Uni. Agri., Faisalabad.

Khan, M.A., M. Irshad, M.B. Ilyas and M. Hussain, 1998. Relationship of environmental conditions with leaf rust and stripe rust development on wheat. *Pakistan. J. Ari. Sci*., 35: 34.

Sheroze, A., A.S. Shakir and A. Rashicd, 2002. Evaluation of some bio-control agents/antagonistic microbes against pustule development of leaf rust of wheat caused by *Puccinia recondita*, f. sp. *Tritici* Rober ex. Desmaz (Erikson and Henn) D.M.. Henderson. *Pakistan J. Pl. Path.*, 1: 51.

Srivastava, A.K., G. Defago and H. Kern, 1985. Hyperparasitism of *Puccinia horiana* and other microcyclic rusts. *Phytopathologische Zeitschrift*, 114: 73.

Steel, R.G.D, and J.H. Torrie, 1980. *Principles and Procedures of Statistics. A Biometrical Approach.* McGraw-Hill, Book Co. Inc., Singapore, pp: 107.

Sultana K., L.R. Batra, M.A. Nasir and J.R. Stavely, 2000. Hyperparasitism of *Verticilium lecanii* and *Cladosporium cladosporioides* on *Uromyces appendiculatus*. *Pakistan J. Phytopath.*, 12: 42.

Whipps, J.M., 1993. A review of white rust (*Puccinia horiana*, disease on chrysanthemum and the potential for its biological control with *Verticillium lecanii*. *Ann. App.Biol.*, 122: 173.

(Received 18 November 2002; Accepted 20 December 2002)