EFFECT OF LEAF EXTRACT AND EXUDATES OF *SOLANUM NIGRUM* L. ON SOME PHYLLOPLANE FUNGI

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Abstract: Leaf serves as an important natural habitat for the microbes by providing the nutrient in the form of organic (sugars, amino acids, etc.) and inorganic substances. The present paper deals with the effect of leaf extracts and exudates of *Solanum nigrum* L.on some phylloplane fungi. More number of amino acids and sugars were detected in extract than exudates of leaves of S.nigrum.The exudate and extract of leaves stimulated the linear growth of dominant phylloplane fungi.

Keywords: Leaf extract, *Solanum nigrum*, Amino acids

INTRODUCTION

There are many evidences which shows that both organic and inorganic materials accumulate in water which is in contact with the plant surfaces. They have 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. The influence of leachates on plant surface microbial population is very complex.Leachate composition varies from plant to plant and also with the age of the organ being leached. Since leachates contain components(minerals and organic compounds) 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 population of plant surfaces(Ruinen, 1961; Last and Deighton, 1965; Blakeman, 1971).The subject was reviewed by Tukey (1971) and Godfrey (1976).The present paper deals with the effect of leaf extracts and exudates of *Solanum nigrum* on some phylloplane fungi.

MATERIALS AND METHODS

Mature blackberries were collected from the field; the seeds were taken out from the blackberries with the sterilized forceps. Seeds were soaked in water for 24 hours, kept in running water for a few hours and then they were sown in four experimental beds of botanical garden of the department. The seeds were sown at a depth of 3-4 cm and plant to plant and row to row distance of 50 cm was maintained. The plants were irrigated with tap water at regular intervals of 7 days.

To study the effect of leaf exudates and extracts, leaves of 15 and 135 days old plants were removed separately .The cut ends of the petioles were sealed with paraffin wax and then the leaves were immersed in polythene bags containing sterilized distilled water.100 g leaves of both the ages were immersed in 100 ml sterilized distilled water and were kept at 20^0C for 12 hr.The individual exudates were filtered through four Whatman No.44 filter papers and evaporated to dryness at 45^0C.This residue was redissolved in 0.5 ml sterilized distilled water.

The leaf extract was obtained first by washing 100 g leaves of 15 and 135 days old leaves by sterilized distilled water and crushing in 50 ml of 80% ethanol with a pestle-mortar and filtering through Whatman filter paper No.44, to remove the leaf debris. The filtrate was then concentrated as for leaf exudates and residual solution was used for spotting the chromatographic paper.

For detection of amino acids and sugars present in the exudates and extracts, ascending paper chromatographic technique was employed. For amino acids, n-butanol-glacial acetic acid-water (3: 1: 1 V/ V) was used as solvent. Ninhydrin (0.1% in n-butanol) was used as spraying reagent. For analysis of sugars, n-butanol-glacial acetic acid: water (4: 1: 5 V/ V) was used as solvent and alcoholic diphenyl amine in acetone was used as detecting reagent. Identification of amino acids and sugars were done by comparing

the chromatograms of known mixtures of amino acids and sugars respectively.

For studying the effect of leaf exudates and extracts on linear growth of some phylloplane fungi, the leaf exudates and extracts were filtered through Seitz filters. The effect of leaf exudates and extracts on linear growth and on conidial germination of *Alternaria alternate, Cladosporium cladosporioides, C.herbarum , Curvularia lunata, Drechslera australiensis* , and *Fusarium oxysporum* were studied on potato dextrose agar medium. Filtered sterilized exudates/extracts (100 ml) were added to 150 ml of sterilized medium which in turn was poured in Petri dishes. Such Petri dishes were inoculated centrally with an inoculum disc (2 mm diameter) containing mycelia and conidia of the test fungus, cut from edges of an actively growing culture of each fungus. Five replicates of each fungus were taken for each set of an experiment as well as control. In place of exudates and extracts, equal amount of sterilized distilled water was added in PDA to serve as control. The radial growths of the test fungi were measured after 6 days of inoculation at 25 $\pm 1^0$C.

To study germination of conidia, the test fungi were obtained from 10-day old culture on PDA.The germination of conidia was studied *in vitro* by hanging drop method in clean sterilized cavity slides. A layer of petroleum jelly was smeared outside the cavity by means of a brush. A drop (approximately 0.01 ml) of exudates/ extract having conidia of the test fungi was placed at the centre of the coverslip.The later was immediately inverted over the cavity avoiding the contact of the drop with slides as well as with the layer of petroleum jelly. Sides of the coverslip were also blocked with petroleum jelly to check any evaporation of the hanging drop. Five replicates were kept for each treatment. Sterilized distilled water was used as control. The slides were incubated at 25± 1^0C for 12 hr and then examined under the microscope. The percentage germination of conidia was calculated and lengths of 10 randomly selected germ tubes were measured for each of the replicated treatments.

Percentage stimulation of fungi was:

$$\frac{\text{Treatment} - \text{Control}}{\text{Control}} \times 100$$

OBSERVATION AND RESULTS

The qualitative analysis of amino acids and sugars in leaf exudate and leaf extract of *Solanum nigrum* L. made by chromatographic technique is presented in Tables 1 and 2.However the effect of leaf exudates and leaf extracts on linear growth and conidial germination of some phylloplane fungi are presented in Table 3.

Table 1. Presence of amino acids in the leaf exudates and leaf extracts of leaves of *Solanum nigrum* L.

Name 0f Amino acid	**Leaf Exudates**	**Leaf Extracts**
Alanine	–	+
Asparagine	+	+
Glutamic acid	+	+
Glycine	+	+
Isoleucine	+	+
Leucine	–	+
Proline	–	+
Tryptophan	+	+
Valine	+	+

Table 2. Presence of sugars in leaf exudates and leaf extracts of leaves of *Solanum nigrum* L.

Name of Sugar	Leaf Exudates	Leaf Extracts
Glucose	+	+
Mannose	□	+
Ribose	□	+
Rhamnose	□	+
Sucrose	+	+
Xylose	+	+

Table 3. Effect of leaf exudates and extracts of *Solanum nigrum* on conidia germination percentage and germ tube length (µm) of some phylloplane fungi.

Name of fungi	Control		Exudate				Extract			
	Conidia Germin--ation (%)	Mean Germ Tube Length (µm)	Young leaves		Mature leaves		Young leaves		Mature leaves	
			Conidia Germin--ation (%)	Mean Germ Tube Length (µm)	Conidial Germin--ation (%)	Mean Germ Tube Length (µm)	Conidia Germin--ation (%)	Mean Germ Tube Length (µm)	Conidia Germin--ation (%)	Mean Germ Tube Length (µm)

Alternaria alternata	48.9	47.0	54.8	68.1	79.2	91	73.1	163.1	80.9	190.1
Cladosporium herbarum	50.4	5.1	52.9	7.2	72.7	20.1	54.8	10.1	79.0	40.2
Dactylinum dendroides	50.4	8.2	56.2	24.1	68.3	50.1	56.3	45.3	73.6	56.0
Curvlaria lunata	36.8	43.0	50.1	105.2	58.2	187.2	48.2	190.1	50.0	190.3
Drechslera australiensis	42.4	39.1	54.2	121.2	60.5	205.3	53.3	197.2	58.4	132.2
Fusarium oxysporum	60.8	60.1	68.1	80.1	90.2	112.1	84.4	93.1	98.5	140.1

DISCUSSION

Leaf surfaces offer an attractive habitat for certain saprophytic fungi due the increased availability of soluble nutrients leaked from plant tissues. These leached substances contain compounds like sugars and amino acids which can be readily utilized as nutrients by microorganisms. In the present study chromatographic analysis of leaf exudates and leaf extracts of *Solanum nigrum* shows that amino acids, viz.alanine, asparagine, glutamic acid, glycine, isoleucine, tryptophan and valine were detected from leaf exudates. The leaf extract contains alanine, asparagine, glutamic acid, glycine, isoleucine, leucine, proline, tryptophan and valine amino acids (Table 1).

Sugars detected from leaf extract are glucose, mannose, rhamnose, ribose, sucrose and xylose.The leaf exudates contain glucose, sucrose and xylose (Table 2).Soluble sugars and amino acids were also reported from leaf leachates by some workers (Ruinen, 1961; Last and Deighton, 1956; Blakeman, 1971; Grover 1971). The amino acids and sugars exuded from the leaves make the leaf surface nutritionally rich which permit better growth of fungi.Mishra and Tewari(1978) observed that leaf extracts and exudates of wheat and barley had stimulatory effect on most fungi and nutritional level of leaf exudates affects the growth of fungi on leaf surface. Singh and Rai (1981) found that extract of mustard and barley, sterilized by filtering through seitz filter as well as autoclaving the medium considerably stimulated the growth of all the tested saprophytic and pathogenic fungi and the autoclaved extract was found to be most effective. It may be seen that the growth performance of each fungus tested in the present study increased in the presence of exudates and extracts in the medium from young to mature leaf stages. Thus increase in population of fungi on ageing leaves may be attributed to variations in amount and composition of water soluble compounds released on surfaces of leaves. In general, leachates are released in higher amount from older than the young leaves (Arens, 1934; Tukey Jr.*et al*., 1958; Tukey 1971; Blakeman, 1971, 1972; Godfrey, 1976; Singh *et al*., 1986).

In general leachates may have a direct effect on microorganisms, the possibility exists that the organic molecules exudated,are able to stimulate or inhibit the germination of fungal spore(Topps and Wain,1957).The conidial germination and germ tube growth is largely attributed to their carbohydrates

and amino acids components or through interactions between them and other chemicals on the leaf surface.Godfrey(1974) showed that a relatively concentrated aqueous leachate from the fronds of *Parthenium aquilinum* stimulated germ hyphal growth of *Botrytis cinerea* although it had no effect on the percentage of germination. This was regarded as direct nutritional effect.Purkayastha and Mukhopadhya(1974) found similar results with *Helminthosporium oryzae* on *Oryza sativa* leaf leachate.It is evident from the present study that exudates and extract of leaves of *Solanum nigrum* stimulated the percentage of conidial germination and germ tube growth of all the test fungi. Schneider and Sinclair (1975) reported inhibitory effect of the exudates of young cow pea leaves with regard to conidial germination and germ tube growth. Sharma and Sinha (1971) recorded that exudates from young leaves were more effective in causing inhibition of germination of conidia of *Colletotrichum gramini-cola* than those from mature leaves. Brillova (1971) reported that germination of *Cercospora beticola* was inhibited by 3-hydroxy tyramine present in sugar beet leaf washings.Godfrey and Clements(1978) reported that leaf leachates of lilac(*Syringa vulgaris*) contained substances which inhibited the germination of *Alternaria alternata* and *Botrytis cinerea*.The inhibition of of conidial germination and germ tube growth may be due to phyllopalne microorganisms which may release some chemicals inhibitory to germination and other inhibitory substances originating from leaf itself. Sadasivam et al. (1976) observed that leaf exudates of tapioca slightly stimulated the germination of conidia of *Curvularia* sp., *Alternaria* sp.and *Helminthosporium* sp. Singh *et al*. (1986) also noted inhibitory effect of *Cercospora beticola* by the exudates of young leaves. Thus the nutrient released from leaf surface have a distinct, selective action on phylloplane microorganisms (Purkayastha and Deverall, 1965; Bhadur and Sinha,1970; Mishra and Srivastava,1970;Sadasivam *et al*.,1976;Godfrey and Clements,1978;Mishra and Tewari,1978;Singh *et al*.,1986).

REFERENCES

Bahadur, P. and Sinha, S. (1970). Studies on spore germination of *Uromyces ciceri- sarietini* under influence of leaf exudated of gram. Indian Phytopath.**23:**6.

Banik, S. and Krishnamurthy, K.V.M. (2004). Effect of phylloplane mycoflora of black gram on the grwoth and conidial germination of *Corynespora cassiicola (Berk.* and *curt) wei. J. of Interacademicia*. **8** : 1-6.

Blakeman, J. P. (1971). The chemical environment of the leaf surface in relation growth of pathogenic fungi. In: T F Preece and C H Dickinson (Eds) *Ecology of Leaf Surface Microorganisms*. pp 225-268, London: Academic Press.

Blakeman, J. P. (1972). Effect of plant age on inhibition of *Botrytis cinerea* spores Bacteria on beet root leaves. *Physiol Pl Path* **2**: 143-152.

Bora, L.C. and Chand, J.N. (1994). Leaf Leachates and leaf surface colonizers of mung bean (*Vigna radiata* L.) cultivars at different growth stages. *Indian. J. of Mycol. and Pl. Path.* **24** : 6-10.

Godfrey, B. E. S. (1976). Leachates from aerial plants and their relation to plant surface microbial populations. In: C H Dickinson and T F Preece (Eds) *Microbiology of Aerial Plant Surfaces*. pp 433-439, London: Academic Press.

Godfrey, B. E. S. and Clements, D. M. (1978). Effect of lilac leaf leachate on Germination of *Alternaria alternata* and *Botrytis cinerea*.*Trans Br mycol Soc* **70**: 163-165.

Grover, R. K. (1971). Participation of host exudates chemicals in appressorium formation by *Colletotrichum piperatum*.In:T F Preece and C H Dickinson(Eds) *Ecology of Leaf-Surface Microorganism*.London: Academic Press.

Kawamata, H.,Narisawa, K. and Hashiba, T. (2004). Suppression of rice blast by phylloplane fungi isolated from rice plants. *J. of Gen. Pl. Path*. **70** : 131-138.

Kishan, Bal.; Navneet and Mehrotra, R.S. (1988). Phyllosphere mycoflora of wheat in relation to leaf leachates and resistance to Helminthosporium blight. *Indian Phytopath.* **41** : 398-405.

Sharma, Indu, (2004). Phylloplane microfungi of sugar cane. *Indian J. of Microbiol.* **44** : 113.115.

Last, F. T. and Deighton, F.C. (1965). The non-parasitic microflora on the surfaces living leaves.*Trans Br mycol Soc* **48**: 83-99.

Mishra, R. R. and Tewari, R.P. (1978). Studis on the phyllosphere fungi: Germination behaviour of certain fungi in leaf extract and exudates of wheat and barley.*Acta Bot Indica* **6**: 21-30.

Monaco, C.I.; Nico, A.I.; Mitidieri, I. and Alippi, H.E. (1999). Saprobic fungi inhabiting tomato phylloplane as possible antagonistas of *Alternaria solani*. *Acta Agronomica Hungarica*. **47** : 397-403.

Nix-stohr, S.; Burpee, L.L. and Buck, J.W. (2008). The influence of exogenous nutients on the abundance of yeasts of the phylloplane of turfgrass. *Microbial Ecology.* **55** : 15-20.

Purkayastha, R. P. and Deverall, R. J. (1965). The growth of *Botrytis fabae* and *B.cinerea* into leaves of bean (*Vicia faba*).*Ann appl Boil* **56** : 139-147

Purkayastha, R. P. and Mukhopadhyay, R. (1974). Factors affecting the colonization of leaves by *Helminthosporium oryzae.Trans Br mycol Soc* **62**: 402-406.

Purkayastha, R. P. and Mukhopadhyay, R. (1974). Factors affecting the colonization of leaves by *Helminthosporium oryzae.Trans Br mycol Soc* **62**: 402-406.

Ranjan S.; Govindjee and Laloraya, M.M. (1955). Chromatographic studies on the amino acid metabolism of healthy and diseased leaves of *Croton sparsiflorus* Morong. *National Institute of Science of India* Proc **21**: 42-47.

Sadasvivam, K. V.; Rangaswami, G. and Prasad, N.N. (1976). Studies on the phyllosphere microflora of tapioca (*Manihot utilissima Pohl.) Zbl Pakt Abt I I Bd.***131**: S 632-643

Sharma, J. K. and Sinha, S. (1971). Effect of leaf exudates of Sorghum varieties varying in susceptibility and maturity on the germination of conidia of *Colletotrichum graminicola* .In: T F Preece and C H Dickinson (Eds) *Ecology of leaf surface Microorganisms*. 597-601, London: Academic Press.

Sharma, Neeta and Verama, **H.N.** (1988). Mycotoxic activity of leaf extracts of *Clerodendrum* spp. on phylloplane mycoflora of tobacco and tomato and growth of fungi. *Indian J. of Pl. Path.* **6**:180-183.

Singh, A.K. and Rai, Bharat (1989). Effect of leaf extract of wheat treated with pollutants on growth behaviour of some phylloplance fungi. *Proc. of Indian National Sci. Acad.* **55** : 133-138.

Singh, D. B. and Rai, B. (1981). Effect of leaf extracts of mustard and barley on growth behaviour of some phylloplane microfungi.*Bull Torrey bot Club* **108 :** 419-421.

Singh, P. N.; Sindhu, I. R. and Gupta, **Kumkum** (1986). Effect of leaf exudates and extracts of spinach on some phylloplane microfungi.*Acta bot Indica* **14 :** 104-110.

Topps, J. H. and Wain, R.L. (1957). Fungistatics properties of leaf exudates. *Nature* **179 :** 652-653.

Tukey, H. B. Jr. (1971). Leaching of substances from plants. In: T F Preece and C H Dickinson (Eds) *Ecology of leaf surface microorganisms*.pp 67-80, London: Academic Press.

Tukey, H. B. Jr., Witter, S. H. and Tukey, H.B. (1958). Loss of nutrients by foliar leaching as determined by radioisotopes. *Am Soc Hort Sci.,* **71**: 496-50.