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Leaf Exudates of *Vicia faba* and their Effects on *Botrytis fabae* and Some Associated Fungi

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Analysis of leaf exudates of *Vicia faba* using paper chromatography to identify individual amino acids and sugars qualitatively was investigated. The results revealed that the number of identified amino acids detected in the leaf exudates of the susceptible plants was more than those of resistant plants. The results also showed an increase in the number of amino acids exuded by infected leaves, but no marked difference in sugars of infected and non infected plants. Lithium chloride application led to decrease in amino acid and sugar contents. The number of amino acids and sugars was also decreased with leaf age. *Botrytis fabae* and the selected fungal species (*Alternaria alternata*, *Fusarium oxysporum* and *Aspergillus niger*) were used to show the effect of individual amino acid and sugar on their spore germination. It was observed that all amino acids stimulated the fungal spore germination except serine which inhibited its spore germination. In case of *A. alternata*, spore germination was stimulated by all amino acids except serine, alanine, glutamic acid, arginine and methionine which caused the inhibition. In case of *F. oxysporum*, aspartic and glutamic acids inhibited spore germination but the other amino acids stimulated its spore germination. Aspartic acid and phenyl alanine inhibited the spore germination of *A. niger*. All the identified sugars (galactose, glucose, fructose and rhamnose) stimulated spore germination of all tested fungi.

KEYWORDS: ***Botrytis fabae*, Leaf exudates, Lithium chloride, Phyllosphere, *Vicia faba***

There is now a wealth of evidence which shows that both organic and inorganic materials accumulate in water which is in contact with plant surfaces. Some of these materials originate outside the plant as deposits from the atmosphere such as mineral particles, pollen grains and rainwater. The greater proportion, however, has their origin within the plant and pass through the outer tissues into water in contact with the surface layers. This process is usually referred to as leaching.

Since leachates contain components which can be utilized as nutrients by microorganisms and may also contain substances which inhibit their germination and growth, they are of considerable importance to the microbial populations of plant surfaces (Blakeman, 1971; Last and Deighton, 1965; Ruinen, 1961). Tukey (1971) reported that all the essential minerals found in plants including both the macro and microelements were found to be exuded from leaves. In addition; the free sugars, pectic substances and sugar alcohols have been recorded. All of the amino acids found in plants and many of the organic acids have been detected in exudates.

Concerning the effect of leaf exudates on the fungal infection of host plants, Tyagi and Chauhan (1982) reported that the leaf exudates of the susceptible cultivar of *Capsicum annuum* (Malna) stimulated spore germination of the pathogen, *Alternaria solani* while leaf exudates of resistant cultivar (Simla) inhibited its spore germination. Moreover, Migahed *et al.* (1991) reported that the leaf exudates of numerous plants produced a stimulatory effect on spore germination of *A. alternata*. Leaf exudates was reported to vary with the host variety, age of host plant and maturity of the leaves (Sharma *et al.*, 1986; Singh *et al.*, 1986).

Conidial germination and germ tube elongation rates of *Bhynchosporium oryzae* were higher in leaf exudates and extracts of the susceptible cultivar Jaya than in those of the resistant Dular. There was a gradual increase in these rates when the conidia were exposed to exudates or extract from host plants of increasing ages (Singh and Gutpa, 1983). On the other hand, Singh *et al.* (2000) reported that the antifungal activity of methanolic extracts of *Centella asiatica* and *Andrographis paniculata* leaves was observed against fourteen fungi. Higher efficacy of active ingredient of these extracts under field conditions is envisaged against plant pathogens.

Amino acid and sugar contents of healthy and *Botrytis fabae*-inoculated bean leaves were determined. Certain qualitative changes were detected as a result of *B. fabae* infection (El-Beih *et al.*, 1988). Kumar (1984) studied amino acid, sugar and organic acid contents in the leaf extracts and leaf leachates of three potato varieties in relation to *Alternaria solani*. He found that the germination of *A. solani* in 100 ppm solution of adipic acid was inhibited. The presence of adipic acid in the leachates of the moderately resistant variety may be a cause of resistance against the pathogen.

However, another approach to disease control started earlier (Kent, 1941) aims to achieve increasing disease resistance by applying chemicals not directly toxic to the plant pathogen, but altering the host metabolism and com-

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position, that disturb the host - parasite relationship sufficiently to prevent the successful establishment of the disease organism. Few results, scattered throughout the literature, have been reported in this field concerning the application of lithium salts. Thus, the present work was undertaken in order to study the effect of lithium chloride on leaf exudates of resistant and susceptible varieties of *Vicia faba* to chocolate spot disease, and also to detect the effect of the individual components of amino acids and sugars of leaf exudates on spore germination of *B. fabae* and some associated phyllospheric fungi.

Materials and Methods

V. faba seeds (resistant 461 and susceptible 402 varieties to chocolate spot disease) were used in this investigation. Different concentrations of lithium chloride (1, 3, 5, 7 and 9 mM) were applied as drench after three weeks of planting. Plants were sprayed with conidial suspension of *B. fabae* until they were wet. Then the plants were covered by plastic bags for 4 days to keep the humidity high and placed in greenhouse of the Faculty of Science at Mansoura University (Shalouf, 1994). Untreated plants (control) were drenched with water only. Sampling of plants occurred 5 and 7 weeks after planting.

Leaves of *V. faba* plants were first sprayed with distilled water and kept in humid atmosphere for 24 hours. They were then washed several times with sterile distilled water at 50 m*l*/gm of leaves. The exudates of the leaves were concentrated under vacuum at 35°C to 10 m*l* (Dunn *et al*., 1971). The soluble sugars and amino acids, in the leaf exudates, were separated using the ascending one dimensional paper chromatography (Simola, 1968). The solvent system for detection of amino acids and sugars are n-butanol: acetic acid: water (4 : 1 : 5 v/v/v) and isopropanol: n-butanol: water (7 : 2 : 1 v/v/v), respectively.

The effects of the individual amino acids and sugars identified in leaf exudates of *V. faba* plants on the germination of washed spores of the pathogen *B. fabae* and the most abundant associated phyllospheric fungi (*A. alternata*, *Fusarium oxysporum* and *Aspergillus niger*) were investigated using the method adopted by Buxton (1962). One m*l* of spore suspension (2×10^7 spores/m*l*) prepared from 10-day old cultures were added to each of 1 m*l* of the tested solutions (authentic) to give a final concentration of 0.5% for the carbon source and 0.1% for the nitrogen source. Control vials contained a modified liquid Czapeck's medium, but the other vials contained different identified sugars and amino acids after 6 hours, the germinated spores were counted using a haemocytometer. The criterion of germination was the formation of a microscopically visible germ tube (Migahed *et al*., 1991).

Results and Discussion

Chromatogrpahic analysis revealed that the number of identified amino acids detected in the leaf exudates of the untreated susceptible plants was more than that detected in resistant plants. Six (serine, glutamic acid, alanine, methionine, arginine and phenylalanine) and five (aspartic acid, serine, glutamic acid, alanine and methionine) amino acids were detected in the leaf exudates of the susceptible and resistant varieties respectively, with the absence of arginine and presence of threonine in the second period in case of susceptible variety. This finding was in agreement with that obtained by Abd El-Kader (1980) and Migahed *et al.* (1991). It was also clear that certain qualitative changes in the amino acids content have been occurred as a result of plant infection by *B. fabae*. In case of resistant variety, aspartic acid disappeared in the first period while arginine was appeared in the second period. In case of susceptible plants, threonine and arginine were detected in first and second period respectively (Figs. 1 and 2). This conclusion can be subtantiated by the results obtained by Abd El-Kader (1980). It has been established that the infection of certain phytopathogenic fungi to the host induced both qualitative and quantitative changes in their nutrients (Singh *et al*., 1986).

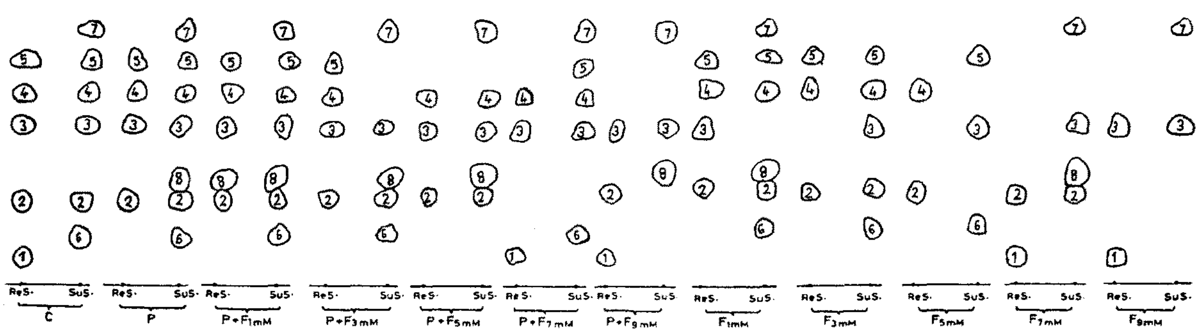

Fig. 1. Chromatographic map showing typical separation of the amino acids by ascending paper chromatography of leaf exudates of the 1st sampling after the treatment with pathogen (*Botrytis fabae*, P) and different concentrations of fungicide (*lithium chloride*, F). (1, Aspartic acid; 2, Serine; 3, Glutamic acid; 4, Alanine; 5, Methionine; 6, Arigine; 7, Phenyl alanine; 8, Threonine). C = Control.

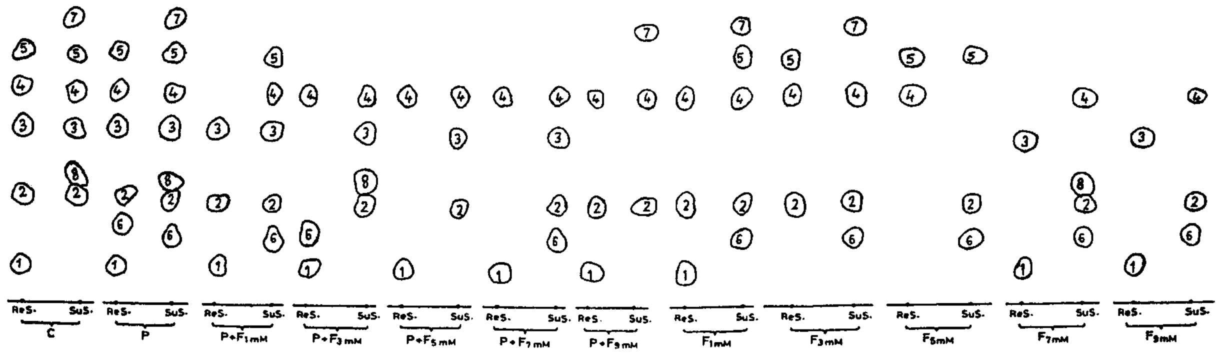

Fig. 2. Chromatographic map showing typical separation of the amino acids by ascending paper chromatography of leaf exudates of the 2nd sampling after the treatment with pathogen (*Botrytis fabae*, P) and different concentrations of fungicide (*lithium chloride*, F). (1, Aspartic acid; 2, Serine; 3, Glutamic acid; 4, Alanine; 5, Methionine; 6, Arigine; 7, Phenyl alanine; 8, Threonine). C = Control.

Application of lithium chloride led to a decrease in the number of amino acids at all concentrations of lithium chloride especially at 9 mM in both varieties at the two sampling periods which showed high reduction in the number of amino acids comparing with the control plants as shown in Table 1. Also, it was observed that the most common amino acids were glutamic acid in case of susceptible plants and alanine in case of resistant plants. However, various physiological effects of lithium have been reported including in solute uptake capacity (Thellier *et al.*, 1980), photosynthesis (Stitt and Schreiber, 1988), thigmomorphogenetic responses (Boyer *et al.*, 1979), stomatal regulation (Brogardh and Johnson, 1974).

Dealing with the sugar components, four sugars were detected in untreated resistant plants, namely, galactose, glucose, fructose and rhamnose while three sugars were recorded in case of susceptible plants with the absence of rhamnose as compared with resistant plants (Figs. 3 and 4). In this connection, Grover (1971) showed that sucrose and glucose were detected in the leaf exudates of *Caspicum fructescens.* Also, Houssa *et al.* (1991) showed that sucrose was the major sugar detected in leaf exudate of *Xanthium strumarium.* The results also revealed that qualitative changes in sugar contents were detected in the leaf exudates of the infected plants as compared with untreated plants, especially at the second period where fructose was absent but rhamnose was present in resistant plants while fructose was detected in infected susceptible plants. No marked differences in sugar content of inoculated and non-inoculated plants were detected in the first period. This conclusion can be substantiated by the results obtained by Abd El-Kader (1980).

Application of lithium chloride to the infected plants caused decreasing in the sugar components with increasing the concentration of the fungicide. Glucose was the most abundant sugar in all treatments in both varieties at the two sampling periods except at concentration 3 mM and 5 mM in case of susceptible and resistant plants respectively in the second period as shown in Table 2. The results showed that the spectrum range of amino acid and sugar components of leaf exudates differed according to the plant species and its varieties. This conclusion can be substantiated by the results obtained by Grover (1971), Blakeman (1972), Godfrey (1972), Purkayostha and Mukopadhyay (1974), and Migahed *et al.* (1991).

Leaf exudates may enhance pathogenesis by direct stimulation of germination of its spores, by furnishing nutrients for the saprophytic growth phase, by overcoming the effects of postulated inhibitors. The spores of most fungi seem to require carbon and nitrogen nutrients for germination. Studying the effects of authentic amino acids and sugars, comparable to those detected in the leaf exudates of the resistant and susceptible varieties, on the spore germination of the tested leaf surface fungi, it was found that aspartic acid, alanine, phenyl alanine, glutamic acid, threonine, arginine and methionine have stimulatory effect on spore germination of *B. fabae*. Arginine gave the highest stimulatory effect while serine inhibited its spore germination. This is in agreement with results obtained by Abd-El Kader (1980).

In case of *A. alternata*, aspartic acid, phenylalanine and threonine induced great stimulatory effects on its spore germination, while serine, alanine, glutamic acid, arginine and methionine showed an inhibitory effects. In this respect, Singh *et al.* (1986) reported that the exudates of both young and mature leaves of *Spinacia oleracea* stimulated the linear growth and conidial germination of *A. alternata*. Migahed *et al.* (1991) also found that the leaf exudates of numerous plants produced a stimulatory effect on spore germination of *A. alternata*. For *F. oxysporum*, aspartic and glutamic acids had an inhibitory effects on the spore germination, but the other amino acids caused stimulation on the spore germination. In case of *A. niger*, all the identified amino acids showed stimulation on the spore germination except aspartic acid and phenylalanine had inhibitory effects on its spore germination (Fig. 5).

Table 1. Amino acid components of the leaf exudates of the two varieties of *Vicia faba* (resistant and susceptible) after treatment with pathogen (*Botrytis fabae*) and different concentrations of fungicide (*lithium chloride*) (1st and 2nd sampling)

Amino acid	Sampling	Control		Pathogen		P + F1mM		P + F3mM		P + F5mM		P + F7mM		P + F9mM		F 1mM		F 3mM		F 5mM		F 7mM		F 9mM	
		Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.
Aspartic acid	1st	+[a]	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	-	+	-
	2nd	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	-	-	-	-	+	-	+	-
Serine	1st	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	-	+	+	-	-
	2nd	+	+	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	-	+	-	+	-	+
Glutamic acid	1st	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	+	+	+
	2nd	+	+	+	+	+	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	+	-	+	-
Alanine	1st	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-
	2nd	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	-
Methionine	1st	+	+	+	+	+	+	+	-	-	-	-	+	-	-	+	+	+	+	-	+	-	-	-	-
	2nd	+	+	+	+	-	+	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	-
Arginine	1st	-	+	-	+	-	+	-	+	-	-	-	+	-	-	-	+	-	+	-	+	-	-	-	-
	2nd	-	-	+	+	-	+	+	-	-	-	-	+	-	-	-	+	-	+	-	+	-	+	-	+
Phenylalanine	1st	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	-	-	-	-	+	-	+
	2nd	-	+	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	-
Threonine	1st	-	-	+	+	-	+	-	+	-	-	-	+	-	-	-	+	-	+	-	+	-	-	-	-
	2nd	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Total	1st	5	6	5	7	4	7	4	5	3	5	3	5	3	3	4	6	3	5	2	3	2	4	2	2
	2nd	5	6	6	7	3	5	3	4	2	3	2	4	3	3	3	5	3	4	2	3	2	4	2	2

[a](+) Presence, (-) Absence, (P) Pathogen, (F) Fungicide.

Table 2. Sugar components detected in the leaf exudates of the two varieties of *Vicia faba* (resistant and susceptible) after treatment with pathogen (*Botrytis fabae*) and different concentrations of fungicide (*lithium chloride*) (1st and 2nd sampling)

Amino acid	Sampling	Control		Pathogen		P + F1mM		P + F3mM		P + F5mM		P + F7mM		P + F9mM		F 1mM		F 3mM		F 5mM		F 7mM		F 9mM	
		Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.	Res.	Sus.
Galactose	1st	+[a]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	+
	2nd	+	+	+	+	+	-	+	-	+	+	-	+	+	+	+	+	-	+	+	+	-	-	+	+
Glucose	1st	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	2nd	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
Fructose	1st	+	+	+	+	+	+	+	-	+	+	-	-	-	-	+	+	+	+	+	+	-	-	-	-
	2nd	+	-	-	+	-	+	-	+	-	-	+	-	-	-	+	-	+	+	+	-	+	+	-	-
Rhamnose	1st	+	-	+	-	-	-	-	-	+	-	+	-	+	-	+	-	+	-	+	-	-	-	-	-
	2nd	-	-	+	-	+	-	+	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-
Total	1st	4	3	4	3	3	3	3	2	4	3	3	2	3	2	4	3	3	2	3	2	2	2	2	2
	2nd	3	2	3	3	3	2	3	2	2	2	2	2	2	2	4	2	3	2	2	2	3	2	2	2

[a](+) Presence, (-) Absence, (P) Pathogen, (F) Fungicide.

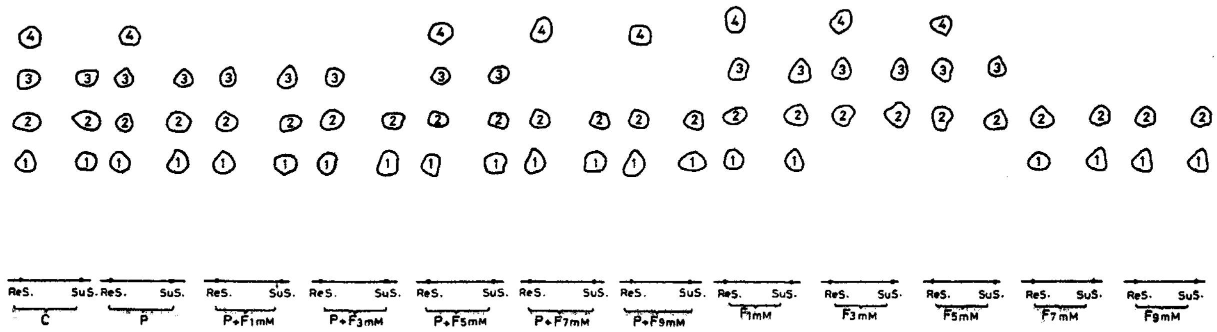

Fig. 3. Chromatographic map showing typical separation of the free sugars by ascending paper chromatography of leaf exudates of the 1st sampling after the treatment with pathogen (*Botrytis fabae*, P) and different concentrations of fungicide (*lithium chloride*, F). (1, Galactose; 2, Glucose; 3, Fructose; 4, Rhamnose). C = Control.

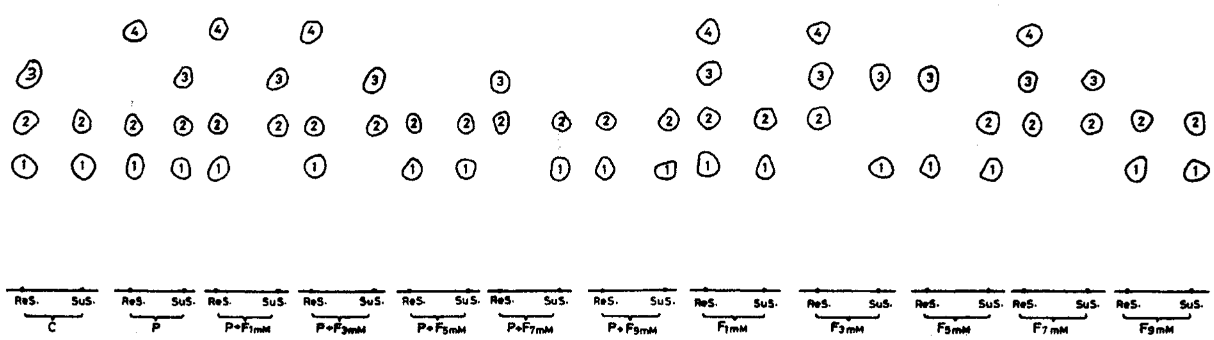

Fig. 4. Chromatographic map showing typical separation of the free sugars by ascending paper chromatography of leaf exudates of the 2nd sampling after the treatment with pathogen (*Botrytis fabae*, P) and different concentrations of fungicide (*lithium chloride*, F). (1, Galactose; 2, Glucose; 3, Fructose; 4, Rhamnose). C = Control.

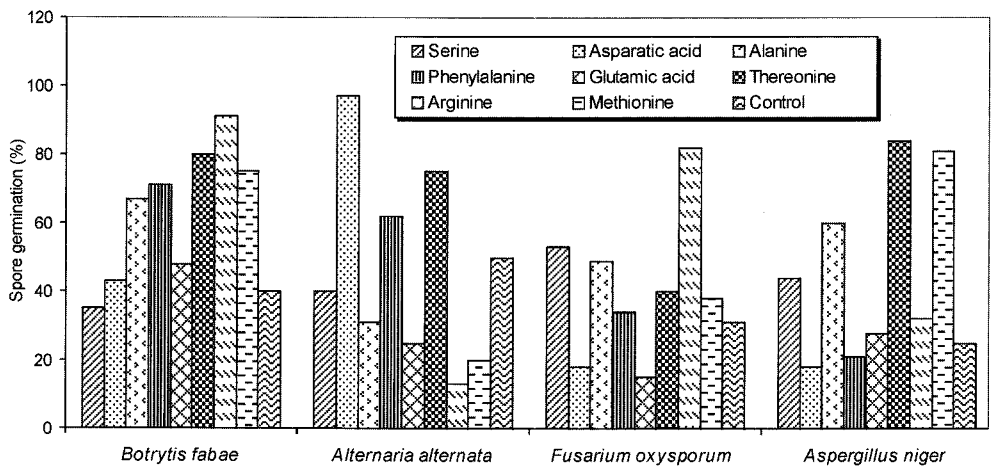

Fig. 5. Effect of amino acid components in the leaf exudates of resistant and susceptible varieties of *Vicia faba* on spore germination (%) of fungal species.

The above mentioned pattern of results was in agreement with the results of Tyagi and Chauhan (1982), Kumar (1984), Singh *et al.* (1986), and El-Beih *et al.* (1988).

Concerning sugars, glucose and galactose had great stimulatory effects and appeared to be the best sugars for the spore germination of the tested fungi, *B. fabae*, *A. alternata*, *F. oxysporum* and *A. niger* followed by fructose and rhamnose (Fig. 6). These results were in agreement with the results obtained by Tyagi and Chauhan (1982), and Kumar (1984).

Since leachates contain components which can be utilize as nutrients by microorganisms and may also contain substances which inhibit their germination and growth, they are of considerable importance to the microbial populations of plant surfaces (Blakeman, 1971; Last and Deighton, 1965; Ruinen, 1961).

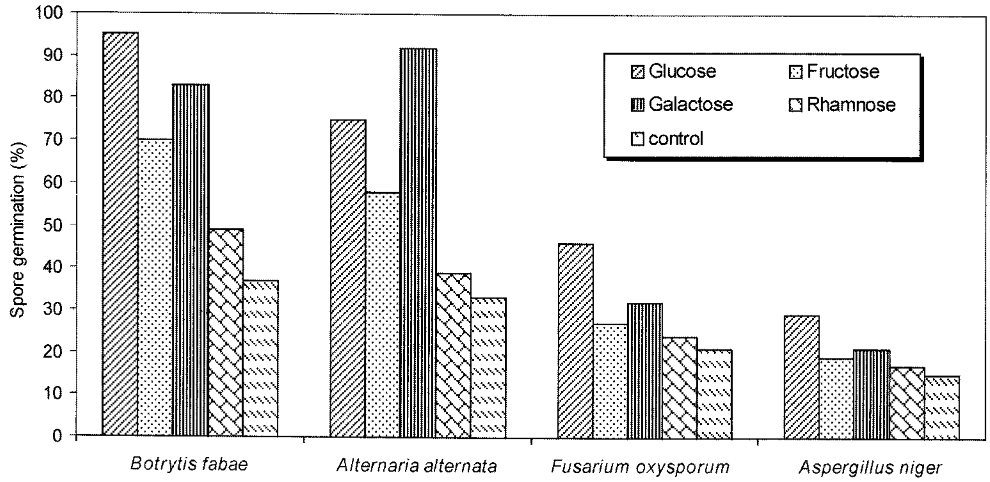

Fig. 6. Effect of sugar components in the leaf exudates of resistant and susceptible varieties of *Vicia faba* on spore germination (%) of fungal species.

In conclusion, the stimulatory effect of leaf exudates is usually regarded as nutritional and attributed largely to the carbohydrates and amino acids present (Godfrey, 1974; Purkayastha and Mukopadhyay, 1974).

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