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Antifungal Activity of Natural Piperitone as Fungicide on Root Rot Fungi

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Abstract: The aims of the present study were comparing the growth inhibiting ability of piperitone as natural chemical against *Fusarium grraminearium*, *Bipolaris sorokiniana Rhizoctonia solani* AG-4 and *Fusarium oxysporum* and evaluat the disease severity of root rot of wheat and alfalfa seedlings. Also to determine the growth characteristics of affected wheat and alfalfa plants after inoculation with tested fungi. *In vitro* and *in-vivo* studies was carried out on the effect of Piperitone as a new natural fungicide, against four phytopathogenic fungi, known to cause root rot in wheat and alfalfa plants. Peperitone caused a significant inhibition of growth of tested fungi at the minimum inhibitory dose on agar medium at 12 ppm. In greenhouse experiment, piperitone also recorded reduction in the disease severity of tested plants, in the presences of each fungus (*Bipolaris sorokiniana, Fusarium graminearum*, *Fusarium oxysporum* and *Rhizoctonia solani* AG-4) with the averages 1.26, 1.00,1.00 and 1.26, respectively, compared with inoculated wheat and alfafa plants in the absence of piperitone with the averages 3.40, 3.10, 2.90 and 3.40 respectively. Plant height, fresh weight of shoot and root of wheat and alfalfa plant were also increased in the presence of piperitone compared with each of tested fungi.

Key words: Piperitone · Fungicides · Phytopathogenic Fungi · Wheat · Alfalfa

INTRODUCTION

Alafalfa (*Medicago sativa* L.) is subject to stand injury and yield loss from several diseases such as damping off and root rot. *Rhizoctonia solani* and *Fusarium oxysporum* are the main pathogens of these diseases [1]. In the Kingdom of Saudi Arabia, Al-Doss *et al*. [2] found that *Rhizoctonia solani* causes root canker and root rot in Lucerne growing areas in Saudi Arabia. Ten Lucerne cultivars were evaluated for damping off diseases severity and resistance to *R. solani* AG-4. All landraces and imported varieties were highly susceptible. The Egyptian cultivar was the most resistant cultivar to root canker, While the locale cultivars Qassimi and Hasawi and the introduced cultivars CUF101, Panner 4860 and Serosal, were moderately resistant. *R. solani* AG-4 affects root fresh weights. *Cymbopogon* species is known as a source of commercially valuable compounds like pipertone which are either used as such in perfumery and allied industries or as starting materials for the synthesis of other products commonly used in perfumery. This genus is distributed in the tropical and subtropical parts of the world, more than 52 types in Africa, 45 in the Indian, six in South America and Australia, four in Europe and two in North America [3-4]. The *in-vitro* and *in- vivo* efficacy of leaf extracts from 5 plants including *Cymbopogon citrates* were evaluated in inhibiting the growth of four plant pathogenic fungi (*Macrophomina phaseolina*, *Fusarium moniliforme*, *Fusarium solani* and *Botryodiplodia theobromae*). Aqueous extracts of *C. citrates* completely inhibited the growth of *M. phaseolina* and *B. theobromae* and also significantly reduced the growth of *F. moniliforme* and *F. solani* [5].

Essential oil extracts from various parts of 11 higher plants were screened *in-vitro* for their fungi toxicity against fungal pathogens: *Colletotrichum falcatum*, *Fusarium moniliforme*, *Ceratocystis paradoxa*, *Rhizotonia solani*, *Curvularia lunata*, *Periconia atropurpuria* and *Epicocum nigrum*. GC-MS analysis of *Lippia alba* essential oil showed Limonene (at 12.6%) and piperitone (at 19%) to be major chemical constituents. Piperitone was strongly toxic even at 200 ppm, (6). Screening of some aromatic plants for fungi toxicity of their volatile oils, (*Cymbopogon pendulus*) showed

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strong activity as fungicidal that completely inhibited the mycelial growth of the tested fungi at its minimum inhibitory concentration of 200ug /ml and the heavy inocula of the tested fungi [11]. The aims of this study were comparing the growth inhibiting ability of piperitone as natural chemical agent against *Fusarium grraminearium*, *Bipolaris sorokiniana Rhizoctonia solani* AG-4 and *Fusarium oxysporum in-vitro* and *in-vivo*, evaluating the disease severity of root rot of wheat and alfalfa seedlings and determining the growth characteristics of affected wheat and alfalfa plants after inoculation with tested fungi.

MATERIALS AND METHODS

Isolation and Identification of Piperitone: The procedure for isolation and identification of piperitone from *Cymbopogon proximus* was carried out according to El-Hussieni *et al*. [7] Pathogens The pathogens used in this study were originally isolated from brown to black discoloration of the sub-crown inter –node of brown colored stem bases or from root rot diseased wheat and alfalfa plants from the commercial field in Riyadh region. The fungal colonies from root tissue emerged were purified and the single spore of each colony was placed on 2% water agar. Fungi were then examined, identified with a light microscope as *Bipolaris sorokiniana* (Sacc) Shoemaker, *Fusarium graminearum* Schwabe, *Rhizoctonia solani* AG-4 and *Fusarium oxysporum* [8-10].

Antifungal Activity Test: Piperitone was tested for its antifungal activity against *Bipolaris sorokiniana*, *Fusarium graminearum*, *Rhizoctonia solani* AG-4 and *Fusarium oxysporum*. Fungal cultures were maintained by regular sub-culture on potato dextrose agar (PDA) medium. For the bioassay, 8-mm disc taken from the edge of actively growing fungal colonies was placed in the center of Petri dish containing specific concentration of piperitone (8ppm and 12ppm). Control Petri dishes contained only the solvent and the medium. All the cultures of tested fungi were incubated in the dark at 27°C. The inhibition of mycelial growth was determined by Singleton, *et al*. [9].

$$\% \text{ Mycelial inhibition} = \frac{\text{Control (area)} - \text{Treatment (area)}}{\text{Control (area)}} \text{ X } 100$$

Greenhouse Experiments: Two experiments design in a randomized complete block with four replicates were carried out in the greenhouse at the College of Food Science and Agriculture, King Saud University. Wheat and alfalfa seeds were surface disinfested in 1.00 % sodium hypochlorite solution for 10 min and washed in sterilize water, 20 wheat seeds/pot and 50 alfalfa seeds/pot were planted in 12 cm diameter plastic pots containing steam sterilized soil. The soil consisted of clay loam and sandy in a 1:1 ratio by volume. Control pots containing autoclaved soil alone. Pots were inoculated with tested fungi and piperitone treatments were also added for comparison between all treatments. Temperatures during the experiment ranged from 18-27°C.

To produce conidia for inoculation of *Fusarium oxysporum, F. graminearium* and *Bipolaris sorokinianna*, each isolate was transferred to plates with PDA and maintained at 25°C in the dark for 5 days [11]. When conidia were formed, 10 ml of sterile distilled water was added to each plate. Conidia were scraped from the agar surface with rubber spatula, suspended in distilled water and filtered through a triple layer of cheesecloth. The concentration of conidia was measured by a hemocytometer and adjusted to 2 X 10^6 conidia per milliliter by adding sterile distilled water. Inoculum of *R. solani* AG-4 was prepared by the whole grain method of mention author (s) [12]. The inoculum was thoroughly mixed and added to the pot soil surface at the rate of 0.5% w/w per pot [13] and then covered with a thin layer of sand. The control consisted of sterilized ground wheat grains with no pathogen.

Disease Severity Index (DSI): Disease severity index on wheat and Alfalfa plants was based on a scale 0-4, where 0= No apparent infection, 1= light discoloration of crown and root tissue approximately 25 %, 2= 26-50 % crown and root tissue covered with dark brown lesion, 3= 51-75 crown and root tissue covered with large dark brown lesion and 4= 76-100 dead plant [14]. At the end of experiment, the different parameters of disease severity and plant growth [plant height (cm), fresh weight (g) of root and shoots] were assessed. Data were analyzed using the Statistical Analysis System [15]. Analysis of variance and least significance difference values (LSD),(P=0.50) were used to detect differences among treatment means.

RESULTS AND DISCUSSION

The results (Table 1) showed significant antifungal activity of piperitone on all tested fungi. Piperitone was found effective in inhibiting the mycelial growth of *Fusarium oxysporum*, *Bipolaris sorokiniana*,

Table 1: Antifungal Activity of piperitone at two concentrations on four plant pathogenic fungi after 5 days of inoculation

Tested Fungi	** % Mycelial inhibition at two concentrations	
Bipolaris sorokiniana	32.88d	69.65b
Fusarium graminearum	55.36b	65.24c
Rhizoctonia solani AG-4	40.20c	68.41b
Fusarium oxysporum.	*65.26a	*72.38a
LSD. 0.05	4.0615	1.9741

*Values within a column or between the columns followed by the same letters (a, b, c, & d) are not significantly at (P= 0.05)

**% Mycelial inhibition = Control (area) - Treatment (area) X 100/ Control (area)

Table 2: Fungistic effect of piperitone on DSI, plant length, fresh shoot and root of wheat plants, 28 days after inoculation with soil- borne fungi

			Fresh Weight (g)	
Treatment	DSI*	Plant length (Cm)	Shoot	Root
Control	00.00	*20.66a	*15.23a	*27.38a
P1 (piperitone 4ppm)	00.00	19.60ab	12.65c	24.52ab
P2 (piperitone 8ppm)	00.00	20.00ab	14.20ab	25.16ab
P1 (piperitone 12ppm)	00.00	20.00ab	14.70a	25.26ab
B. sorokiniana alone	3.40a	15.00d	7.67e	12.66g
B. sorokiniana + P1	2.20b	18.33bc	12.83c	20.13de
B. sorokiniana + P2	1.70c	18.66bc	13.16bc	21.30cd
B. sorokiniana + P3	1.20e	19.33ab	15.28a	22.37cd
F. grraminearium alone	3.10b	14.00d	9.42d	16.93f
F. grraminearium + P1	1.50d	17.33c	14.39ab	16.90f
F. grraminearium + P2	1.20e	19.00abc	14.90 *a*	18.29ef
F. grraminearium + P3	1.00e	19.66ab	13.35bc	21.24cd
LSD.0.05	0.2352	1.8389	1.2514	2.4935

*Values within a column or between the columns followed by the same letters (a, b, c, & d) are not significantly at (P= 0.05)

** DSI: Disease Severity Index

Table 3: Fungistic effect of piperitone on DSI, plant length, fresh shoot and root of alfalfa plants, 28 days after inoculation with soil- borne fungi

			Fresh Weight (g)	
Treatment	DSI**	Plant length (Cm)	Shoot	Root
Control	00.00	*12.00a	*8.74a	*8.86a
P1 (piperitone 4ppm)	00.00	12.00a	8.32a	8.44a
P2 (piperitone 8ppm)	00.00	12.00a	8.40a	8.42a
P1 (piperitone 12ppm)	00.00	12.00a	8.55a	8.12a
F. oxysporum alone	2.90b	8.00c	4.98e	4.03e
F. oxysporum + P1	2.03c	10.66ab	5.65de	4.51ed
F. oxysporum + P2	1.23e	11.33ab	6.61cd	4.91ed
F. oxysporum + P3	1.00e	12.00a	6.80cd	6.58c
R. solani AG-4 alone	3.40a	6.00d	4.00e	6.88d
R. solani AG-4 + P1	2.66b	10.33b	7.13bc	8.12bc
R. solani AG-4 + P2	1.66d	11.33ab	7.30bc	8.12ab
R. solani AG-4 + P3	1.26e	12.00a	8.32ab	8.50a
LSD.0.05	0.2969	1.3721	1.2748	1.2474

*Values within a column or between the columns followed by the same letters (a, b, c, & d) are not significantly at (P= 0.05)

** DSI: Disease Severity Index

Rhizoctonia solani AG-4 and *Fusarium graminearum* (at 12ppm) with average of 72.38, 69.65, 68.41 and 65.24 respectively. Other studies support these results in which *Cymbopogon pendulus*, exhibited strong antifungal activity and completely inhibiting the mycelial growth of the tested fungi at its minimum inhibitory concentration of 200ug /ml [10]. The *in-vitro* and *in- vivo* efficacy of leaf extracts from 5 plants including *Cymbopogon citrates* were evaluated in inhibiting the growth of four plant pathogeni fungi (*Macrophomina phaseolina*, *Fusarium moniliforme*, *Fusarium solani* and *Botryodiplodia theobromae*). Aqueous extracts of *C. citrates* completely inhibited the growth of *M. phaseolina* and *B. theobromae* and also significantly reduced the growth of *F. moniliforme* and *F. solani* [5].

Data in (Table 2) show the fungistic effects of piperitone product on diseases severity index, plant length (cm), fresh and dry weight (gm) of wheat plants after inoculation with tested fungi. The disease severity index showed highly significant differences among all treatments. The treatments of *B. sorokiniana* plus piperitone and *F. grraminearium* plus piperitone [at 12ppm (P3)] were the most effective to control root rot wheat diseases with the average of disease severity of 1.20 and 1.00, respectively, compared with inoculated wheat plants with *B. sorokiniana* alone and *F. grraminearium* alone with an average of the disease severity index of 3.40 and 3.10 respectively. Significant differences in plant height (cm) were also observed; plants inoculated with *B. sorokiniana* alone and *F. grraminearium* alone showed reduction in plant height with an average of 15.00 and 14.00 (cm) respectively, compared with inoculated wheat plants in the presence of piperitone (at 12 ppm) with an average of 19.33 and 19.66(cm) respectively. Reduction in fresh weight of shoot and root in the absence of piperitone was recoded (*B. sorokiniana* alone) with an average of 7.67 and 12.66 (g) compared with inoculated wheat plants in the presence of piperitone at (12ppm), with an average of 15.28 and 22.37(g) respectively.

Data in (Table 3) show the Fungistic effects of piperitone product on Diseases Severity Index (DSI), plant length (cm), fresh and dry weight (gm) of alfalfa plants after inoculation with tested fungi. The disease severity index showed highly significant differences among all treatments. The treatments of *F. oxysporum* plus piperitone and *R. solani* AG-4 plus piperitone [at 12ppm (P3)] were the most effective to control root rot alfalfa diseases with an average of disease severity index of 1.00 and 1.26, respectively, compared with inoculated wheat plants with *F. oxysporum* alone and *R. solani* AG-4 alone with the average of the of 2.90 and 3.40 respectively. Significant differences in plant height (cm), were also recorded, plants inoculated with *F. oxysporum* alone and *R. solani* AG-4 alone showed reduction in plant height (cm) with an average 8.00 and 6.00 (cm) respectively, compared with inoculated wheat plants in the presence of piperitone (at 12 ppm) with the average of 12.00 and 12.00 (cm) respectively. Reduction in fresh weight (g) of shoot and root in the absence of piperitone was recoded (*R. solani* AG-4 alone) with the average of 4.00 and 6.88 (g) compared with inoculated wheat plants in the presence of piperitone at (12 ppm), with the average of 8.32 and 8.50 (g) respectively.

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