Mycobiology 32(3): 134-138 (2004)

In vitro Effects of Plant Extracts, and Phytohormones on Mycelial Growth of Anthracnose Fungi

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(Received June 12, 2003)

Water extracts of six plants, such as *Allium sativum*, *A. cepa*, *Zingiber officinale*, *Platycodon grandiflorum*, *Oenanthe javanica*, and *Capsella brusapastoris*, were tested *in vitro* for inhibitory activity against mycelial growth of anthracnose fungi, *Colletotrichum gloeosporioides*, *C. dematium*, and *C. coccodes*. Among the plant extracts, an *Allium sativum* extract has good inhibitory effects in all the fungi. Four phytohormones namely, IAA (indole-3-acetic acid), NAA (á-Naphthyl acetic acid), 2,4-D (2,4-Dichloro phenoxy acetic acid) and BAP (Benzyl adenine purine) were used to find out the role over mycelial growth of these fungi. All the concentrations of BAP have good inhibitory effect against mycelial growth of these fungi than that of other tested plant hormones.

KEYWORDS: Anthracnose pathogen, Phytohormones, Plant extracts

Anthracnoses are devastating diseases of the foliage, stems, or fruits that typically appear as small or large, dark-colored spots or slightly sunken lesions with a slightly raised rim, caused by *Colletotrichum* spp. The fungal genus *Colletotrichum* is composed of a number of saprophytic and plant pathogenic species of worldwide importance on a wide range of economic crops and ornamentals (TeBeest *et al.*, 1997; Templeton, 1992). Anthracnose of pepper (*Capsicum annuum* L.) is one of the most destructive diseases in pepper-growing areas of Korea (Park and Kim, 1992). The disease produces symptoms on leaves, stems, and fruits (Hong and Hwang, 1998). Five anthracnose fungi, *Colletotrichum gloeosporioides*, *C. dematium*, *C. coccodes*, *C. acutatum*, and *Glomerelle cingulata*, are pathogenic to different tissues of pepper (Park and Kim, 1992). *C. coccodes* can infect pepper seeds, seedling leaves and stems, mature leaves, and sometimes green but not red fruits (Oh *et al.*, 1988; Park and Kim, 1992; Yu *et al.*, 1987). Anthracnose of ginseng (*Panax* ginseng Meyer) was reported by Nakata and Takimoto (1922), and Whetzel and Rosenbaum (1912) confused it with leaf blight of ginseng in Korea. Few reports on ginseng are available except that the disease was found in ginseng seeds or on plants obtained from North Korea by Russian scientists (Bunkina, 1960; Edel'Shtein, 1960) after the World War II. Our present observation confirmed *Colletotrichum* spp., such as *C. gloeosporioides*, *C. dematium*, *C. coccodes*, are responsible for anthracnose disease on ginseng in Korea (Han *et al.*, 2004, unpublished).

Due to increasing awareness of negative effects of synthetic fungicide on human and animal health and to the agroecosystem, research efforts on alternative and more environmentally friendly methods of controlling pests and diseases have proliferated. Besides biocontrol agents, the use of plant products in plant disease control seems to be a logical approach. Using extracts from plants containing natural antifungal compounds for plant disease control is considered to be one of the desirable methods for plant protection in agriculture (Kim *et al.*, 2002). Antifungal compound has been tested from *Allium sativum* that inhibits fungi including *Aspergillus* (Yoshida *et al.*, 1987). In recent past, several plant species have been screened for antifungal activity (Grayer and Harborne, 1994) and control pre-harvest (Tewari, 1995) and post-harvest diseases of several plant species (Mishra and Dubey, 1994). The plant extracts exhibited a marked effect on germination of fungal spores as well (Singh and Singh, 1981; Singh *et al.*, 1983; Dubey, 1991; Islam *et al.*, 2003) and it inhibited the fungal growth (Khair *et al.*, 1995).

The role of plant growth regulators (PGRs) in plant diseases is not clearly identified (Al-Masri *et al.*, 2002). Alterations in the levels of PGRs and in disease susceptibility or resistance reaction are associated with plant pathogen interaction (Singh *et al.*, 1997). Some investigations indicated that naphthalene acetic acid (NAA) is a potential antifungal agent (Nakamura *et al.*, 1978; Tomita *et al.*, 1984; Michniewicz and Rozej, 1988). Auxin strongly inhibited mycelial growth, sporulation, and spore germination of *Fusarium culmorum in vitro* (Michniewicz and

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Rozej, 1987).

The objectives of this study was to find out the probable plants, which has antifungal activities including plant hormones against anthracnose.

Materials and Methods

Fungi used. *Colletotrichum gloeosporioides*, isolated from diseased pepper (GP4) and ginseng (Kgsp), *C. dematium* (G20) and *C. coccodes* (G14) from diseased ginseng leaves, were used in all experiments. Fungi were cultured on PDA and maintained at 28°C.

Plant extraction. The water extraction of *Allium sativum* (Garlic), *Allium cepa* (Onion), *Zingiber officinale* (Zingiber), *Platycodon grandiflorum*, *Oenanthe javanica*, and *Capsella brusapastoris* were done following the method described by Mahadevan and Sridhar (1982). For each plant extracts, 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0 g tissues were cut into pieces and immediately plunged in sterilized distilled water in a beaker. The tissues were crushed thoroughly in a mortar with a pestle and then passed through two layers of cheesecloth. The ground tissues were re-extracted in sterilized distilled water of the said plant materials. The extracts were passed through cheesecloth and mixed both the extracts. The ratios of extracts were adjusted to 2.5, 5.0, 7.5, 10.0, 12.5 and 15.0% added with in sterilized distilled water and the extracts were used for experiment.

Media with plant extracts.

Mycelial growth in plant extracts medium: Experiments were carried out on potato dextrose agar (PDA) amended with each plant extract to obtain a final concentration of 0, 2.5, 5.0, 7.0, 10.0, 12.5 and 15% in PDA medium. All media were adjusted to pH 6.5 by using 0.1 N HCl and 0.1 N NaOH and autoclaved at 15 lb/in.^{2} pressure for 20 min. Twenty milliliter quantity of each medium was poured in Petri plates (90 mm). After solidification, triplicate plates of each plant extracted agar medium were used for inoculating experiment. The experiment of mycelial growth inhibition was carried out following Miah *et al.* (1990). The 5 mm agar discs cut from the margins of actively growing colonies of the seven days old fungal cultures on PDA medium were placed at the center of each Petri plates and kept them at 28°C for incubation. The measurement of radial growth of mycelium was taken after 13 days of incubation. The diameter of the colony was measured in two directions at right angles to each other, whereas in case of irregular colonies the measurement was taken along the longest and the shortest directions and the average was taken as the growth of the colony (Brown, 1923).

Mycelial growth inhibition test in different phytohormones: Experiments were carried out on potato dextrose agar (PDA) amended with each phytohormone to obtain a final concentration of 0, 10, 20, 30, 40, and 50 $\mu g/ml$. All media were adjusted to pH 6.5 by using 0.1 N HCl and 0.1 N NaOH and autoclaved at 15 lb/in.^{2} pressure for 20 min. Twenty milliliter quantity of each medium was poured in Petri plates (90 mm). After solidification, triplicate plates of each plant hormone containing media were used for inoculating experiment. The mycelial growth of fungi was recorded as described earlier data were taken after 10 days of incubation. Statistical analysis of data given as percentage was carried out from angular transformed values and performed using Microsoft Excel Software. LSD were determined, whenever, the calculated F values were significant at 5% level (Snedecor and Cochran, 1980).

Results and Discussion

Six locally available plants (*A. sativum* (Garlic), *A. cepa* (Onion), *Z. officinale* (Zingiber), *P. grandiflorum*, *O. javanica*, and *C. brusapastoris*) were investigated on the antifungal activities against *Colletotrichum* spp. Plant extracts were made using water only. In this experiment, garlic extracts showed good inhibitory effect against the mycelial growth of all the 4 isolates of *Colletotrichum* spp. In case of isolate Kgsp (*Colletotrichum gloeosporioides*), 4.3 mm mycelial growth was measured after 13 days of incubation at 25°C on comparison with control (8.5 mm) with the application of 15% garlic extracts. Except onion extracts, increase of extract concentrations decreased mycelial growth also. Onion extracts has no inhibition effect against mycelial growth of isolate Kgsp. The rest of the plant extracts showed intermediate effects against mycelial growth of the tested anthracnose fungi (Table 1). When the plant extracts were treated against the isolate G14 (*C. coccodes*), garlic extracts showed excellent inhibitory effect on mycelial growth. After 13 days of incubation, only 2.5 mm mycelial growth was recorded in 15% garlic extracted medium on comparison with control (8.5 mm). Next to garlic extracts, zingiber extracts has inhibitory effect against the isolate G14. The remaining plants extracts have no inhibitory effect against the isolate G14 (Table 1). In case of isolate G20 (*C. dematium*), zingiber extracts has good effect against the mycelial growth of the fungus. After 13 days of incubation, 4.4 mm mycelial growth was measured at 15% concentration of zingiber-extracted medium on comparison with control (8.5 mm). Garlic extracts has also good inhibitory effect against the mycelial growth of this isolate. Except this two plant extracts, all other used plant extracts have no inhibitory effects against the mycelial growth of G20 (Table 1). On the other hand, when the isolate GP4 (*C. gloeosporioides*) was tested with these plant extracts, the highest

Table 1. Effect of plant extracts on the inhibition of mycelial growth of antrachnose fungi *Collectotrichum* spp. after 13 days of incubation at 25°C

Isolates	Plant extracts	Radial growth of mycelium in different concentrations (mm)					
		2.5	5.0	7.5	10.0	12.5	15.0
Kgsp	*Allium cepa*	7.6bc	7.9b	8.5a	8.5a	8.5a	8.5a
	Zingiber officinale	6.6d	6.2c	6.0c	5.5ab	5.5c	5.4d
	Allium sativum	7.5c	5.1d	4.9d	4.7b	4.6d	4.3e
	Platycodon grandiflorum	7.5c	7.5b	7.5b	7.4ab	7.3b	7.3b
	Oenanthe javanica	8.5a	7.5b	7.4b	7.3ab	7.3b	6.4c
	Capsella brusapastoris	7.8b	7.5b	7.5b	7.4ab	7.3b	7.0bc
	Control	8.5a	A	A	A	A	A
G14	*Allium cepa*	7.7b	8.5a	8.5a	8.5a	8.5a	8.5a
	Zingiber officinale	6.2c	6.0b	5.5b	4.8b	4.1b	3.6b
	Allium sativum	6.2c	6.2b	5.6b	4.0c	3.2c	2.5c
	Platycodon grandiflorum	8.5a	8.5a	8.5a	8.5a	8.5a	8.5a
	Oenanthe javanica	8.5a	8.5a	8.5a	8.5a	8.5a	8.5a
	Capsella brusapastoris	8.5a	8.5a	8.5a	8.5a	8.5a	8.5a
	Control	8.5a	A	A	A	A	A
G20	*Allium cepa*	8.5a	8.5a	8.5a	8.5a	8.5a	8.5a
	Zingiber officinale	7.1c	7.0b	6.4d	6.1d	5.4c	4.4c
	Allium sativum	8.5a	8.5b	7.2c	7.1c	6.6b	5.8b
	Platycodon grandiflorum	8.5a	8.5a	8.5a	8.5a	8.5a	8.5a
	Oenanthe javanica	8.5a	8.5a	8.5a	8.5a	8.5a	8.5a
	Capsella brusapastoris	8.5a	8.5a	8.5a	8.5a	8.5a	8.5a
	Control	8.0b	A	B	B	A	A
GP4	*Allium cepa*	5.5b	5.5c	5.5c	5.4c	5.3c	5.3c
	Zingiber officinale	5.4b	5.4c	5.3c	5.1c	5.1c	4.9d
	Allium sativum	4.9b	4.7d	4.6d	4.2d	3.7d	3.4e
	Platycodon grandiflorum	6.9a	7.4ab	7.5a	7.6a	7.6a	7.6a
	Oenanthe javanica	7.4a	7.5.ab	7.6a	7.6a	7.7a	7.8a
	Capsella brusapastoris	7.2a	7.1b	6.8b	6.7b	6.7b	6.6b
	Control	7.9a	A	a	a	A	A

*Means within the same column followed by the same letter are not significantly different ($P<0.05$. LSD one-way ANOVA).

mycelial growth (3.4 mm) inhibition recorded against 15% garlic extracts and the lowest (7.8 mm) was in 15% *Oenanthe javanica*, on comparison with control (7.9 mm). Inhibition rate of mycelial growth of isolate GP4 as treated with 15% concentration of extract of *A. cepa*, *Z. officinale*, *P. grandiflorum*, and *C. brusapastoris* was 5.3, 4.9, 7.6 and 6.6 respectively recorded after 13 days of incubation (Table 1). LSD value at 5% level show that garlic and zingiber extracts have significant inhibitory effects on mycelial growth in all the isolates. Alam *et al.* (2002) tested the antifungal effect of ten plant extracts against conidial germination of *C. gloeosporioides* and found that *Tagetes erecta* (leaf) and *Azadirachta indica* (bark) extracts were most effective. Alam *et al.* (2002) reported that *Vinca rosea* root extract and leaf, root and seed extracts of *A. indica* inhibited 100% spore germination of *Bipolaris sorokiniana* and *Rhizopus artocarpi.* Alam *et al.* (1999) reported the antifungal effects of leaf and root extracts of *V. rosea* and leaf, root and seed extracts of *A. indica* against chilli fruit rot pathogen *Alternaria tenuis*. Singh *et al.* (1990) reported, ajoene, a compound derived from garlic, inhibited spore germination of some fungi, *Alternaria solani*, *A. tenuissima*, *A. triticina*, *A.* sp., *Colletotrichum* sp., *Curvularia* sp., *Fusarium lini*, *F. oxysporum*, *F. semitectum* and *F. udum*, which cause serious disease in some important crop plants in India. The compound was very effective in inhibiting spore germination at concentration of 25 *μg/ml* in some fungi and, in most cases there was 100% inhibition of germination at 100 *μg/ml*. Powell and Ko (1986) reported that root extracts of garlic in the genus *Allium* inhibited the germination of zoospores and chlamydospores of *P. palmivora.*

Four phytohormones (IAA, NAA, 2,4-D and BAP) were tested to study the role against the mycelial growth of anthracnose fungi. When the hormones were treated against the isolate GP4 (*C. gloeosporioides*), the highest (5.9 mm) and the lowest (7.1 mm) mycelial growth inhibition were recorded at 50 *μg/ml* BAP and 10 *μg/ml* IAA and 2,4-D on comparison with control (8.5 mm) after 10 days of incubation at 25°C. With the increase of concentrations, all hormones inhibited the mycelial growth of the isolate GP4 (Table 2). On the other hand, the mycelial

Table 2. Effect of phytohormones on the inhibition of mycelial growth of antrachnose fungi *Collectotrichum* spp. after 10 days of incubation at 25°C

Isolates	Name of phytohormones	Radial growth of mycelium (mm) in different $\mu g/ml$ concentrations				
		10	20	30	40	50
GP4	IAA	7.1b	6.9b	6.9ab	6.8b	6.7b
	NAA	6.9bc	6.8bc	6.6ab	6.6b	6.4b
	2,4-D	7.1b	6.8bc	6.8ab	6.8b	6.7b
	BAP	6.7c	6.6c	6.2b	6.0b	5.9c
	Control	8.5a	A	A	A	A
Kgsp	IAA	6.5b	6.4b	6.3b	6.3b	6.2b
	NAA	6.4b	6.4b	6.4b	6.1b	6.0b
	2,4-D	6.5b	6.4b	6.3b	6.2b	6.2b
	BAP	6.3b	6.1b	5.9c	5.6c	5.4c
	Control	8.5a	A	A	A	A
G20	IAA	7.9b	7.8b	7.5b	7.0b	6.6b
	NAA	8.5a	8.5a	8.4a	8.3a	8.2a
	2,4-D	8.5a	8.5a	8.5a	8.5a	8.5a
	BAP	7.7c	7.5c	7.3c	6.8b	6.6b
	Control	8.5a	A	A	A	A
G14	IAA	8.5a	8.5a	8.5a	8.2a	8.1b
	NAA	8.5a	8.1b	8.0a	7.8b	7.2c
	2,4-D	8.5a	8.5a	8.5a	8.5a	8.5a
	BAP	8.5a	8.5a	7.8a	7.2c	6.3d
	Control	8.5a	A	A	A	A

*Means within the same column followed by the same letter are not significantly different (P<0.05. LSD one-way ANOVA).

growth of isolate Kgsp (*C. gloeosporioides*) was inhibited more with the application of BAP than that of other used plant hormones. The concentration 50 $\mu g/ml$ showed the highest mycelial growth inhibition (5.4 mm) and the lowest was 6.5 mm at 10 $\mu g/ml$ in both IAA and 2,4-D (Table 2). In case of isolate G20 (*C. dematium*), the highest mycelial growth inhibition (6.6 mm) was recorded both in IAA and BAP at 50 $\mu g/ml$ concentration. 2,4-D has no growth inhibitory effect against the isolate G20. More or less similar inhibitory effect was also observed incase of the isolate G20 when treated with different concentrations of NAA (Table 2). Inhibitory effect of phytohormones was not satisfactory against the isolate G14 (*C. coccodes*). The highest 3.6 mm and the lowest 8.1 mm mycelial growth inhibition was recorded with the treatment of BAP and IAA at 50 $\mu g/ml$ concentration after 10 days of incubation. NAA has a little effect and at 50 $\mu g/ml$ concentration; 7.2 mm mycelial growth was recorded after 10 days on comparison with control (8.5 mm). The hormone 2,4-D has no inhibitory effect against the isolate G14. This study indicates that BAP has more or less good inhibitory effects against mycelial growth in all the tested anthracnose fungal isolates. LSD at 5% shows that BAP has a significant activity against mycelial growth of all the isolates, except isolate G20. This study also showed that higher concentrations of plant hormones inhibited better mycelial growth of anthracnose fungi than that of lower concentrations (Table 2). Michniewicz and Rozej (1987) and Melinda and Stevenson (1991) have pointed out that auxin acts as a fungal growth and development controlling factor, while its role in the growth and development processes may vary in different species. NAA reduced mycelium growth rate of fungi (Al-Masri *et al.*, 2002; Michniewicz and Rozej, 1987). NAA increase the resistance of potato plants to early blight (Melinda and Stevenson, 1991).

Acknowledgment

Authors are very much grateful to Dr. Chung-Duk Kim, President, Korea Science and Engineering Foundation (KOSEF) for granting Postdoctoral Fellowship to the 1st author of this research work under which finance this work was carried out. Thanks are also extended to the President, Dongguk University and Head, Department of Biology, Dongguk University for giving permission to do this work providing all kinds of laboratory facilities.

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