The past study on the isolated strain with Nitrogen, Phosphorus and Potassium (NPK) fertilizer and vermicompost on nodulation, crop yield, bacterial population and nutrient status in soils of pigeon pea especially in medium black soil of Madhya Pradesh were found negligible. However, it has been tried to collect published as well as unpublished information, so that the study can be interpreted in the light of the past findings.

**Characteristics of isolated strain of Rhizobium**

Inoculated *Rhizobium* species often fails to compete with indigenous soil rhizobia and not enough to increase nodulation as these strains have to complete with native rhizosphere community for nutrients (Bromfield et al., 1986) and (Singleton and Tavares, 1986). Therefore, effective inoculants strains have been selected which are able to compete with the native *Rhizobium* and thus from a high percentage of nodulation (Hafeez et al., 1991), (Hafeez et al., 2000) and (Hafeez et al., 2001).

There are different type of legumes from various size and shapes of nodules on their roots. Although many more differences have been even found within the same species of legumes. Hence, nodules are collected when plants are in flowering stage and one can make out an effective nodule which is large in size and red in colour. Such nodules are used for separation of *Rhizobium* in the laboratory. An isolation of *Rhizobium* is made by following usual techniques. Yeast Extract Manitol Agar (YEMA) is the special medium used to get the *Rhizobium* growth. Following tests are performed in the laboratory.

i. Complete Plant cover test

ii. Hennaed Jar test
iii. Test in earthen pots.
iv. Separation of root and their testing.
v. Tissue culture test.
vi. Field experiment.

This cross inoculation groups as a new concept that is separate *Rhizobium* species only nodulate specific related legumes. For example *Bradyrhizobium Japonicum* nodulating soya bean cannot infect groundnut plants and vice versa. The cross inoculation groups have naterbigul compartment and no exceptions are found from the published information so far it is not known what are the colonies grown on a medium may not be only of *Rhizobium* they can be even of Agrobacterium for definite conclusion one has to inoculates seeds of particular sequence and wait for formation of nodules on such plants isolated colonies are of *Rhizobium* for this purpose one has to see that inoculated plants form effective nodules on roots. For further combination following tested are performed in the laboratory.

i. Growth on yeast mannitol agar.
ii. Examination under microscope
iii. Congored test
iv. Alkaline mixture test of Hoffer
v. Lactose agar test

Besides, there are several tests of nodule formation on roots of legume conducted, of which following are most important reasons for existence of cross inoculation groups in the nature.

**Affected crop by Rhizobium species**
<table>
<thead>
<tr>
<th>Group</th>
<th>Rhizobium species</th>
<th>Crop infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chanvali</td>
<td><em>Rhizobium species</em></td>
<td>Chavali, Moong,</td>
</tr>
<tr>
<td>Chickpea</td>
<td><em>Rhizobium species</em></td>
<td>Chick pea.</td>
</tr>
<tr>
<td>Peas</td>
<td><em>Rhizobium species</em></td>
<td>Peas, Masoor</td>
</tr>
<tr>
<td>Beans</td>
<td><em>Rhizobium phaseoli</em></td>
<td>All types of beans</td>
</tr>
<tr>
<td>Soyabean</td>
<td><em>BradyRhizobium taporicum</em></td>
<td>Soyabean</td>
</tr>
<tr>
<td>Alfalta</td>
<td><em>Rhizobium milileti</em></td>
<td>Methi, Alfalfa</td>
</tr>
<tr>
<td>Barseem</td>
<td><em>Rhizobioum trifoli</em></td>
<td>Barseem</td>
</tr>
<tr>
<td>Lupin</td>
<td><em>Rhizobium lupi</em></td>
<td>Lupin</td>
</tr>
</tbody>
</table>

**Table 2.1**

Above cross inoculation groups are recognized everywhere and accordingly *Rhizobium* species is selected to inoculate a particular crop.

The grouping of *Rhizobium* is based on cross inoculation which is the only well accepted and authentic classification included in Bergey's manual of determinative bacteriology.

The plant inoculated with homologous strain of rhizobia had lower phenols but higher concentration of reducing sugars in the contrast to plants inoculated with heterologus rhizobia. The biochemical changes in plant after inoculation could be responsible for differentiating the resistance of a host nodulation by a heterologus *Rhizobium* and its susceptibility to infection by homologues *Rhizobium* suggested by Rao and Iswaran (1976), Roy (1982) and Lindeman *et al.* (2007). Lange (1961) pointed out the weakness of the present
scheme of nodule bacteria. In recent years Gaur et al. (1974) have reported the existence of double promiscuity in groundnut rhizobia-symbiosis.

Promiscuity between cow pea rhizobia and \textit{R. Japonicum} has been observed by Vincent (1970). They have reported that isolated from soybean usually nodulate both soyabean and cow pea whereas most cowpea bacteria do not nodulate soybean. Graham and Parker (1964) could not differentiate strains of \textit{R. Japonicum} and \textit{Rhizobium} species using morphological, biochemical and plant infectivity tests. It was further supported by Bromfield and Roughly (1980) who observed close relationship of \textit{R. Japonicum} isolated from nodules of locally adapted soybean towards \textit{Rhizobium} species Ahmed et al. (1981) in serological examination observed greater affinity of cowpea rhizobia with \textit{R. Japonicum}. Nodulation of pea nut (\textit{Arachis hypogea}) by \textit{R. Japonicum} and vice-versa has been demonstrated by Pueppke et al. (1980).

Nodulation of pigeon pea (\textit{Cajanus cajan}) by \textit{R. japonicum} has also been reported Roughly et al. (1980), Singh and Subba Rao (1981).

The system of cross inoculation grouping of rhizobia is not free from limitations because rhizobia have been often found to cross infect or interchange between groups. However, there is no better alternative to this system. Therefore many rhizobiologists have come to regard the cross inoculation grouping as a convenient and satisfactory way to classify rhizobia in to species.

The primary prerequisite for successful establishment of legume-\textit{Rhizobium} symbiosis leading to nodulation is the entry of the lower symbiosis
into the root of the higher symbiosis, which takes place in a series of events as under :-

1. Recognition of the host.
2. Attachment and curling of root hairs.
3. Formation of infection thread and liberation of rhizobia in the root cortex.

Infection and nodule formation are host specific phenomena. It has recently been suggested that the host specificity of legume Rhizobium symbiosis may be determined by the binding of host plant lectins to characteristic carbohydrate receptor on the Rhizobium cells surface by Bohlool and Schmidt (1974), Dazzo and Hubbell (1975) and Broughton (1978).

Verma (1978), Hubell et al. (1978) and Martinez et al. (1979) have reported involvements of two cell wall hydrolyzing enzymes cellulose and pectinase. Pectinase is present in bactericides while cellulose is in the host cytoplasm. Both host and Rhizobium take part in the infection process. Thus hydrolytic enzyme produced by bacteria might allow the bacteria either to penetrate the root hair wall or perhaps produce an elongated invigilation on imagination of the root hair surface.

The Rhizobium legume symbiosis has made a vital contribution to soil fertility and productivity. It was found that the symbiotic fixation of atmospheric nitrogen by bacteria ranged from a few to a few hundred kilogram nitrogen per hectar per year according to the conditions reported by Iswaran and Apte (1970) and Iswaran (1973).
Rhizobium legume symbiosis is recognized as one of the most important nitrogen fixing system. The isolation of superior Rhizobium is very important because the effective rhizobial strains are used as inoculants to ensure effective nodulation (Moxley et al., 1986), (Asad et al., 1991), and (Shah et al., 1995). The competitive ability of an inoculants strain is a major factor determining the success of rhizobial inoculation. Inoculated Rhizobium sp. often fails to compete with indigenous soil rhizobia and do not increase nodulation as these strains have to compete with native rhizosphere community for nutrients (Bromfield et al., 1986), (Singleton & Tavares, 1986). Therefore, effective inoculant strains have been selected which are able to compete with the native rhizobia and thus form a high percentage of nodulation (Hafeez et al., 1991), (Hafeez et al., 2000), and (Hafeez et al., 2001).

Medicago sativa (alfalfa) and Trifolium subterraneum (clover) are most important forage legumes in Pakistan. Successful establishment of alfalfa and clover involve an effective symbiotic association between Rhizobium strains and compatible host plants. Inoculation with superior Rhizobium strains is required to increase the yield of legume crops through nitrogen fixation (Ather, 1988) and also to cut the input price of these fodder crops. Host plant competing rhizobial strains show their high specificity for the respective crops (Hafeez et al., 2000), (Naeem et al., 2004).

Rhizobia may be introduced to legumes by inoculation of the seed or soil. Seed may be inoculated by farmers immediately prior to sowing or custom inoculated by local seed merchants with coating facilities to be sown within a week. Alternatively, legume seed may be commercially inoculated and stored prior to its sale. This product is commonly referred to as pre-inoculated seed. However, despite a growing demand for pre-inoculated seed
since its introduction in Australia in 1971, testing in both 1972–1974 and 1999-2002 revealed poor survival of the rhizobial inoculum raising questions as to the value of this technology (Brockwell *et al.*, 1975), (Gemmell *et al.*, 2002). Alternative methods to seed coating include direct inoculation of the soil using peat inoculants suspended in water or inoculants formulated as liquids or granules (Deaker *et al.*, 2004).

The effectiveness of the bacteria depends on the inorganic or mineralizable nitrogen content of the soil the level of available phosphorous and potassium, pH, sodium salt and the presence of useable from a number of secondary nutrients (Alexander, 1977). The competitive ability of a strain appears to be intrinsic to the organism; it is greatly influenced by the environmental factor but which are not yet fully understood (Gaur and Lowther, 1982). For a large part of their existence rhizobia survive as free living bacteria before they get involved in the process of nodule formation. The environmental factors have a critical role to play during this phase of their life cycle. The factors which affect the rhizobia population and the process of nodulation have critically been reviewed by Dart (1974), Danso and Alexander (1975), Lie (1974) and Brockwell *et al.* (1982). Rhizobia is the common name given to a group of small, rod-shaped, gram negative bacteria, which collectively have the ability to produce nodules on the roots (or, in some cases, the stems) of leguminous plants.

In early studies with these organisms, it was established that no strain could nodulate all legumes, but that each could nodulate some legumes though not others. This led to the concept of cross- inoculation groups with organisms grouped according to the hosts they nodulated. For a time this was
the basis on which rhizobia was identified. Thus, rhizobia isolated from one species of clover would usually nodulate other species of clover and, were then collectively called *Rhizobium trifolii*, while rhizobia isolated from medics would also nodulate lucerne and fenugreek, and were called *R. meliloti*. This specificity is still very important in selecting the appropriate inoculants or your crop or planting, and the wrong choice of inoculants is still a common cause of nodulation failure.

Rhizobia consist of 57 species found in 12 genera. Most belong to the *Rhizobiaceae*, a probably - monophyletic group of proteobacteria within that group, however, they are scattered among several different families.

**Family of *Rhizobium* species**

<table>
<thead>
<tr>
<th>Family</th>
<th>Genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizobiaceae</td>
<td><em>Rhizobium (including Allorhizobium)</em></td>
</tr>
<tr>
<td>Brady Rhizobiaceae</td>
<td><em>Brady Rhizobium</em></td>
</tr>
<tr>
<td>Hyphomicrobiaceae</td>
<td><em>Azorhizobium, Devosia</em></td>
</tr>
<tr>
<td>Phyllobacteriaceae</td>
<td><em>Mesorhizobium, Phyllobacterium</em></td>
</tr>
<tr>
<td>Brucellaceae</td>
<td><em>Ochrobacterium</em></td>
</tr>
<tr>
<td>Methylobacteriaceae</td>
<td><em>Methylobacterium</em></td>
</tr>
<tr>
<td>Burkholderiaceae</td>
<td><em>Burkholderia, Cupriaridus</em></td>
</tr>
<tr>
<td>Oxalobacteraceae</td>
<td><em>Herbaspirillum</em></td>
</tr>
</tbody>
</table>

**Table 2.2**

Atmosphere is major reservoir of nitrogen on earth, yet only 8% of net primary production of organic matter involves nitrogen derived from
nitrogen, the remaining primary production uses nitrogen from fixed forms of nitrogen. Microbial involvement in the nitrogen cycle is of great importance. Only a small number of bacteria are able to fix atmospheric nitrogen, they must be aerobic and anaerobic, free fixers on a symbiotic basis. Almost 85% of fixed nitrogen available to plants comes from biologically fixed processes.

Intercropping provides an insurance against calamities and helps in the maximization of productivity and profit by efficient utilization of natural resources like land, light, and water. Dwivedi and Bajpai (1997) reported that groundnut (*Arachis hypogaea* L.) could be grown with crops of several rainy seasons but the most suitable crop appears to be pigeon pea (*Cajanus cajan* (L.) Millisp). It is because of its slow growth in the early stages of development.

Determinate plant type pigeon pea cultivators have been found to show low pod damage by pod fly and pod borer than those of indeterminate plant type mainly due to non-availability of undeveloped pods for a longer duration. Now day urgent need for developing determinate type high yield cultivars resistant to pod borer complex (Lateef and Reed, 1981) and (Lal, 1998).

The genus *Rhizobium*, together with *Agrobacterium* and *chromobacterium* comprise the family Rhizobiaceae (Breed et al., 1957). The six species of *Rhizobium* are recognized and are distinguished by difference in effectiveness coupled with some biochemical tests. Apart from the non-infective rhizobia, the host plant reaction still remains to be valid test in the identification of rhizobia and its differentiation from *Agrobacteria*.
Many of the biochemical and physiological characteristics like activities of catalase, oxidase, urease, and liquefaction of gelatin, indole production, and reduction of methylene blue and methyl red, production of levan, Vogespraskauer reaction and fomentation of carbohydrates were not suitable for being used to identify *Rhizobium* Graham and Parker (1964), Kleczlowska *et al.* (1968) and Vincent (1970).

The litmus milk test has been used in *Rhizobium* characterization Breed *et al.* (1957). But the long incubation times necessary, coupled with the variable result obtained make it of doubtful taxonomical value for species separation (Graham and Parker, 1964). Nevertheless, the usefulness of certain other cultural and biochemical characteristics which are reviewed below has been reported from time to time.

Rhizobia show little or no growth within 24 hours on Yeast Extract Mannitol Agar at 25 - 28°C (Kleczlowska *et al.*, 1968). Where as allied contaminants showed good growth. with the notable exceptions, strains of *R. trifolii*, *R. leguminosarum*, *R. phaseoli* and *R meliloti* are fast growing showing development of visible colonies in 3 – 5 days, whereas strains of cow pea miscellany, soybean and lupin rhizobia are slow growing and take 7-15 days to achieve comparable development (Graham and Parker, 1964).

The well isolated colonies of rhizobia appear raised, wet, shining and somewhat translucent with smooth edge. The consistency of the colonies may vary depending upon the origin or the species of *Rhizobium* (Vincent, 1970).

Norris (1965) tested 717 strains of rhizobia or growth and mannitol yeast extract medium containing bromothymol blue. Strains of *R. Trifolli*, *R.*
Leguminosarum and R. phaseoli produced acid in this media while most other strains of Rhizobium of soybean, lupin and cow pea groups produced alkalinity. Norris (1965, 1970 and 1972) proposed differences in Rhizobium evolution the basis of production of acid or alkalinity and suggested the need for further work especially with Rhizobia from tropical legumes. In the case of sesbanias rhizobia, both alkali and acid production was observed by Johnson and Allen (1952) and Norris (1965).

Except few strains from media, none of the rhizobia were found to grow in moderately alkaline (pH 10.0) medium. At pH 11.0 Agrobacteria but not media - rhizobia could grow. This characteristic was considered suitable for differentiating rhizobia from Agrobacteria (Allen and Allen, 1950). Graham and Parker (1964) observed that many of the Agrobacteriaceae also did not tolerate pH 9.5.

Pillai and Sen (1966) and Norris (1970) suggested the use of peptone - glucose solution for the isolation of root nodule organism which was subsequently found Agrobacterium radiobacteria, A. tumefactions and a few strains of media rhizobia were able to grow on the medium having concentration of 2 percent Nacl and while the other rhizobia did not grow. Several exceptions with rhizobia from Indian soils are also reported Pillai and Sen (1973).

Veal infusion broth containing peptone and salt was found to support the growth of Agrobacterium but not of Rhizobium Kleczlowska et al. (1968) proposed a modification of the above medium by replacing glucose in place of veal infusion. All rhizobia except a few strains of media rhizobia show no or
little growth whereas Agrobacteria grow well. Oken et al. (1972) showed that the two of the four strains of cicer rhizobia tested, grow well on this medium.

Graham and Parker (1964) observed that compared the growth of *Rhizobium* sp. on calcium glycerophosphate agar. Only strains of *R. Meliloti* and *Agrobacterium radio bacteria* produced a precipitate in this medium, though *R. leguminosarum* strains also grew well. Slow growing rhizobia showed poor or no growth.

Graham and Parker (1964) found that on citrate medium majority of media and a few of cowpea group rhizobia grew but not rhizobia of other groups.

The reduction of Nitrate to Nitrite by rhizobia has been reported by Allen and Allen (1950), Graham and Parker (1964) and Konde and Monis (1967). Graham and Parker (1964) reported those *Agrobacterium radio bacteria, A. tumefactions & medic rhizobia* in variable reduced Nitrate to Nitrite, while in other groups certain strains did not reduce nitrate.

Bergersen (1961) proposed a new medium and found that agrobacteria tolerated 20 m.e. mn / but rhizobia of media or clovers, which were only tested could not do so.

The primary pre-requisite for successful establishment of legume - *Rhizobium* symbiosis leading to nodulation is the entry of the lower symbiotic into the root system of the higher symbiotic, which takes place in a series of events as given below.
REVIEW OF LITERATURE

1- Recognitions of host.
2- Attachment and curling of root hair
3- Formation of infection thread and liberation of rhizobia into not cortex.

Since symbiotically fixed nitrogen is in organic form, there would be little loss and therefore would be equivalent to an effective application of 100 kg nitrogen as ammonium sulphate. Legumes play unique role in the plant kingdom and rank third amongst flowering plants in number of species they contain (Allen and Allen, 1961).

Among different legumes examined in Indian soils, excepting the species of Cassia and Glycine max, all nodulate well although they vary in extent of nodulation (Rangaswamy and Oblisamy, 1962), (Satyanarayana and Gaur, 1965) and (Sundara Rao et al., 1972).

The cross-inoculation grouping system is not perfect, since rhizobia have often been found to cross-infect between groups, Gaur et al. (1974) have reported the existence of double promiscuity in groundnut rhizobia symbiosis. Promiscuity between cow pea rhizobia and R. Japonicum has been observed by Barua and Bhaduri (1967). They reported that rhizobia isolated from Soybean usually nodulate Soybean and cowpea, whereas most cowpea rhizobia do not nodulate soybean. Nodulation of pigeonpea (Cajanus cajan) by R. japonicum has also been reported by Singh and Subba Rao (1981).

The genus Rhizobium along with the genera Agrobacterium and Chromobacterium comprise the family Rhizobiaceae (Breed et al., 1957). The six species of Rhizobium are recognized by differences in ineffectiveness associated with certain biochemical tests. Apart from the non-ineffective
rhizobia, host-legume reaction still remains to be valid test in the identification of rhizobia and its differentiation from agro bacteria.

Except few strains from medic, none of the rhizobia was found to grow immoderately alkaline (pH 10.0) medium. At pH 11.0 Agrobacteria could grow but not medic rhizobia. This characteristic was considered suitable for differentiating rhizobia from Agrobacteria (Allen and Allen, 1950).

Majority of rhizobial strains except few strain of medic rhizobia showed poor or no growth on glucose-peptone medium, whereas Agrobacteria grew well (Klaczkowska et al., 1968). However, Okon et al. (1972) reported that two of the four strains of Cicer rhizobia tested grew well on this medium.

Graham and Parker (1964) observed that Agrobacterium radiobacter, and A.tumefaciens and a few strains of medic rhizobia were able to grow on yeast extract mannitol medium containing 2% NaCl, whereas other rhizobia did not grow. They suggested this attribute as a diagnostic characteristic. Several exceptions with rhizobia from Indian soil sere also reported by Pillai and Sen (1966).

Infection and nodule formation are host specific phenomena. Legume species or cultivars which are nodulated by some rhizobial isolated are not nodulated by other isolates. It has been reported recently that host - bacterium symbiosis may be determined by the binding of host legume lectins (glycero-proteins) to characteristic carbohydrate receptors on the Rhizobium cell surface. The election is present on root their which serves as target cells for infection (Dazzo et al., 1979), (Stacey et al., 1980). But not all legume seed lections specifically recognize the corresponding rhizobial symbiont (Dzzo
and Hubbell, 1975). The accumulation and subsequent polar attachment of *Rhizobium* to legume root is likely to be one of the first steps in the required sequence of interactions leading to infection and nodulation.

Curling of root hairs after inoculation with *Rhizobium* is the first obvious response of the host. Root hair curling is caused by secretion of the bacteria. Several workers suggested the production of indol acetic acid (IAA) in the rhizosphere of legumes. Tryptophan produced in the roots of legumes Rovira (1959), Vasantha and Subba Rao (1965) is converted into IAA by rhizobia Beltra *et al.* (1980).

A mature nodule consists of a central "bacteroid zone", which is surrounded by the nodule cortex. This zone is made up of host cells containing 'bacteroids' enclosed in membrane envelopes of host origin. The volume and number of bacteroids have a direct relationship with the nitrogen fixation (Chopra and Subba Rao, 1967)

Simultaneous with the formation of bacteroids, red pigment haemoglobin accumulates between the bacteroids and the membranous envelopes. This pigment is known as 'leghaemoglobin', the prefix 'leg' indicating its presence in leguminous root nodule. The globulin is produced by the plant, whereas the is produced by the bacterium. Leghaemoglobin is only present in infected cells and its location is confined to the host cytoplasm. The amount of leghaemoglobin and bacteroid production has direct relationship with the amount of nitrogen fixed by legumes (Chopra and Subba Rao, 1967), (Tilak and Saxena, 1976).
The difference in nitrogen fixation due to the varietal differences has been reported by Burton (1967). Due to strain specificity only a few percent of the infected root hairs may give rise to nodules.

It is not uncommon to find that rhizobial strains of approximately equal efficiency as judged by nitrogen fixation in green-house tests may vary greatly in ability to enhance growth or improve yields under field conditions. One of the factors responsible for this effect is the variation in the ability of rhizobial strains to endure antagonistic micro flora (Burton, 1964). The aerobic spore-forming bacteria and the Streptomycetes are the most active antagonists to the rhizobia (Allen and Allen, 1961).

It has been reported by several workers that the presence of organic matter in soil had a favorable influence on the number of rhizobia. Heavy nodulation of certain pulses in soil enriched with charcoal was also reported by Iswaram et al. (1979) and Singh and Tilak (1977). Some workers, however, have pointed out that activated vermiculite or phosphorus and potassium fertilizers are better than any of the organic materials. However, Dev and Tilak (1976) reported that farmyard manure proved better than organic cakes like neem-cake, mahua-cake, and alsi-cake for the nodulation of legumes.

The prime objective of legume inoculants manufacture is to provide highly efficient Rhizobium culture. Attempts have been made by several workers to correlate efficiency with (i) cultural and physiological characteristics, (ii) antigen structure Date et al. (1965), (iii) mutations Jordan (1962), (iv) phage sensitivity Schwinghamer (1964), and (v) sensitivity towards antibiotic action Graham (1963), Schwinghamer (1964). But the most ideal way of determining efficiency is to conduct growth tests in Leonard jars,
pot culture and field evaluation. With the knowledge of cross-inoculation group, it has become easier to select the efficient strains and has also been possible to produce composite culture.

The nitrogen fixing ability of a rhizobial strain can be determined by growing plants in sand cultures devoid of nitrogen source. Under these circumstances dry matter and protein contents of plants are directly related to the ability of rhizobia to provide nitrogen. However, several workers observed that strains showing equal efficiency under the laboratory conditions may show marked differences in ability to nodulate under field conditions. Burton (1967) suggested the following reasons for it:

i. Differences of strains related to soil fertility level and pH of the soil.

ii. Inherent variations in rhizobial efficiency that come in the view point under the nitrogen stress provided by the plant.

iii. Variations in the ability of the nodulating bacteria to resist the antagonistic micro flora.

iv. Differences in competitive qualities of the rhizobial strains.

In India, many workers have reported the response to sulphur application in pigeon pea Tandon (1986). Research conducted at Rachi during rainy season revealed significant interaction effect between the levels of phosphorus and sulphur on the seed and stalk yield of pigeon pea. Maximum seed (1590 kilogram per hectare) and stalk (7200 kilogram per hectare) yields of pigeon pea were obtained at 26.2 kilogram phosphorus along with 20 kilogram sulohur per hectare Srivastava and Srivastava (1993). Further, studies conducted by Singh and Masood Ali (1994) at Kanpur indicated a significant response of pigeon pea to applied sulphur up to 40 kilogram per
hectare. Oke (1969) revealed significant improvement in the growth and nodulation of pigeon pea due to sulphur application in pot culture experiment. At ICRISAT, Hyderabad, omission of sulphur in a pot culture experiment caused drastic reduction in the growth of a short duration pigeon pea genotype (ICP-87), which exhibited classic sulphur deficiency symptoms (unpublished). Therefore, the present investigation was carried out to study the effect of different sources and levels of sulphur on the seed yield of pigeon pea Pujari et al. (1998).

Nodulation

Sarkar et al. (1993) reported higher number of nodules plant-1 with Rhizobium inoculation. Among the different fertilizer treatments the highest number of nodules plant-1 was observed 24.10, 48.70 and 31.69 at 30, 45 and 60 DAS respectively in the crops grown with basal application of recommended dose of NPK closely followed by vermicompost (60 kg P2O5/ha) treatment. The lowest nodules number plant-1 was recorded in untreated control. Maximum number of nodule plant-1 of 28.53, 42.59 and 36.50 at 30, 45 and 60 DAS respectively were recorded in the plants grown with the treatment combination inoculation and basal application of NPK (recommended dose) while the corresponding lowest numbers, (17.75, 40.84 and 23.90 at 30, 45 and 60 DAS respectively) were obtained with the treatment combination of untreated control plot. In general, number of nodules plant-1 increased up to first 45 DAS and thereafter started decreasing, irrespective of all the treatments. This is mainly due to the reduction in nutrient demand of the crop as it steps forward towards maturity.
Residual fertility status of soil: Use of manures and fertilizers is the kingpin of our strategy of achieving the enhanced productivity and assured sustainability. In this strategy one aspect, which has been neglected, in part is the balance use of fertilizers. The combined application of sulphur and phosphorus has significantly influenced the residual fertility status of soil (Kene et al., 1990).

Gupta and Namdeo (1999) reported that application of 50% RDF with organic manure, *Rhizobium* and PSB significantly increases the symbiotic traits over control in pigeon pea crop.

Rajkhowa et al. (2000) reported that the application of nitrogen through vermin compost produced the highest nodule and dry weight per plant and was at par with the treatment that that received 75 per cent N through urea along with 5t/ha vermin compost. The highest root weight per plant was recorded with application of N as vermin compost followed by FYM in green gram over control.

Tiwari et al. (2000) reported that the beneficial effect of applied phosphatic fertilizers along with *Rhizobium* inoculation was reflected in effective nodules and number and dry weight of nodules. The number of nodules at 45 days after sowing (DAS) increased from 10-18 nodules/plants with *Rhizobium* inoculation. Dry weight of nodules also significantly increased with phosphorus fertilizer and *Rhizobium* inoculation.

Tarafdar and Rao (2001) reported that inoculation of cluster bean with *Rhizobium* and AMF coupled with the application of FYM significantly improved nodule number and nitrogen age activity. Inoculation with
Rhizobium or AMF increased nodule number significantly and their combination in the presence of FYM was the most effective (86%) among different treatments.

Gupta and Namdeo (2001) conducted an experiment and found that the data on mean nodule number, nodule dry weight and grain yield showed that all Rhizobium strains under test could significantly increase the nodule number by 3.0 to 4.2 folds, nodule dry weight by 2.5 to 3.3 folds and grain yield by 17.5 to 24.2% over uninoculated.

Chinnaswamy and Dhar (2005) Nodulation and nitrogenous activity were observed only in Bradyrhizobium and co-inoculated treatments. Among PGPR strains, KB 133 exhibited maximum increase on root mass and total plant dry weight. Co-inoculation of KB 133 with Bradyrhizobium strain ASR 011 showed significant superior performance on nodulation, nitrogenase activity and total N content per plant. Symbiotic efficiency of strain ASR-11 along with PUR171 and USDA 123 with K 133 increased significantly compared to either Bradyrhizobium or PGPR strain inoculation. Studies suggest that PGPR strains not only influences root growth, but also show compatible synergistic effect with B. japonicum strain on nodulation and nitrogen fixation in soybean.

Gupta (2005) found that 50% RDF+5t/ha organic manures + Rhizobium inoculation + PSB inoculation recorded the highest nodule number/plant (28) and nodule dry weight (42 milligram per plant) in pigeon pea crop.

Rahul et al. (2007) studied the effect of biofertilizer and sulfur levels on the growth and yield of black gram (Vigna mungo) Cv. Type 9. The results
revealed that application of sulfur at 20 kg/ha + dual inoculation with Rhizobium and PSB significantly increased the growth characters (Plant height, nodules and dry weight) and grain and straw yields of black gram.

Jain (2008) conducted an experiment at Gwalior and reported that application of *Rhizobium* and PSB as co-inoculants with and without different sources of organic increased the nodule number, nodules dry weight and root dry weight of chickpea. The symbiotic traits (nodule number, nodules dry weight and root dry weight) at 55 DAS was higher than 35 DAS.

Choudhary *et al.* (2013) reported that application of RDF – NPK + *Rhizobium* + VAM + PSB in groundnut recorded highest (19.23) effective nodules/plant followed by Vermiconpost 1.25 t/ha + PM 1.25 t/ha + *Rhizobium* + VAM Vascular Arbuscular Mycorrhizee + PSB phosphate solubilizing bacteria (17.55).

**Crop yield**

Gupta and Namdeo (1999) reported that application of 50% RDF with organic manure, *Rhizobium* and PSB significantly increases the seed yield over control in pigeon pea crop.

Dubey (2000) reported that yield of crop increased significantly due to *Rhizobium* inoculation.

Khoja *et al.* (2002) observed that seed inoculation with *Rhizobium* + PSB significantly increased the plant height, number of branches and dry matter accumulation per plant as well as pods/ plant, seed/ pod and test weight, grain and straw yields over un inoculation treatments.
Lakpale et al. (2003) reported that the application of 2.5 tones of FYM per hactare and fertilizer resulted in significantly higher number of branches/plant, pods/plant, seeds/pod and 100- seed weight and seed yield of grain than the no application of FYM.

Zaidi et al. (2003) the interactive effect of rhizotrophic micro organisms on the yield and nutrient uptake of chickpea plant and soil was determined in a sandy clay loam soil, deficient in available phosphorus (P). Plant yield and nutrient uptake significantly enhanced as a result of inoculation with Rhizobium sp. and phosphate solubilizing micro organism (PSM). Pseudomonas strata or penicillium sp. were variable. In addition, the available P status of the soil improved by the addition of P. straita with Rhizobium sp. and AM fungus. The nitrogen content of the soil did not showed appreciable changes after the inoculation.

Gupta (2005) conducted an experiment at Sehore and found that application of 50% recommended dose of fertilizer + PDS compost + Rhizobium + PSB recorded significantly higher yield of pigeon pea and also the available N, P and organic carbon in soils as against the existing practices of recommended dose of fertilizer + Rhizobium + PSB. Net profit was also higher in this treatment which was greater by Rs. 1682/ha compared with the existing practices.

Basu et al. (2006) reported that pod yield of groundnut was the highest for inoculation with Rhizobium which was about 7.04 and 11.06% higher than that of inoculation with phosphorbacterium and control, respectively. Higher uptake of nitrogen and phosphorus was noted under inoculation with
Rhizobium. Likewise, inoculation with Rhizobium and phosphobacterium had about 47.90 and 28.6% higher K uptake as compared to no inoculation.

Gupta (2006) reported that Rhizobium + PSB + Azotobacter inoculation recorded maximum crude protein content, N and P uptake followed by Rhizobium + phosphate solubilizing bacteria (PSB). Highest balance of available N and P was recorded under treatment Rhizobium + PSB + Azotobacter over control and PSB alone. Likewise, balance sheet of nutrients, highest economic viability was recorded with Rhizobium + PSB + Azotobacter inoculation.

Kumar et al. (2007) reported that maximum nitrogen uptake was noted with dual inoculation like Rhizobium and PSB which was significantly higher to single inoculation of Rhizobium and PSB. There was increase in P and K uptake of legume crop with dual inoculation. The P and K uptake due to dual inoculation was significantly higher than Rhizobium and PSB. It showed synergistic effect of both the inoculants on P and K uptake by legumes.

Jain (2008) reported that application of Rhizobium and PSB as co-inoculant with and without different sources of organic increased the yield attributing characters, yield and uptake of N, P and K in chickpea.

Sharma et al. (2010) revealed that application of RDF + 15 kg ZnSo4 significantly higher number of pods per plant, number of seeds per pod, 100-seed weight and seed yield of pigeonpea (13.78 q ha-1) followed by RDF + 25 kg ZnSo4 (13.53 q ha-1) and RDF + seed treatment with sodium molybdenum @ 4 g kg-1 (12.42 q ha-1) as compared to control (7.78 q ha-1). Ramesh et al. (2006) conducted a field experiment at Bhopal applying different organic
manures (cattle dung 4 t ha$^{-1}$, vermicompost 3 t ha$^{-1}$ and poultry manure 2 t ha$^{-1}$) to pigeonpea and reported that the highest protein content in seed was recorded with the application of cattle dung (21.25%) followed by vermicompost (20.90%) and poultry manure (20.87%).

Choudhary et al. (2013) conducted an experiment at Jobner and reported that application of RDF – NPK + *Rhizobium* + Vascular Arbescular Mycorrhizee (VAM) + phosphate solubilizing bacteria (PSB) in groundnut recorded highest haulm yield (46.56 q/ha) followed by Vermiconpost 1.25 ton per hector + PM 1.25 per hector + *Rhizobium* + VAM + PSB (38.70 quintal per hector).

**Rhizobium, vermicompost and nutrients on soil available nutrients**

Gupta and Namdeo (1999) reported that application of 50% RDF with organic manure, *Rhizobium* and phosphate solubilizing bacteria (PSB) significantly increases the available N, P, and K in soil over control in pigeonpea crop.

Tolanur and Badanur (2003) the effect of integrated use of organic manure, green manure and fertilizer N on nutrients status of soil and productivity of *rabi* sorghum, chick pea system was studied in a field experiment conducted during 1996-97 with various treatment combination including FYM, compost, vermin compost, green manure and fertilizer N. The differences in organic carbon, available N, P and K were significantly influenced. The organic carbon and available N status declined with application of fertilizer N alone and increased with conjunctive use of fertilizer N and organic manure. The combined application of organic and
inorganic N sustained the productivity. Soil available nutrients like N, P and K increased significantly with the application of various organic sources of nutrients in combination with fertilizer over the fertilizer alone. The highest grain yield of *rabi* sorghum and chickpea was obtained with 50 per cent N through green manure plus 50 per cent fertilizer N.

Gajalakshmi and Abbasi (2004) conducted four species of deteritivorous (humus-former) earthworms were tested for their ability to vermicompost paper waste blended with cowdung in 6:1 (w/w) ratio. The species used were *E. eugeniae, P. excavates, L. mauritii* and *Drawida willsi* Michaelsen.

Gupta (2005) conducted an experiment at Sehore and found that application of 50% recommended dose of fertilizer + PDS compost + *Rhizobium* + PSB recorded available N, P and organic carbon in soils as against the existing practices of recommended dose of fertilizer + *Rhizobium* + phosphate solubilizing bacteria (PSB). Net profit was also higher in this treatment which was greater by Rs. 1682 per hectar compared with the existing practices.

Sharma (2007) carried out an experiment at Gwalior and found that higher consumption of nutrients from soil which rescue the lower amount of nutrients in soil. On the other hand lower consumption of nutrients from soil which rescue the higher amount of nutrients in soil.

Jain (2008) found that application of *Rhizobium* and phosphate solubilizing bacteria (PSB) as co-inoculants with and without different sources of organic increased the available nutrients and balance sheet of nutrients in soil and economic viability of chickpea.
Rhizobium population

Pal (2002), observed that the seed inoculation of crops significantly increased the MPN count of PSB both in rhizosphere and non-rhizosphere soils of crops. The same was also tune in of total count of bacteria, fungi and actinomycetes. The effect was highest in legumes followed by pseudo-cereals and cereals.

Singh and Tarafdar (2002) reported that the *Rhizobium* inoculation significantly influenced total bacteria as well as actinomycetes population besides the improvement in *Rhizobium* population. The fungi and Azotobacter population remained unaffected due to *Rhizobium* inoculation.

Prasad and Chandra (2003) observed that *Rhizobium* sp. Indicated highest population at 6 days while PSB and PGPR showed their maximum population at 3 days. Co-culturing of phosphate solubilizing bacteria (PSB) or/and PGPR with *Rhizobium* sp. favored the growth of *Rhizobium* sp. at both 3 and 6 days, highest (2.1x10^{12} gram) being at 6 days with phosphate solubilizing bacteria (PSB).

Jain (2008) conducted an experiment and reported that application of *Rhizobium* and phosphate solubilizing bacteria (PSB) as co-inoculants with and without different sources of organic increased the microbes’ population in soil.

Dalal and Nandkar (2010) observed that *Rhizobium* sp. and *Azotobacter chroococcum*. Phosphorus can be made soluble in boil by phosphate solubalizing bacteria (Pseudomonas striate). They possess ability to bring insoluble phosphate in to soluble from by secreting organic acids. NPK is most
important elements in controlling the normal growth and yield of crops including Pigeon pea. Generally the crops need recommended doses of 25kg P: 50 kg/ha and to get the 1.2 a tonnes/ha yield, the crop plant required 8.5, 8.0 and 16kg NPK/ha respectively.

Singh et al. (2013) observed that *Rhizobium* spp. are gram-negative soil bacteria that have a profound scientific and agronomic significance due to their ability to establish nitrogen-fixing symbiosis with leguminous plants, which is of major importance to the maintenance of soil fertility. For this reason and taking into consideration the importance of legumes in animal and human consumption, some attention has been given to the effects that heavy metals exert on *Rhizobium* isolates as free-living organisms or symbiotically associated with legumes.