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STUDIES OF ANTAGONISTIC EFFECT OF SEED MICRO-FLORA ON RHIZOBIUM

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ABSTRACT

Analysis of seeds of chickpea (*Cicer arietinum* L.) var.PG -114 were performed for the presence of microflora on its surface *viz*. bacteria, actinomycetes and fungi and its antagonistic effect on inoculated Rhizobium were studied. Although, bacteria, fungi as well as actinomycetes were present on seed surface of chickpea var. PG-114 used in the study. Antagonistic effect of seed microflora towards inoculated Rhizobium could not be seen as the inoculated Rhizobium strains failed to grow on the top layer of medium.

Key words : Antagonistic effect, Inoculation, Microflora, Rhizobium, Chickpea.

INTRODUCTION

The microflora present on seed surface and its effect on the pre emergence of the seedlings have been studied by Salisbury (1957). Gibson (1957) working with seeds of Pinus patula reported that some genera of fungi in particular saprophytic fungi present on seed surface i.e. seed coat under favourable conditions invade tissues of the germinating seeds and kill the seedlings. Lawrence and Rediske (1961) with isotope tagged seeds demonstrated that the seed coat microflora was directly responsible for weakening of seed vigour. However, the study of the effect of seed microflora on inoculated Rhizobium has been neglected. The present study was planned to study the seed microflora as well as its antagonistic effect if any on the Rhizobium inoculation and over the performance of inoculated strains in terms of nodulation and nitrogen fixation.

MATERIALS AND METHODS

To study the antagonistic effect of seed micro-flora to Rhizobium an experiment was conducted in laboratory using triple layer technique (Panthier *et al*., 1979) with certain modifications. Seeds of chickpea var. PG-114 were obtained from G.B. Pant University of Ag. and Tech.

Preparation of dilutions

The dilutions were made by adding 10 seeds in 100ml sterile water in conical flask aseptically. The flasks were shaken for 10minutes on rotatory shaker. Tenfold serial dilution upto 10^{-3} was prepared. Both 10^{-2} and 10^{-3} were used in study.

Actinomycetes

Bottom layer

2 ml both the dilutions were poured in sterilized plates separately in 5 replicates. Then 10 ml Ken-Knight medium at 40-45^{0}C. Temperature was poured in each plate. Each plate was mixed gently to mix inoculum and was allowed to cool until the agar was firm.

Intermediate layer

An intermediate layer consisting of 5ml of 2% sterile water agar was poured over the bottom layer of each plate.

Fig. 1a : Culture plates representing the growth of actinomycetes on seed surface. The red circle represents fully grown actinomycetes colony.

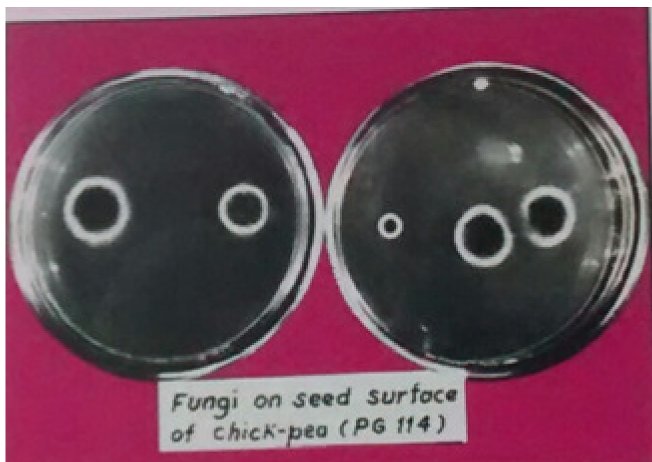

Fig. 1b : Culture plates representing the growth of fungi on seed surface. The red circle represents dividing fungi.

Fig. 1c : Culture plates representing the growth on seed surface. The red circle represents bacterial colony.

The plates were incubated ay 26^0C. for 6 days to permit sufficient development of actinomycetes.

Top layer

After incubation at 26^0C for 6 days, 15ml of modified

Table 1 : Microbial population on seed surface of chick pea population X10^3/seed.

Bacteria	Fungi	Actinomycetes
13.8	5.2	6.8

Table 2 : Composition of different media used in the study.

I Yeast extract Mannitol Agar	
Mannitol	10.0g
di-potassium hydrogen orthophosphate (K_2HPO_4)	0.5g
Magnesium sulphate ($MgSO_4.7H_2O$)	0.2g
Sodium Chloride	0.1g
Yeast extract	1.0g
Calcium carbonate ($CaCO_3$)	2.0g
Agar	20.0g
Congo-red	10.0ml (0.25% solution)
Distilled water	1000ml
pH	7.2
Ken-Knight medium	
Dextrose	1.0g
Potassium di-hydrogen phosphate (KH_2PO_4)	0.1g
Sodium nitrate ($NaNO_3$)	0.1g
Potassium chloride (KCl)	0.1g
Magnesium sulphate ($MgSO_4.7H_2O$)	0.1g
Agar	20.0g
Distilled water	1000ml
Rose-Bengal Agar (Martin, 1950)	
Glucose	10.0g
Peptone	5.0g
Potassium di-hydrogen phosphate (KH_2PO_4)	1.0g
Magnesium sulphate ($MgSO_4.7H_2O$)	0.5g
Streptomycin	30.0g
Agar	20.0g
Rose-Bengal	0.035g
Distilled water	1000ml
Nutrient agar	
Beef extract	3.0g
Peptone	5.0g
Agar	20.0g
Distilled water	1000ml
Modified YEMA medium	
Mannitol	10.0g
di-potassium hydrogen orthophosphate (K_2HPO_4)	0.5g
Magnesium sulphate ($MgSO_4.7H_2O$)	0.2g
Yeast extract	1.0g
Sodium chloride (NaCl)	0.2g
Ferric chloride ($FeCl_3$)	4.88mg
Agar	7.0g
Distilled water	1000ml

YEMA medium of 40-50^{0}C temperature containing Rhizobium culture (48 hours old) was poured on a top layer. These plates were incubated at 30^{0}C. Seven days after incubation, observation were recorded to see the antagonistic effect of actinomycetes present on the seed surface of Rhizobium.

Fungi

Bottom layer

Fungi streptomycin Rose Bengal Agar medium (annexure III) was used. Bottom layer was preparedas mentioned above.

Intermediate layer

As described for actinomycetes.

Top layer

As described for actinomycetes.

Bottom layer for bacteria nutrient agar medium (annexure IV) was used. Bottom layer was prepared as mentioned above.

Intermediate layer

As described for actinomycetes.

Top layer

As described for actinomycetes.

RESULTS AND DISCUSSION

Mainly the actinomycetes, bacteria and fungi were found on seeds. Data presented in Table 1 and Fig. 1 showed number of actinomycetes, number of bacterial and fungal colonies present on the seed surface. On the top layer of plates, our Rhizobium strains (TAL- 620, G-3) fail to grow. Therefore, antagonistic effect of seed microflora towards inoculated Rhizobium could not be seen as the inoculated Rhizobium strains failed to grow on the top layer of medium. However, seeds with surface sterilization before inoculation could give better results in terms of performance of used Rhizobium strains.

Composition of different media used in the study are given in Table 2.

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