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Sensitivity among *Pestalotiopsis* spp. Against the Phytoalexins of Three Rosaceae Plants

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Abstract: The experiment was aimed to know differential sensitivity of four *Pestalotiopsis* spp., *P. funerea* (*Eriobotrya japonica*), *Pestalotiopsis* sp. (*Rhaphiolepis umbellata*), *P. photinia (Photinia glabra)* and *P. breviseta* (*Viburnum awabuki*), to the five phytoalexins isolated from three Rosaceae plants. The names of two kind biphenyl phytoalexins accumulated in *E. japonica* (loquat), *R. umbellata* and *P. glabra* (photinia), were aucupurin and eriobofuran, 4′-methoxyaucuparin and rhaphiolepsin, 2′-methoxyaucuparin and 4′-methoxyaucuparin, respectively. Four *Pestalotiopsis* spp. showed different sensitivity to the phytoalexins tested. *P. funerea* was less sensitive to aucuparin in comparison with the other fungi. The less sensitive fungi to eriobofuran was obtained with *P. photiniae.*, P. breviseta was unable to germinate in the medium containing rhaphiolepsin in concentration of 10 ppm and 50 ppm. Of the five phytoalexins tested, 2′-methoxyaucuparin was less toxic to the fungi.

Key words: Phytoalexins, *Pestalotiopsis*, rosaceae plants

Introduction

The damage of plants caused by infection diseases are some times enormous and the nurseries in many countries had to be abandoned in the past owing and the ravages of the diseases as well as in the landscape. Many different groups of organisms including the fungi, bacteria and viruses attack rosaceae plant parasitically. One of the important disease of Rosaceae is Pestalotiopsis leaf spot disease. Symptoms usually brown, circular, sunken spots up to 3 mm in diameter developed scattered over the upper surfaces of the leaves.

Plants have various mechanisms for resistance to injection by pathogens. One of the dynamic resistance mechanisms is the production of antimicrobial compounds called as phytoalexin, that kill the pathogens or restrict their intracellular development. In previous reports the number of phytoalexins that had been isolated from three Rosaceae plants were biphenyl compounds (Widyastuti *et al.*, 1992). The objectives of the present study was to know the pathogenicity and differential sensitivity of *Pestalotiopsis* spp., one of the important fungal pathogens of plant, to the phytoalexins of three Rosaceae plants.

Materials and Methods

Plants: Seedlings of *Eriobotrya japonica* Lindl. (loquat) and *Rhaphiolepis umbellata* Makino were grown in a green house at about 25°C (six months to two years before treatment with several elicitors).

The plant materials of *Photinia glabra* Maxim. (photinia) were prepared vegetatively by using healthy softwood cuttings. The cuttings were grown in greenhouse (average temperature 25°C, natural light) in a coarse sand until the roots and shoots are developed. After having grown as small plants, they were transplanted to the pots containing a mixture of soil and organic fertilizer (1:1) and grown for 3 to 6 months.

Fungi: Three Pestalotiopsis were isolated from the respective host plants. To obtain the good result, it was necessary to perform the assay in the optimum temperature for growth of the three *Pestalotiopsis* spp. For determination of the optimum temperature for mycelia growth, 7.5 mm plugs were taken from edges of actively growing mycelium of fungi tested on potato sucrose agar (PSA, Potato 200 g, Sucrose 20 g, Agar 20 g, water 1000 ml). The plugs were then inverted in the center of petridishes poured with 10 ml of PSA.

Toxicological assay: The phytoalexins were purified from three plants used, as described before (Widyastuti *et al.*, 1991a). The purified phytoalexins were assayed for toxicity by measuring its effects on the spore germination and hyphal elongation of pathogenic fungi of three Rosaceae plants *(P. funerea, Pestalotiopsis* sp. and *P. photinia)* and one pathogenic fungi of non-Rosaceae plant *(P. breviseta* isolated from *Viburnum awabuki*) with a method as described by Kiraly *et al.* (1974). An ethanolic solution of each phytoalexin was added to water so that the final volume of ethanol was 2%. A drop of the solution was added to water agar (agar 20 g, water 1000 ml) block containing spore suspensions (105 spores/ml) and then incubated at 20–25°C in moist chamber until germination rate of the control was over 80%. The germination rate and germ tube length were then measured under a light microscope.

Optimum temperature: The results were given in Fig. 1 . These data shown that the optimum temperature for three *Pestalotiopsis* spp. tested was 25°C. Sizes of conidia produced on PSA were measured and the data are shown in Table 1. There were no significant differences in the size of conidia among species tested.

Data in Table 2 showed that *P. breviseta,* which was isolated from non-rosaceous plant (*V. awabuki*) did not cause any symptom on loquat, *R. umbellata* and photinia. Isolate of Pestalotiopsis from loquat was strongly virulent on the loquat and *R. umbellata* but not on photinia. Starting about 7 days after inoculation, the fungi induced brown, circular, sunken spot up to 1.0 mm in diameter on the leaf. Reisolation from the spot always yielded an isolate with characteristics, similar to the one employed to inoculate the leaves.

Sensitivity to the phytoalexins: Susceptibility and resistance of host plants to pathogens comprise a series of many integrated processes that involve e.g. enzymes,

Table 1: Sizes of konidia of four *Pestalitiopsis* spp. produced on media

Fungi (Host plants)	No of isolates tested	Length (μm)			Wide (μm)		
		Min.	Max.	Av.	Min.	Max.	Av.
Pestalotiopsis funerea (loquat)	3	20.8	28.8	23.1	5.0	10.4	7.7
Pestalotiopsis sp. (*R. umbellata*)	3	15.0	27.5	21.2	3.8	9.1	6.4
Pestalotia photiniae (photinia)	3	17.5	27.5	22.5	5.0	10.4	7.5
Pestalotiopsis breviseta (*V. awabuki*)	3	17.5	27.5	22.4	6.3	7.5	7.3

a) Each value represents an average of three exoeriment, 100 spores were observed

Table 2: Pathogenicity of four *Pestalotiopsis* spp. against three Rosaceous plant

Fungi (Host plants)	Kinds of plants inoculated		
	Eriobotrya japonica	*Rhaphiolepis umbellate*	*Photinia glabra*
Pestalotiopsis funerea (loquat)	+	+	-
Pestalotiopsis sp. (*R. umbellata*)	+	+	±
Pestalotia photiniae (photinia)	±	±	+
Pestalotiopsis breviseta (*V. awabuki*)	-	-	-

Table 3: Effect of five phytoalexins on the spore germination rate of four *Pestalotiopsis* spp.

Fungi (Host plants)	Conc.[a]	Germination rate (%)[b]				
		AU[c]	Er[d]	Fm[e]	Rh[f]	Tm[g]
Pestalotiopsis funerea	0[h]	100.00	100.00	100.00	100.00	100.00
(loquat)	10	91.00	31.00	87.00	30.00	100.00
	50	21.00	0.00	32.00	0.00	94.00
Pestalotiopsis sp.	0	76.00	23.00	78.00	20.00	96.00
(*R. umbellata*)	10	75.00	37.00	55.00	16.00	93.00
	50	15.00	0.00	8.00	0.00	77.00
Pestalotia photiniae	0	100.00	100.00	100.00	100.00	100.00
(photinia)	10	75.00	37.00	55.00	16.00	93.00
	50	15.00	0.00	8.00	0.00	77.00
Pestalotiopsis breviseta	0	100.00	100.00	100.00	100.00	100.00
(*V. awabuki*)	10	73.00	30.00	93.00	0.00	92.00
	50	18.00	0.00	34.00	0.00	85.00

a) Concentration of phytoalexin (ug/ml)
b) Each value represents an average of three experiments. In each experiment, 100 spores were observed
c) AU: aucuparine; d) Er: eriobofuran
e) FM: 4' -methoxyaucuparin f) Rh: rhaphiolepsin
g) TM: 2'- methoxyaucuparin h) Control (treated with 2% ethancl only)

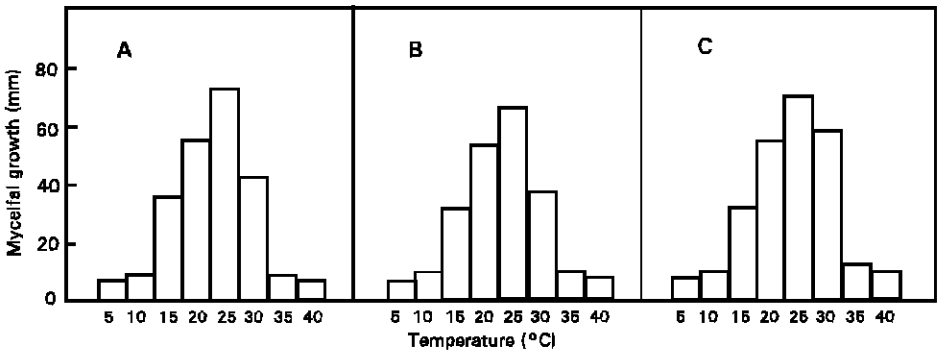

Fig. 1: Mycelial growth of three *Pestalotiopsis* spp. at several temperatures. A: *Pestalotiopsis funerea*, B: *Pestalotiopsis* sp., C: *Pestalotia photiniae*

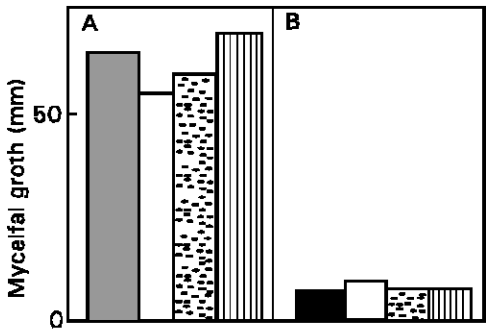

Fig. 2: The inhibitory effects of two fungicides against four *Pestalotiopsis* spp.
A: Copper oxychloride (100 ppm), B: Benomyl (1 ppm)
: *Pestalotiopsis* funerea,
: *Pestalotiopsis* sp.,
: *Pestalotia photininae*,
: *Pestalotiopsis braviseta*

toxins and phytoalexins (Bailey, 1982). An attempt was also made to know the correlation between the host-pathogen specificity and the sensitivity of the pathogens against two phytoalexins from loquat namely aucuparin and eriobofuran (Miyakado *et al.*,1984), two those from *P. umbellata*, namely 4'-methoxyaucuparin and 2'-methoxyaucuparin (Widyastuti *et al.*, 1991a; 1991b) and two from photinia, named 4'-methoxyaucuparin and 2'-methoxyaucuparin (Widyastuti *et al.*, 1992).

The plants in the family of Rosaceae have been known to be associated with many kinds of pathogenic *Pestalotiopsis* spp., which in certain conditions cause severe diseases. Understanding the characteristics of the fungi isolated from different hosts, is important to make strategy in controlling the diseases, for example, to provide a basic consideration in selecting the isolate used in the resistance study. There have been several results of experiments which indicate that several isolates of fungi causing the same diseases could differ in their characteristics (Jones and Belmar, 1989). It was difficult to differentiate the *Pestalotia* from four hosts based on the morphological colony, conidia dimension and temperature response. The average size of conidia was about 22 x 7 μm (Table 1). The similarity in the morphological characteristicts obtained in this experiment could be used to support the previous workers who named *Pestalotia funerea* instead of *Pestalotiopsis funerea* (Miyakado *et al.*, 1984). The earlier studies with *Entomosporium spp.* isolated from *E. japonica, P. glabra* and *R. umbellata* failed to find the reguler correlation between the origin of isolates and variation of the conidial size (Horie and Kobayashi, 1979).

The four isolates seemed to have difference in their host specificity (Table 2). This information should be considered in controlling the diseases. For example when the environment factors are good for stimulating the *Pestalotiopsis* leaf spot on *E. japonica*, it is not wise to cultivate *E. japonica* together with *R. umbellata* and *P. glabra* in the same area, because *E. japonica* will be infected by the conidia from several other host plants.

There have been several studies concerning the specificity of phytoalexin to the related pathogens. One of the most famous studies was made by Cruickshank (1962), who concluded that pathogens were often sensitive to phytoalexins from plants other than their hosts. The results of the present experiment could not completely agree with the above conclusion. For example, the germination rate of *P. funerea* treated with 10 μm/ml of aucuparin (loquat), eriobofuran (loquat), 4'-methoxyaucuparin (*R. umbellata*) and rhaphiolepsin (*R. umbellata*) was 91, 41, 87 and 30%, respectively. Thus in agreement with Cruickshank's results, it could be concluded that *P. funerea* was more sensitive to 4'-methoxyaucuparin and rhaphiolepsin than to aucuparin. However, it could not be said that the same fungi was more sensitive to 4'-methoxyauparin than to eriobofuran. This phenomenon indicates that in the plants having multi-phytoalexins, the specificity of each phytoalexins should be analyzed separately.

Data in Fig. 2 showed that benomyl (1 ppm) was very effective for inhibiting the mycelial growth of the fungi tested. Thus, for direct application, benomyl seems to be better than the phytoalexins. As suggested by Mansfield and Bailey (1982), though phytoalexin can inhibit several pathogens, their usefulness is stiil limited. However, the problem may be overcome by preparation of analogous phytoalexin.

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