GROWTH AND LONGEVITY OF RHIZOBIAL STRAINS IN DIFFERENT BIPHASIC CARRIERS

Thesis
Submitted in
Partial Fulfilment of the
Requirements for the Degree of
MASTER OF SCIENCE IN AGRICULTURE
(SOIL SCIENCE AND AGRICULTURAL CHEMISTRY)

By
MANINDER SINGH JOHAR

DEPARTMENT OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY
JAWAHARLAL NEHRU KRISHI VISHWA VIDYALAYA
JABALPUR (M. P.)

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J.N.Dube,
M.Sc., Ph.D.,
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Jawaharlal Nehru Krishi Vishwa Vidyalaya,
JABALPUR-4 (M.P.)

CERTIFICATE

I hereby certify that this thesis embodies a record of bonafide research work done by SHRI MANINDER SINGH JOHAR, on the problem entitled "GROWTH AND LONGEVITY OF RHIZOBIAL STRAINS IN DIFFERENT BIPHASIC CARRIERS" under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma. All the assistance and help received during the course of investigation and source of literature have been duly acknowledged by him.

Jabalpur, Dated 30-6-1971

(J.N.DUBE)
Chairman
Advisory Committee

APPROVED BY :

Members of Advisory Committee:

1. Dr. J.N.Dube
2. Dr. C.L.Sanoria
3. Dr. L.K.Joshi

External Examiner

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M. S. Johar

Dated: 30-6-71 (M.S.JOHAR)

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Dedicated to my beloved father

SARDAR RAJINDER SINGH JOHAR

C O N T E N T S

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INTRODUCTION

INTRODUCTION

Rhizobial cultures survive very well in a culture collection but rank correlation coefficients for age of strain and decreasing nitrogen fixing ability (Means and Erdman, 1963) differed very much. From the culture collection to the production of legume inoculants is in essence a technological step as described by Burton (1969). Biphasic system of solid and liquid carrier has been found by Australian and American workers to be very effecient with respect to growth and longevity of rhizobial strains and peat is rated an ideal solid providing favourable conditions for the organisms to multiply and survive for ample time. There is a burning evidence for the view point that the shelf life of rhizobium culture can be prolonged provided the inoculants are preserved at low temperatures. Nitragin Co. inoculant which was over a year under cold storage exhibited an excellent performance in increasing the yield of soybean (Dube, 1971). The carrier of this inoculant is peat which contains high organic matter and substantial quantities of inorganic material to support the growth of rhizobia.

Peat of high organic matter content is not available in India, which warrants the microbiologists to search the substitute for peat. Indigenous materials easily available in the country were employed to study their suitability to act as a carrier, which may not only support rhizobia for their survival but may also prolong their effective existence in the prepared inoculant. Four primary materials viz. peat (Indian), compost, lignite and teak leaf meal were selected for study, taking peat (USA) as standard for the production of the inoculant. The survival pattern of serologically different proven strains of Rhizobium japonicum, the shelf life, physicochemical characteristics and the efficiency exhibited by each carrier have been investigated under laboratory and field conditions.

Effect of sunlight is another environmental variable that governs not only the market life of the inoculant itself but also the survival of rhizobia as an epiphytotic on legume seed. This important variable has a provision of UV light and so has been examined in greater detail to find performance of inoculant in a pragmatic situation wherein the inoculated seeds are often liable to get spontaneous or otherwise exposure to insolation.

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REVIEW OF LITERATURE

REVIEW OF LITERATURE

General :

The rhizobia are soil bacteria of simple morphology and are easily cultured. There is nothing of greater interest in the effect of these bacteria when free living in soils or in the rhizosphere of plants. The interest in the genus Rhizobium lies, above all, in its ability to form a symbiotic association with legumes in which nitrogen is fixed, this latter fact being of greater agronomic importance. The problems (Dixon, 1969) encountered in the study of the interaction of the bacteria with their hosts and the production of nodules are sufficiently fascinating to encourage research even in the absence of any economic return.

Legume Inoculants-Technology and Microbiology :

Exploitation of the symbiotic association for the benefit of crop production involves the application of microbiology in soil science, and this exercise is as complex a problem as any other in the realm of applied microbiology. From the strain selection to production of legume inoculant on pilot

or commercial scale a number of technological and microbiological problems are in vogue. A few are solved and a few await solution.

To inoculate the seeds with rhizobium, various methods have been developed from time to time, and various carriers have been used. A sufficiently large number of bacterial cells should be present on the seed to induce nodulation. Though leguminous seeds are the habitats for rhizobia, but quantitative studies by Vincent (1958, 1965) on survival of rhizobia on seed showed that seed provides a poor substrate for sustaining them. This fact was known quite early since Thornton (1919). Inoculation of seeds with broth culture reduces the bacterial number on seed (Burton, 1965; Dowson, 1967; Thompson, 1961). Thompson (1960, 1961) and Bowen (1961) showed that seed coat of certain leguminous crops contains some water soluble antibiotic which is particularly effective against rhizobia.

To overcome this problem, seed pelleting with inert materials like gum arabica, molasses, milk, and $CaCO_3$ were used but they were not quite effective.

Biphasic System for Inoculants:

To overcome this problem, then various types of "carrier" are developed. These carriers serve as storage media for rhizobia, as well as seed pelleting material, thus protecting the bacteria from coming in direct contact with seed testa, which have some antibiotic materials. Thornton (1929) used skim milk containing 0.1% calcium phosphate, rhizobium culture was added to it and the suspension was then spread uniformly on seeds and mixed well. The inoculated seeds were dried for a few hours before planting. This material though was better than broth culture, but was not much effective. Jensen (1961) found that Rhizobium meliloti survived well in air dried garden soil to which 0.6% mannitol and some calcium carbonate had been added. Norris (1963) found that rhizobium could be stored for long time on unglazed procelain beads dried over silica gel. Fraser (1966) stated, "for preinoculation to be successful, sufficient rhizobia must survive storage to provide a nucleus for rapid multiplication in the presence of germinating seed."

She further states,

> "It was thought that initial inoculum carried on dry substrate with nutrients present might meet these requirements and various substances were considered as carrier."

Finally milled peat is a commonly used carrier for rhizobia for seed inoculation. Peat based inoculum promotes better survival of rhizobia on seed surface than either cells suspended broth or freeze dried cultures (Vincent, 1965). Burton (1965) found that when peat based inoculum was used in comparison to liquid base inoculant for seed inoculation, both were equally effective when the seeds were planted within one day after inoculation, but as this period increased, the effectiveness of liquid base inoculant decreased. Radcliffe et al. (1967) also found similar results in case of Rhizobium trifolii carried in peat, in comparison to soil. Fraser (1966) used plaster of paris granules with good success. Compost medium has been used by Indonesian microbiologist (Jutono (1968). He found that after one week incubation, the medium contained 7.5×10^8 viable cells of rhizobium. The advantages of this medium were that

it was cheap and required no special implementation for its prepartion.

Growth and Longevity of Rhizobia under Environmental Conditions, Temperature, Moisture and Oxygen Tension :

Rhizobia are soil bacteria which utilise materials secreted into the soil by plant roots and also possibly by the other microflora associated with the plant rhizosphere. The extent to which rhizobia may survive in soils in the absence of legume hosts depends upon the soil conditions, the strain of rhizobium and the kinds of plants which are growing in the particular soil. The complexity of the system wherein many factors interact, has made it difficult to decide which factors affecting the survival of rhizobia in natural soils are critical.These difficulties are compounded by the fact that under identical conditions different strains of rhizobia can be found to react differently. As with so much else in the study of rhizobia, no generalisation may be made. Many experiments of agronomic importance have been done which have shown the degree of survival of rhizobia under laboratory conditions. These experiments are reviewed by Vincent (1958). Later work showed that vegetative cells of rhizobia may survive

in autoclaved soil for as long as 30 to 45 years when provided with mannitol (Jensen, 1961). Growth and survival of rhizobium in a carrier is greatly determined by various factors, such as temperature, moisture content, aeration, nutritional status, and the nature of the carrier itself. The influence of temperature on rhizobia in peat culture has been studied by Spencer and Newton (1953) with culture in screwcapped jars, by Gunning and Jordan (1954) using heated peat. Fred, Baldwin and McCoy (1932) quoted early reports of loss of viability of rhizobia on the surface of inoculated seeds due to the effect of temperature to which they were exposed. All these workers concluded that low temperatures restrict the multiplication but improve the survival during storage. Studies by Vincent (1958) indicated that after 45 days of storage, highest counts were obtained at 5°C and lowest at 37°C and thus confirmed the earlier findings. Temperature and desiccation affect the survival of rhizobia in soils. In study of these factors, Marshall (1964) found that Rhizobium trifolii and Rhizobium meliloti were susceptible to high soil temperatures in light soils although they were able to survive at the same temperatures in soils of higher texture. Rhizobium lupini and Rhizobium japonicum were comparatively

resistant to the high temperatures at which the two former species failed to survive. The temperature sensitivity of Rhizobium meliloti and the survival of native rhizobia capable of nodulating Acacia, Lotus, and Psoralea species, had been attributed to the adaptation of the native tropical rhizobia to the high temperatures (Wilkins, 1967). Effect of desiccation of peat cultures on the survival of rhizobia was studied by Hedlin and Newton (1948) and Vincent et al (1954). These workers found that high moisture content adversely affected the survival of Rhizobium meliloti. According to Roughley and Vincent (1967) too little moisture (30%) and as much as (60%) were generally deleterious to the survival of rhizobia. Studies carried out on humus carrier by Hedlin and Newton (1948) indicate that the viability of nodulating bacteria was affected by moisture content of medium in which they were present. Rhizobia did not survive in dried out humus cultures. van Schreven et al (1954) also showed that the growth and viability of rhizobia in soil peat mixture was injured by too low and too high moisture content.

Gaseous exchange of the culture is another important factor which determines the growth multiplication of bacteria in carrier. There are few conflicting

reports on the effect of aeration in peat culture. Heldin and Newton (1948) found that rhizobia grew better in containers allowing free exchange of gases, whereas Newbould (1951), Spencer and Newton (1953) and Gunning and Jordan (1954) found satisfactory results in either screw capped jars or sealed cans. According to Hedlin and Newton (1948), in restricted aeration only pure cultures grew better than contaminated ones, but in contrast to those, Newbould (1951) found a count of Rhizobium meliloti as higher as 58 x 10^6/g of nonsterile peat culture after four months of storage in sealed cans.

Longevity of Rhizobia under Radiation:

There are no extensive data available which can show the effect of radiation on survival of rhizobia in carrier, except solar radiation. The antiseptic and germicidal properties of sunlight are well known. Alexander and Chamblee (1965) have demonstrated detrimental effect of exposing inoculated trefoil seeds to 16 hours of sunlight on both a moist and dry soil, which implies a rhizobial species by irradiation interaction. Further they observed differential susceptibility amongst rhizobia to insolation that resulted in ineffective inoculation.

According to Fred et al (1932), sunlight is harmful to rhizobium organisms. No information is available regarding the effect of other types of radiations on rhizobium survival in carriers. Radiations may affect adversely in a indirect way such as by increasing the temperature.

Nature of carriers plays some important role in survival of rhizobia. According to Dart et al(1969), the success of peat as a good carrier is due to the structure of its particles, organic matter and amorphous nature which favour both growth and survival of rhizobium under adverse conditions.

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MATERIALS AND METHODS

MATERIALS AND METHODS

Field Experiment :

An experiment was laid out during kharif, 1970 at Livestock Farm, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur under the Soybean Research Project with an objective to study the suitability of different indigenous carriers for the production of soybean inoculants.[a]

Four serologically different and proven strains from Culture Collection of Agricultural Microorganisms Jawaharlal Nehru Agricultural University (CCAM-JNAU), (S 176, S 177, S 179 and S 188) were selected for the study.

The strains were grown in yeast extract mannitol (YEM) broth (Appendix Table - I) at 25°C for four days, and the broths were used for the inoculum production in different carriers according to the method of Burton (1967).

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a - All India Coordinated Research Project on Soybean Fourth Workshop Conference held at U.P.Agricultural University, Pantnagar, February, 1971. Report presented by J.N.Dube.

Five indigenous materials such as Indian peat, lignite, mixture of lignite and teak (9:1), compost, teak leaf meal were selected using American peat as a standard. Besides these treatments, one positive control (Nitragin Co. inoculant,1970) and one negative control (no inoculum) were also included in order to have the comparative picture of the performance of the legume inoculants produced in different carriers. The pH of each of these carriers was adjusted to 7.00 by the addition of calcium carbonate. The composition of the carriers is given in Appendix Table-II. The modified carriers were autoclaved separately for 12 hours at 0.7 kg cm^{-2} so as to make the solid carriers free from microbes.

The separately produced broth cultures of four single strains were tested according to the method of Date (1969), (Table - 1).

The broths were mixed manually in sterile carriers on 40% moisture basis. The solid cultures were then subjected to minimal drying at 24°C for 24 hours. The final product was packed in polythene bags. Four to five fine punctures were done in the bags in order to facilitate oxygenation. The individual solid cultures of different strains were

mixed togather so as to formulate a composite culture of four strains. This inoculant was branded as JNKVV Composite Culture.

A replicated field trial on Bragg Soybean was laid out in simple randomized block design. Besides the bean yield the uptake of nitrogen was studied for which 10 soybean plants were randomly sampled from each plot after 35 and 55 days of planting. Nodules borne on the root crown of the plants were counted in order to assess the total nodule number per plant. The nodules from five plants were seperated out, oven dried at 80°C for 24 hours, and the dry nodule weight so obtained was recorded. The plant and seed nitrogen was determined with the conventional micro-Kjeldahl procedure.

Laboratory Studies :

1. Carriers, finished and unfinished:

Indigenous carriers under study belonged to two groups, finished and unfinished. To first group belonged the existing carriers like peat, compost and lignite and to the other the teak leaf meal. The unfinished carriers were subjected to humification process and so inoculated with the following humus forming microflora:

Metarrhizium brunneum, Helminthosporium geniculatum, Humicola grisea,(obtained from Commonwealth Mycological Institute, Kew, Surrey, England),Thermomonospora viridis, Strentomyces violaceous rubar (from Institute of Microbiology, Rutgers State University New Brunswick, NJ USA). The material was inverted at monthly intervals to facilitate through decomposition, and the decomposition was permitted to progress upto twelve months.

As the particle size of a carrier is of primary importance with respect to moisture retention capacity, both types of products were subjected to sieve analysis of mesh 50, 100 and 200. In composted teak leaf meal the size of the particles reflected grossly the degree of decomposition by particular organism. The moisture holding capacity and apparent density of each carrier were also determined.

2. Chemical Composition:

Indigenous carriers under study were analysed for pH; (Piper,1950); total nitrogen (microKjeldahl's); total phosphorus (Koeing and Johnson method) as described by Jackson,1965; total potassium by Flame photometrically, as described by Black (1965) available N, P and K (Subbiah and Asija, 1956),

Bray and Kurtz (1945); Hanway and Heidal (1952); Organic matter and ash content (Jackson, 1965) and cation exchange capacity (Piper, 1950).

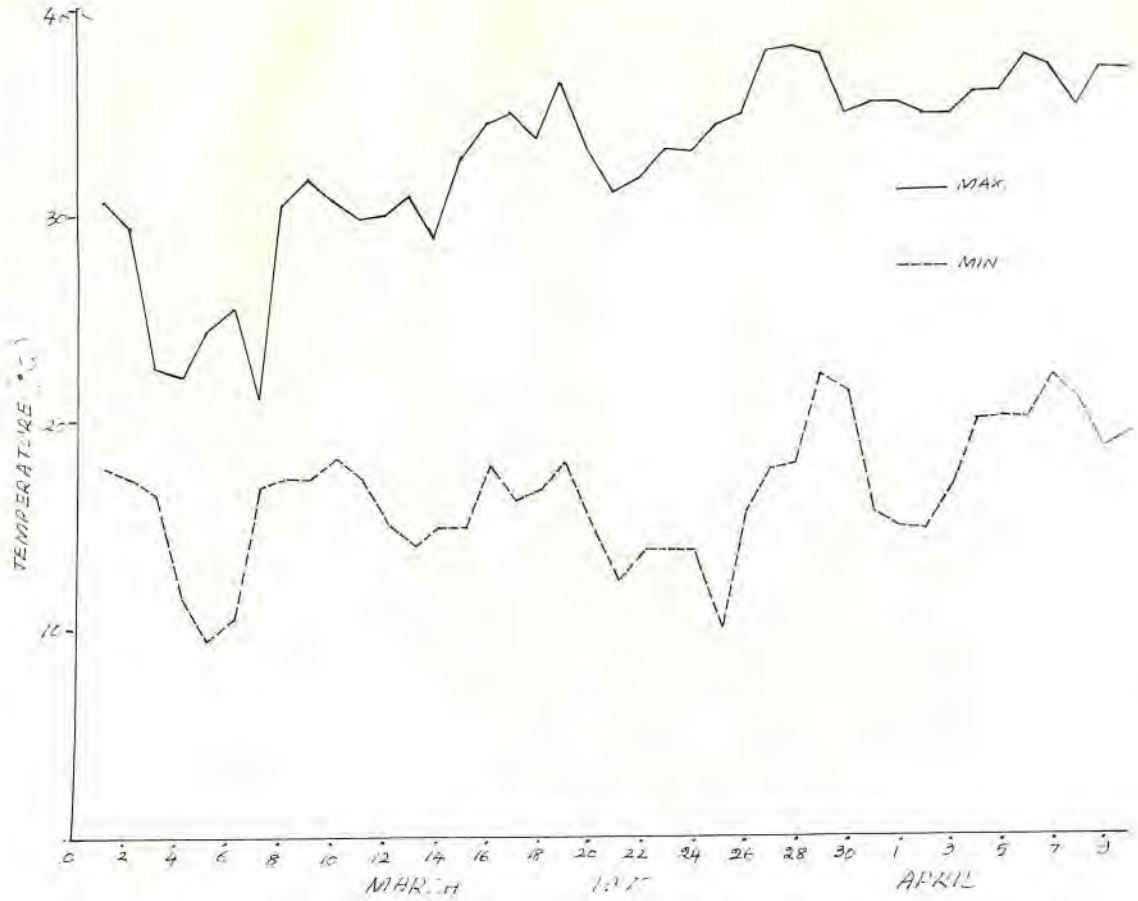
3. Longevity of Inoculants in Biphasic Carriers:

Rhizobium japonicum serotype AGI of (CCAM-JNAU) was used as an inoculum in the above carriers. The cultures prepared were packed in polythene bags, as described in 1. To find out the growth and longevity of the strain, freshly prepared inoculant, in different carriers were stored in two temperatures, refrigerator temperature (4°C) and at room temperature for 40 days. The room temperature prevailing during March-April, 1970 at Jabalpur is shown graphically (Fig.No.1). The population density was determined in the fresh cultures by the pour plate method, (Harrigans and McCance, 1966).

Samples from both the temperatures were drawn at the interval of 10 days, and the viable rhizobial counts alongwith contaminants were monitored.

4. Viability of rhizobial cells on seed surface under spectral radiations :

Soybean seeds treated with effective soybean inoculants (AHH serotype) were exposed to fluorescent,


40
30
20
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TEMPERATURE (°C)
MAX
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2
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26
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1
3
5
7
9
MARCH
APRIL

ultraviolet and sunlight. All the samples were drawn with the interval of 2 hours except the first one to estimate the viable count of rhizobia per seed. Ten inoculanted seeds were sampled for monitoring the population density on the surface. These seeds were transferred in the flask having 100 ml sterile Ringer solution and were shaken in the rotary shaker for one and a half hour. After thorough shaking, 1 ml of suspension was drawn with the sterile syringe and transferred to the test tube containing 9 ml of Ringer solution. Serial dilutions were made successively upto 10^5. 0.1 ml of suspension was used from the last dilution for plating with YMA congored medium. The petridishes were incubated at 28°C for 3 days and the counts of rhizobial colonies were recorded. The data on epiphytotic rhizobia were reckoned to be based on split plot design and so analysis of variance was calculated and the interactions: Carrier x Radiation, and radiation x duration were obtained vide Appendix (Table - XIII and XIV).

5. Nodulation studies of Inoculated Seeds Subjected to Solar Radiation :

Soybean seeds treated with effective inoculant (S 400) in lignical were packaged in low density polythene bags and exposed to sunlight in two series

A and B as described in(Table - 2). The samples of exposed seeds were drawn at the interval of three hours upto 48 hours. The diurnal temperature was recorded at the time of each sampling. The exposed seeds were tested for viable rhizobial count and the nodulating ability. The method as detailed by Darbyshire (1966) was followed for testing the nodulating ability of the inoculant.

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Table - 1

Norms of Quality Control for broth and solid phase carriers.

Medium	Rhizobia/ml.	Range
Broth	500×10^6	Satisfactory
Broth	$< 500 \times 10^6$	Doubtful
Solid Culture	$< 10 \times 10^6$	Doubtful
Solid Culture	10 to 100×10^6	Satisfactory
Solid Culture	1000×10^6	Very satisfactory

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Table - 2

Time Intervals for Exposure of Inoculated Soybean Seed to Sunlight.

SERIES[c]

A				B			
March 1971.	Seed sample No.	Time	Tempera-ture.	March 1971.	Seed sample No.	Time	Tempe-rature
26	A1[a]	8 AM	31[b]	25	B1[a]	8 PM	24
	A2	11 AM	41			11 PM	23
	A3	2 PM	40			2 PM	21
	A4	5 PM	38				
		8 PM	29	26	B2	8 AM	31
		11 PM	28		B3	11 AM	41
		2 PM	24		B4	2 PM	40
					B5	5 PM	38
27	A5	8 AM	32			8 PM	29
	A6	11 AM	41			11 PM	27
	A7	2 PM	42			2 PM	25
	A8	5 PM	36				
		8 PM	29	27	B6	8 AM	32
		11 PM	26		B7	11 AM	42
		2 PM	23		B8	2 PM	42
					B9	5 PM	36
28	A9	8 AM	36		B10	8 PM	29

a - Seeds samples were picked up.

b - 0°C (Temperature in 0°C).

c - Soybean seeds were sterilized and inoculated with culture 5400. 19 packets containing 10 seed each were made and exposed to sunlight in two series A and B. In A sampling start from morning and in B from night. Samples were picked up only at day hours in both the series except two samples in B series, these two samples were picked up in night hours.

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

Physico-Chemical Characteristics of Carriers :

The physicochemical analysis (vide table-3) of different carriers showed that out of all the carriers except TLM (60%) passed through 50 mesh. The seiving with 100 and 200 mesh showed that the lignite, peat (USA), peat (Indian) and compost are having the fine particle size (93, 86, 83 and 76% respectively) and the TLM possesses the coarser particles.

The apparent density of lignite, compost and TLM was found to be of low(0.70, 0.74, 0.86 g/cc) respectively where as peat (USA) and peat (Indian) were observed to have this constant to a slightly higher degree (1.04 and 1.10 g/cc).

Peat (USA) and lignite were at par with respect to water holding capacity (47%) followed by compost, TLM and peat (Indian) 42, 41 and 39% respectively.

Total N content in peat (USA) and TLM was observed to the tune of 1.93% and 1.00%, peat(Indian), compost and lignite were observed to have very low N content (0.64, 0.39 and 0.33%). Only TLM and compost

were tested for total P and K. The P and K content of compost was 1.70% and 1.14% respectively, where as TLM contained P and K to the extent of 0.13 and 0.85% respectively.

Peat was found to be superior in available N, P and K followed by other carriers. Lignite, peat (USA) and TLM were observed to have organic matter content to the extent of 94.24%, 74.70% and 70.75% respectively, compost and peat (Indian) were poor in organic matter content.

As far as pH is concerned peat (USA), peat (Indian), lignite and TLM except compost (pH 7.05) fell in the acidic range (pH 3.80 to 6.30).

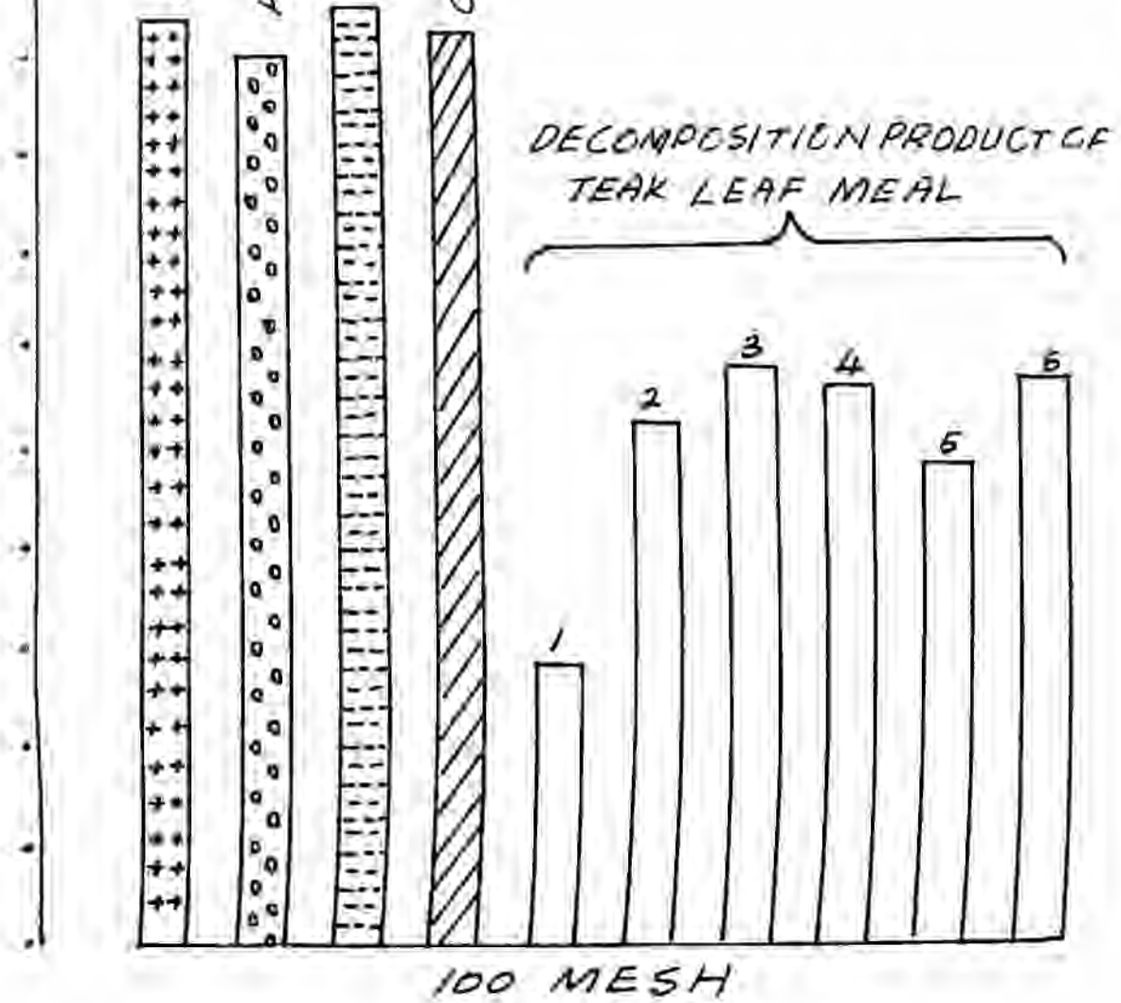
Lignite exhibited the highest cation exchange capacity (C.E.C.) 177.9 me/100 gm.followed by peat (USA), TLM, peat (Indian) and compost.

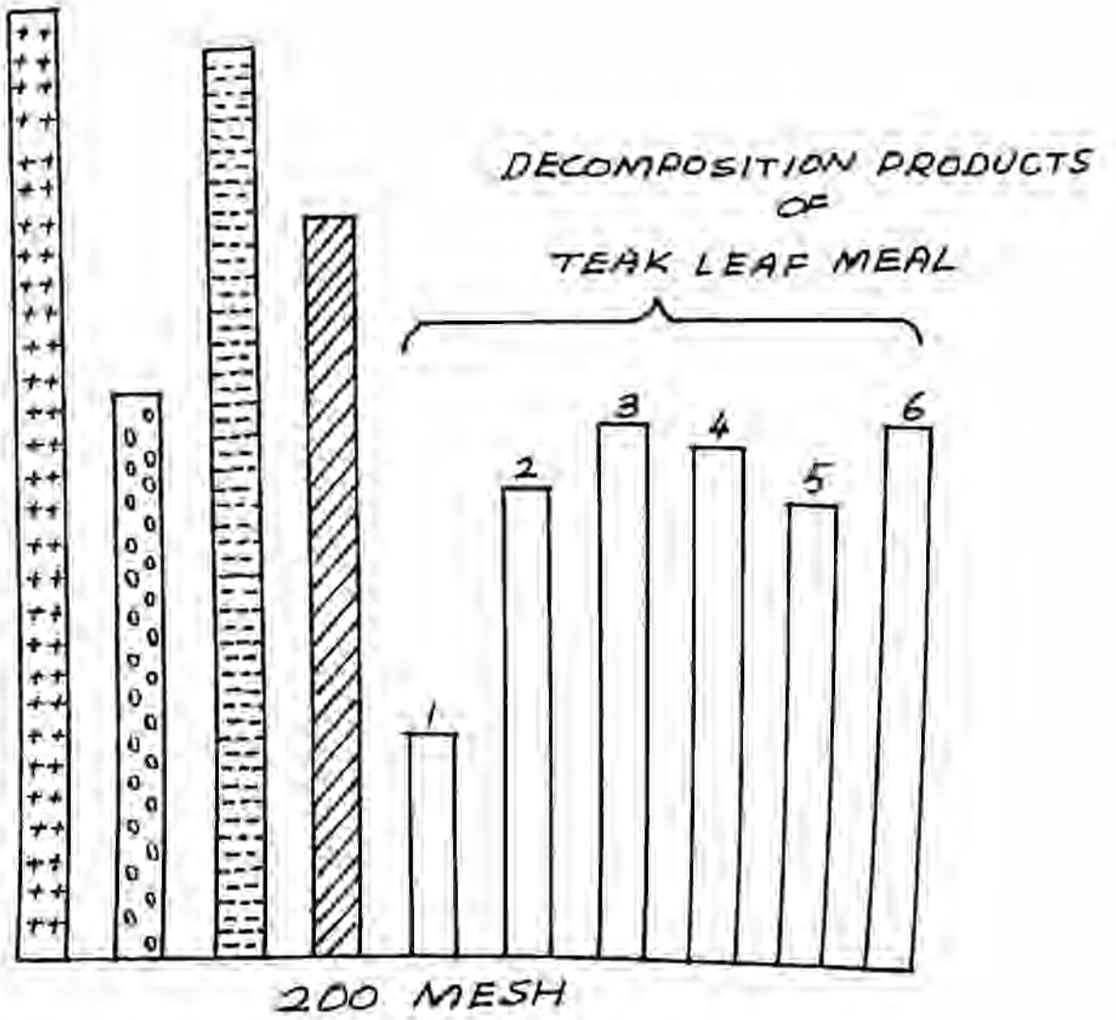
Decomposition of unfinished carriers (Berseem leaf meal (BLM) and Teak leaf meal (TLM) by humus forming microorganism :

In general (Table - 4 and Fig.No.1) BLM was decomposed faster than TLM as judged by the increase in the amount of 100 mesh particles, Metarrhizium brunneum was most effective upto 271% and 200 mesh particles to 376% over control, whereas it was least

PEAT (AMERICAN)
PEAT(INDIAN)
LIGNITE
COMPOST
TEAK LEAF MEAL

1. ORIGINAL
2. HUMICOLA GRISEA
3. HELMINTHOSPORIUM GENICULATUM
4. THERMOMONOSPORA VIRIDIS
5. METARRHIZIUM BRUNNEUM
6. STREPTOMYCES VIOLACEOUS RUBER





effective on TLM where it increased 100 mesh and 200 mesh particles to 69% and 98.9% respectively.

For the BLM the decomposing effeciency was in the following order:

Metarrhizium brunneum > Strentomyces violaceous ruber (227% of 100 mesh particles and 319% of 200 mesh particles) > Helminthosporium geniculatum (217% and 307% of 100 and 200 mesh particles) > Humicola grisea (154% and 203% of 100 mesh and 200 mesh particles respectively) and Thermomonospora viridis (184% and 270% of 100 mesh and 200 mesh particles).

The organisms which could decompose BLM very effectively were not so effective on TLM and the pattern was :

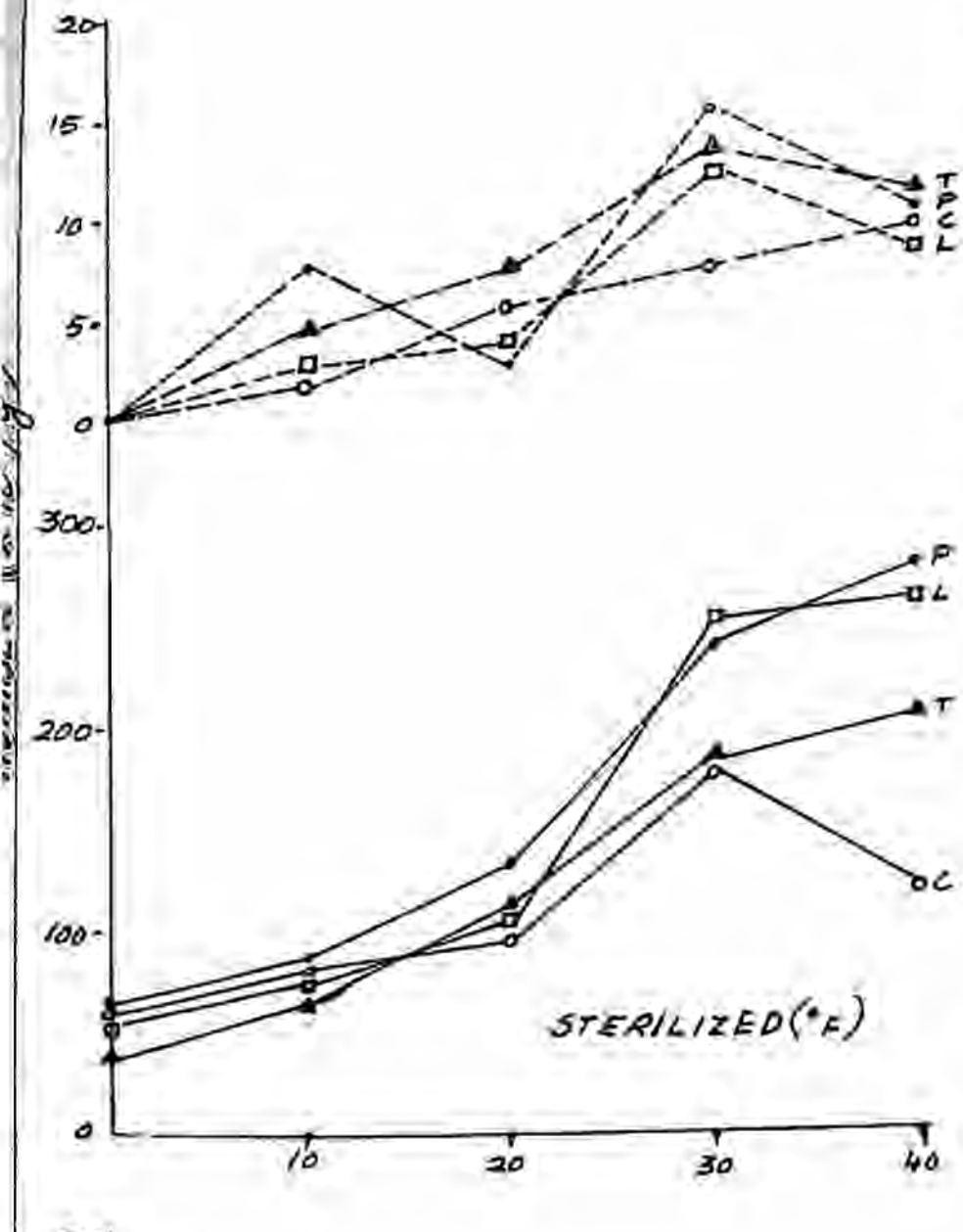
Helminthosporium geniculatum (100 mesh particles to 100% and 200 mesh particles to 137%)> Strentomyces violaceous ruber (100 mesh to 96% and 200 mesh to 136%)> Thermomonospora viridis (100 mesh to 93% and 200 mesh to 126%)> Humicola grisea (100 mesh to 81% and 200 mesh to 114%)> Metarrhizium brunneum (100 mesh to 69% and 200 mesh to 100%).

Growth of Rhizobium japonicum, E-179:

The growth of Rhizobium japonicum E-179, one of the component strains of composite culture in three indigenous carriers, lignical, compocal and teak leaf meal was studied for 40 days at room temperature (θ_R) and refrigeration (θ_F). Fig.No.2 illustrates that initial sterilization of carriers had no appreciable advantage as far as the development of rhizobium was concerned.

Growth of the bacterial strain in sterilized and nonsterilized lignical in 40 days was indentical to that in peat (USA) at room temperature, but at refrigeration the viable count increased more in lignical than in peat (USA). The growth curve of Rhizobium in lignical was more sharp than in Peat(USA). TLM was less favourable for growth of the strain than lignical and peat (USA)at both the temperatures, but was more than compocal.

The carrier compocal behaved similar to other carriers when the storage temperature was θ_R but at θ_F it was divergent from others as the rhizobial density started declining after 30th day. Sterilization and no sterilization did not make any difference with respect to this depression.


20
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STERILIZED (°F)
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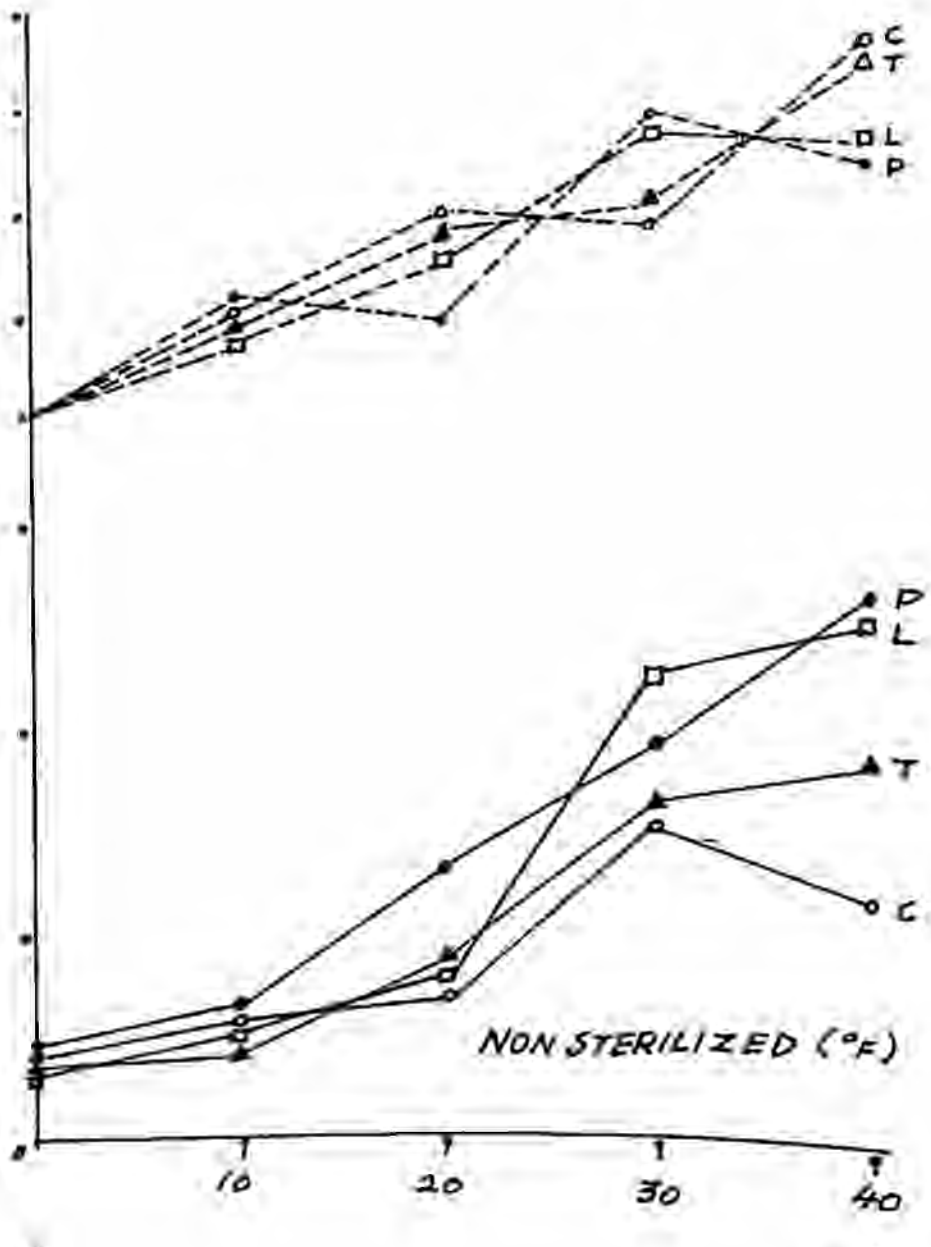
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T
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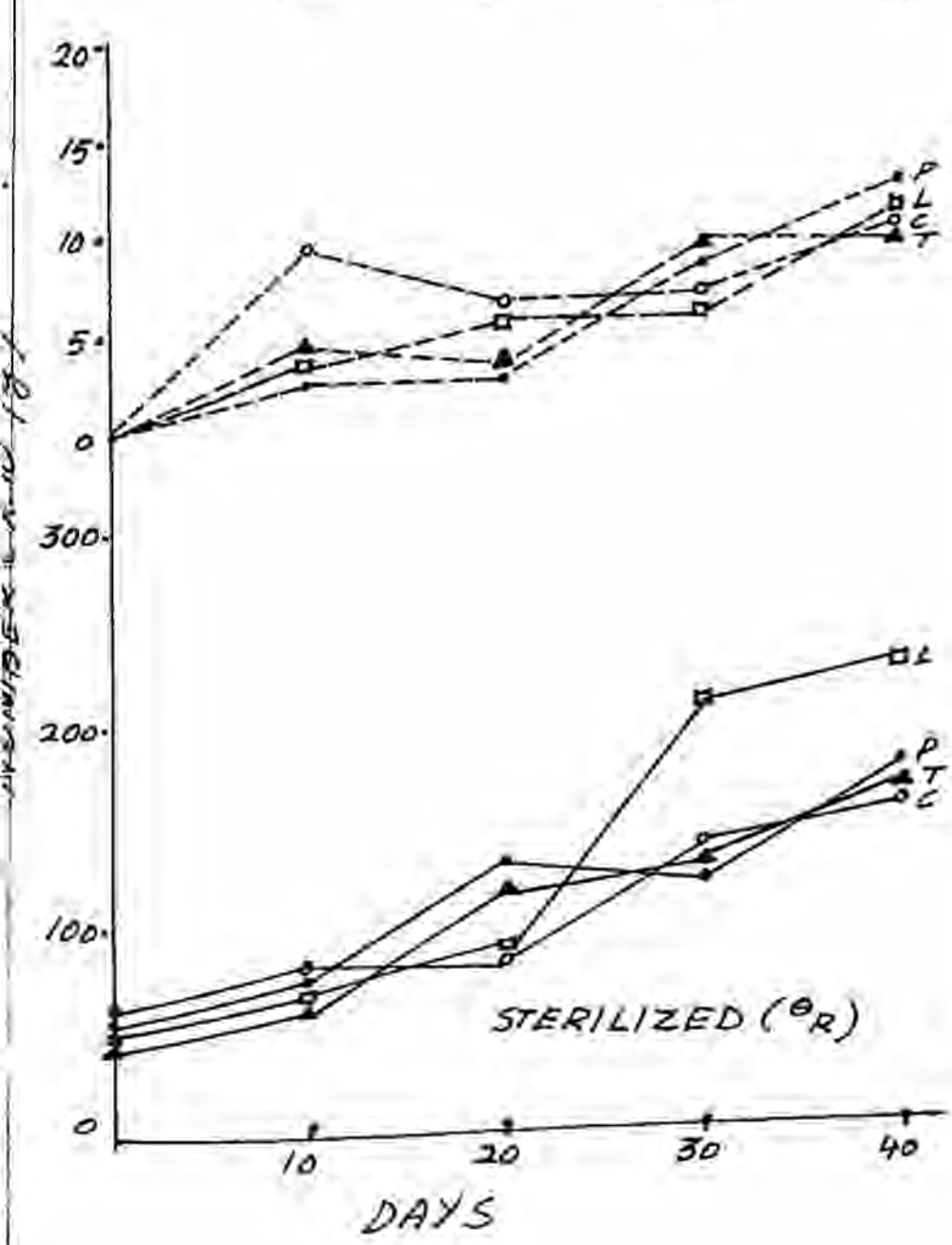
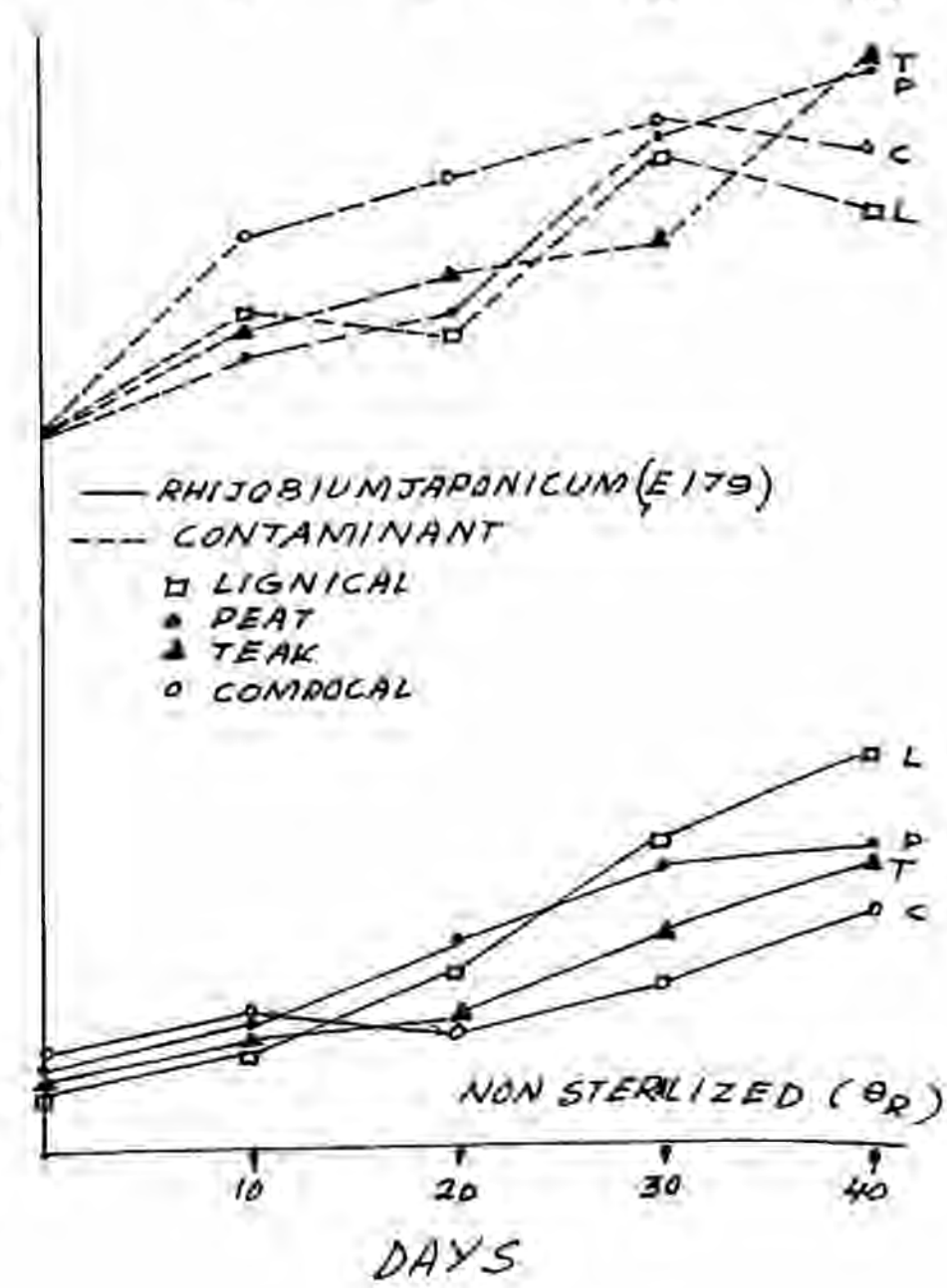
NON STERILIZED (°F)
10
20
30
40


20
15
10
5
0
300
200
100
0
P
L
C
T
L
P
T
C
STERILIZED (θR)
10
20
30
40
DAYS


T
P
C
L
RHIZOBIUM JAPONICUM (E 179)
CONTAMINANT
LIGNICAL
PEAT
TEAK
COMPOCAL
L
P
T
C
NON STERILIZED (θR)
10
20
30
40
DAYS

The growth rate of contaminants was unaffected by sterilization and storage temperatures. The population of the contaminants in compocal was higher than in other carriers at all intervals during the period of study.

Effect on Nodulation:

To see the effect of carriers having same strains of Rhizobium, the seeds were treated, and sown in the field with randomized block design. All the carriers proved inferior to positive control concerning number of nodules formed per plant, but were all better than negative control in which no nodule was formed at 35th day. Table 6 indicates that compocal, ligniteak and peat (Indian) were similar statistically and were better than teak leaf meal. Peat (USA) and lignical were statistically similar and were better than other carriers except positive control.

Observations on dry weight of nodules (odw), responded in a different way. Lignical and positive control proved similar regarding dry weight of nodules, whereas less number of nodules were formed by lignical. Both differed from and proved superior significantly to all other carriers regarding dry weight of nodules. Statistically compocal, peat (Indian) and ligniteak were the same.

Nodule number per plant at 55 days, as indicated by (Table - 6) was same in peat (USA), lignical, ligniteak and positive control. Teak leaf meal increased the nodule number by 134% over negative control whereas lignical increased to 441%. Similarly in dry weight of nodules at 55 days peat (USA),lignical, ligniteak and peat (Indian) did not differ from each other but were significantly superior to negative control teak leaf meal and compocal.

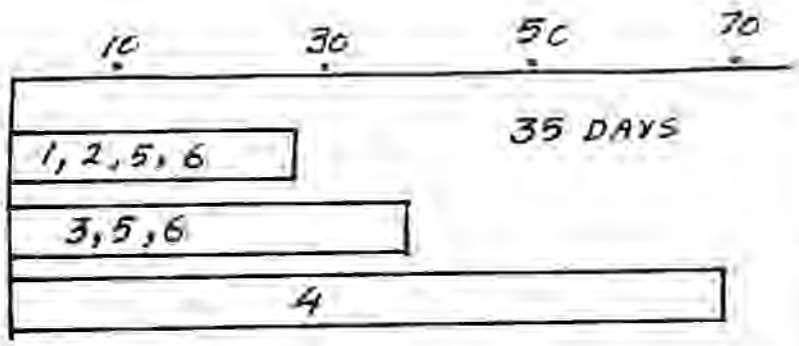
According to percentage increase in number of nodules per plant at 55 days over control the carriers could be grouped as table -5.

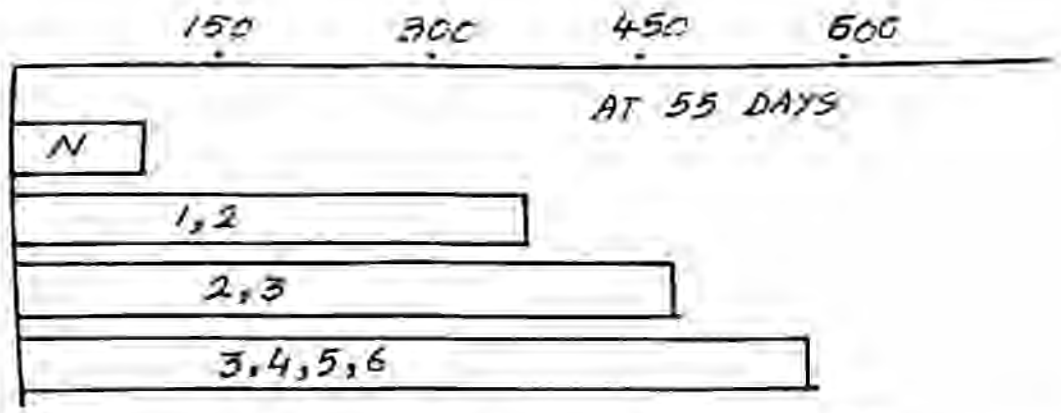
According to increase in odw. over control at 55 days the carriers could be grouped as given in the table - 5.(Fig.No.3).

Effect of carrier on Nitrogen uptake:

A significant effect of carriers was observed on the nitrogen uptake by plants during growth period and at maturity. (Table - 8). Carrier, lignical and positive control (Nitragin) were statistically similar and were superior to all other carriers. Lignical and positive control increase the nitrogen uptake to 66.8 and 74.8% respectively. Carrier, teak leaf meal was the same as negative control and compocal. But compocal differed from control (Negative).

NODULATION (Mg./Plant, Odw)

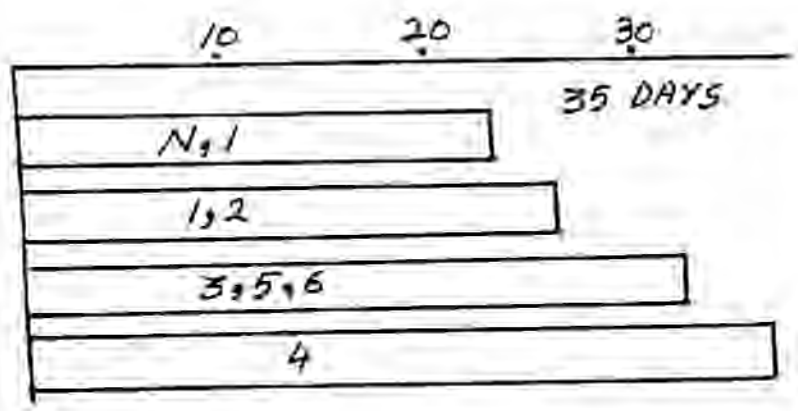


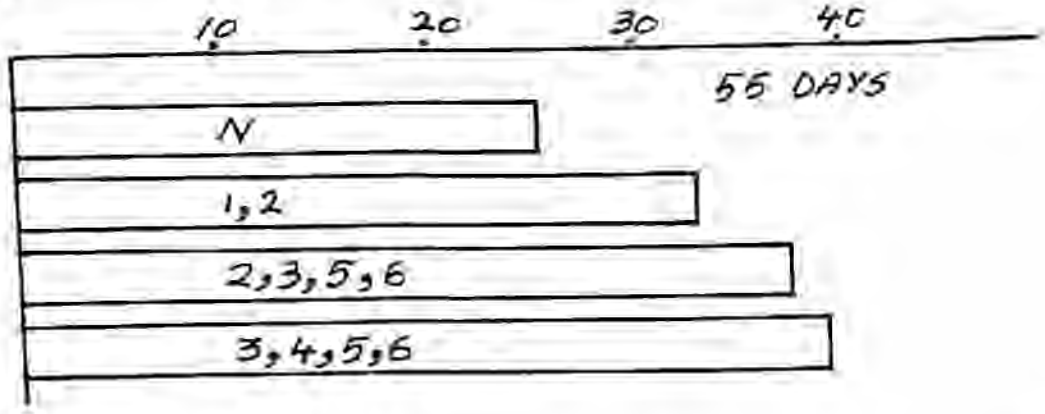


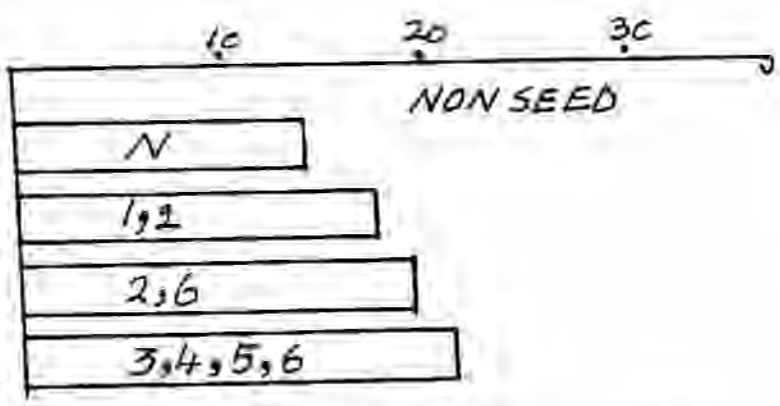
DURING GROWTH ↑

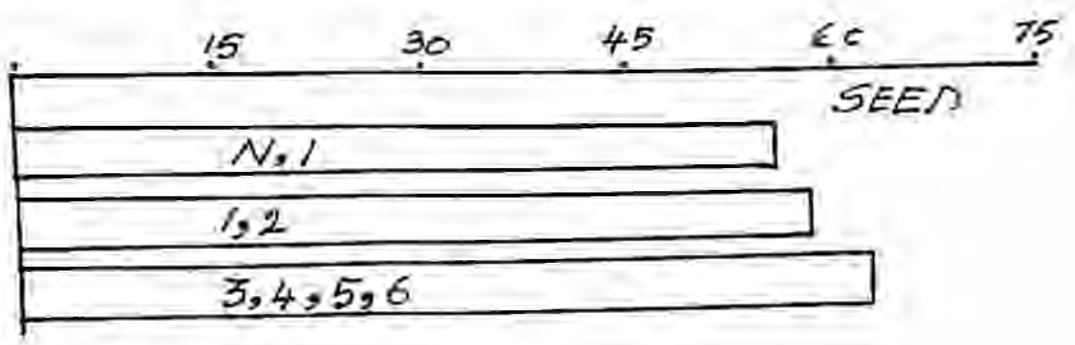
NITROGEN UPTAKE mg./plant

↓ AT MATURITY

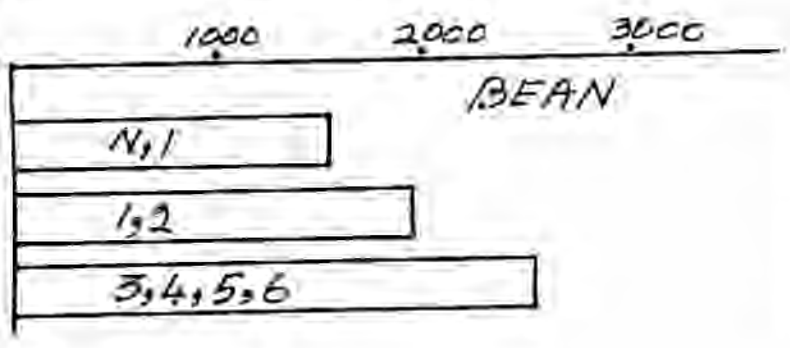


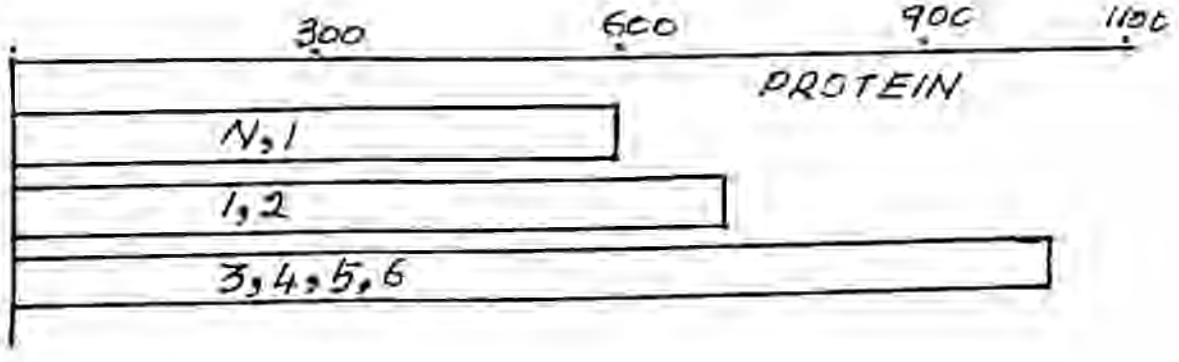






YIELD (Kg./ha)





Ligniteak, peat (USA) and peat (Indian) were equally effective but were statistically superior to teak leaf meal and compocal inferior to lignical and positive control at 35 days. At 55 days peat (USA), lignical, ligniteak (9:1), peat (Indian) and positive control (Nitragin) became statistically same but better than teak leaf meal and compocal. The carriers, compocal, peat (USA), ligniteak, peat (Indian) were in the same group. According to increase in percentage nitrogen uptake, the carrier grouped as given in Table 7 (Fig. 3).

The nitrogen content of seed and nonseed was also influenced by the carriers. Teak leaf meal and compocal increased nitrogen content in nonseed by 21.4% and 30.3% and in seed by 3.2 and 7.2% respectively. All other carriers increased the nitrogen content of both nonseed and seed significantly over control, teak leaf meal and compocal, ranging from 44.6% to 60.6% in nonseed and 14.6% to 19.5% in seed nitrogen respectively.

According to the effect on nitrogen content at maturity, the carriers could be classified into groups as given in Table 9.

Protein yield was affected by the effect of different carriers (Fig.3). Teak leaf meal and compocal were statistically not better than control, and similar to each other. But all other carriers showed a significant increase in protein yield ranging from 75.6% to 96.0% over control, teak leaf meal and compocal (Table 8).

Survival of Epiphytotic Rhizobia in Carriers Exposed to Different Radiations

As the strain No.S 188 seretype No.ANH was applied at normal rate through normal procedure to the soybean seeds and epiphytotic rhizobial population were recognized by the characteristic appearance of the colonies on YEMA plates, the enumeration data invariably represented the actual number of the applied inoculant strain. In the initial stage peat (USA) and lignite were equally good and peat (Indian) and compost were equally good but ranked medium, and TLM was poor. All carriers (Fig.No.4) behaved to all types of radiation in a similar manner. Amongst the various sources of radiation diffused light (DL) was most conducive for viability of rhizobia (Table -10), Sunlight (SL) and fluorescent light (FL) were deliterious and UV radiation was extremely detrimental. Exposure to radiation beyond 6 hours led to considerably lower population of rhizobia on the seed. The population level beyond 5 hours fell to such a degree

COMPOCAL

TEAK LEAF MEAL

PEAT (U.S.)

LIGNICAL

RHIZOBIAL DENSITY/SEED

10^6 10^5 10^4 10^3 10^2 10^1

8 11 13 15 17 19 8

HOURS

X REFRIGERATED
• ROOM TEMPERATURE
Δ ULTRA VIOLET
○ FLUORESCENT
⊙ SUN LIGHT

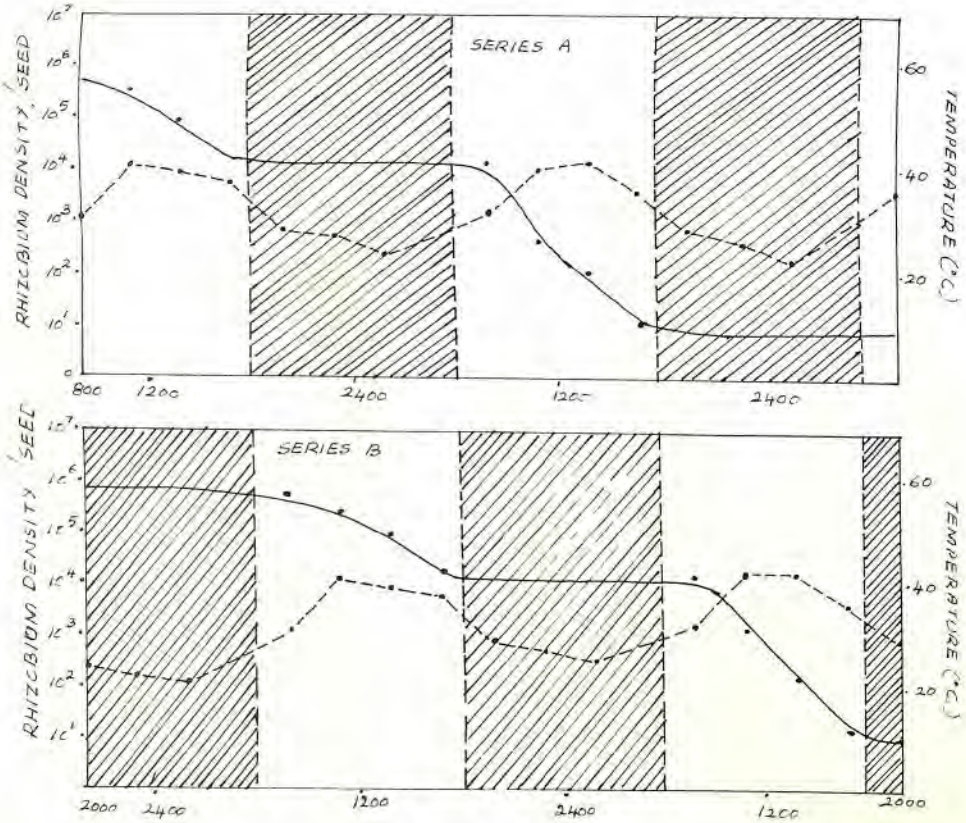
that either the survived bacteria did not nodulate or if nodulated then ineffectively. Radiation by duration interaction (Appendix table -XIV) revealed that diffused light had no adverse influence, amongst the other two continuous radiation sources, UV was not harmful upto 5 hr. but became lethal to rhizobia beyond this. The FL, also behaved like UV upto 5 hr. but there-after caused a continuous fall in rhizobial population.

The sunlight invariably was discontinuous and so upto 5 hr. there was no decline in rhizobial population but its adverse influence appeared only at 7th hour when night came in so the rhizobial numbers persisted at this level even upto 24 hours.

Initial rhizobial population in different carriers was of the range, 12 to 21×10^5 . In case of diffused light DRT (Decimal Reduction Time) could not be calculated obviously because of no reduction. In SL it was 2 hr. 30 mt. for the range of 10^5 to 10^4 in FL it was 2 hr 12 mt to 10^5 to 10^4 and 1 hr 14 mt for 10^4 to 10^3. The comparative DRT in 10^5 to 10^4 was only marginally more in sunlight than UV. UV and FL both compared very well in 10^4 to 10^3 range.

Exposure Effect of Sunlight

The rhizobial density on the seed exposed to two series of sunlight treatment was observed. The


SERIES A
SERIES B
RHIZOBIUM DENSITY / SEED
TEMPERATURE (°C.)
800
1200
2400
1200
2400
2000
2400
1200
2400
1200
2000
TIME IN HOURS
MARCH 1971

results are tabulated in Table 11 and illustrated in Fig. 5.

The table indicates a definite effect of sunlight on rhizobium population and nodule formation. A relation was also observed between density of rhizobium on seeds and number of nodules formed per plant.

Night provided absence of any radiation and so the rhizobial density remained constant. In the series A were the exposure was immediately done after the inoculation the population density at (9th and 24th hr) and in the series B where the exposure of the sunlight was not done immediately after seed treatment, the rhizobial population at (8 PM and 8AM, 0 and 12th hour) was the same. Furthermore the nodulation was also in the two series of seed treatments.

During forenoon exposure for 3 hr of samples A_1 and A_2 (8 AM and 11 AM), B_2 and B_3 (8AM and 11 AM) no appreciable reduction was observed in density as well as on nodulation. But as the degree of insolation increased, the density of rhizobium on seeds decreased continuously in both series, and consequently with the decrease in the density, number of nodules per plant also dropped.

The customary finding is that whenever the density of viable cells per seed reaches below 10^3 the modulation is absent as it happened similarly in the two series of exposure to solar radiation.

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Table - 3

Physicochemical characteristics of carriers.

Contents	Peat (USA)	Peat (Indian)	Lignite	TLM	Compost
Physical					
Sieve analysis Mesh					
∠60	100%	100%	100%	60%	100%
∠100	94%	90%	95%	29%	92%
∠200	86%	83%	93%	23%	76%
Apparent density					
7 100 and ∠ 200	1.04 g/cc	1.10 g/cc	0.70 g/cc	0.86 g/cc	0.74 g/cc
Water holding capacity	47%	39%	47%	41%	42%
Chemical					
Total Nitrogen	1.93%	0.64%	0.33%	1.00%	0.39%
Total P.	-	-	-	0.13%	1.70%
Total K.	-	-	-	0.85%	1.14%
Available nitrogen.	427 ppm	325 ppm	125 ppm	-	-
Available P.	10 ppm	6 ppm	6 ppm	-	-
Available K.	63 ppm	47 ppm	48 ppm	-	-
Organic Matter	74.70%	41.13%	94.24%	70.75%	52.97%
Ash (500°)C.	25.30%	68.87%	5.76%	29.25%	47.03%
pH.	3.80	4.65	4.95	6.30	7.05
C.E.C.	148.8 me/100g	88.8 me/100g	177.9 me/100g	112.7 me/100 g.	70.5 me/100g

Table - 4

Longterm Microbial Decomposition of Berseem and Teak Leaf Meals.

Mesh	Original	H.grisea[b]	Helminthosporium geniculatum[b]	Thermomonospora viridis[b]	Metarrhizium brunneum[b]	Streptomyces violaceus ruber.[b]
			BERSEEM LEAF MEAL (BLM)			
∠ 50	61[a]	77	80	76.6	90	80
∠ 100	18.5	47.0	59.7	52.6	68.7	61.0
∠ 200	13.5	41.0	55.0	50.0	64.3	56.6
			TEAK LEAF MEAL (TLM)			
∠ 50	60.0	90.0	91.0	90.0	89.0	88.0
∠ 100	29.0	52.5	58.0	56.0	49.0	57.0
∠ 200	23.0	49.3	54.5	52.0	46.0	54.5

a = Figure represents percentage material of the particular mesh.

b = Time of decomposition for 12 months and 12 days (18.4.69 to 1.5.70).

Table - 5

Ranking of Carriers according to total nodule number and oven dry weight (odw.) in soybean.

Group	Carrier	Nodule number in %.	Carrier	Odw.
Group I.	Teak leaf meal.	134%	Teak leaf meal and compocal.	230-319%
Group II.	Compocal and Peat (Indian)	270-301%	Compocal & peat (USA)	319-426%
Group III	Peat (Indian), peat (USA), ligniteak.	301-381%	Peat (USA), ligniteak, peat(Indian) lignical.	426-517%
Group IV.	Peat (USA), lignical, & ligniteak.	369-441%		

Table - 6

Nodulation

Period of samples (days).		CARRIERS							L.S.D. 5% P.
35		N	1	2	5	6	3	4	P	
	x	00	1.03	4.30	4.30	4.80	6.15	6.36	24.3	0.826
		N	2	1	5	6	3	4	P	
	odw	00	18.66	21.83	32.50	33.30	46.66	69.16	73.50	21.99
55		N	1	2	6	3	5	P	4	
	x	25.75	61.25	97.0	105.25	122.00	125.00	126.25	141.00	23.23
		N	1	2	3	5	6	P	4	
	odw.	25.50	325.50	415.00	521.00	563.75	576.75	601.25	612.75	128.85

Negative control N, Teak leaf meal -1, Compocal -2, Peat(USA) -3, Lignical - 4, Ligniteak (Ind) - 5., Peat (Indian) - 6, Positive control Nitrazin-P.

x -- Nodule number per plant.

odw. -- Dry weight of nodules in mg.per plant.

Table - 7

Ranking of carriers according to nitrogen uptake by soybeans.

Group	Carriers	Percentage
	At 35 days	
Group I.	Negative control and teak leaf meal.	12.3%
Group II.	Teak leaf meal and compocal.	12.3-29.8%
Group III.	Ligniteak, peat (USA), and peat (Indian).	48.1-55.0%
Group IV.	Lignical.	66.5-74.5%
	At 55 days	
Group I.	Teak leaf meal and compocal.	23.8-36.7%
Group II.	Compocal, Peat (USA), ligniteak(9:1) and peat (Indian).	36.7-53.5%
Group III.	Peat (USA), lignical, ligniteak and peat (Indian).	46.4-62.5%

Table - 8

Plant nitrogen in soybeans grown treated with culture.

Sample	CARRIER								L.S.D. 5% P.
Plant N. (35 days) g/kg.	N	1[a]	2	5	3	6	4	P	3.91
	21.3	24.5	29.3	32.3	33.1	33.8	36.6	38.0	
Plant N. (55 days) g/kg.	N	1	2	3	5	6	4	P	5.45
	25.83	32.00	35.33	37.83	38.00	39.66	41.83	42.00	
Non seed N. g/kg.	N	1	2	6	5	3	4	P	2.26
	14.00	17.00	18.25	20.25	21.00	21.50	21.75	22.50	
Seed N. g/kg.	N	1	2	3	4	5	6	P	3.8
	55.0	56.2	59.0	63.1	63.1	64.0	65.6	65.8	
Protein yield kg/ha.	N	1	2	3	5	4	6	P	167
	558	631	767	980	1002	1019	1068	1094	

a = N. 1, 2, 3, 4, 5, 6, P vide table - 6.

Table - 9

Ranking of carriers according to nonseed and seed nitrogen

Group	Carriers	Percentage
	Non seed nitrogen	
Group I.	Teak leaf meal and compocal.	21.4 - 30.3%
Group II.	Compocal and peat (Indian).	30.3 - 44.6%
Group III.	Peat (USA), lignical, ligniteak and peat (Indian)	44.6 - 66.6%
	Seed nitrogen	
Group I.	Teak leaf meal and compocal.	3.2 - 7.2%
Group II.	Peat (USA), lignical; ligniteak and peat (Indian)	14.6 - 19.8%

Table - 10

Survival Pattern of Epiphytiotic Rhizobia in Carriers Exposed to Different Radiation

	Peat (USA)	Lignite	Peat (Indian)	Compost	TLN	L.S.D.5% P
Carriers.	4.836[a]	4.793	4.776	4.693	4.625	Non significant.
	Diffused Light.	Sunlight	Fluorescent	UV		L.S.D.5% P.
Radiation.	6.134	5.461	4.017	3.367		0.054
	0[b]	3	5	7	9	24	L.S.D.5% P.
Duration.	6.189	5.882	5.632	3.953	3.615	3.199	0.210

a = Bacterial density transformed to log (n+1).

b = Numbers represent hours since the commencement of the exposure.

Table - 11

Exposure effect of sunlight on Epiphytotic rhizobial densities and nodulation.

SERIES - A			SERIES - B		
Sample	Density	Nodulation.	Sample	Density	Nodulation
A1	22×10^5 a	+(7)	B1	26×10^5 a	+(6)
A2	13×10^5	+(6)	B2	23×10^5	+(6)
A3	46×10^4	+(4)	B3	12×10^5	+(5)
A4	67×10^3	+(3)	B4	66×10^4	+(3)
A5	80×10^3	+(3)	B5	78×10^3	+(3)
A6	20×10^2	-	B6	77×10^3	+(1)
A7	5×10^2	-	B7	31×10^2	-
A8	20		B8	7×10^2	-
A9	-	-	B9	50	-

For series A & B vide table - 2.

Figure in parenthesis show number of nodules borne laterally.

a = Rhizobium japonicum S400 was applied to the seeds and the number of typical rhizobial colonies (average of 3 estimates) from the dilution plate are presented.

DISCUSSION

DISCUSSION

What should be the physicochemical characteristics of the materials likely to serve as carriers for rhizobia one can only guess at present, and so only empirical determinations are available for the suitability of a particular material for the purpose. Chemical composition of the carriers differed in availability of N, P, K and organic matter, and cation exchange capacities. Superiority of peat lies in the available mineral status and of lignite in the organic matter content. The cation exchange properties of plant lignins (Thompson, Chesters and Engelbert, 1964) isolated by fractional extraction has been found to vary with the source. Lignin like soil organic matter has higher absorptive capacity for copper than for calcium. Lignite contains abundant amount of lignins derived from plant remains of geological age and, therefore, its high CEC value is highly valuable (Dart, Roughley and Chandler, 1969) for holding the macro and micronutrients for the rhizobia which are impregnated within the carrier.

The microbial decomposition of the teak leaf meal (TLM) and the berseem leaf meal (BLM) was studied for producing humus. The organisms which could decompose BLM effectively were ineffective on

TLM. This is possibly due to the differences in the lignins, the physical properties of the two meals with respect of imbition of water and other physical properties of indeterminate type. Based on its degradation products it is obvious that plant lignin varies with species, with age of the plant and often between different portions of the same plant. The TLM lignin probably is of different type so that humus forming microorganisms could effect the degradation to a lower level than in the case of BLM. The fact that the degree of microbial attack differed with the microorganisms indicates the possibility of a selectivity approach by the microorganisms on the plant lignins.

The manufacture of legume inoculants required a prerequisite of strain selection (Vincent, J.M. 1968). The strains of rhizobia included in commercial inoculants should possess the following characteristics as outlined by Brockwell et al (1968) in IX International Congress of Soil Science Transactions, Australia.

(A) Ability to compete with any naturalized strains for infection sites and so form sufficient nodule tissue to permit maximum nitrogen fixation.

(B) Ability to nodulate the host, or hosts, both promptly and at a high level of effectiveness over a range of environmental conditions;

(C) Ability to persist in the soil for at least several years after their introduction.

The strains used in the current investigations were obtained from (CCAM - JNAU) a registered collection in the World Catalogue of Collection of Rhizobia under IBP-PP, Theme No.8. All strains of Rhizobium japonicum used had specific properties with respect to colonial morphology and congo red absorption capacity (cra⁺/cra⁻) and belonged to distinct serogroups. From the bacteriological point of view there is no doubt about the superiority of a system that secures and maintains freedom from other microorganisms (van Schreven, 1958; Hamatova,1962; Roughley and Vincent, 1967). There are difficulties, however, when production is attempted on large scale. Whether sterilization of the carriers is necessary is only partly replied through the current studies since sterilization does not seem to extend the storage life of the cultures prepared under laboratory conditions. The growth rate of contaminants was unaffected both by sterilization and by storage temperature, and this leads to dispensing away of the

steps of sterilization and of maintaining the cultures at refrigeration temperature. The carrier, lignical proved equivalent and identical to peat with respect to the growth rates of impregnated rhizobia. TLM was not a favourable medium which means that some toxic substances within the plant materials are possibly inhibiting the growth. Thus lignical can be substituted for peat and the step of sterilization is unnecessary because the carrier itself does not carry any viable rhizobial cells unless it becomes casually or incidentally contaminated in the manufacturing process. The advantages of sterilization with respect to contaminants were not even marginal in the slow growing rhizobia, and hence this step is technologically redundant. In sterilized infertile sandy soil (Chowdhury Marshall and Parker, 1968). Rhizobium trifolii grew at a faster rate than Rhizobium lupini at all temperatures up to 30°C. The presence of the appropriate host plants in the sterilized infertile sand resulted in faster growth rates in both species, particularly Rhizobium lupini. However, for cowpea rhizobia sterilization of carrier is essential (Roughley and Vincent, 1967).

Allen and his associate (1968) at the University of Wisconsin have worked out a correlation

analysis of criteria of symbiotic nitrogen fixation. Correlation coefficients show an inverse relationship between number of nodules and plant height, dry matter yield, nitrogen content, nodule weight and nodule nitrogen respectively. Plant height was positively correlated with dry matter yield and nitrogen content but not with weight and nitrogen content of the nodules. Increased nodule weight corresponded to increased nodule nitrogen, thereby indicating a greater efficiency of nitrogen fixation following an increase in nodule mass. Increased nodule nitrogen was related to an increase in nitrogen content of the soybean plants and dry matter yield but not to plant height. Sensitivity of different criteria as a major efficiency was in the following order: nodule weight > nodule nitrogen > nitrogen content > dry matter yield > plant height > nodule number. The three criteria: nodule weight, nitrogen content and dry matter yield were used to evaluate the field performance of the composite strains of Rhizobium japonicum in different carriers. The evaluation trial proved conclusively that TLM and compocal were inferior and therefore, unsuitable as carriers. Lignical and the peats are identical and thus the former can prove itself to be a good substitute for the latter.

Scanning reflection electron microscopy of peat cultures of Rhizobium trifolii (Dart and Roughley, 1969) showed that rhizobial cells were enmeshed in the matrix of the remaining plant cell walls in the peat. The shielding effect of peat for rhizobia in biphasic carriers, (Burton and Curley, 1965) towards radiation was shared by all types of carriers. This important characteristic of an ideal carrier is thus duly fulfilled by the carriers under test.

Sunlight may be considered to be a special type of radiation with energy equivalents of 0.09 $W.cm^{-2}$ at 540 nm effective for photochemical reactions. Unfortunately sunlight is unreliable and uncontrollable, its intensity varies drastically over a 24 hour period, and the useful intensities are available only during about 30% of a summer day.

Interestingly enough the UV light intensity increases with altitude as wavelengths become shorter, the relative proportion of UV is greatest in morning and evening, and varies inversely with wavelength, the water vapour or clouds exert relatively little effect on the UV light impinging on the earths atmosphere. The diffused light is shorn partially of the UV component due to absorption process. Fluorescent

light contains UV light at 253.7 nm in their emmission spectra but to a low degree. The declining trend in epiphytotic rhizobial population is spontaneous and common with different sources of radiation because the UV component is present in all but differs only in the degree of intensity of the component. Insolation means UV exposure and it beyond 3 hours resulted (Alexander and Chamblee, 1965) in a less effective inoculation.

Radiation whether continuous or interrupted reduces viability of spores in Bacillus puritus and Clostridium tetani (Borick and Fogarty, 1967) or inhibits oxygen utilization and destruction of ubiquinone in Thiobacillus thiooxidans (Adair 1968). Such effects except oxygen utilization in nonsporing nonphotosynthetic rhizobia are uncommon. Respirometric studies might be very useful in throwing light on the oxygen consumption by rhizobia in the biphasic carriers.

The data on decimal reduction time and on the nodulation test of inoculated seeds showed convincingly that there exists a lower numerical limit for rhizobial density. Inoculation of seeds is a strategic placement of rhizobia to occupy a predominant and principal position as epiphytotics, so that they may have an edge over others in colonizing

rhizosphere and later on to infect and nodulate. Extrapolation of nodulation test currently done in Jensen nutrient agar to field conditions is to be kept reserved because the field variables include drying winds (Alexander and Chamblee, 1965) that lead to a greater reduction in rhizobial number much below the lower numerical limit.

However, one can safely conclude that in order to make the legume inoculant a success the inoculated seeds should be prevented as far as possible totally from insolation but if unavoidable, an exposure beyond three hours will mean less effective or absence of nodulation, as a one eventuality in the practical attainment of legume-rhizobium symbiosis.

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SUMMARY

SUMMARY

An attempt was made to find a substitute for peat (USA) used in the production of legume inoculant, among indigenous materials, like compost, peat (Indian), lignite and teak leaf meal. All carriers were examined for their physicochemical properties. Lignite, peat (Indian) and compost had finer particles than other; peat (Indian) was heavier and lignite lighest, and lignite had the maximum water holding capacity whereas the peat (Indian) had minimum. In general peats were found to be superior in available N, P and K. Lignite had highest organic matter and CEC followed by peat and compost. The primary materials such as berseem leaf meal (BLM) and teak leaf meal (TLM) were decomposed by humus forming microorganisms, and it was found that microorganisms were more effective on BLM than on TLM.

The lignite, compost and TLM were modified by addition of $CaCO_3$ to serve as vehicle and named as lignical, compocal and TLM respectively. Composite culture of four different Rhizobium japonicum strains were raised in these carriers. Maximum population of rhizobia was obtained in lignical whereas minimum in TLM. Contaminants were highest in compocal, and

were unaffected by previous sterilization of carriers and storage temperatures. Evaluation of field performance showed that after 35 days of planting development of nodules was similar in lignical and peat(USA) based cultures. After 55 days lignical based culture gave maximum nodule number as well as nodule weight. Lignical based culture proved to be the best with respect to plant N whereas compocal and TLM based cultures were poor. TLM and compocal based cultures were also poor in increasing N content of seed and nonseed as compared to other carrier based cultures. As far as radiation effect was concerned all carriers behaved similar to all types of radiation. Five hr. exposure to UV and FL lights reduced the rhizobial population below the lower critical limit of 10^3 so that no nodules were formed. In case of insolation upto seven hr. did not affect materially the nodulation potential of the inoculants. When inoculated seeds were exposed to sunlight for 24 hr., a reduction in epiphytotic rhizobial population occurred leading to absence of nodulation.

Thus lignical proved as the best substitute for peat, because it promoted growth of rhizobia and helped comparatively a good longevity, a criterion so essential in the market or shelf life of the legume inoculant.

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* Original not seen.

APPENDIX

Table-I

Composition of Yeastextract -Mannitol-Agar (YMA)

Yeastextract	1.0 g
Mannitol	10.0 g
NaCl	0.1 g
K_2HPO_4	0.5 g
$MgSO_4\ 7H_2O$	0.2 g
$CaCO_3$	1.0 g
Agar	15.0 g
Distilled water	1000 ml
Congored	3-4 ml of 1% congored

Table - II

Composition of different carriers

Carriers	Original pH	For pH 7
Peat (USA)	3.80	20%[a]
Peat (Indian)	4.65	5%
Lignite	4.95	15%
Teak	6.30	10%
Compost	6.80	15%

a = Percent $CaCO_3$ added to get the pH 7.00.

Table - III

Growth Curves of Rhizobium japonicum in Sterilized Carriers.

Carriers	Temperature of storage.	Population a/ (10^8/g) period of sampling (days)				
		0	10	20	30	40
Peat (USA)	A	65	87	133	248	291
	B	50	74	136	151	184
Lignical	A	56	76	104	257	263
	B	46	71	92	222	235
Compocal	A	58	84	92	179	118
	B	53	81	87	145	165
Teak	A	40	75	112	182	203
	B	43	73	121	140	175

A = Refrigeration Temperature (4°C).

B = Room Temperature prevailing in March-April 1970.

Rhizobium japonicum S179 was incorporated in different carriers.

a/ = Rhizobial density determined in duplicate. Average values are presented.

Table - IV

Growth curves for Rhizobium japonicum in Nonsterile carriers.

Carriers	Temperature of storage.	Population a/ (10^8/g) Period of sampling (days). 0	10	20	30	40
Peat (USA)	A	49	67	130	193	271
	B	43	63	106	140	157
Lignical	A	34	55	80	227	257
	B	31	49	90	152	202
Compocal	A	50	57	63	153	117
	B	49	72	58	82	123
Teak	A	40	62	81	167	189
	B	36	56	65	109	150

For details vide footnote of Table-III.

Table - V

Effect of different carriers upon Nodule number in soybean.
(1st observation after 35 days of planting)

Treatment	R-I	R-II	R-III	R-IV	R-V	R-VI
Control	00	00	00	00	00	00
Nitragin	21.5[a]	25.1	34.6	21.6	21.7	21.4
JNKVV (A.P.)	6.8	5.6	10.4	2.4	6.1	5.7
JNKVV (I.P.)	2.4	5.2	3.3	3.2	5.8	6.2
JNKVV (Lignical)	9.2	6.0	7.2	5.6	6.7	3.5
JNKVV (Teak)	0.6	1.4	3.4	0.3	0.7	0.2
JNKVV (Compocal)	4.5	4.2	4.2	4.5	3.3	4.5
JNKVV (Ligniteak)	2.3	4.0	7.6	4.5	4.3	3.1

a = Nodule number per plant.

Table - VI

Effect of different carriers upon nodule weight in soybean.
(1st observation after 35 days of planting).

Treatment	R-I	R-II	R-III	R-IV	R-V	R-VI
Control	000	000	000	000	000	000
Nitragin	134[a]	051	113	039	051	053
JNKVV (A .P.)	050	047	071	035	047	030
JNKVV (I.P.)	037	055	040	019	055	034
JNKVV (Lignical)	142	067	063	033	066	044
JNKVV (Teak)	012	023	033	011	030	022
JNKVV (Compocal)	027	018	018	016	019	014
JNKVV (Lignitesk) 9 :1	068	038	048	016	012	015

a = Nodule weight mg/plant.

Table - VII

Effectivity of different carriers upon nodule number in soybean.
(2nd observation after 55 days of planting)

Treatment	R-I	R-II	R-III	R-IV	R-V	R-VI
Control	11[a]	16	17	2	39	18
Nitragin	19	51	116	103	118	98
JNKVV (A.P.)	92	54	114	58	92	82
JNKVV (I.P.)	53	71	88	65	96	48
JNKVV (Lignical)	69	83	138	114	93	62
JNKVV (Teak)	20	37	64	54	35	35
JNKVV (Coopocal)	65	56	81	96	34	56
JNKVV (Lignitcak) 9 d	54	80	88	113	92	83

a = Nodule number per plant.

Table - VIII

Effectivity of different carriers upon nodule dry weight (odw) in soybean
(2nd observation after 55 days of planting)

Treatment	R-I	R-II	R-III	R-IV	R-V	R-VI
Control	35[a]	80	100	60	58	93
Nitragin	227	262	447	556	332	480
JNKVV (A.P.)	219	308	573	324	313	347
JNKVV (I.P.)	334	324	733	216	336	322
JNKVV (Lignical)	238	279	483	719	471	211
JNKVV (Teak)	122	242	313	207	217	195
JNKVV (Compocal)	230	351	360	277	170	274
JNKVV (Lignitcak) 9 :1	194	439	288	611	383	340

a = Nodule Dry Weight (odw) in mg. per plant.

Table - IX

Effect of different carriers upon Nitrogen Content in Soybean (after 55 days of planting) Plant.

Treatment	R-I	R-II	R-III	R-IV	R-V	R-VI	Total	Mean
Control	26[a]	28	26	23	27	25	115	25.83
Nitragin	43	39	46	41	43	40	252	42.00
JNKVV (A.P)	47	34	37	40	32	37	227	37.83
JNKVV (I.P)	33	39	41	46	41	38	238	39.66
Lignical	48	37	46	42	35	44	251	41.83
Teak leaf-meal	25	29	37	31	36	34	192	32.00
Compocal	32	35	40	38	33	34	212	35.33
Ligniteak (9:1)	35	40	36	37	41	39	228	38.00
Total	289	281	306	298	288	291	1755	

a = Nitrogen content in gram per kg.

Table - X

Effect of different carriers upon nonseed nitrogen in soybean (at maturity)

Treatment	R-I	R-II	R-III	R-IV	R-V	R-VI	Total	Mean
Control	11[a]	9	8	10	7	11	56	14.00
Nitragin	13	15	17	14	19	12	90	22.50
JNKVV (A.P)	14	16	13	12	14	17	86	21.50
JNKVV (I.P)	15	11	14	16	13	12	81	20.25
Lignical	17	13	16	12	15	14	87	21.75
Teak leaf-meal.	11	10	12	14	11	10	68	17.00
Compocal	13	10	12	11	14	13	73	18.25
Lignitoak (9:1)	13	14	18	14	13	12	84	21.00
Total	107	98	110	103	106	101	625	

a = Non seed Nitrogen gram per kg.

Table - XI

Effect of different carriers upon seed Nitrogen in Soybean.

Treatment	R-I	R-II	R-III	R-IV	R-V	R-VI	Total	Mean
Control	56[a]	51	53	55	57	58	330	55.5
Nitragin	66	63	64	60	69	73	395	65.8
JHKVV (A.P)	67	68	62	63	60	59	379	63.1
JHKVV (I.P)	70	64	66	63	69	62	394	65.6
Lignicol	66	61	62	59	68	63	379	63.1
Teak leaf-seal.	56	56	60	59	53	55	341	56.8
Compocol	60	56	63	59	63	54	354	59.0
Lignitesk (9:1)	67	61	62	61	68	65	394	64.0
Total	508	483	492	478	507	489	2958	

a = Seed Nitrogen in gram per kg.

Table - XII

Effectivity of different carriers upon protein yield

Treatment	R-I	R-II	R-III	R-IV	R-V	R-VI	Total	Mean
Control	0.373[a]	0.215	0.260	0.364	0.338	0.426	2.028	0.373
Nitragin	0.734	0.671	0.689	0.586	0.819	0.938	4.436	0.739
JNKVV (A.P)	0.773	0.801	0.629	0.663	0.579	0.531	3.976	0.662
JNKVV (I.P)	0.837	0.652	0.721	0.675	0.825	0.616	4.326	0.721
Lignical	0.750	0.646	0.666	0.488	0.792	0.671	4.013	0.668
Teak leaf meal	0.393	0.473	0.545	0.488	0.266	0.391	2.556	0.426
Compocal	0.526	0.414	0.710	0.438	0.655	0.366	3.109	0.518
Lignitenk(9 d)	0.787	0.615	0.629	0.572	0.777	0.686	4.066	0.677
Total.	5.173	4.437	4.849	4.273	5.101	4.625	28.508	

a -- Protein yield kg/plot (in grain).

Note :- Protein yield kg/plot x 1431.48 (factor) = Protein yield kg/hectare.

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TABLE - XIII

Carrier x Radiation Interaction for Survival of Rhizobia

Carrier	L I G H T				Average
	Df.	UV	Fluor-escent	Sunlight	
Peat (USA)	6.242	3.419	4.102	5.581	4.836
Peat (Indian)	6.184	3.377	4.038	5.506	4.776
Lignite	6.202	3.394	4.063	5.512	4.793
Compost	6.071	3.341	3.963	5.387	4.693
Teak leaf -meal.	5.971	3.302	3.897	5.310	4.625
Average	6.134	3.367	4.017	5.461	

Table - XIV

Radiation x Duration interaction for survival of rhizobia

Duration (hr.)	Light*				Average.
	Df.	UV	Fl.	Sl.	
D_1 (0)	6.189	6.189	6.189	6.189	6.189
D_2 (3)	6.248	5.539	5.619	6.122	5.882
D_3 (5)	6.138	5.474	5.532	6.025	5.632
D_4 (7)	6.102	1.000	3.837	4.872	3.953
D_5 (9)	6.054	1.000	2.612	4.794	3.615
D_6 (24)	6.030	1.000	1.000	4.766	3.199
Average	6.134	3.367	4.017	5.461	

* Df = Diffused light
UV = Ultra violet
Fl = Fluorescent light
Sl = Sunlight

Table - XV

Protocol of Analysis of Variance for Survival of Rhizobia on seed exposed to various radiation upto different duration.

Source of Variance.	Df	S.S.	M.S.S.	"F" value calculated	"F" table value.5% P.
Replication S.S.	2	0.010	0.005	0.03	4.46
Carrier S.S.	4	1.10	0.275	2.02	3.84
Error (a)	8	1.09	0.136	--	
Light S.S.	3	437.57	145.85	4289.07	2.62*
Duration S.S.	5	507.96	101.59	2987.94	2.23*
Interaction					
Light x Carriers	12	0.27	0.22	0.64	1.72
Light x Duration	15	327.46	21.83	642.05	1.67*
Light x Duration x Carriers.	60	1.34	0.02	0.58	1.32
Error (b)	250	8.54	0.034	-	

C.D. at 5% P.

Light S.S.	0.054
Duration S.S.	0.21
Interaction Light x Duration.	0.294.

* Significant at 5% P.