Research Paper :

In vitro antifungal effect of crop root exudates against *Sclerotium rolfsii* Sacc. causing stem rot in groundnut

N.B. BAGWAN

International Journal of Plant Protection (October, 2010), Vol. 3 No. 2 : 298-305

SUMMARY

Crop root exudates of 20 crops like groundnut, soybean, pigeonpea, green gram, black gram, chickpea, pea, cowpea, mustard, cotton, castor, sunflower, safflower, sesamum, sorghum, pearl millet, maize, wheat, onion, and garlic were used in this study. Large variations of inhibitory effect of root exudates on *S. rolfsii* were observed. Low concentrations of root exudates (5% and 10%) had no effect on inhibition of mycelial growth and germination of sclerotia while, at high concentration (20%) inhibited the mycelial growth and germination of sclerotia. Mycelial growth, dry mycelium weight and sclerotial germination were recorded lowest in root exudates of sunflower, maize, pearl millet, sorghum, safflower, garlic, and onion. Mycelial growth, dry mycelium weight and sclerotial germination was recorded highest in root exudates of soybean, groundnut, green gram, black gram, pigeonpea, chickpea, pea and cowpea. It was observed that the root exudates of maize, sunflower and pearl millet showed a highest percentage of inhibition of mycelial growth and sclerotial germination. Another interesting of thing was observed that root exudates of groundnut, soybean and pea stimulate the mycelial growth and germination of sclerotia as compared to control. The results of this study suggested that the intercropping or crop rotation of safflower, maize, pearl millet, sorghum, sunflower, garlic, and onion with groundnut may be useful for the management of stem rot of groundnut and also for reduction of soil population of *S. rolfsii* in groundnut field. Similarly intercropping or crop rotation of soybean, green gram, black gram, chickpea, pea and cowpea with groundnut should be avoided. Based on these findings, it is hypothesized that root exudates of some crops contain antifungal compounds, while other stimulate the growth of fungal pathogens. Cultivation of safflower, maize, pearl millet and sorghum with groundnut could lead to a reduction in the occurrence of stem rot disease, especially when chemical control is not effective and economically costly. However, further investigation is necessary for isolation and identification of antifungal compounds in root exudates related to host-pathogen interaction.

Correspondence to :
N.B. BAGWAN
Department of Plant Pathology, Directorate of Groundnut Research (ICAR), JUNAGADH (GUJARAT) INDIA

Key words :
Root exudates, Mycelial growth, Sclerotial germination, *Sclerotium rolfsii*

Accepted :
August, 2010

In Saurashtra region of Gujarat, farmers have a tendency to grow identical crop in the same field for several years. Continuous cropping of the same crop results in the accumulation of soil populations of specific plant pathogens, and leads to a decline in crop quantity and quality. Farmers therefore are recommended crop rotation with specific crop species to minimize the accumulation of soil populations of soil borne plant pathogens. The relation between crop exudates and soil-borne fungal diseases is a challenging problem in the field of Plant Pathology. Direct monitoring of antifungal compounds at the site of interest, the rhizosphere, remains a difficult task (Bonsall *et al.,* 1997, Keel *et al.,* 1992, Maurhofer, 1995, Notz *et al.,* 2001). Over the past decade, enormous steps have been taken toward understanding these different types of interactions (Hirsch *et al.,*2003.) and recently the field of plant biology has recognized the importance of root exudates in mediating these biological interactions (Bais *et al.,* 2003, Walker *et al.,* 2003, Weir *et al.,* 2004). The rhizosphere represents a highly dynamic front for interactions between roots and pathogenic and beneficial soil microbes, invertebrates, and root systems of competitors (Hirsch *et al.,* 2003). Although the functions of most root exudates have not been determined, several compounds present in root exudates play important roles in biological processes (Bais *et al.,* 2003, Bais *et al.,*2002, Kneer *et al.,* 1999). The plant defenses induced by root exudates simply reduce susceptibility to pathogen infection, whereas in other cases these defenses initiate production and release of leafy volatiles that attract predators of plant enemies.

Up to now, no control strategies are field effective against stem rot and the only way to

produce groundnut safely is the chemical control. Therefore, the objective of this study was to evaluate the influences of root exudates produced by some crops on mycelial growth and germination of sclerotia of *S. rolfsii* causing stem rot in groundnut.

MATERIALS AND METHODS

Sowing of seeds in test tube:

Seeds of twenty crops *viz.,* groundnut, soybean, pigeonpea, green gram, black gram, chickpea, pea, cowpea, mustard, cotton, castor, sunflower, safflower, sesamum, sorghum, pearl millet, maize, wheat, onion, and garlic were surface sterilized with 0.1% $HgCl_2$ and after three washes with sterile distilled water placed into in test tubes (size) containing 25 ml of hydroponic nutrient solutions. One seed was placed in each test tube and for each crop 10 replications were maintained. These test tubes were placed in BOD at 28^{0}C for two weeks. After 2 weeks of growth the seedlings were removed and the solution containing root exudates was collected. The solution was centrifuged and kept at 4^{0}C for further study. All these process were carried out under aseptic conditions.

Standardization of different concentrations:

After collection of root exudates, the root exudates were filtered through Whatman No. 1 filter paper and standardized at four different concentrations *viz.,* 5, 10, 15 and 20% by adding sterile distilled water. These concentrations were used in solid and liquid media to study the mycelial growth and sclerotial germinations of sclerotia *of S. rolfsii.*

Influence of root exudates on mycelial growth on solid medium:

This was done on Potato dextrose agar medium using poisoning food technique. Four different concentrations *viz.,* 5, 10, 15 and 20% were amended in the 20 ml PDA medium after autoclaving in 90 mm culture plates. For each concentration and each crop root exudate, five replications were maintained. After solidification the agar medium in the culture plates was seeded with the highly virulent (NRCG-SR-07) isolate of *S. rolfsii* (5 mm culture discs of three days old culture) in the centre of Petriplates. The plates without any amendment served as control. The plates were incubated at 28^{0}C in BOD. After 3 days of incubation, the diameter of the mycelial growth of *S. rolfsii* was measured and average mycelial growth was recorded. The data from the replicated plates were averaged and the result was expressed as per cent inhibition of mycelial growth over the control.

The percentage growth inhibition *S. rolfsii* was obtained by using the formula:

$$\text{Percentage growth inhibition} = \frac{A - B}{A} \times 100$$

where,

A = Area covered by test pathogen in control (mm)

B= Area covered by test pathogen in different treatments and concentrations (mm)

Influence of root exudates on mycelial growth liquid medium:

Influence of root exudates on mycelial growth was studied in *S. rolfsii* special liquid medium. 100ml of *S. rolfsii* special liquid medium was amended with four different concentrations *viz.,* 5, 10, 15 and 20% of root exudates in 250ml capacity conical flasks. For each concentration and each crop root exudates, five replications were maintained. Five mm diameter mycelial plugs of highly virulent (NRCG-SR-07) isolate of *S. rolfsii* were cut from the periphery of PDA plates and used to inoculate 250ml capacity conical flasks containing 100ml of *S. rolfsii* special liquid medium. The flasks without any crop root exudates amendment were served as control. These flasks were incubated at 28^{0}C in BOD. After 10 days of incubation the fresh mycelium of *S. rolfsii* was harvested and dried at 40^{0}C in oven. Average dry mycelial weight was recorded.

Influence of root exudates on sclerotial germination on solid medium:

Influence of root exudates on sclerotial germination was studied on PDA medium. Two hundred sclerotia of highly virulent (NRCG-SR-07) isolate of *S. rolfsii* were surface sterilized with 0.1% $Hgcl_2$ and washed with sterile distilled water thrice. These sclerotia were soaked in the different concentrations of root exudates for 24 hours at 28^{0}C. Thirty sclerotia thus soaked in root exudates were distributed evenly on the surface of each PDA plate in five replications and incubated at 28^{0}C in BOD. After 72 hr. the percentage germination of sclerotia was recorded. Sclerotia soaked in sterile distilled water were served as control. Sclerotia were considered to have germinated if the white, threadlike mycelium characteristic of *S. rolfsii* were observed on sclerotia and medium.

RESULTS AND DISCUSSION

The results obtained from the present investigation have been presented in the following sub heads :

Influence of root exudates on mycelial growth on solid medium:

The experimental results in Table 1 indicated that the low concentrations of root exudates (5% and 10%) had no effect on inhibition of radial growth of mycelium. The radial growth of *S. rolfsii* was inhibited on the media containing 20% crop root exudates of safflower, maize, pearl millet, sorghum, sunflower, garlic and onion. On the other hand mycelial growth of *S. rolfsii* was recorded highest on the media containing root exudates of soybean, groundnut, green gram, black gram, pigeonpea, chickpea, pea, cowpea. It was observed that the root exudates of maize, sunflower and pearl millet showed a highest percentage inhibition of radial growth. The radial growth of *S. rolfsii* in root exudates of maize, sunflower and pearl millet was 25.33, 26.67and 29.67mm, respectively. Another interesting thing was observed that root exudates of groundnut, soybean and pea stimulated the mycelial growth. *S. rolfsii* grew well in root exudates of groundnut, soybean and pea where, the radial growth was 90 mm (Fig. 1).

Influence of root exudates on mycelial growth in liquid medium:

Similar results were obtained from growing *S. rolfsii* in liquid medium. 20% root exudates of safflower, pearl millet, sorghum, sunflower, garlic and onion inhibited the growth of *S. rolfsii* in special liquid medium. Average dry mycelium weight in root exudates of, sunflower, pearl millet, safflower, sorghum, onion, garlic and maize was 11.3, 13.0, 21.3, 29.3, 21.7, 33.0, and 57.6 mg, respectively. The growth of *S. rolfsii* in root exudates of groundnut, soybean, green gram, black gram, pigeonpea, chickpea, pea, cowpea, sesamum, castor, and wheat was more

Table 1 : Influence of root exudates on radial growth of mycelium on solid medium

Crop root exudates	Colony diameter (mm)* of *S. rolfsii* in different concentrations				
	5%	10%	15%	20%	% Inhibition (at 20%)
Sorghum	89.33	86.04	82.04	47.67	47.04
Pearl millet	87.33	81.33	54.67	29.67	67.04
Maize	86.08	79.76	52.74	25.33	71.85
Wheat	89.33	89.00	86.00	79.00	12.22
Garlic	83.00	83.33	81.67	58.67	34.81
Onion	89.00	84.33	79.67	48.67	45.93
Sesamum	90.00	89.67	88.67	80.33	10.74
Sunflower	86.67	80.67	55.33	26.67	70.37
Safflower	88.00	82.67	68.67	47.00	47.78
Mustard	90.00	89.67	81.33	80.67	10.37
Cotton	90.00	89.33	83.67	73.33	18.52
Castor	90.00	89.33	80.33	68.67	23.70
Groundnut	90.00	90.00	90.00	90.00	0.00
Soybean	90.00	90.00	90.00	90.00	0.00
Pigeonpea	90.00	90.00	90.00	90.00	0.00
Green gram	90.00	90.00	90.00	90.00	0.00
Black gram	90.00	90.00	90.00	90.00	0.00
Chickpea	90.00	90.00	90.00	90.00	0.00
Pea	90.00	90.00	90.00	90.00	0.00
Cowpea	90.00	90.00	90.00	90.00	0.00
Control	90.00	90.00	90.00	90.00	
C.D. (P=0.05)	0.09	0.09	0.22	0.43	
S.E.M $\pm$	0.03	0.03	0.08	0.15	
C.V. %	0.57	0.58	1.47	3.20	

* Mean of five replications

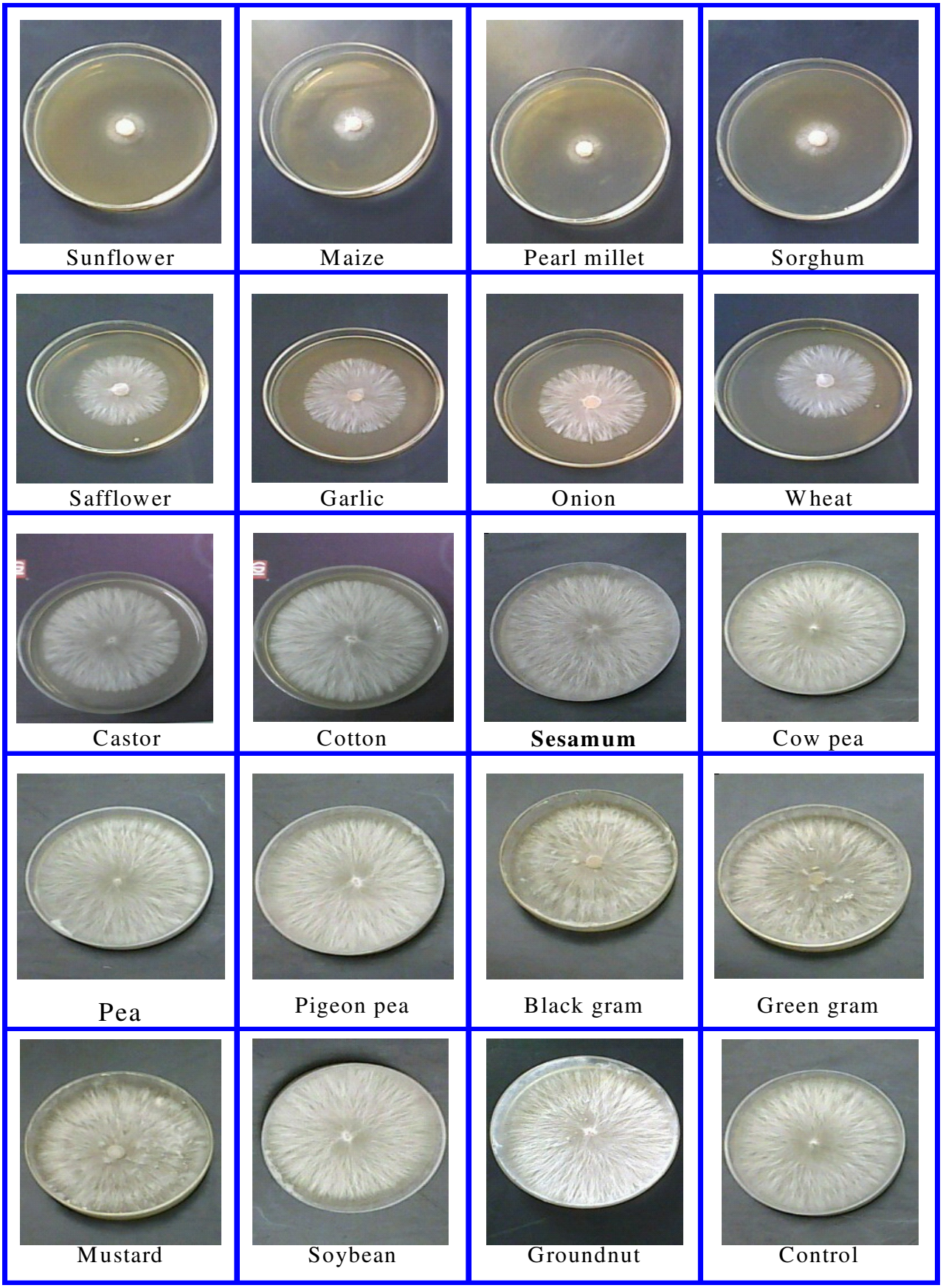

Fig. 1 : Influence of root exudates on radial growth of mycelium on solid medium

(Table 2 and Fig. 2).

Influence of root exudates on sclerotial germination on solid medium:

Germination of sclerotia at 5 and 10% was 100%, but a noteworthy reduction in germination of sclerotia was recorded in 20% root exudates of safflower, maize, pearl millet, sorghum and sunflower. The root exudates of pearl millet, sunflower, and maize showed only 27.33, 28.00 and 31.00% germination of sclerotia. Maximum germination of sclerotia was recorded in root exudates of soybean, groundnut, green gram, black gram, pigeonpea, chickpea, pea and cowpea. The germination of sclerotia in exudates of soybean and groundnut was 100% (Table 3).

Based on these findings, twenty crop root exudates can be classified into three groups, such as 1. Highly antifungal, this group includes maize, sunflower and pearl millet. 2. Moderately antifungal, this group includes safflower, sorghum, garlic, and onion. 3. Enhancing fungal growth, they are soybean, groundnut, green gram, black gram, pigeonpea, chickpea, pea and cowpea.

Results of this study suggested that the intercropping or crop rotation of safflower, maize, pearl millet, sorghum, sunflower, garlic, and onion with groundnut may be useful for the management of stem rot of groundnut and also for reduction of soil population of *S. rolfsii* in groundnut field. Similarly intercropping or crop rotation of soybean, green gram, black gram, chickpea, pea and cowpea with groundnut should be avoided.

Asghari and Mayee (1997) reported that onion and garlic crop rotation with groundnut crop reduced stem rot incidence considerably and gave higher pod yield and kernel weight. Rotation of groundnut with cotton, wheat

Table 2 : Influence of root exudates on mycelial growth in liquid medium

Crop root exudates	Average dry mycelium weight (mg)* of *S. rolfsii* in different concentrations				
	5%	10%	15%	20%	% Inhibition (at 20%)
Sorghum	188.00	139.67	46.00	29.33	91.92
Pearl millet	213.33	111.00	33.33	13.00	96.42
Maize	227.00	216.33	69.33	57.67	84.11
Wheat	371.00	340.67	225.33	153.00	57.85
Garlic	222.33	119.67	50.67	33.00	90.91
Onion	157.33	101.33	30.00	21.67	94.03
Sesamum	300.33	284.00	196.00	155.00	57.30
Sunflower	243.67	81.33	29.67	11.33	96.88
Safflower	246.33	86.33	36.67	21.33	94.12
Mustard	319.67	273.67	239.33	173.00	52.34
Cotton	336.67	312.33	173.33	99.00	72.73
Castor	294.33	241.33	179.00	118.33	67.40
Groundnut	334.33	360.67	375.00	329.33	9.27
Soybean	408.33	412.33	416.00	394.67	-8.72
Pigeonpea	374.67	388.67	390.00	328.67	9.46
Green gram	420.33	427.00	427.67	361.67	0.37
Black gram	342.33	349.00	355.67	291.33	19.74
Chickpea	370.33	376.33	377.33	284.33	21.67
Pea	324.00	328.67	330.67	243.67	32.87
Cowpea	361.00	367.33	368.00	307.00	15.43
Control	353.33	350.00	354.67	363.00	0.00
C.D. (P=0.05)	1.51	1.48	1.49	1.26	
S.E.M $\pm$	5.27	5.62	6.51	6.30	
C.V. %	0.57	0.58	1.47	3.20	

* Mean of five replications

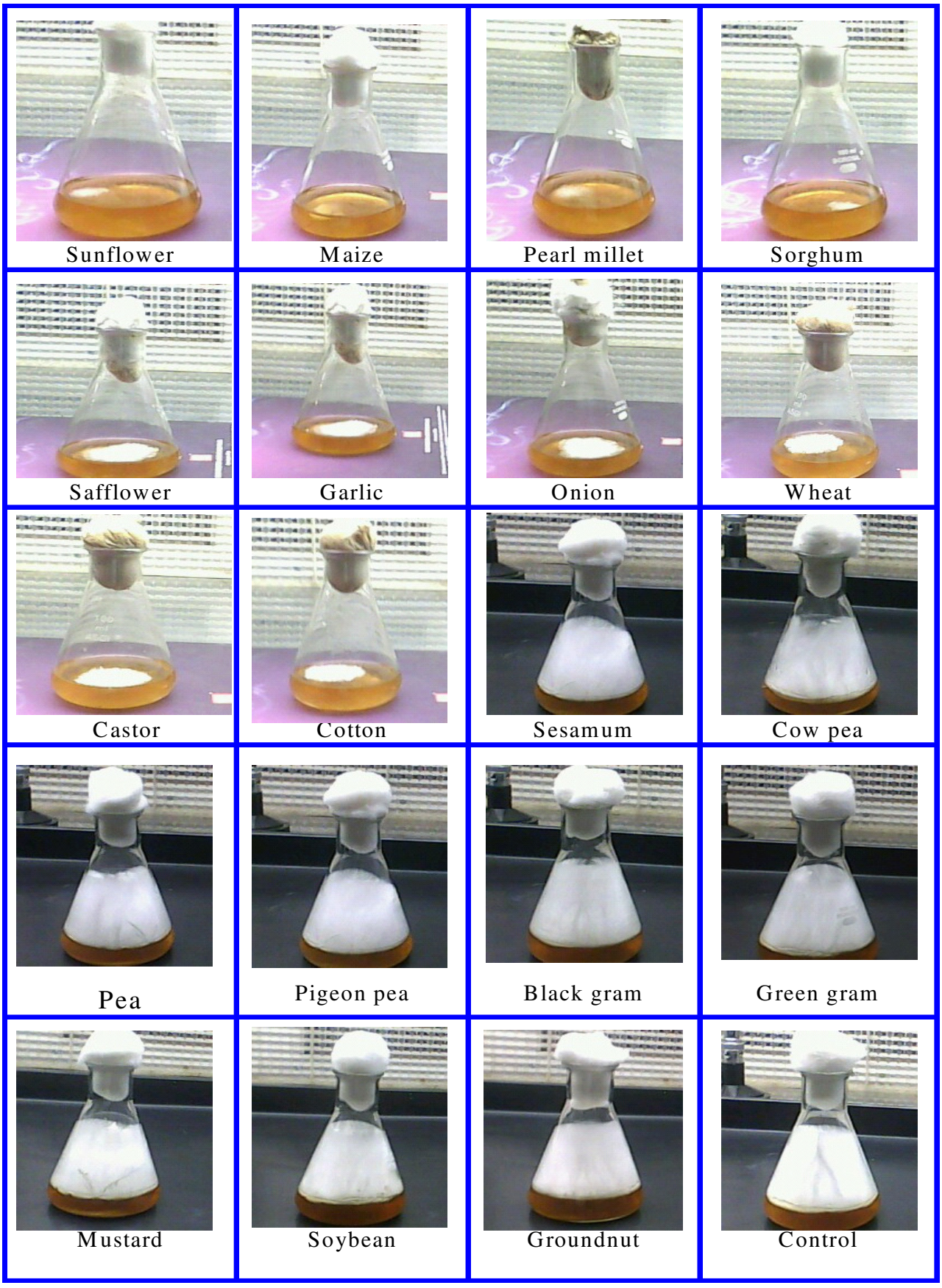

Fig. 2 : Influence of root exudates on mycelial growth in liquid medium

Table 3 : Influence of root exudates on percentage germination of sclerotia of *S. rolfsii*

Crop root exudates	Percentage germination of sclerotia of *S. rolfsii* in different concentrations				
	5%	10%	15%	20%	% Inhibition (at 20%)
Sorghum	100	86.04	82.04	41.00	54.44
Pearl millet	100	81.33	45.33	27.33	69.63
Maize	100	79.76	33.67	31.00	65.56
Wheat	100	89.00	73.33	78.67	12.59
Garlic	100	83.33	56.00	56.67	37.04
Onion	100	84.33	63.00	51.00	43.33
Sesamum	100	89.67	66.67	74.67	17.04
Sunflower	100	80.67	37.33	28.00	68.89
Safflower	100	82.67	60.67	46.67	48.15
Mustard	100	89.67	67.00	77.00	14.44
Cotton	100	89.33	68.67	69.00	23.33
Castor	100	89.33	81.00	68.33	24.07
Groundnut	100	100	100	100	0.00
Soybean	100	100	100	100	0.00
Pigeonpea	100	100	100	100	0.00
Green gram	100	100	100	100	0.00
Black gram	100	100	100	100	0.00
Chickpea	100	100	100	100	0.00
Pea	100	100	100	100	0.00
Cowpea	100	100	100	100	0.00
Control	100	100	100	100	0.00
C.D. (P=0.05)		0.09	0.48	0.50	
S.E.M $\pm$		0.03	0.03	0.17	
C.V. %		0.57	3.38	3.64	

* Mean of five replications

and maize was effective against stem rot (Garren, 1961). Schroth and Snyder (1961) proved that amino acids and sugars of bean exudates favourably influenced germination of chlamydospores of *Fusarium solani* f.sp. *phaseoli* in soil. Park *et al.* (2004) isolated the antifungal compounds from the root exudates of corn and discussed for their potential to control soil borne pathogens. However, further studies should be carried out to clarify the role of root exudates for building up resistance to soil borne diseases.

REFERENCES

Asghari, M.R. and Mayee, C.D. (1997). Biological control of *Sclerotium rolfsii* Sacc. by *Trichoderma harzianum* Rifai in groundnut. Abstr. In: Indian Phytopathological Society –Golden Jubilee International Conference on integrated plant disease management for sustainable agriculture. November 10-15, 1997, New Delhi, India. P. 258.

Bais, H.P., Loyola-Vargas, V.M., Flores, H.E. and Vivanco, J.M. (2001). Root-specific metabolism: the biology and biochemistry of underground organs. *In vitro Plant,* **37**:730–41.

Bais, H.P., Park, S.W., Stermitz, F.R., Halligan, K.M. and Vivanco, J.M. (2003). Exudation of fluorescent beta-carbolines from *Oxalis tuberosa* L. roots. *Phytochemistry,* **61**:539–43.

Bais, H.P., Walker, T.S., Schweizer, H.P. and Vivanco, J.M. (2002). Root specific elicitation and antimicrobial activity of rosmarinic acid in hairy root cultures of sweet basil (*Ocimum basilicum* L.). *Plant Physiol. Biochem.,* **40**:9837.

Bonsall, R. F., D. M. Weller, and L. S. Thomashow (1997). Quantification of 2,4-diacetylphloroglucinol produced by fluorescent *Pseudomonas* spp. *in vitro* and in the rhizosphere of wheat. *Appl. Environ. Microbiol*., **63** : 951–955.

Garren, K.H. (1961). Control of *Sclerotium rolfsii* through cultural practices. *Phytopathology,* **51**: 120-124.

Hirsch, A.M., Bauer, W.D., Bird, D.M., Cullimore, J., Tyler, B. and Yoder, J.I. (2003). Molecular signals and receptors: controlling rhizosphere interactions between plants and other organisms.*Ecology,* **84**:858–68.

Keel, C., Schnider, U., Maurhofer, M., Voisard, C., Laville, J., Burger, U., Wirthner, P., Haas, D. and fago, G. De´ (1992). Suppression of root diseases by *Pseudomonas fluorescens* CHA0—importance of the bacterial secondary metabolite 2,4-diacetylphloroglucinol. *Mol. Plant Microbe Interact*., **5** : 4–13.

Kneer, R., Poulev, A.A., Olesinski, A. and Raskin, I. (1999). Characterization of the elicitor-induced biosysnthesis and secretion of genistin from roots of *Lupinus luteus*. *J. Exp. Bot*., **50**:1553–59.

Maurhofer, M., Keel, C., Haas, D. and Defago. G. (1995). Influence of plant species on disease suppression by *Pseudomonas fluorescens* strain CHA0 with enhanced antibiotic production. *Plant Pathol*., **44** : 40–50.

Notz, R., Maurhofer, M., Schnider-Keel, U., Duffy, B., Haas, D. and Defago, G. (2001). Biotic factors affecting expression of the 2,4-diacetylphloroglucinol biosynthesis gene *phlA* in *Pseudomonas fluorescens* biocontrol strain CHA0 in the rhizosphere. *Phytopathology,* **91**:873–881.

Park, S., Takano, Y., Matsuura, H. and Yoshihara, T. (2004). Antifangal effect and compound of *Zea mays*. *Biosci. Biotechnol. Biochem*., **68** : 1366-1368.

Schroth, M.N., and Snyder, W.C. (1962). Exudation patterns of bean seeds and hypocotyls and their effect on *Fusarium solani* f.sp. *phaseoli* (Abstr.) *Phytopathology,* **52** : 751.

Walker, T.S., Bais, H.P., Grotewold, E. and Vivanco, J.M. (2003). Root exudation and rhizosphere biology. *Plant Physiol*., **132** : 44–51.

Weir, T.L., Park, S.W. and Vivanco, J.M. (2004). Biochemical and physiological mechanisms mediated by allelochemicals. *Curr. Opin. Plant Biol.,* **7** : 472–79.

****