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**RESEARCH ARTICLE**

# Effect of Different Salt Concentrations and pH on the Growth of Rhizobium Isolated from Groundnuts (*Arachis hypogaea*).

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## *Abstract*

Groundnut nodules shapes, sizes and colour inside denote that the crop is being nodulated by 4 strains. It is expected that those nodules having different shapes small round, double, triple and single large, may not have the same efficiency in fixing nitrogen. Four isolates were isolated from four different shapes of nodules and laboratory experiment was conducted to find out the effect of different salts concentration ( 0,1,2,3,4,5,and 6% w/v), various pH levels (5,6,7,8,9, and 10) and different carbon sources on the growth of these isolates. The results indicated that the four isolates have different response to salinity, acidity and growth on different carbon sources.



## Introduction:-

Rhizobia are soil bacteria characterized by their unique ability to infect root hairs of legumes and induce effective nitrogen fixing nodules to form on the roots. Rhizobia commonly occur in soils but often fail to produce effective nodulation, either because too few are present or because those present cannot work effectively with the particular legume.

The process in which the rhizobia colonize the rhizosphere, infect the roots and fix nitrogen leads to plant development and grain yield improvement (Deshwal, et al, 2013). The effectiveness of rhizobia populations in fixing nitrogen is correlated with soil fertility status where acidic soils have been reported to contain less effective rhizobia strains (Graham, et al.,1994). Legumes can grow in much degraded soils because they have the ability to fix nitrogen in association with rhizobia (Freitas, et al.,2004). Legumes provide a multiple benefits to both soil and other crops through intercropping (Ndakidemi, et al., 2011). Despite vast and potential uses of grain legumes like groundnuts, common bean and peas as human food, animal feed and soil fertility enhancer, they can be grown in different agro-ecological zones (Hendawey, and Younes, 2013). Small-scale farmers who are the major legume producers in many developing countries rarely apply fertilizers during legume production due to their low income; hence the crop is largely dependent on fixed nitrogen from native nitrogen fixers (Dakora and Keya., 1997). In most cases, native nitrogen fixers are competitive to inoculants but not efficient strain and possibly incompatible to the host plant (Wilkinson, et al., 1996). Therefore, relying on native nitrogen fixers without prior information on its efficiency and compatibility with host legume leads to crop production failure.

Rhizobia infect the roots of legumes and induce the formation of nodules in which the rhizobia fix nitrogen. Many environmental factors such as drought, acidity, alkalinity, salinity can affect the process of nitrogen fixation. Selection of indigenous and most tolerant bacteria could be more beneficial and efficient for high nitrogen fixation. It has been observed that ground nuts has different shapes of nodules which are expected to be belong to different

rhizobium species, therefore this study was carried out to examine the effect of salinity and pH stress on four isolates of rhizobium isolated from groundnuts based on the shape of nodules.

## Materials and methods:-

**Isolation, purification and preservation of the rhizobia isolates:-**

Groundnut nodules having different shapes and sizes, small round, double, triple and large round were coded GNI 1, GNI2, GNI3 and GNI4 and they were collected from root system from one groundnut plant and then a healthy and undamaged nodules were separated carefully from the groundnut roots, immersed for 10 seconds in 95% ethanol, then soaked in 0.3% sodium hypochlorite solution for 3 minutes and rinsed several times with sterilized distilled water. A few nodules from each shape were separated and crushed in 1ml sterile distilled water. Suspension from each nodule type was taken by loop and streaked onto plates of yeast extract mannitol agar (YEMA) medium (cent,1970) containing congo red. Sub-culturing was made and single colonies were selected and each isolate was re-streaked for purification (Somasegaran and Hoben. 1994). The bacteria isolates were stored on YEMA slant tubes for further investigation.

**Effect of salinity:-**

Conical flasks 250-ml containing 100 ml broth culture supplemented with various levels of NaCl (0, 1, 2, 3, 4, 5, and 6%w/v) was sterilized at 121 °C for 20 minute. The flasks were inoculated with 1ml (about $10^9$ rhizobia cells) of a four-day-old culture of the rhizobia isolated under test. The flasks were incubated at 28°C for 7 days with frequent shaking, and then rhizobia plate count was made.

**Effect of pH values:-**

Conical flasks volume 250ml containing 100ml of yeast extract broth culture were adjusted at different pH levels 5, 6, 7, 8, 9, and 10 using 1N HCl or NaOH., pH values measured at the start of the experiment. Conical flasks contained 100 ml of YEMB medium were inoculated separately with 1 ml of each isolate under test (about $10^9$ rhizobia cells/ml) as a standard inoculum. Flasks were kept in incubator with frequent shaking at 28 °C for 7 days. The rhizobia growth was determined by a plate count technique (Vincent, 1970) using YEMA with congo-red.

**Effect of different carbon sources:-**

The objective of this test was to examine the growth of Rhizobium isolates in different carbon and energy sources media. The test included sucrose, $C_{12}H_{22}O_{11}$ (Fred et al.,1932), mannitol, $C_6H_{14}O_6$ (ISI approved-YEMA), glycerol, $C_3H_8O_3$ (Arias & Martinez-Derts. 1976) and glucose, $C_6H_{12}O_6$ (Dextrose) (GPA). The treatments were arranged on RCBD with 3 replicates. Sterilized solid media from the five media mentioned above were prepared in petridishes. One ml broth culture from each isolate under test containing approximately 108 bacterial cell/ml was placed onto petridishes with 3 replications. All the petridishes were incubated for 3-5 days at 28°C with daily observations. The growth was scored by visual inspection as (+) for heavy growth and (-) for poor growth since the colonies were uncountable.

## Results and discussion:-

**Effect of salinity:-**

The number of rhizobia cells/ml showed an inverse relationship with respect to increasing NaCl concentration (0-6% w/v). All the isolates were able to withstand up to 4 % NaCl whereas 6 % affected the growth diversely. Isolates GNI3 and GNI4 in concentration 1, 2, 3, and 4 were able to tolerate salinity stress better than isolates GNI1 and GNI2 but the differences were not considerable. In both concentration 5 and 6 % the growth of all isolates were dropped down and retarded. This indicated that the groundnut isolates tested are variable in their response to higher salinity. Marked variation in salt tolerance has been found previously in *Rhizobium* and *Bradyrhizobium* strains from other species.

Growth of a number of rhizobia was inhibited by 100 mM NaCl (Yelto *et al.*,1983), while some rhizobia, e.g., *Rhizobium meliloti*, were tolerant to 300 to 700 mM NaCl (Embalomatis *et al.*,1994). Strains of *Rhizobium leguminosarum* have been reported to be tolerant to NaCl concentrations up to 350 mM NaCl in broth culture (Breedveld *et al.*,1991).Soybean and chickpea rhizobia were tolerant to 340 mM NaCl, with fast-growing strains being more tolerant than slow-growing strains (El-Sheikh and Wood. 1995). *Rhizobium* strains from *Vigna unguiculata* were tolerant to NaCl up to 5.5%, which is equivalent to about 450 mM NaCl (Mpepereki *et al.*,1997). It has been found that the slow-growing peanut rhizobia are less tolerant to than fast-growing rhizobia (Ghittoni and Bueno. 1996). Rhizobia from woody legumes also showed substantial salt tolerance: strains from *Acacia, prosopi*,

and Leucaena are tolerant to 500 to 850 mM NaCl (Lal and Khanna. 1995). Successful *Rhizobium*-legume symbiosis under salt stress requires the selection of salt-tolerant rhizobia from those indigenous to saline soils (Zahran, 1991).

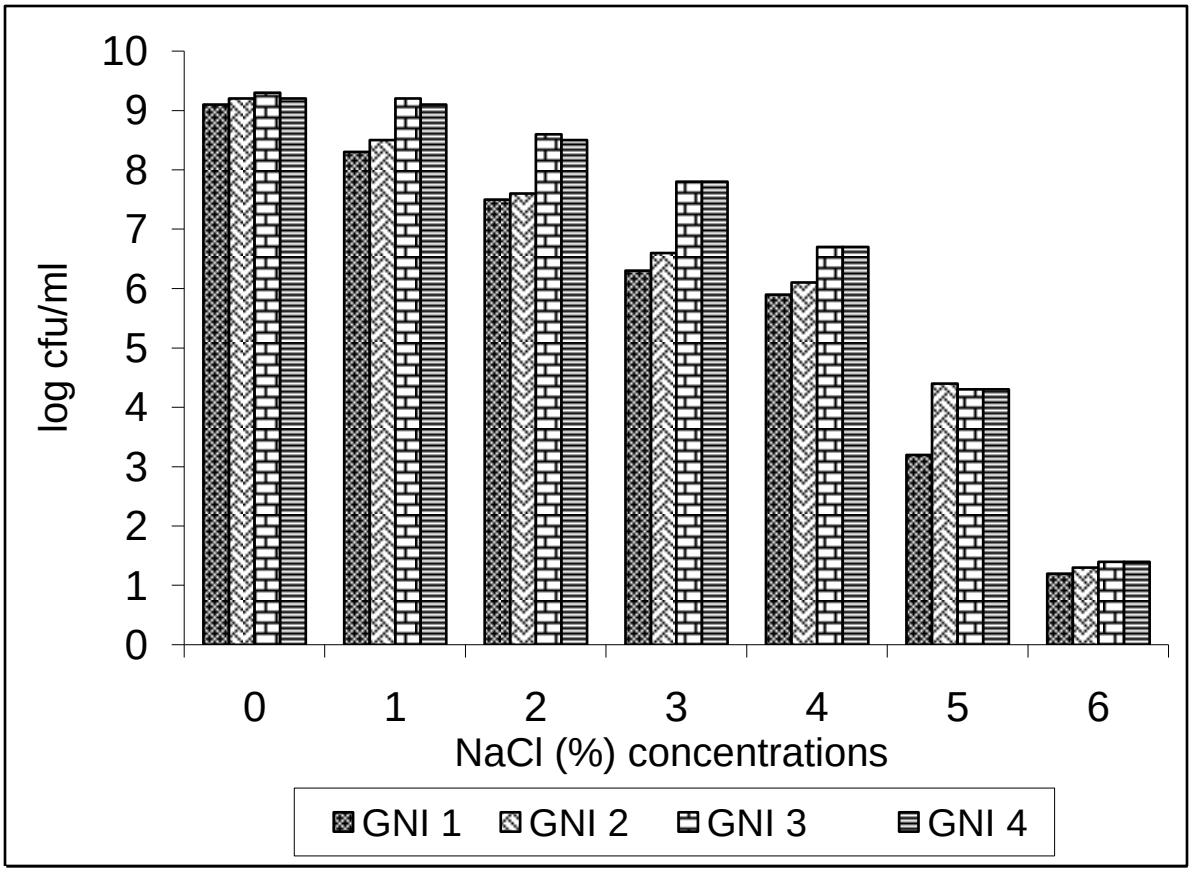


**Figure 1: Effect of NaCl concentration (%) on survival and growth of the isolates**

**Effect of pH values:-**

The results indicated that the isolates were tolerant to low and high pH since they grew over a range of pH 5 to 10 (figure 2). All groundnut isolates responded to varying pH with an optimum growth at pH 7 and 8. Isolates didn't show differences at pH 7, while at pH 8 GNI3 and GNI4 were far better than GNI1 and GNI2. There was a great inhibition in the counts of all isolates tested at pH 10 down to $10^3$ cell/ml compare to counts amounting to $10^8$ and $10^9$ at pH 8 and 9 respectively

Tolerance of rhizobium strains to high alkalinity has been observed in many previous research, Jordan, (1984) reported tolerance up to pH 9.5 for *Rhizobiaceae.* Nour *et al.,* (1994), Showed that that the pH tolerance of rhizobial strains isolated from chickpea (*Cicer arietinum*) to be 10.0, and Shenbagarthi, (1993) has reported that rhizobial strain SBS-R100 isolated from *Sesabania procumbens* was capable of growth at pH 11.

Soil acidity constrains symbiotic $N_2$ fixation in both tropical and temperate soils (Munns, 1986), limiting *Rhizobium* survival and persistence in soils and reducing nodulation (Ibekwe *et al.,*1997). *Rhizobium* with a higher tolerance to acidity has been identified (Graham, P. H *et al;* 1982). The basis for differences in pH tolerance among strains of *Rhizobium* and *Bradyrhizobium* is not clear (Correa and Barneix. 1997).

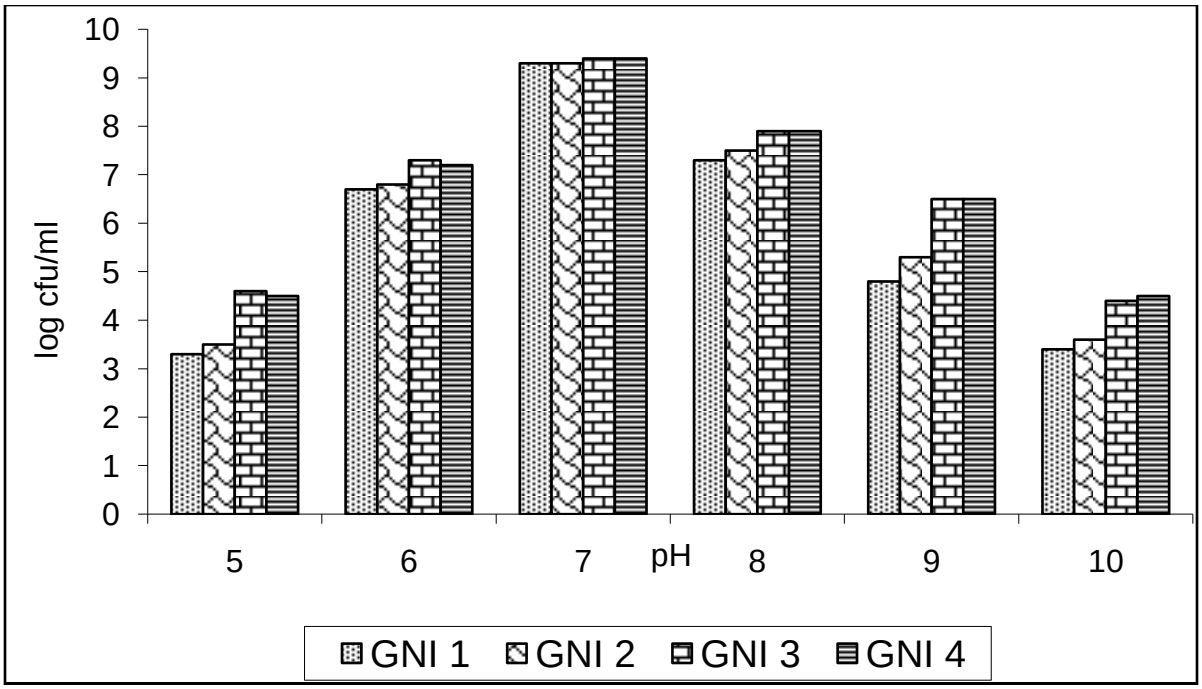


**Figure 2: Effect of different pH values on growth and survival of the isolates**

**Effect of carbon sources:-**

This table shows the growth of the isolates in media with different carbohydrates. The growth of isolates was different in all the tested carbon sources. GNI 1 and GNI 2 grew similarly well on mannitol and glycerol and poorly in sucrose and glucose. GNI 3 grew well on all tested carbon sources.

GNI 4 grew well on sucrose and mannitol and poorly in glucose and glycerol. Previous study conducted by Jordan (1984) found that the genus *Bradyrhizobium* rarely utilized sucrose, while Hamdi (1985) affirms that the slow growing rhizobia *(Bradyrhizobium)* do not metabolize this carbohydrate. In this study, all isolates showed a poor growth on glucose and sucrose in comparison with mannitol or glycerol. Generally al the isolates showed a better growth on mannitol than other carbohydrates.

**Table 1: Growth of groundnuts isolates in different media of rhizobia**

| | **Fred et al (1932)** | **ISI approved (1986)** | **(YMA)** | **Glucose Peptone Agar** | **Arias & Martinez-Drets(1976)** |
|---|---|---|---|---|---|
| **Carbon source** | Sucrose | Mannitol | Mannitol | Glucose | Glycerol |
| **GNI1** | - | + | + | - | + |
| **GNI2** | - | + | + | | + |
| **GNI3** | + | + | + | + | + |
| **GNI4** | + | + | + | - | - |

## Conclusion:-

Four rhizobia isolates of groundnuts nodules were examined under salinity stress, pH and different carbon sources. All isolates are capable of growing under different salinity concentration and various pH values, however 0-1 % salinity and neutral pH are the optimum for better bacterial growth. The four isolates have ability to grow at different sources of carbon. Further advanced techniques for identification of rhizobia isolates within one host leguminous crop are required.

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