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Elicitation of phytoalexin in non-infected soybean leaves by ajmalicine, an alkaloid

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Foliar spray of ajmalicine solution (100μg/ml) reduced *Myrothecium* infection of soybean leaves significantly and also induced glyceollin accumlation in treated, non-inoculated leaves. Similar concentration (100μg/ml) of ajmalicine was not inhibitory to the mycelial growth of the pathogen. Possible involvement of phytoalexin in reduction of leaf spot disease of soybean has been discussed.

Key words : Phytoalexin, Soybean leaves, Ajmalicine, *Cantharanthus roseus*

Some biotic and abiotic agents are capable of activating host's defence by eliciting phytoalexin production/accumulation (Ghosal and Purkayastha, 1987 ; Purkayastha and Ghosh, 1983). Induction of soybean phytoalexin (glyceollin) by various chemicals and their involvement in disease resistance have also been reported earlier (Chakraborty and Purkayastha, 1987 ; Ward *et. al.*, 1980). This communication, however, reports the elicitation of phytoalexin (glyceollin) production in healthy soybean leaves (Svoboda *et. al.*, 1959) by *Cantharanthus roseus* (L) G. Don (Syn. *Vinca rosea* L.) and its effect on the development of leaf spot disease of soybean caused by *Myrothecium roridum* Tode. ex. Fr.

MATERIALS AND METHODS

Soybean seeds [*Glycine max* (L.) Merrill] were obtained from Pulses and Oilseeds Research Station, Berhampore, West Bengal and the seeds were disinfected with 0.1% $HgCl_2$, sown in earthenware pots (22 cm. diam.) and kept under ordinary conditions of light and temperature (28-34°C) of the Experimental Garden of the Department.

M. roridum was collected from the stock culture of the Department and maintained on potato dextrose agar medium.

Pathogenicity of *M. roridum* was tested on intact plants (3-week-old) and disease intensity was assessed following the method of Purkayastha and Raha (1979). The leaf spots were graded into 4 size groups, viz. very small (0.5 mm diam.), small (1 mm diam.), medium (2 mm diam.) and large (>2 mm diam.) with respective values 0 1, 0.2, 0.5 and 1.0. The total number of spots in each size group was multiplied by the value assigned to it. The summation of such values taken together for all leaves divided by the number of plants per treatment gave the disease index for a plant.

Ajmalicine, an alkaloid of *C. roseus* was tested on soybean leaves. Pure crystalline ajmalicine was dissolved in a known volume of warm methanol and diluted with sterile distilled water to obtain desired concentrations (0 1, 1, 10 and 100 μg/ml) of ajmalicine which were sprayed separately of 2-week-old plants twice at an interval of 3 days. The plants were inoclulated after 48 h of second spray. Control plants were sprayed with sterile distilled water containing similar amount of methanol but without ajmalicine.

RESULTS AND DISCUSSION

The pathogenicity test on soybean plants (cv. PK-327) treated with different concentrations of ajmalicine reveals that 100 μg/ml of ajmalicine reduced disease significantly (Table 1).

Table 1. Effect of foliar application of ajmalicine on disease development

Ajmalicine (μg/ml)	DI/plant*	%Reduction in disease in relation to control
0 1	41.10	5.84
1.0	36.25	16.95
10	34.20	21.65
100	14.54	66.69
Control (without ajmalicine)	43.65	—

DI=Disease index ; *7 days after inoculation

The disease indices were 14.54/plant and 43.65/plant for treated and control plants respectively. The remaining three concentrations did not reduce disease significantly.

Since ajmalicine reduced disease significantly, it was considered worthwhile to determine whether induction of phytoalexin was anyway involved in the reduction of disease symptoms. Hence, 'Facilitated Diffusion Technique' of Keen (1978) was followed for the extraction of phytoalexin from treated and untreated, inoculated leaves. Ethyl acetate extracts of leaves were spotted on T.L.C. plates (Silica gel G), developed in chloroform : acetone : conc. NH_4OH (50 : 50 : 1), dried and sprayed with Fast Blue Salt B. The presence of glyceollin was confirmed by co-chromatography with authentic glyceollin. For quantification of glyceollin, unsprayed reacting zone of TLC was scraped and eluted in spectral grade absolute ethanol. Glyceollin was measured by UV-spectrophotometry. Ajmalicine cused glycoeollin accumulation in healthy soybean leaves (Table 2).

Table 2. Effect of ajmalicine on production of glyceollin in soybean leaves (cv. PK-327)

Treatment	Glyceollin content (μg/g fresh weight of leaf)
Ajmalicine treated (non-inoculated) (100μg/ml)	84.80
Inoculated with *Myrothecium roridum* (Ajmalicine untreated)	194.12
Ste·ile distilled water (Control)	0

Glyceollin was extracted 48 h after inoculation/treatment

Significant reduction in leaf spot disease of soybean by ajmalicine is also noteworthy because this natural product could be used as an inducer of resistance. The induction of resistance in host may be due to systemic action of ajmalicine because this active compound (100 μg ml) did not significantly inhibit the mycelial growth of the pathogen *in vitro* (Table 3). This finding supports the

Table 3. Effect of ajmalicine on growth and sporulation of *M. roridum in vitro*

Ajmalicine (μg/ml) in P.D.A.	Diameter of mycelial mat* (mm)	Sporulation*
0.1	34 33±0 66	+++
1.0	33.00±0 58	+++
10	32 67±0.33	+++
10)	31 67±0.33	+
Control (P.D A. without ajmalicine)	33.33±0.66	+++

Incubation period—6 days ; Temperarure 30±1°C ; *Sporulation +++ — abundant
+ — few spores

earlier report of Doubrava *et. al.* (1988). They demonstrated that oxalate, an active principle in the extracts of spinach leaves, induced systemic resistance of cucumber plants against anthracnose caused by *Colletotrichum lagenarium*.

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