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Effect of graded levels of potassium on the *in vitro* production of hydrolytic enzymes by *Rhizoctonia solani* of rice

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Abstract
The production of Polygalacturanase (PG), Polygalacturanase transeliminase (PGTE) and Pectic Trans Eliminase (PTE) were measured and the results showed that, all the enzymes production reduced significantly in all the treatments with increasing levels of potassium when compared to control. The maximum and minimum inhibition was observed at 100-kg k2o ha^{-1} levels respectively. PG production was considerably inhibited in all the treatments. Maximum inhibition of 44.44 per cent was noticed in 100-kg k2o ha^{-1} when compared to control. Minimum inhibition of 14.37 per cent was noticed in 25 kg k2o ha^{-1} level. PGTE and PTE production also followed the same pattern, recording the maximum and minimum percentage of reduction of 73.33 and 26.66, 72.17 and 20.00 per cent respectively over control.

Keywords: Potassium, *Rhizactonia solani*, *in vitro*

Introduction
Rice is an important cereal crop, which is the primary food for half of the human population.. The average annual production of rice in India is 2.8 t ha^{-1}. Rice crop suffers from a number of fungal, bacterial and viral diseases. Among the fungal diseases, sheath blight is a major disease caused by *Rhizoctonia solani* Kuhn. (*Thanatephorus cucumeris* (Frank) (Donk.).Several workers reported that potassium fertilization reduced the susceptibility of rice to diseases, hastened the maturity and increased the yield. Yamada (1959) reported that the deficiency of potassium and excess of nitrogen were responsible for the incidence of diseases like sheath blight, brown spot, blast and stem rot of rice. Kannaiyan and Prasad (1978) reported that potassium application reduced the sheath blight disease of rice.

Materials and Methods
Modified Czapek's broth in which the carbon source was substituted by three per cent pectin for pectinolytic enzymes was prepared. Potassium chloride at 0, 25, 50, 75 and 100 kg ha^{-1} was incorporated. The pH of the medium was adjusted to 6.5 - 7.0, distributed in 50 ml quantities in 250 ml Erlenmeyer flasks, sterilized and cooled to room temperature. The flasks were inoculated with eight mm discs of the fungal growth and incubated at room temperature (28±1 °C) for 15 days.
At the end of incubation period, the biomass from the culture solution was removed by filtration under suction in a previously dried and weighed filter paper. The filter paper with biomass was dried, at 105 °C for 25 hrs. and the dry weight was determined. The culture filtrates were centrifuged at 2100 rpm. for 30 minutes, examined microscopically for the presence of contaminating fungal spores and retained for the various enzyme assays.

Enzyme assay
Pectinolytic enzymes
(a) Polygalacturonase (PG)
The loss in viscosity of sodium polypectate was employed to estimate PG activity. Sodium polypectate of 0.75 per cent was prepared in buffer. To four ml of sodium polypectate, one ml of sodium acetate-acetic acid buffer at pH 5.2 and two ml of the culture filterate was added. The pH of the reaction mixture was adjusted to 5.2 and immediately transferred to a 150 size Ostwald-Fenske viscometer placed in a water bath at 28±1°C. Viscosity losses were measured and per cent loss in viscosity was calculated, employing the following formula:

Percentage loss in viscosity = $\frac{T_0 - T_1}{T_0 - T_w} \times 100$

Where,
T_0 = flow time at 0 time (seconds)
T_1 = flow time at 1 intervals (seconds)
T_w = flow time of double distilled water (seconds)

Pure culture filtrates without test compounds served as controls (Mahadevan *et al*., 1966) [3].

(b) Polygalacturonase transeliminase (PGTE)
The activity of PGTE was determined by the viscosity loss of sodium polypectate. To four ml of freshly prepared 1.2 per cent sodium polytpectate dissolved in boric acid-borax buffer at pH 8.6, one ml of the buffer (at pH 8.6) and two ml of the filtrate was added. The pH was adjusted to 8.6 and immediately transferred to Ostwald-Fenske viscometer size 150 and loss in viscosity was determined as detailed earlier.

(c) Pectin trans-eliminase (PTE)
The activity of PTE was determined by the viscosity loss of one per cent citrus pectin. The reaction mixture consisted of four ml of one per cent citrus pectin in 0.2 M Boric acid - Borax buffer at pH 8.6. The pH of the reaction mixture was adjusted to 9.6, and immediately transferred to Ostwald-Fenske viscometer size 300 and loss in viscosity was determined as detailed above.
In vitro production of pectinolytic enzymes

Result and Discussion
The production of polygalacturonase (PG), polygalacturonase transeliminase (PGTE) and pectin trans-eliminase (PTE) were measured and the results are presented in Table 1. The results showed that the pectinolytic enzymes production reduced significantly in all the treatments with increasing levels of potassium when compared to control. The maximum and minimum inhibition was observed at 100 kg K_2 O ha^{-1} and 25 kg K_2O ha^{-1} levels respectively.
Polygalacturonase (PG) production was considerably inhibited in all the treatments. Maximum inhibition of 44.44 per cent was noticed in T_5 treatment when compared to control. Minimum inhibition of 14.37 per cent was noticed in T_2 treatment at 25 kg K_2 O ha^{-1} level.
Polygalacturonase transeliminase (PGTE) production also followed the same pattern, recording the maximum and minimum percentage of reduction of 73.33 and 26.66 per cent respectively over control.
The production of pectin trans-eliminase (PTE) was also influenced by the potassic levels. Slight reduction of 20.00 per cent was observed at 25 kg level whereas the reduction was more with the concentration of K and it reached a maximum value of 72.17 per cent at 100 kg level.
The results showed that the pectinlytic enzyme production was found to be reduced significantly in all the treatments with increasing levels of potassium when compared to control.
Penetration of the pathogen into host is more dependent on the maceration of the tissues and disintegration of the cell walls of the host, which are achieved through hydrolytic enzymes (Rosenberg and Wilkers, 1952; Bateman, 1964; Kannaiyan and Prasad, 1974; Prabakar, 1991 and Murugesan, 1993) [7, 6, 4].

Table 1: Effect of graded levels of potassium on the *in vitro* production of pectinolytic enzymes* by *R. solani*

K_2O levels kg ha^{-1}	PG**	Percent decease over control	PGTE***	Percent (-) decrease are control	PTE****	Per cent decrease (-) over control
0	30.6		7.5		11.5	
25	26.2	+14.37	5.5	+26.66	9.2	+20.00
50	22.1	+27.77	4.1	+45.33	7.1	+38.26
75	19.0	+37.90	3.0	+60.00	5.0	+56.52
100	17.0	+44.44	2.0	+73.33	3.2	+72.17
SE	0.3095		0.3093		0.2503	
CD (p=0.05)	0.8792		0.8792		0.7116	

* Mean of three replicates
**Per cent loss in viscosity of 0.75 sodium polypectate at the end of 120 min.
***Per cent loss in viscosity of 1.25% sodium polypectate at the end of 120 min.
****Per cent loss in viscosity of 1.0% citrus pectin at the end of 120 min.
Activity expressed per g of oven dry tissue

The above results confirm the findings of Ramasamy (1974) [7] with *Fusarium oxysporum* f.sp. *melonis*, Prabakar (1986) [5] with *Pyricularia oryzae*, Jayaraj (1989) [2] with *Sclerotium oryzae*, Prabakaran (1991) [6] with *Pyricularia oryzae* and Murugesan (1993) [4] with *Pyricularia grisea* and Alagappan (1992) [1] with *Colletotrichum capsici*.
The reduced activities of pectinolytic enzyme in the sheath portions taken from the plants applied with different levels of potassium might be due to

a. Increased potassium levels in the host
b. The inhibitory effect of phenolic compounds on pectinolytic and cellulolytic enzymes has been reported by Mahadevan (1966) [3].
c. Increased uptake of Ca^{++} (Anonymous, 1966 and Ramasamy, 1974) [7].
d. Decreased establishment of the pathogen as revealed in the present investigation.

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