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# Survival of inoculated *Rhizobium leguminosarum* bv. *viciae* on lentil seed as influenced by soil type and moisture levels

**RAJIB KARMAKAR* and RAMESH CHANDRA**

*Department of Soil Science, College of Agriculture, G.B. Pant University of Agriculture & Technology, Pantnagar 263145, Uttarakhand, India; *Pesticides Residue Analysis Laboratory, NHRDF, Nashik 422 011, Maharashtra, India*

## ABSTRACT

**Survival of seed inoculated *Rhizobium leguminosarum* bv. *vicae* on lentil was examined in sandy loam and sandy clay loam soils at 30 and 50% moisture of soil water holding capacity (WHC) under green house conditions. Irrespective of moisture levels, sandy clay loam soil gave significantly more counts of inoculated *R. leguminosarum* on the germinating seed upto 8 days. Soil moisture of 50% of WHC supported significantly more population (47.8%) on seed than at 30% moisture irrespective of the soil type and days of observation. Interaction between soil × moisture × germination periods was significant and indicated that 30% moisture in sandy loam soil reduced the viable counts at different germination periods more drastically compared to sandy clay loam soil.**

**Key words:** *Rhizobium*, Soil moisture, Soil texture, Survival

The survival of rhizobia in soil is an important factor for root colonization and successful nodulation of host plants. Factors such as soil texture, temperature, moisture content, pH, *etc.*, influence the survival of *Rhizobium* in soil (Zahran 1999). Athar (1998) reported that strains of *Rhizobium leguminosarum* nodulating lentil survived well up to maximum of -1.5 M Pa soil moisture potential. The adverse effects of soil moisture differ with soil type and are attributed mainly to desiccation (Trotman and Weaver 2000, Vriezen *et al.* 2006). Al-Rashidi *et al.* (1982) and Moawad *et al.* (2005) reported that soil with more clay and organic carbon maintained higher population during desiccation. In most of these studies, survival of rhizobia was studied in soil without seed. However, seeds have definite influence on the survival of inoculated rhizobia because of the variation in the composition of seed exudates and spermospheric effect. The interactive effect of moisture, soil type and seed appears important in survival of inoculated *Rhizobium* on seed and rhizosphere for root colonization. The present study was, therefore, undertaken to examine the influence of soil moisture on the survival of seed inoculated *R. leguminosarum* bv. *viciae* on lentil seed in two soils of varying texture.

## MATERIALS AND METHODS

The experiment was carried out in the green house at G.B. Pant University of Agriculture and Technology, Pantnagar, India. Elite *Rhizobium leguminosarum* bv. *viciae* strain LB-4 $^{str\ -rif\ +\ Hg}$ having intrinsic resistance of 200 µg ml$^{-1}$ streptomycin, 15 µg ml$^{-1}$ rifampcin and 20 µg ml$^{-1}$ mercury was obtained from culture collection of Pulse Microbiology Laboratory, Department of Soil Science of the University. The resistance of the obtained culture was confirmed by growing on Yeast Extract Mannitol Agar (YEMA) medium containing above quantity of the antibiotics and mercury. The culture was multiplied in YEM broth for 4 days at 28 ± 2°C and its carrier based inoculant was prepared by mixing with neutralized charcoal (12.5% $CaCO_3$, pH 7.3) in 1: 2 ratio. Soils of sandy loam and sandy clay loam textures were collected in bulk from Crop Research Centre of the University, processed by passing through 2 mm sieve and analysed for their physico-chemical properties (Table 1). The presence of indigenous population of rhizobia having resistance of 200 µg ml$^{-1}$ streptomycin, 15 µg ml$^{-1}$ rifampcin and 20 µg ml$^{-1}$ mercury was also examined by plating 100 times diluted suspension of both the soils with YEMA containing above concentrations of the antibiotics and mercury. The petri plates on incubation for 7 day at 28 ± 2°C did not form rhizobia like colonies, indicating absence of rhizobia in the soil having resistance of 200 µg streptomycin + 15 µg rifampcin + 20 µg mercury ml$^{-1}$ medium.

Plastic columns of 30 cm length and 10 cm diameter, prepared using plastic pipe, were filled up with 4 kg soil and compacted by tapping them gently. Two moisture regimes of 30 and 50% of their water holding capacity (WHC) in both the soils were created and maintained throughout the experiment gravimetrically. Columns were weighed regularly at 3 - 4 days interval to observe the water loss and accordingly, the sterilized water was supplied from outside to the columns to maintain the same moisture regimes. Ten seed of lentil (*Lens culinaris* L.) cultivar PL 4 were inoculated by mixing the

**Table 1. Physico-chemical properties of experimental soils**

| Property | Sandy loam | Sandy clay loam |
|---|---|---|
| pH (1:2 soil: water ratio) | 7.2 | 7.5 |
| Organic carbon (g/kg) | 6.9 | 8.4 |
| Available P (kg/ha) | 15.6 | 13.4 |
| Available K (kg/ha) | 212.8 | 210.0 |
| Total nitrogen (mg/kg) | 966 | 866.8 |
| Available N (kg/ha) | 254.0 | 219.5 |
| Mechanical analysis | | |
| Sand (%) | 63.6 | 51.6 |
| Silt (%) | 22.0 | 28.0 |
| Clay (%) | 14.4 | 20.4 |

moist seed with 200 mg carrier based inoculant and were sown at a depth of 2 cm in each of the 10 columns of both the soils maintained at two moisture regimes. Germinating seeds from the 2 soil columns of each treatment were taken out aseptically at every 48 hour interval with adhered soil upto 8 DAS and transferred to 99 ml sterilized water. Ten-fold serial dilutions were prepared and one ml of suitable dilutions was plated on YEMA medium containing 200 μg $ml^{-1}$ streptomycin + 15 μg $ml^{-1}$ rifampicin + 20 μg $ml^{-1}$, mercury. The plates were then incubated at 28 ± 2°C for 7 days and characteristic colonies of the marked strain were counted to compute the population per seed or seedling.

## RESULTS AND DISCUSSION

The viable counts of inoculant *R. leguminosarum* strain on germinating seed were significantly influenced by soil type, moisture level and time (Table 2). Irrespective of moisture levels, sandy clay loam soil supported significantly more viable population of inoculated strain on the germinating seed in soil. Similarly, 50% moisture favoured significantly more population (47.8%) on seed than at 30% moisture, irrespective of the soil type and days of observation. The viable population of inoculated strain progressively declined on germinating seed and maximum decline by 12.5% was observed between 6 and 8 days. Similar decline in *Rhizobium* population on chickpea seed was also observed by Kumar *et al.* (1986) which could be due to desiccation or toxic seed exudates (Singh and Khurana 1988 and Trotman and Weaver 2000). Significant soil × moisture interaction indicated that difference in the viable counts due to moisture level was more discernible in sandy loam soil, which showed 71.2% higher viable counts at 50% moisture than 30% moisture (Table 2). Sandy clay loam soil showed only 28.8% more viable counts at 50% moisture than 30% moisture. It suggested that soil moisture was more critical in sandy loam soil compared to sandy clay, which is an established fact and has been reported by several workers (Al-Rashidi 1982, Moawad *et al.* 2005).The interaction between moisture × germination periods was also significant and the difference in the viable counts due to moisture level went on widening with the germination time and the highest difference of 153.3% in the viable population on seed due to moisture level was recorded on the 6th day of observation (Table 2). The 50% moisture level, in general, gave significantly more viable counts at all the intervals of observations. The poor survival of microorganisms at low moisture may be the case of desiccation of the cells (Kumar 1986, Trotman and Weaver 2000). Soil × germination period interactions were also significant, however, indicated no definite trend in the influence of both the soils on viable counts on germinating seed at different periodic intervals (Table 2). Sandy loam soil recorded significantly more viable counts on 0 and 6 days while sandy clay loam soil gave more viable population on 2, 4 and 8th day. Interaction between soil × moisture × germination period was also significant and indicated that 30% moisture

**Table 2. Effect of soil and moisture on survival of *Rhizobium leguminosarum* bv. *viciae* on lentil seeds**

| Treatment | *R. leguminosarum* bv. *viciae* population (x $10^4$ cfu/seed) |
|---|---|
| **Soil** | |
| Sandy loam | 5.5 |
| Sandy clay loam | 5.9 |
| SEm ± | 0.01 |
| CD at 5% | 0.04 |
| **Moisture** | |
| 30 % of WHC | 4.6 |
| 50 % of WHC | 6.8 |
| SEm ± | 0.01 |
| CD at 5% | 0.04 |
| **Germination period (day)** | |
| 0 | 11.3 |
| 2 | 7.9 |
| 4 | 5.6 |
| 6 | 2.7 |
| 8 | 1.2 |
| SEm ± | 0.01 |
| CD at 5% | 0.04 |

| Soil type | Interaction: Soil moisture X soil type | |
|---|---|---|
| | 30% of WHC | 50% 0f WHC |
| Sandy loam | 4.03 | 6.9 |
| Sandy clay loam | 5.2 | 6.8 |
| SEm ± | 0.02 | |
| CD at 5% | 0.03 | |

| Soil moisture | Interaction: Moisture X germination period (day) | | | | |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 |
| 30% of WIIC | 11.3 | 6.0 | 3.4 | 1.5 | 0.9 |
| 50% of WHC | 11.7 | 9.7 | 7.8 | 3.8 | 1.4 |
| SEm ± | 0.01 | | | | |
| CD at 5% | 0.06 | | | | |

| Soil type | Interaction: Soil type X germination period (day) | | | | |
|---|---|---|---|---|---|
| | 0 | 2 | 4 | 6 | 8 |
| Sandy loam | 11.3 | 6.9 | 5.2 | 2.9 | 1.1 |
| Sandy clay loam | 11.7 | 8.9 | 6.0 | 2.5 | 1.2 |
| SEm ± | 0.02 | | | | |
| CD at 5% | 0.05 | | | | |

| Soil type | Soil moisture | Interaction: Soil type X moisture X germination period (day) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 |
| Sandy loam | 30% of WHC | 10.9 | 4.1 | 2.5 | 1.5 | 1.1 |
| | 50% of WHC | 11.7 | 9.5 | 7.8 | 4.3 | 1.2 |
| Sandy clay loam | 30% of WHC | 11.6 | 7.9 | 4.2 | 1.5 | 0.80 |
| | 50% of WHC | 11.8 | 9.8 | 7.8 | 3.4 | 1.6 |
| SEm ± | 0.0278 | | | | | |
| CD at 5% | 0.0796 | | | | | |

in sandy loam soil reduced the viable counts along with the germination period more drastically as compared to sandy clay loam soil. The maximum difference in the viable population at 30% moisture between the soils was registered on 2nd day of sowing (Table 2). At 50% moisture level the population in both the soils, though was significantly different, did not vary as much as at 30% moisture. It suggests that adverse effect of low moisture was more pronounced in sandy loam soil than sandy clay loam soil which could be due to relatively low clay content in the soil. Clay soils retain moisture for longer period and also have favaourable matrix for microbial population as compared to sandy loam soil. As reported earlier (Al-Rashidi 1982, Moawad *et al.* 2005), higher clay content support better population during desiccation.

The present study suggests that moisture and soil texture influenced the survival of inoculated *R. leguminsarum* on lentil seeds. Sandy clay loam at 50% of the soil water holding capacity supported better survival than in sandy loam soil or at 30% moisture.

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