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Effect of leaf leachates of potato plants on germination of *Phytophthora infestans* sporangia and zoospores

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Abstract

Leaf leachates of two cultivars of potato i.e. Kufri Chandramukhi (KCM) and Kufri Jyoti (KJ) contain nine amino acids. Total sugar content of KCM and KJ leachates varied between 0.10 and 0.37 µg per sq. cm and 0.02 to 0.23 µg per sq. cm. respectively. The sugars exuded more by KCM. Total phenol content of KCM and KJ leachates varied between 1.00 to 2.0 µg per sq. cm and 1.05 to 3.16 µg per sq. cm respectively. The percentage germination of *P. infestans* sporangia in KCM and KJ cultivars was more in mature leaf leachates as compared to young leaves, moreover it was less in KJ cultivar as compared to KCM. The germ tube length of *P. infestans* zoospores in leaf leachates was inhibited in both the cultivars except in mature leaf leachate of 94 days old Kufri Chandramukhi.

Introduction

Leaf leachates play a vital role in stimulating or inhibiting the growth of propagules settled down on leaf surface. The nature of leaf leachates varies from plant to plant and is influenced by the age of the organ.This paper deals with the composition of leaf leachates of two cultivars of potato i.e. Kufri Chandramukhi (KCM) and Kufri Jyoti (KJ) and their role in germination of *Phytophthora infestans* sporangia and zoospores.

Material and Method

Leaf leachates of young leaves (second leaf from top) and mature leaves (fifth/sixth leaf from top) of each variety were collected in sterilized plastic boxes by the method of Godfrey (1975) in 22nd, 222rd and 5th sampling the age of plants were 42, 67 and 94 days respectively. Amino acids were qualitatively analysed by unidirectional ascending paper chromatography (Block *et al*. 1958). Total sugar contents of leachates were estimated by anthrone reagent test (Morris, 1948). Total phenols were estimated by the method of Farkas and Kiraly (1962).Germination of *P. infestans* sporangia were estimated by placing 0.02 ml of leachate plus 0.02 ml of sporangial suspension in one concavity of sterilized double concavity slide. The other concavity served as control was inoculated by 0.02 ml of sterilized distilled water plus 0.02 ml of sporangial suspension. The slides were incubated at 8 ^{0}C for 1-1/2 hours, 2 hours and 2-1/2 hours. Five non overlapping fields were observed for percentage sporangial germination.Germination of *P. infestans* zoospores were estimated by placing 0.02 ml of leaf leachate plus 0.01 ml of zoospore suspension in one concavity and the other concavity which served as control was inoculated with 0.02 ml of sterilized distilled water and 0.01 ml of zoospores suspension. The slides were incubated at 25 ^{0}C for hours. The germ tube length was measured.The present inhibition in germ tube length was calculated by using the formula suggested by Vincent (1947).

Results

Nine amino acids viz.. L-glutamic acid, DL-serine, DL- threonine, L-ornithine monohydrochloride, L-arginine

monohydrochloride, DL-α-alanine, L-proline, L-cystine and DL-valine were detected in the leaf leachates of two cultivars of potato i.e. KCM and KJ. Two amino acids, DL-serine and L-arginine monohydrochloride were exclusively detected in the leachates of KJ cultivar. While the rest seven were common in both the cultivars. Four amino acids viz. DL-threonine, Dl- α-alanine, L-proline and DL-valine and five amino acids viz. DL-serine, DL-threonine, L-arginine monohydrochloride, L-cystine and DL-valine were detected from young leaves of KCM and KJ cultivars respectively.

Total sugars and phenol content of leachates of young and mature leaves of KCM and KJ cultivars of potato were given in Table 1. Total sugar content of KCM leachates varied between 0.10 and 0.37 µg per sq. cm. The lowest amount of sugars was exuded by leaves of 2^{V} sampling. In 22^{nd} and 25^{th} sampling the sugar content was more in young leaf leachates as compared to mature ones. Similarly the total sugar content of KJ leaf leachates varied between 0.02 to 0.23 µg per sq. cm. The maximum amount of sugar was present in the leachates of mature leaves of 22^{nd} sampling. The sugar content was more in the leachates of young leaves of the 22^{nd} and 222^{rd} sampling. On comparing the sugar content of leaf leachates of cultivars it was found that sugars was exuded more by KCM.

Total phenol content of KCM leachates varied between 1.00 to 2.0 µg per sq. cm the phenols were present more in young leaf leachates of 22^{nd} and 222^{rd} sampling as compared to mature leaf leachates, while the reverse was true for 25^{th} sampling. The phenol content of KJ leachates varied between 1.05 to 3.16 µg per sq. cm. In all the samplings total phenol content was more in young leaf leachates and the maximum amount was found in the young leaf leachates of 25^{th} sampling. On comparing the phenolic content of KCM and KJ leachates we found that in 22^{nd} and 222^{rd} sampling more phenols were exuded by young leaves of KCM and mature leaves of KJ. In the 25^{th} sampling phenolic content was much higher in young leaves of KJ cultivar as compared to other samples of leaf leachates.

The percentage germination of *P. infestans* sporangia in the leachates of KCM and KJ cultivars of *Solanum tuberosum* at different time intervals is given in Fig.1. After 1-1/2 hours percent sporangial germination was found enhanced except young leaf leachates of 22^{nd} sampling when compared to control in both the cultivars, germination While, sporangial germination was inhibited when compared to control after 2 hours and 2-1/2 hours. Moreover, it was found that percent sporangial germination was more in mature leaf leachates in comparison with young leaf leachates in all the samplings. It was found that percent sporangial germination of *P. infestans* was less in the leaf leachates of KJ cultivar as compared to that of KCM cultivar.

Germ tube length of *P. infestans* zoospores in leaf leachates of two cultivars of potato is given Fig.1. The germ tube length was inhibited in the leaf leachates of both the cultivars except in case of mature leaf leachates of 25^{th} sampling of Kufri Chandramukhi (Table.2) where the germ tube exceeds that of control. In Kufri jyoti leaf leachates the germ tube length never exceeds those of control. In 25^{th} sampling when environmental conditions were favourable for late blight of potato the leaf leachates caused more inhibition of germ tube length as compared to control.

Discussion

The influence of leachates on plant surface microbial populations is very complex. Microorganisms

themselves respond in different ways to leachates from different plants (Chet *et al.*, 1973). Purnell (1971) provided evidence to suggest that leachates may contain some components which stimulate growth and at the same time others which are inhibitory.

The amount of sugar exudation changes from sampling to sampling and between young and mature leaves of the same cultivar. Sugar exudation was more on the surface of KCM cultivar and hence microflora was more on this cultivar as also reported by Collins (1976) that *Antirrhinum majus* Nanum exuded more sugar than *A.majus* Fi hybrid which supported less phylloplane microflora. Bansal *et al*. (1988) reported that sugar exudation was more on the surface of WH 157 cultivar as compared to NP 830 cultivar of wheat at any given time hence microflora was more on WH 157 cultivar.

The percentage germination of *P.infestans* sporangia in the leachates of KC and KJ cultivars of potato were found enhanced after 1-1/2 hours of incubation except in young leaf leachates of 22nd sampling, while sporangial germination was inhibited as compared to control after 2 hours and 2-1/2 hours of incubation. The stimulating effect of sporangial germination in leaf leachates is usually regarded as nutritional and attributed largely to the presence of carbohydrates and amino acids. Godfrey (1974) showed that a relatively aqueous leachates from fronds of *Pteridium aquilinum* increase the germination hyphal length of *Botrytis cinerea.* The nutrients released from leaf surface have a stimulatory effect on phylloplane microorganisums (Bahadur and Sinha, 1970;Sadasivam *et al.*, 1976; Mishra and Tewari, 1978). Wang and Pinckard (1973) found leachates from cotton bolls inhibited the germination of *Diplodia gossypina* spores and suggested that this inhibitory effect was due to phenolic compounds in leachates of lilac *(Syringa vulgaris)* contained tree phenolic substances which inhibited the percentage germination *Alternaria alternata* and *Botrytis cinerea* when compared with deionized water control. Singh *et al*. (1986) found exudates of leaves of *Spinacia oleracea* stimulated the percentage of conidial germination and germ tube growth of all test fungi except *Cercospora beticola* where inhibitory effect was observed by the exudates of young leaves. The percent sporangial germination was less in the leaf leachates of KJ cultivar as compared to KCM cultivar. Particularly in the 25th sampling when the environment conditions were favourable for the disease development, the sporangial germination was much higher than disease development the spoirangial germination was much higher in the leaf leachates of KCM cultivar. Similarly the germ tube length of *P. infestans* zoospores in mature leaf leachates of 25th sampling of KCM exceeds that of control while the same was inhibited in case of KJ. This indicated the resistance of KJ against *P. infestans*. Link and Walker (1933) found resistance of red or yellow pigmented varieties of onion to *Colletotrichum circinans* was due to higher concentration of phenolic acids, catechol and protocatechuic acid in the scale leaves. Germination of *Peronospora tabacina* was greatly reduced on leaves of *Nicotiana tobacum*, compared to other tobacco species (Shepherd and Mandryk, 1963). The inhibition in germination was attributed to the presence of phenolic compounds in the droplets removed directly from the leaves.

Sharma and Sinha (1971) recorded that exudates from young leaves were more effective in causing inhibition of germination of conidia of *Colletrichum graminicola* than those from mature leaves. Schneider and Sinclair (1975) found that leachates from leaves of resistant varieties of *Vinca unguiculata* inhibited germination of *Cercospora varieties* of *Vinca unguiculata* inhibited germination of *Cercospora canescens*

spores. They suggested the presence of a specific inhibitor in the leaf washings. Singh and Gupta (1983) found the stimulatory effect of leaf exudates on conidial germination of rice leaf scald fungus, *Rhynchosporium oryzae,* was higher in those collected from the susceptible cultivar (Jaya) than the resistant cultivar (Dular). The significance of amino acids in the growth and development of *P.infestans*

Table 1: Total sugars and total phenols in the leaf leachates of two cultivars of *Solanum tuberosum*

Sampling number		Sugars µg/cm^2		Phenol µg/cm^2	
		KCM	KJ	KCM	KJ
II	Young	0.37	0.20	2.80	1.84
	Mature	0.12	0.23	1.09	1.50
III	Young	0.20	0.11	1.91	1.22
	Mature	0.31	0.02	1.00	1.05
IV	Young	0.14	0.22	1.23	3.16
	Mature	0.10	0.15	1.44	1.21

Table 2: Percentage inhibition in germ tube length of *P. infestans* zoospores in the leachates of cultivers of *Solanum tuberosum*

Sampling number		KCM	KJ
II	Young	3.81	38.16
	Mature	45.03	19.08
III	Young	26.71	20.61
	Mature	54.19	28.24
IV	Young	26.71	11.29
	Mature	-16.48	5.64

was emphasized by French (1953) and Child and Fothergill (1967). No definite correlation could be established between amino acids and resistance of potato plants to infection by *P.infestans*. However, differences have been observed in the composition of amino acids in the leacheats of KCM and KJ cultivars.

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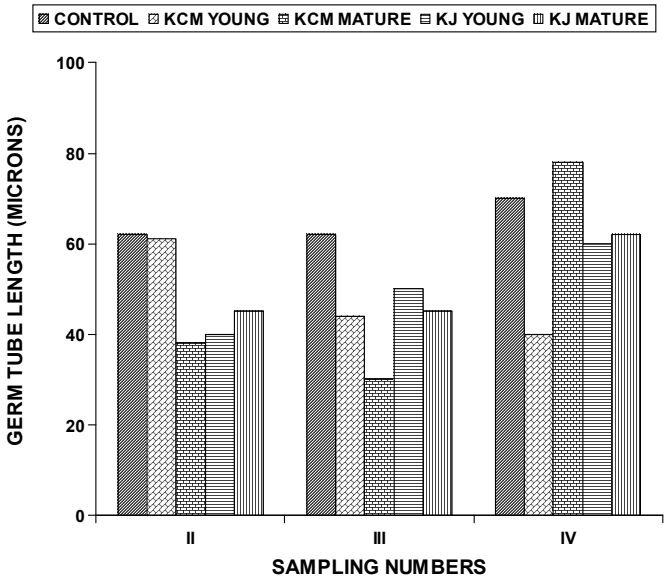

Fig 1: Growth of germ tube length in control and leachates of Kufri Chandramukhi and Kufri Jyoti cultivars.