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ISOLATION OF CROP SPECIFIC INDIGENOUS *Rhizobium* STRAINS AND STUDY THEIR EFFECTS ON SEED GERMINATION

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ABSTRACT

Use of chemical nitrogen (N) in N deficient soil and Seed treatment with fungicides including Benomyl, Carbendazim, Carboxin, Thiabendazole, Thiram, Tolylfluanid and Mancozib etc. has been broadly practiced to good yield and cheap insurance against seed-and soil-borne pathogens resulting deterioration of soil quality and poor nodulation because most fungicides toxic to *Rhizobium* bacteria. The chemical fungicides also leaves residue on soil and crops. Beyond chemical N and fungicide spray of plant growth promoting substance is also a common practice in legume crops causing excess expenses on farming. Use of efficient crop specific indigenious *Rhizobium* strains might be a good alternative to reduce economic and organic farming because it has already reported that *Rhizobium* species fixes sufficient quantity of atmospheric nitrogen which range up to 25% of the requirement of the crop, it has also been reported that indigenous Rhizobium not only protect seeds from root rots causing fungal plant pathogens but also produces plant growth promoting substances. In view of above there were 5 root nodules samples of each Soybean (*Glycine max*), Chickpea (*Cicer arietinum*) (Chana) and Fenugreek (*Trigonella foenum-graecum or Methi*) were collected from the organic fields of individual crops where there were no use of N-fertilizer and fungicides. Among the several isolates efficient and most competent strains were selected and their formulation applied for seed germination test and we found better seed germination in relative *Rhizobium* treated seeds comparative to the control. Pot nodulation confirms that isolates are true *Rhizobium* and positive seed germination confirms that isolates are Plant Growth Promoting Rhizobacteria (PGPR).

KEYWORDS : Indigenious *Rhizobium* strains, seed germination test, pot nodulation

Rhizobia are symbiotic nitrogen-fixing bacteria and are the most important contributors of fixed nitrogen to soil. Legume inoculation with *Rhizobium* is an aged practice that has been carried out for more than a century in agricultural systems (Qureshi et al., 2009). Nitrogen fixation by these bacteria can take place only when they grow in association with the host plant; they fail in fixing nitrogen when living free of the host. Each kind of leguminous crops require specific kinds of Rhizobium to cause nodulation in particular crops(Dowling and Broughton, 1986). Baldwin et al.,1932 have classified all *Rhizobium* in seven cross inoculation group on the basis of their ability to form nodulation (Table, 1).

Therefore presence of indigenous and crop specific *Rhizobium* bacteria are environmentally significant in soil N fertility management of cultivated lands. Various

Table 1: Cross Inoculation groups of *Rhizobium*

S.No.	Cross inoculation Group	*Rhizobium species*	Leguminus crops
1.	Alfalfa group	*Rhizobium meliloti*	Alfalfa, Sweet clover, Fenugreek
2.	Clover group	*Rhizobium trifolii*	Clover, Berseem
3.	Pea group	*Rhizobium leguminosarum*	Pea, Lentil, tewda
4.	Bean or Phaseoli group	*Rhizobium phaseoli*	Phaseoli, Moong (green gram), Urd (Black gram)
5.	Lupini group	*Rhizobium lupini*	Lupinus
6.	Soybean group	*Rhizobium japonicum*	Soybean
7.	Cow pea group	*Rhizobium spp.*	Cow Pea, Guar bean, Ground nut, Redgram

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researchers have reported the synergistic effects of auxin producing plant-growth promoting rhizobacteria and *Rhizobium* on nodulation and yield of legume crops (Tilak et al., 2006).

Rhizobium can also control crop specific fungal root rot disease (Omar et al .,1998 and Jain et al., 2011). Some species of *Rhizobium* produces plant growth promoting hormones- gibberellins and Indole-3 Acetic acid (Atzorn et al., 1988), Cytokinins (Badenoch-Jones et al.,1987) are called plant growth promoting rhizobacteria (PGPR).

Thus present studies were aiming to isolate efficient and competitive strains of indigenous strains with evaluation their nodulating efficiency and presence of plant growth promoting substances specialty by different Indigenous seed germination tests for Soybean (*Glycine max*), Chickpea (*Cicer arietinum*) (Chana) and Fenugreek (Trigonella foenum-graecum or Methi). Among the five isolates of each one strains of each found more competent than other.

MATERIALS AND METHODS

5 samples of each soybean root nodules, Chick pea (Kabuli Chana and Desi Chana), and Fenugreek were taken from organically grown fields near Indore fields of Madhya Pradesh India. Borosil make Agrade glassware, Biotechniques makes B.O.D. Incubator, Steelmet Pune India make rotary shaker, Olympus CHI 20 Trinocular Microscope with camera, Labomed sterio zoom microscope were used respectively. Yeast extract mannitol agar media (YMA) Hi-Media with 0.0025% Congo red (Hi-Media) was used to isolation of *Rhizobium.* Isolated pure culture slants maintained on Yeast Mannitol Agar (YMA). Organic non chemical treated seeds were used for seed germination test and pot nodulation test.

Isolation

Only powerful, effective and pink large size indigenous root nodules were selected and sterilized in 0.1% (w/v) sodium hypo chloride for 5 min immersed in 95% (v/v) ethanol for 10 seconds and then washed five to six times with sterile water than crushed with sterile rods and this preparation were streaked onto sterilized Yeast extract Mannitol Agar (YEM) plates containing 0.0025% (w/v) congo red (Vincent, 1970) and incubated for 3 days at 30°C. After an inoculation of 3 days at 30°C, single colonies were selected and re-streaked on YEM for purify.

Isolates were also tested for their ability to nodulate indigenous crop (Cregan and Keyser, 1986) pot nodulation test.

Isolates also observed to morphological characterization by colony morphology and Gram staining technique.

Seed Germination Test

Effects of indigenous *Rhizobium* strains on germination of particular seed were tested for the ability of

Fig.1:Soybean (*Glycine max*)

Fig.2: Chickpea (*Cicer arietinum*)

Fig .3: Fenugreek (*Trigonella foenum-graecum*)

indigenous strain to produce effective PGPR as per method given by Abdul Baki and Anderson, 1973. 20 seeds of each treatment with three replication of each Soybean (*Glycine max*), Chickpea (*Cicer arietinum*) (Chana) and Fenugreek (*Trigonella foenum-graecum or Methi*) were surface-sterilized with 0.02% sodium hypochlorite for 2 min, and rinsed thoroughly in sterile distilled water. For inoculation seeds were dipped of indigenous *Rhizobium spp* broth culture (10^8 cfu ml^{-1}) in petri dish until uniformly soaked, control seeds soaked in sterile water. Germination took place in an incubator at 25°C, in the dark. The seeds were considered to be germinating at the moment of radicle emergence. The number of germinated seeds was recorded daily final quantity of germinated seeds were calculated after 8 days.

RESULTS AND DISCUSSION

There root nodules of leguminous have inherent difference in size shape and crop in accordance with crop. Nodules are small oval, round, or lobed bumps protruding from the roots with a whitish color. Nodule size varies; many forage legumes have small nodules: 15 20 nodules will fill a spoon. Some clover nodules are very small; 100 nodules could fit on the same spoon. The nodules should be obvious and abundant, although the actual number depends on many factors. In general there should be a minimum of 20 well-developed nodules per plant. Note that nodules may be in clusters. Fig.,1, 2, and 3 representing three different legumes root nodules.

Fig .4: Isolated colonies of R. Soybean

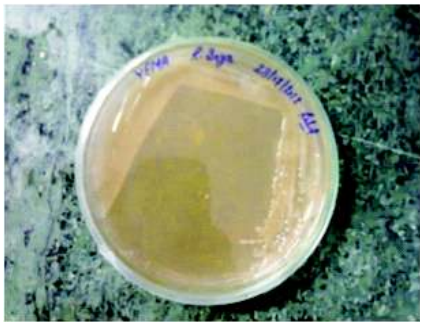
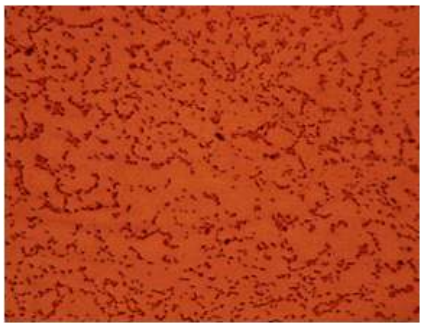
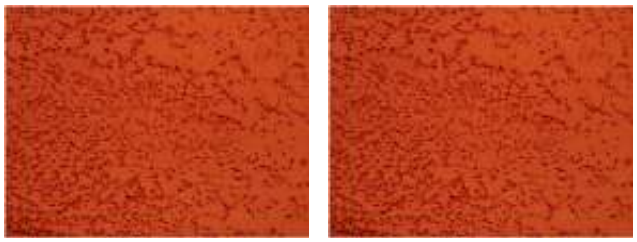


Fig. 5: R. Chick Pea



(a)

(b)



(c)

Fig. 6: *Rhizobium* isolated from (a) Soybean (b) Chickpea (c) Fenugreek

Colonies of *Rhizobium* and other bacteria grows on YEMA media containing 0.0025% (w/v) Congo red after 2-3 days of incubation. Isolates originating from a surface-contaminated nodule were discarded.

The *Rhizobium* colonies were identified by the morphology sticky appearance showing the production of mucous though at lower levels. Analysis of colony

morphology indicated round colonies, with white color or opaque. The non *Rhizobium* colonies were identified by their red appearance on incubation of streaked plates. Isolates were observed to be transient growers as colony becomes visible after 24 h of inoculation. Fig., 4 and 5 represents the isolated colonies of different *Rhizobium* strains.

General microscopic view of the isolates showed that isolated *Rhizobium* were rod cells and gram negative in nature. Fig, 6 represents gram staining slides of *Rhizobium*. Effect of Indigenous *Rhizobium* strains on the seed germination of related crops found early germination. The details of the result are given in the table, 2.

CONCLUSION

Use of Chemical N, fungicide and plant growth promoting tonics are the common practices, these practices may be replaced using indigenous *Rhizobium* culture. 5 samples of root nodules of each Soybean, Chick Pea and Fenugreek were taken and isolate 5 strains from each of which one strain of each found more competitive than other were selected and confirmed as true *Rhizobium* by Pot nodulation test. Indigenous strain also checked for their PGPR producing potential by seed germination test and found that early and almost 100% seed germination compared with control, confirms that in field in Vivo condition use of these indigenous strain as soil or seed treatment will beneficial in terms of atmospheric N fixation, protection against soil borne root pathogens and their potential as effective PGPR.

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Table 2: Details of seed germination observed are given below

S.No.	Details of seed and treatment	Nos of seed germination after days								Total Nos of seeds used
		1st	2nd	3rd	4th	5th	6th	7th	Total	
1.	Soybean Control	0	0	2	7	6	2	0	17	20
2,	Soybean with Indigenous *Rhizobium* culture	0	2	13	5				20	20
3.	Chickpea Control	0	0	0	11	4	2	0	17	20
4.	Chickpea with Indigenous *Rhizobium* culture	0	2	13	5				20	20
5.	Fenugreek Control	0	0	2	11	5	2		20	20
6.	Fenugreek with Indigenous *Rhizobium* culture	0	2	15	3				20	20

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