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Antifungal Activity of 2-Hydroxy 4,4'6'Trimethoxy Chalcone

P. K. Mishra, B. K. Sarma*, P. K. Singhai and U. P. Singh

Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi –221005, India
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Antifungal activity of 2-hydroxy 4,4'6'trimethoxy chalcone individually was tested against spore germination of ten fungi of different genera. Efficacy of the chemical was also tested against conidial germination and other growth parameters of *Erysiphe pisi* on excised pea leaves. 2-Hydroxy 4,4'6'trimethoxy chalcone inhibited spore germination at all the concentrations. Maximum inhibition was observed at 2000 ppm where more than 78 per cent inhibition of spore germination was observed in *Ustilago cynodontis*, *Alternaria brassicicola*, *A. solani* and *Aspergillus flavus*. It also reduced conidial germination of *E. pisi* significantly, when applied as pre-inoculation treatment.

KEYWORDS: 2-Hydroxy 4,4'6'Trimethoxy chalcone, Spore germination inhibition

Synthetic fungicides have been used successfully for the control of various fungal diseases of crop plants. However, their indiscriminate use resulted in resistance, resurgence and replacement of pathogens. Besides these effects, the use of fungicides also resulted in environmental pollution posing potential risk to animal and human health (Prithiviraj and Singh, 1996). Recent awareness of these negative effects warrants the use of environmentally safe alternative methods of plant disease control. The approaches that are presently being persuaded are biological control, genetic engineering and induced resistance through use of biotic and abiotic agents (Lyon *et al.*, 1995). More importantly use of biodegradable natural products, especially from medicinal plants is another aspect gaining importance currently (Prithiviraj and Singh, 1996).

Various active principles isolated from plants were also found effective against plant pathogenic fungi *in vitro* (Maillard *et al.*, 1987; Prithiviraj *et al.*, 1997; Sarma *et al.*, 1999; Singh *et al.*, 2000; Maurya *et al.*, 2002), as well as in glasshouse (Reimers *et al.*, 1993; Singh *et al.*, 1995) and field condition (Prhiviraj *et al.*, 1998). Neemazal, a product of neem (*Azadirachta indica*), and ajoene, a constituent of garlic (*Allium sativum*), have been successfully used for control of powdery mildew of pea (*Pisum sativum*) under field conditions (Singh *et al.*, 1995). Ajoene has also been tested against some other fungal and bacterial diseases under glasshouse and/or growth chamber conditions (Reimers *et al.*, 1993). Some plant alkaloids are known to be antimicrobial (Atta-ur-Rahaman *et al.*, 1997; Maurya *et al.*, 2002) and affect biological functions at very low concentrations. Similarly, several flavonoids are also known to be antimicrobial (Harborne and Grayer, 1993; O'Neill *et al.*, 1982; Baptista and Siqueira, 1994; Dixon *et al.*, 1995; Siqueira *et al.*, 1991; Van Etten, 1976). Looking in to the potentiality of plant alkaloids and plant flavonoids, we have tested the antifungal activity of 2-hydroxy-4,4'6'timethoxy chalcone, a flavonoid isolated from bark of *Pterocarpans marsupium* (Maurya, 1984) was tested against spore germination of some plant pathogenic as well as saprophytic fungi. The germination and development of *Erysiphe pisi* on excised pea leaves were also observed in presence of the chemical.

*Corresponding author <E-mail: birinchi_ks@yahoo.com>

Materials and Methods

Test fungi. The fungi were isolated from their respective hosts (Table 1) from the experimental farm of Banaras Hindu University, Varanasi, India and maintained on slants of potato dextrose agar (PDA) (250 g peeled potato + 20 g dextrose + 15 g agar and one liter distilled water) medium. *E. pisi* was multiplied on 21-d-old pea (cv. *Arkel*) seedlings in glasshouse grown in plastic pots (9 cm diameter) and by tapping heavily infected pea leaves collected from near by areas.

Test chemical. The chemical 2-hydroxy-4,4'6'trimethoxy chalcone (Fig. 1) is a synthetic compound prepared in connection with the synthesis of a naturally occurring benzofuranone, marsupsin, which was isolated from *P. marsupium* (Maurya *et al.*, 1982).

Effect of 2-hydroxy-4,4'6' trimethoxy chalcone on spore germination. The flavonoid 2-hydroxy-4,4'6'trimethoxy chalcone was dissolved first in sodium hydroxide (NaOH) solution and then required concentrations (500, 1,000, 1,500 and 2,000 ppm) were prepared by diluting the stock solution with distilled water. A drop of

Table 1. Effect of 2-hydroxy 4,4'6'trimethoxy chalcone on spore germination of some fungi

Fungus	Host	Concentration (ppm)				
		0	500	1000	1500	2000
		Spore germination (%)				
Fusarium udum	*Cajanus cajan*	94.78	52.56**	36.75**	29.56**	20.33**
Helminthosporium sativum	*Triticum aestivum*	95.00	62.78**	44.83**	41.89**	21.66**
Curvularia lunata	*Oryza sativa*	94.89	82.83	43.66**	32.00**	13.33**
Cercospora blumea	*Blumea species*	94.33	44.28**	51.95**	39.73**	32.89**
Ustilago cynodontis	*Cynodon dactylon*	71.83	36.68**	30.83**	23.34**	10.66**
Colletotrichum capsici	*Capsicum annum*	96.33	60.00**	49.33**	39.71**	13.00**
Alternaria brassicicola	*Brassica campestris*	89.91	45.22**	33.34**	23.55**	10.89**
Alternaria triticina	*Triticum aestivum*	89.78	72.17	52.83**	40.33**	28.76**
Alternaria solani	*Solanum tuberosum*	76.33	24.84**	18.33**	13.83**	09.34**
Aspergillus flavus	*Saprophyte*	86.69	9.66**	7.28**	2.66**	0.33**

Row data with ** varies significantly ($p \leq 0.01$) from their respective control by student *t*-test

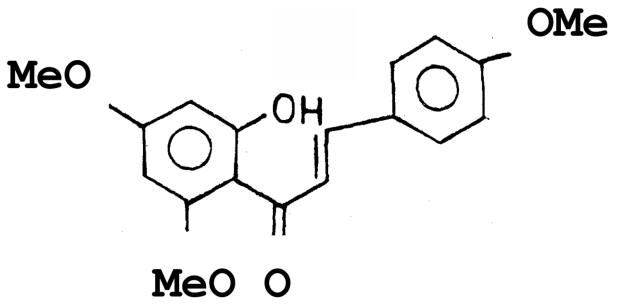

Fig. 1. Structural formula of 2-hydroxy-4,4',6'trimethoxy chalcone.

about (30~40 μl) of each solution was placed separately on grease-free slides. Fungal spores (as listed in Table 1) (approx. 300~400) were lifted from freshly sporulating individual culture and mixed in the solution placed on glass slides with the help of an inoculation needle. The moist chamber was prepared by placing a filter paper moistened with sterilized distilled water on the base of the Petri plates. The slides were then kept on the moist filter paper and covered with the lead of the Petri plates. The slides thus in moist chambers were incubated at 25 ± 2°C for 24 h. A drop of cotton blue prepared in lactophenol was placed on the spore suspension, mixed and covered with a cover glass. The percent germination was observed under a light microscope. A similar experiment was conducted with distilled water, which served as control. All the experiments were conducted in triplicates and subjected to students *t*-test for significance.

Effect of 2-hydroxy 4,4'6'trimethoxy chalcone on germination and development of *Erysiphe pisi* conidia on excised pea leaves. Second nodal leaves from 20-day-old pea plant (cv. *Arkel*), were excised with the help of a sharp scissors and placed on filter paper towels. The leaves were then seeded with *E. pisi* conidia by taping heavily infected plant parts over the adaxial surface of the leaves. The seeded leaves were then floated on sterilized distilled water in Petri plates, which served as control. All the experiments were conducted in triplicates.

Pre-inoculation Treatment. Different dilutions of the chemical (500, 1,000, 1,500 and 2,000 ppm) were sprayed thoroughly with the help of a hand atomizer 24 h prior to inoculation of conidia on excised pea leaves of 20-day-old plants placed on filter paper towels. These leaves were later floated on distilled water keeping adaxial surface upward. After 24 h of spraying, the leaves were seeded with *E. pisi* conidia by taping heavily infected leaves.

Post-inoculation treatment. Excised pea leaves of 20-day-old plants were placed on filter paper towels and seeded with *E. pisi* conidia by taping heavily infected leaves on adaxial surface. The leaves were then floated on distilled water. After 24 h of inoculation, the floated leaves were taken out and placed on filter paper towels and required concentrations (500, 1,000, 1500 and 2,000 ppm) of the chemical were sprayed gently with the help of a hand atomizer on the inoculated leaves, which were then again floated on distilled water in Petri dishes. All the pre-and post-inoculation treated leaves including control were incubated for 24 and 48 h at 25 ± 2°C. The leaves were then fixed and stained after inoculation according to the method of Carver and Adaigbe (1990). Briefly a pad of filter paper was placed in Petri plates containing the fixative (ethyl alcohol-acetic acid, 3 : 1). The leaves were then placed on filter paper with adaxial side up to minimize displacement of conidia on the leaf surface. The chlorophyll was completely removed after 48 h of fixation. The leaves were then removed and placed on filter paper pads containing lactophenol for another 24 h to soften the leaf tissue, and mounted in lactophenol-cotton blue for microscopic observation. Observations regarding percent germination, number of primary branches, number of secondary branches, number of apperssoria and growth of colony (germ tubes length) were made under light micro-

Table 2. Effect of 2 hydroxy 4,4'6'trimethoxy chalcone on growth of *Erysiphe pisi* on detached pea leaves

Concentrations (ppm)	24 h					48 h				
	% Germination	Number of primary branches	Number of secondary branches	Number of appressoria	Length of germ tube (μm)	% Germination	Number of primary branches	Number of secondary branches	Number of appressoria	Length of germ tube (μm)
Control	88.00	1.94	1.08	1.84	232.14	96.00	2.08	0.96	1.36	266.06
Pre-inoculation										
500	78.00	1.34	0.92	1.20	160.00**	69.00	1.60	0.84	1.04	198.36**
1,000	66.66	1.16**	0.56*	0.90**	123.54**	60.00	1.33**	0.68	0.96	178.54**
2,000	50.33	0.98**	0.40**	0.64**	110.28**	54.00	1.00**	0.46**	0.62**	116.65**
Post-inoculation										
500	83.00	1.42	1.04	1.42	230.00	72.00	2.00	1.08	1.52	233.33
1,000	70.00	1.26	0.84	1.28	217.33	66.00	1.60	0.96	1.44	228.00
2,000	52.00	1.08	0.64	0.88	200.99	57.00	1.36	0.68	1.04	213.33

Column data with **varies significantly ($p \leq 0.01$) from their respective control by student *t*-test

scope. All the experiments were conducted in triplicates and subject to student "t" test for statistical significance.

Results

Effect of 2-hydroxy 4,4'6'trimethoxy chalcone The flavonoid 2-hydroxy 4,4'6'trimethoxy chalcone showed significant inhibition in spore germination of all the test fungi. *Alternaria solani*, *A. brassicicola*, *Colletotrichum capsici*, *Ustilago cynodontis*, *Curvularia lunata*, *Fusarium udum* and *Aspergillus flavus* showed maximum sensitivity to the chemical at 2,000 ppm, where more than 78 per cent inhibition in spore germination was recorded. However, out of all the fungi tested *U. cynodontis*, *A. solani* and *A. flavus* were highly sensitive to 2-hydrodxy 4,4'6'trimethoxy chalcone, as it was highly inhibitory even at 500 ppm (Table 1).

Erysiphe pisi also showed sensitivity towards 2-hydroxy 4,4'6'trimethoxy chalcone. There was marked reduction in germination of *E. pisi* conidia on excised pea leaves. The compound also affected colony development of *E. pisi* on excised pea leaves. There was significant reduction in germ tube length at concentrations 500, 1,000 and 2,000 ppm in both pre-inoculation treatments incubated for 24 and 48 h. As compared to 232.14 μm and 266.06 μm germ tube lengths in control only 110.28 μm and 116.65 μm germ tube length was observed at 2,000 ppm pre-inoculation treated leaves incubated for the same period. However, the other parameters, viz., number of primary branches, number of secondary branches, and number of appressoria were not affected significantly by 2-hydroxy 4,4'6'trimethoxy chalcone (Table 2).

Discussion

The effect of flavonoids have been investigated against insects and several micro-organisms i.e. fungi and bacteria by several workers (Harborne and Grayer, 1992; Baptista and Siqueira, 1994). Several groups of chemicals belonging to the class of flavonoids are also reported to be antifungal (Dixon *et al.*, 1995; O'Nell, 1982; Siqueira *et al.*, 1991; Van Etten, 1976). Several workers have also studied the antifungal properties of many *Pterocarpans* (Pueppke and Van Etten, 1976; Van Etten, 1976). But 2-hydroxy 4,4'6'trimethoxy chalcone was tested for the first time for its antifungal activity. The present investigation revealed that, 2-hydroxy 4,4'6'trimethoxy chalcone has antifungal property as it was found effective against several fungi viz., *F. udum*, *Helminthosporium sativum*, *Cercospora blumea*, *U. cynodontis*, *C. capsici*, *A. brassicicola*, *A. triticina*, *A. solani*, *C. lunata* and a saprophytic fungus *Aspergillus flavus*. Further, the same compound showed excellent activity in inhibiting spore germination and development of *E. pisi*. From the excised leaf experiment, 2-hydroxy 4,4'6'trimethoxy chalcone appears to posses the characteristics of a contact fungicide, rather than systemic fungicide as its efficacy is reduced along with the increase of incubation period. Moreover, the compound showed high efficacy when applied as pre-inoculation treatment and thus advocates for its prophylactic uses.

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