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Management of Brown Leaf Rust, *Puccinia recondita* of Wheat Using Natural Products and Biocontrol Agents

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Abstract: The effect of biocontrol agents and natural products on brown leaf rust, *Puccinia recondita* f.sp. *tritici*, of wheat were studied under greenhouse and field conditions in 1998/99 and 1999/2000 seasons. Under greenhouse conditions, application of Plant guard (*Trichoderma harizianum*), Rhizo-N (*Bacillus subtilis*) and the yeast (*Saccharomyces cerevisiae*) gave reasonable control of leaf rust severity with disease reduction percentages of 64.29, 57.14 and 19.14%, respectively. Field application of formulated natural products during two successive seasons gave effective control of brown rust disease. Natural oil, peppermint oil, jojoba oil, eucalyptus oil and chenopodium oil were the most effective treatments in reducing leaf rust severity and also in improving grain yield. All natural products significantly reduced the rust disease incidence in wheat by 55.5-98.2% and subsequently led to an increase in the grain yield that ranged from 8.5-51.8%. Of the biocontrol agents applied in the field, Plant guard was the most effective treatment followed by yeast and then Rhizo-N. These bioagents significantly improved grain yield and increased 100 kernel weight relative to the untreated control. Sumi-8 fungicide (diniconazole) showed complete protection against rust disease incidence in both greenhouse and field trials.

Key words: Biocides, natural products, wheat, brown rust disease

Introduction

Natural products and non-phytopathogenic fungi, bacteria and yeasts have proved to be potential sources of environmentally-safe antimicrobial agents useful in plant protection (Eldoksch, 1984; Biles and Hill, 1988; Bar-Nun and Mayer, 1990; Abdel-Moity *et al.*, 1993; Eldoksch and Abdel-Moity, 1997; Hassanein and Eldoksch, 1997 and Hammouda *et al.*, 1999). Leaf rust disease of wheat caused by *Puccinia recondita* f.sp. *tritici* is considered the most serious disease of wheat in Egypt. This disease develops on leaves causing great losses in yield and grain quality (Abdel-Hak *et al.*, 1980 and Shafik *et al.*, 1992). Control of rust diseases is usually carried out using resistant varieties (Johnson, 1992 and Kolmer, 1995) and application of synthetic fungicides (Dalal and Singh, 1994; Harko *et al.*, 1994; Hofle *et al.*, 1995 and Imbaby *et al.*, 2000). However, leaf rust disease is able within a short time to form new races that are capable of overcoming the resistance of the newly produced commercial cultivars.

Pesticide hazards and resistance problems as well as effects on non-target organisms have produced renewed interest to naturally occurring pesticides and biocontrol agents. These natural compounds are often less toxic and less persistent, so, they are assumed to be environmentally more acceptable and less hazardous to humans and animals.

The present investigation aimed to study the antifungal activity of some formulated natural oils and bioagents against the brown leaf rust, *Puccinia recondita* f.sp. *tritici*, of wheat under both greenhouse and field conditions. The effect of tested natural products and biocontrol agents on grain yield of wheat and 100 kernel weight were also investigated.

Materials and Methods

Plant materials: The tested plant materials were extracted from fruits of caraway, *Carum carvi* L.; leaves of peppermint, *Mentha piperata* L.; leaves of blue gum, *Eucalyptus globulus*; leaves of chenopodium, *Chenopodium ambrosiodes*. The plant oils of clove, *Eugenia caryophyllus*; jojoba, *Simmondsia chinensis*; Natural (93% vegetable oil, 7% emulsion compounds, Stoller Chemical Company); sweet basil, *Ocimum basilicum* and Musk from Musk deer, *Moschus moschiferus* were obtained from certified local market.

Preparation of extracts and bioagents: Samples of about 100-150 g each of dried plant materials were ground into fine powder and extracted by soaking for three days with 80% EtOH (2x1.5 L). The combined ethanol extract was filtered and concentrated under reduced pressure at 45-50°C and then it was partitioned with petroleum ether (3x300 ml). The petroleum ether extract was filtered, concentrated and evaporated to dryness to obtain the crude extracts. Preparing emulsions of plant oils and crude extracts usually require the addition of 0.03% Tween-40 to emulsify the oily material in water to be used for the field application. Control plots were treated only with water + Tween-40. The biocides, Plant guard (*Trichoderma harizianum*) and Rhizo-N (*Bacillus subtilis*) were provided from El-Nasr Company for Fertilizers and Biocides, El-Sadat City, Egypt and the yeast, *Saccharomyces cerevisiae* was obtained from Arab Company for Medicines & Medicinal Plants (Mepa Co), Egypt. The fungicide Sumi-eight 5% EC (active ingredient, Diniconazole) was supplied by Sumitomo Chemical Company, Japan. The biocides, Plant guard and Rhizo-N were adjusted to 0.1% and 0.4% concentrations, respectively in distilled water supplemented with 0.03% Tween-40. The yeast *Saccharomyces cerevisiae* was grown on PDA medium for 48-72 h and incubated at 28°C. Cells were collected by rinsing colonies with 15-25 ml of sterilized distilled water supplemented with 0.03% Tween-40. The resultant suspension was strained through two layers of cheesecloth. The yeast concentration was adjusted to 10^7 colony forming units per ml (cfu/ml) as suggested by Redmond *et al.*(1987).

Greenhouse experiments: Wheat plants (cv. Giza 160) in booting stage were used in this experiment. Five plants per pot (15 cm in diameter) were sown in each replicate. Three replicates were used for each treatment and each treatment was repeated twice. At booting stage (Large, 1954), artificial inoculation with a mixture of freshly collected prevalent leaf rust races and a talcum powder at a ratio of 1:20 (v/v) was carried out using baby cyclones to ensure rapid and equal deposition of spores on all wheat leaves (Tarvet and Cassel, 1951). The plants were sprayed with bioagents at two different times of application, one week prior to inoculation and 30 min. after inoculation. The leaves were sprayed with spore and cell suspensions of the antagonestis as suggested by D'Ercole (1985). Infected plants were kept in moist chamber for 48 h to maintain enough humidity during the incubation period. Plants were kept at 20-22^{o}C for two weeks. Control plants were sprayed only with *P. recondita* f.sp. *tritici* spores. The following equation was used to calculate the percentage of treatment effectiveness:

$$\text{Treatment effectiveness \%} = \frac{\text{Rust severity (control)} - \text{Rust severity (treatment)}}{\text{Rust severity (control)}} \times 100$$

Field experiments: Field trials on wheat plants were carried out at Alexandria University Experimental Station in 1998/99 and 1999/2000 seasons, to evaluate the efficacy of some biocontrol agents and natural products in controlling brown leaf rust, *Puccinia recondita* of wheat as well as their potential effects on grain yield and 100 kernel weight. The experiment was conducted in a Randomized Complete Block Design consisting of 14 treatments in 1998/99 season and 13 treatments in 1999/2000 season including the controls and each treatment was replicated three times.

The field experiment was divided into plots each 1/400 acre. The variety of wheat used in this study was Giza 160, and sowing dates were November 25 and 30 in the first and second growing seasons, respectively. Plots were surrounded by rust spreader border sown with a mixture of highly susceptible varieties to rust infection. Seeding rate was 60 kg/ acre. All other cultural practices were applied as recommended for wheat production.

At the booting stage, artificial rust inoculation was carried out according to Tarvet and Cassel (1951). The natural products and biocontrol agents used in this experiment were applied one week after rust inoculation using a Solo back sprayer (mist blower) at the rate of 500 ml/plot (200 L/acre). Rust severity was determined on flag leaves of 10 plants randomly selected from each plot at the full rust development stage according to the modified Cobb's scale (Peterson *et al.*, 1948). At the harvest stage, grain yield (as ton/acre) on whole plot basis and weight (g) of 100 kernels were recorded.

Treatment effectiveness was calculated according to the previously mentioned equation. Data were statistically analyzed according to Snedecor and Cochran (1971).

Results and Discussion

Effect of biocontrol agents on leaf rust severity of wheat under glasshouse conditions: Two biocides namely Plant guard (*Trichoderma harizianum*) and Rhizo-N (*Bacillus subtilis*) in addition to yeast (*Saccharomyces cerevisiae*) were evaluated for their efficacy to reduce leaf rust severity of wheat under glasshouse conditions in comparison with Sumi-8 fungicide. The data in Table 1, generally, showed that wheat treatments with bioagents and Sumi-8 fungicide one week before inoculation were less effective than wheat treatments after 30 min of inoculation. Plant guard and Rhizo-N were found to be the best bioagents and significantly reduced leaf rust on wheat. There were no significant differences between the treatment of yeast (*S. cerevisiae*) and the control. The fungicide Sumi-8 was more effective than the bioagents used. The inhibition of rust severity by the antagonistic species when they were applied to leaves may be due to inhibitory substances produced by these bioagents or to competition for nutrients and space. Application of biocide Plant guard (*T. harizianum*) conidia on wheat leaves after 30 min of inoculation resulted in a significant reduction in rust severity of about 64.3% compared with the untreated check. *T. harizianum* is known for parasitizing the mycelium of several important plant pathogens, e.g., *Sclerotium rolfsii* and *Rhizoctonia solani* (Papavizas and Lumsden, 1980). The present data are in agreement with that reported by Biles and Hill (1988), who found that sporulation capacity of the fungus *Cochliobolus sativus* was reduced on wheat seedling leaves when treated with *T. harizianum*. Concerning wheat treatment with Rhizo-N (*B. subtilis*), the data showed that the application of Rhizo-N 30 min after inoculation exhibited a significant inhibitory effect on leaf rust *P. recondita* f.sp. *tritici* with 57.14% reduction in leaf rust severity. Similar results were obtained by other researchers using this bacterium for inhibiting the growth of various fungi (Pursey and Wilson, 1984; Abdel-Moity *et al.*, 1993 and Korsten *et al.*, 1997). The biological activity of *B. subtilis* may be due to induction of antibiotics production. Loeffler *et al.*(1986) reported that *B. subtilis* produced different antibiotics namely subtilin, bacillin, bacillomycin, subtenolin, mycosubtilin, toximycin, bacitracin, xanthobacidin, iturin, subtilosin and subsporin complex. The present data indicated that the application of bioagents 30 min after rust inoculation gave better control than when applied one week before inoculation. So, it could be concluded that the tested active biocontrol agents, Plant guard and Rhizo-N, possessed eradicative ability than protective action.

Efficacy of natural products and bioagents on leaf rust severity under field conditions: The effects of natural products and bioagents, in comparison with Sumi-8 fungicide, on reduction of wheat rust severity under field conditions during two successive seasons 1998/99 and 1999/2000, are illustrated in Tables (2 and 3). Results showed that all treatments significantly reduced rust disease incidence on Giza 160 wheat cultivar throughout the two growing seasons. Concerning natural products treatments during the first season (Table 2), the data showed that Natural oil treatment was relatively the most effective and resulted in 98.2% reduction in leaf rust severity followed by jojoba oil (96.3%), peppermint oil (94.4%), chenopodium oil (90.8%), carawy and clove (74.1%) and then basil oil (55.5%). The data also indicated that grain yield (ton/acre) was significantly improved by the application of certain natural products and carawy oil treatment was the best in this respect, giving about 47.1% increase in grain yield followed by Natural oil, jojoba oil, clove oil, eucalyptus oil and then chenopodium oil with 44.0%, 40.1%, 33.2%, 29.3% and 27.0% increase in grain yield, respectively. Data also revealed that most of the applied natural products and bioagents increased the 100 kernel weight (g) over the check control.

Regarding bioagents treatments, Plant guard (*Trichoderma harizianum*), yeast (*Saccharomyces cerevisiae*) and Rhizo-N (*Bacillus subtilis*) exhibited significant reduction in leaf rust severity of wheat with percentages of reduction about 96.3, 81.5 and 71.1%, respectively. The application of yeast and Plant guard caused significant increase in grain yield of wheat by about 53.28 and 45.55%, respectively. There were also significant increases in 100 kernel weight (g) as shown in Tables (2 and 3). Sumi-8 fungicide treatment exhibited complete protection against leaf rust of wheat during the two successive seasons 1998/99 and 1999/2000 with 37.1 and 50.4% increase in grain yield, respectively, compared with the untreated control.

In 1999/2000 growing season, results indicated mostly the

Table 1: Effect of biocontrol agents* on leaf rust severity of Giza 160 wheat variety under glasshouse conditions.

	Control	Plant guard	Rhizo-N	Yeast	Fungicide (Sumi-8)
One week before inoculation					
Leaf rust severity (%)	73.30	36.66	55.00	70.00	26.60
Reduction (%)	00.00	49.99	24.96	4.50	59.62
30 minutes after inoculation					
Leaf rust severity (%)	70.00	25.00	30.00	56.60	00.00
Reduction (%)	0.00	64.29	57.14	19.14	100.00

$L.S.D_{0.05}$ = 19.18 * Plant guard (*Trichoderma harizianum*) Rhizo-N (*Bacillus subtilis*)
Yeast (*Saccharomyces cerevisiae*)

Table 2: Effect of formulated natural products and bioagents* on leaf rust severity, grain yield and 100 kernel weight of wheat cv. Giza 160 in 1998/99 growing season

Treatment	Rate of application (ml/L)	Rust severity (%)	Reduction (%)	Grain yield ton/acre	Increase (%)	100 kernel weight (g)	Increase (%)
Natural products:							
Basil oil	10.0	40.0	55.5	2.81	8.5	4.76	1.27
Chenopodium oil	5.0	8.0	90.8	3.29	27.0	5.03	7.02
Clove oil	10.0	23.3	74.1	3.45	33.2	4.99	6.17
Caraway oil	10.0	23.3	74.1	3.81	47.1	4.92	4.68
Eucalyptus oil	10.0	5.0	94.4	3.35	29.3	4.90	4.25
Jojoba oil	10.0	3.3	96.3	3.63	40.1	5.14	9.36
Musk oil	5.0	8.3	90.8	3.12	20.5	4.87	3.61
Natural oil	6.3	1.6	98.2	3.73	44.0	4.99	6.17
Peppermint oil	10.0	5.0	94.4	3.14	21.2	4.79	1.91
Bioagents:							
Plant guard	1.0	3.3	96.3	3.77	45.5	5.08	8.08
Rhizo-N	4.0	26.0	71.1	2.93	13.1	5.12	8.93
Yeast	10^7 cfu/ml	16.6	81.5	3.97	53.2	5.00	6.38
Fungicide (Sumi-8)	0.35	0.0	100.0	3.55	37.1	4.95	5.31
Control	-	90.0	0.0	2.59	0.0	4.70	0.00
$L.S.D_{0.05}$		29.5	-	0.61	-	0.19	-

* Plant guard (*T. harizianum*); Rhizo-N (*B. subtilis*); Yeast (*S. cerevisiae*)

Table 3: Effect of formulated natural products and bioagents on leaf rust severity, grain yield and 100 kernel weight of wheat cv. Giza 160 in 1999/2000 growing season

Treatment	Rate of application (ml/L)	Rust severity (%)	Reduction (%)	Grain yield Ton/acre	Increase (%)	100 kernel weight (g)	Increase (%)
Natural products:							
Basil oil	10.0	30.66	60.0	2.80	23.9	4.49	2.98
Chenopodium oil	5.0	18.33	76.1	2.69	19.0	4.74	8.71
Clove oil	10.0	20.66	73.0	2.96	31.0	4.56	4.58
Eucalyptus oil	10.0	16.33	78.7	2.66	17.7	4.96	13.76
Jojoba oil	10.0	26.00	66.0	2.96	31.0	4.69	7.56
Musk oil	5.0	10.00	86.9	3.10	37.8	4.87	11.70
Natural oil	6.3	3.33	95.6	3.40	50.4	4.88	11.92
Peppermint oil	10.0	24.66	67.8	3.43	51.8	4.73	8.89
Bioagents:							
Plant guard	1.0	26.00	66.1	2.70	19.5	4.81	10.32
Rhizo-N	4.0g	22.33	70.9	2.66	17.7	4.81	10.32
Yeast	10^7cfu/ml	16.66	78.3	3.06	35.4	4.87	11.69
Fungicide (Sumi-8)	0.35	0.00	100.0	3.40	50.4	4.76	9.17
Control	-	76.66	0.0	2.26	0.0	4.36	0.00
$L.S.D_{0.05}$		15.04	-	0.52	-	0.21	-

* Plant guard (*T. harizianum*); Rhizo-N (*B. subtilis*); Yeast (*S. cerevisiae*)

same trend as in 1998/99 growing season. Of natural products tested, Natural oil formulation gave the best activity in reducing leaf rust severity under field conditions with percentage reduction of rust disease by about 95.6% and basil oil treatment was relatively the least effective with 60% reduction. The rest of the treatments ranged between 86.9% reduction for Musk oil and 66.1% for jojoba oil. These results are in accordance with those reported by Sajid *et al.*(1995), who found that neem oil extracted from *Azadirachta indica* completely inhibited germination of *Puccinia recondita* urediospores in the lab, but in the field neem oil at 4% checked leaf rust on wheat after four applications.

The data also showed that field application of yeast *S. cerevisiae* gave the best control among bioagents tested with 78.3% reduction in leaf rust severity followed by Rhizo-N (70.9%) and then Plant guard (66.1%). These results are in a good agreement with those obtained by Biles and Hill (1988), who found that *Trichoderma harizianum* was effective in reducing sporulation capacity of the fungus *Cochliobolus sativus* on excised wheat seedling leaves. Also, Cook and Baker (1983) indicated that several *Trichoderma* spp. have proved to be effective mycoparasites. The biological control mechanism of *Trichoderma* may be hyperparasitism and/or competition (Elad *et al.*, 1980 and Chet *et al.*, 1981).

Concerning grain yield in 1999/2000 growing season, the significant yield increase ranged between 51.8% (3.43 ton/acre.) in case of peppermint oil treatment and 23.9% (2.8 ton/acre.) in case of basil oil treatment. Concerning bioagents, yeast treatment exhibited significant increase in grain yield (3.06 ton/acre.) while Plant guard and Rhizo-N increased grain yield over the unsprayed control, but this increase failed to reach the 5% level of significant.

It is of interest to mention that while yeast treatment was not significant in glasshouse experiments, it gave good results in field trials, not only in reducing the rust severity but also significantly increasing the grain yield. Hammouda *et al.*(1999) found that yeast extracts resulted in accumulating high concentration of phenolic compounds in leaves of faba bean and these compounds are considered antifungal substances (Reglinski *et al.*, 1993) and consequently might have the ability to inhibit rust infection.

Although Sumi-8 fungicide was more effective in reducing leaf rust severity than natural products and bioagents, they are considered environmentally less persistent and more safe than synthetic fungicides which may leave chemical residues in soil, water and grains (Singh *et al.*, 1994) and subsequently may affect animal and human health. It could be concluded that the use of selected natural products and bioagents as a non-chemical approaches to managing wheat brown rust disease exhibited effective control in reducing leaf rust severity on wheat which led to a significant increase in grain yield and improvement in grain quality. These natural oil products and bioagents can be used effectively and safely in wheat rust disease management program and could be readily helped in supporting the recent emphasis on organic and sustainable agriculture.

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