

ASSESSMENT OF FILTERMUD AS A CARRIER
FOR LEGUME SEED INOCULANTS

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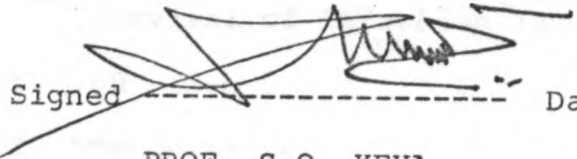
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ABBREVIATION AND SYMBOLS

C	-	Carbon
Ca	-	Calcium
C/N	-	Carbon/Nitrogen Ratio
cv	-	Cultivar
C.V.	-	Coefficient of Variation
cm	-	Centimetre
d.w.	-	dry weight
Fig.	-	Figure
g	-	gram
K	-	Potassium
kg	-	kilogram
Krad	-	Kilo radiation
LSD	-	Least Significant Difference
M	-	Molar
m.e.	-	milliequivalent
mg	-	magnesium
ml	-	millilitre
mm	-	milimetre
Mrad	-	Mega radiation
N	-	Nitrogen
N	-	Normality
Na	-	Sodium
nm.	-	Nanometre

No.	-	Number
NUM	-	Nairobi University MIRCEN
$^{\circ}\text{C}$	-	Degree Centigrade
P	-	Probability
P	-	Phosphorus
Pers.	-	Personal
pF	-	Water Potential
ppm	-	parts per million
P_2O_5	-	Phosphorus pentoxide
spp	-	species
YEMA	-	Yeast Extract Mannitol Agar
YR	-	Munsell Clour Notation
V/W	-	Volume/Weight
w/w	-	weight/weight
%	-	percentage
<	-	Less than
>	-	Greater than
μ	-	micron
μm	-	micrometre

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ABSTRACT

Peat is the most commonly used carrier for *Rhizobium*. Although it is the best carrier identified to date, it has some disadvantages: its suitability varies depending on the source and it is not readily available in many parts of the world especially the tropics. Alternative carriers of uniform composition would therefore be of considerable importance. Filtermud, a byproduct of white sugar factories, is one such material which shows a lot of promise.

In this study the suitability of filtermud as a carrier for legume inoculants has been assessed. Five samples each of fresh and decomposed filtermud were collected from five sugar cane factories in Kenya namely, Ramisi, Miwani, Muhoroni, Mumias and Nzoia. Australian peat used as a standard was supplied by the Agricultural Laboratories, Sefton, New South Wales, Australia.

The physico-chemical properties of filtermud and peat were investigated. Fresh filtermud contained high levels of ash and exhibited high water holding capacity compared to decomposed filtermud. Peat and decomposed filtermud had higher pH values (6.7 to 7.9) than fresh samples (5.3 to 7.0). Extractable phosphorus, organic carbon content, C/N ratio, calcium and magnesium content were all

significantly higher ($P = 0.05$) in fresh filtermud than in decomposed filtermud. The total N level in decomposed filtermud was higher than in fresh samples. There was however no significant difference between the levels of K and Na in the two types of filtermud. Compared to filtermud, peat exhibited a very low P content. However, the levels of total N, organic carbon, K and Na were highest while the C/N ratio, Ca and Mg values were similar to those of fresh and decomposed filtermud.

The plate count method was used for the enumeration of rhizobia. Irrespective of the degree of decomposition or source, filtermud-based inoculants compared well as a carrier with peat-based inoculants. This was judged from the growth and survival of *R. phaseoli* strain NUM 406 within a five to six months period during which cell densities of 10^8 /g of carrier were sustained for all carriers.

The effect of storage temperature on growth and survival of *R. phaseoli* strain NUM 406 and *R. japonicum* strain NUM 504 was also determined using only decomposed filtermud from Muhoroni and peat as carriers. Storage at 4°C did not significantly increase rhizobial numbers while a maximum viable count of 10^9 rhizobia/g of carrier was obtained when inoculants were stored at 28°C for three months. No viable cells were however recovered from inoculants

stored at 40°C for one month.

Based on rhizobial survival on seeds, it was observed that 40% gum arabic was a better sticker than 10% sucrose solution with both carriers when sprinkler and slurry methods of seed inoculation were adopted.

Tests on effectiveness were performed using *Rhizobium* cultures applied as broth, peat-based and filtermud-based inoculants. Nodulation and N₂-fixation were also assessed in Leonard jars under greenhouse conditions. Soybean (*Glycine max* L. Merrill) cv. "Bossier" and the common bean (*Phaseolus vulgaris* L.) cv. "Canadian wonder" were planted in N-free vermiculite in Leonard jars. Measurements of dry matter yield indicated that the effect of inoculation with filter mud based inoculant did not differ significantly (P = 0.01) from peat-based inoculant.

From these investigations based on carrier materials from Kenya, it has been concluded that filtermud is a good carrier for inoculant production. Filtermud is easily available in large quantities at very low cost.

CHAPTER ONE

INTRODUCTION

The family *Leguminosae* has a fundamental role in global agricultural productivity. The productivity is made possible because of the ability of some of the members in this family to fix atmospheric nitrogen in association with appropriate root nodule bacteria of the genus *Rhizobium*. Exploiting the full potential of legume-*Rhizobium* symbiosis has a special relevance to developing countries because of the fact that effective legume-*Rhizobium* symbiosis could reduce dependence on costly nitrogenous fertilizers and enhance food and fodder production. Consequently, the provision of cheap plant proteins to the poor masses in these countries may become possible.

Legumes are routinely used in the tropics as green manure and cover crops and are often rotated or mixed with other crops. It is quite likely that several hundred kilograms of nitrogen are fixed per hectare each year where optimal conditions prevail (Delwiche, 1970; Hardy & Holsten, 1972). This becomes important because expansion of small scale farming in the tropics is often limited by failure to maintain adequate level of available N in the soil. Effectively nodulated legumes offer a possible solution to this problem.

The family *Leguminosae* comprises more than 12000 spp. of leguminous plants although fewer

than 100 of these are currently being used for food production. These cultivated species are chiefly in the sub-family *Papilionoideae* which consists predominantly of nodulating species with the ability to work symbiotically with rhizobia to fix atmospheric nitrogen (Burton, 1979).

The nodule-bacteria, *Rhizobium* spp. is one of the three genera in the family *Rhizobiaceae* and are characterised by their ability to infect and induce nodule formation on the roots and in one case the stem of leguminous plants. *Rhizobium* spp. are differentiated by the kinds of plants they nodulate. Plants mutually susceptible to nodulation by a particular species of *Rhizobium* constitute a cross-inoculation group. There are six recognised *Rhizobium* spp. and their respective hosts. *Rhizobium phaseoli*, Dangeard, 1926 has *Phaseolus vulgaris* L. and *P. coccineus* as the major hosts. *Rhizobium leguminosarum* (Frank) Frank 1989, nodulates *Pisum*, *Vicia*, *Lathyrus* and *Lens* spp. *Rhizobium trifolii* Dangeard 1926, is specific to *Trifolium* spp. while *R. meliloti*, Dangeard 1926, has *Medicago*, *Melilotus* and *Trigonella* spp. as the major hosts. *Rhizobium japonicum* (Kirchner) Buchanan, 1926 nodulates *Glycine max* (L.) Merrill. and *R. lupini* (Schroeter) Eckhardt, Baldwin and Fred 1931, nodulates *Lupinus* and *Ornithopus* spp. Successful legume - *Rhizobium* symbiotic system involve mutual compatibility

between the symbionts all the way from multiplication of bacteria in the rhizosphere to nodule initiation and function.

While soil is the natural habitat for rhizobia, they are not universally present, moreover many of those present are often of low effectiveness to respective legumes. Therefore, the need to supply superior bacteria artificially led to the development of artificial inoculation. Since then, several forms of inoculants and carrier materials have been investigated. These forms of inoculants include agar slants, liquid cultures, freeze dried cultures, frozen concentrates, oil dried and those based on carrier materials like peat. Whilst it is possible to successfully inoculate legume seeds using all the above types of inoculants, peat-based inoculant offers some outstanding advantages. These include increased protection for the rhizobia in harsh soil conditions and improved post-inoculation survival on seed, (Vincent, 1958).

Peat, though the universally accepted carrier, is not easy to come by in many parts of the world especially the tropics. The suitability of peat depends mainly on its quality which has been shown to be very variable even from one deposit (Roughley & Vincent, 1967). This has prompted a world wide search for alternative carrier materials. Soil

supplemented with materials like lucerne meal, ground straw, yeast and sugar have been reported (Strijdom & Deschodt, 1976). Various materials other than soil have also received attention as potential carriers. These include vermiculite, decomposed saw dust, coffee husk and perlite (Vincent, 1965), and rice husk compost (Urio & Chodhury, 1979). Soil-bagasse mixture has also proved a suitable carrier for *Rhizobium* inoculants (Vencatasamy & Peerally, 1981). In Zimbabwe, cob-earth carrier (Corby, 1976) and bagas-silo (Ryder, M.R. & Grant, P.M., personal communication) are being used for inoculant production. Tilak & Subba Rao (1978) had good success with a charcoal-filtermud mixture 1:1 (w/w). Filtermud, an industrial waste from sugarcane mills, has shown a lot of promise (Philpotts, 1976; Urio & Chowdhury, 1979). The suitability of coal as a carrier has also been investigated by many research workers (Strijdom & Deschodt, 1975; Paczkowski & Berryhill, 1979; Halliday & Graham, 1978).

The choice of a carrier material depends on its availability as well as its ability to support a large viable count during the shelf-life of the incorporated rhizobia. The material should also have good absorptive capacity and high stickability to the seed. There is no commercial inoculant

production in Kenya but some are being produced on an experimental basis using a locally available carrier material, filtermud. The present pilot production of inoculants is being carried out by Nairobi *Rhizobium* MIRCEN of the University of Nairobi.

Filtermud, a byproduct from white sugar factories, is the finest deposit obtained during the filtration and clarification processes of crushed cane. It consists of very fine fibre particles, mud solids and chemical substances. Since it is readily available in Kenya, filtermud has been used in this investigation in order to assess its suitability as a carrier for *Rhizobium*.

The objectives of this study were therefore:

1. to investigate the physical and chemical properties of fresh and decomposed filtermud sampled from different sugar factories in Kenya.
2. to establish the growth and survival of selected *Rhizobium* spp. in laboratory prepared filtermud-based inoculant.
3. to determine the survival of *Rhizobium* on selected legume seeds inoculated with filtermud-based inoculants.
4. to assess the effectiveness of *Rhizobium* inoculants prepared with filtermud in terms of nodulation and nitrogen fixation under green house conditions.

CHAPTER TWO

LITERATURE REVIEW

Rhizobia are natural soil microorganisms which in symbiotic association with appropriate legumes can fix atmospheric nitrogen. Although rhizobia may be present in a particular soil, the resident rhizobia could be of low effectiveness or at times some soils may lack specific rhizobial groups for certain legumes. In order to maximise crop yield, there is therefore a need for artificial inoculation of legumes with effective rhizobia.

Rhizobium inoculants exist in various forms such as agar, liquid, lyophilized and peat-based cultures. However, the most commonly used is the peat-based type due to its superiority in promoting post-inoculation survival of rhizobia on legume seed. Peat serves as a carrier for rhizobia. Such carrier materials should have certain qualities which can support the growth and survival of rhizobia for a given period of time without losing their nodulation ability and effectiveness. Such qualities include: non-toxicity to the rhizobia and good water holding capacity. The carrier should be easy to pulverise and sterilize, it should have good adhesion to the seeds and be readily available

at moderate cost (Burton, 1979).

2.1. Physico-chemical Properties of Filtermud and Peat.

2.1.1. Physical characteristics:

The physical nature of a carrier will influence some of its qualities like stickability to the seed and absorption of liquids. To date, there is very little information on physical properties of filtermud otherwise known as filterpress mud, filtercake or pressmud. Abu-Idris *et al.* (1979) observed that fibrous materials comprised about 24% of the total dry weight (coarse and fine fibres averaged 7% and 17% respectively) and mud solids consisted of 76% dry weight.

Philpotts (1976) reported 7.8% fibre, 11.6% mud solids, 78.8% water and 81% dry matter loss on ignition. There is very little that has been reported on the physical properties of peat. Burton (1967) reported an ash content for peat of 13.2% while Stridjdom & Deschodt (1976) observed ash contents of 33.1% and 47.5% from Putfontein and Barrydale, S. Africa, respectively.

2.1.2. Chemical characteristics

Unlike the physical characteristics, quite a bit of work has been done to establish the chemical properties of filtermud and peat. There are increasing interests in the chemical properties of filtermud because of the possibilities of using it as a fertilizer. Filtermud has been used to fertilize sugarcane in several countries (Prasad, 1974; Alexander, 1971&1972; Wood, 1981; Mutanda, 1978).

The most important chemical properties of filtermud and peat that have been reported by most of the workers include organic matter, organic C, total N, extractable P and exchangeable bases like K, Na, Ca and Mg.

Organic matter values in filtermud show a lot of variation from one country to another. Mutanda (1978) recorded 19.90% in Kenya while Alexander (1972) obtained 63.90% and Wood (1981) reported 68.10% in South Africa. In Trinidad, Abu-Idris *et al.* (1979) observed 68.9% of organic matter in filtermud. Organic matter content of peat used for inoculant production by Nitragin Company, Milwaukee, USA was given as 86.80% (Burton, 1967). In South Africa, Strijdom & Deschodt (1976) reported 58.30% and 45.70% organic matter in peat from Putfontein and Barrydale, respectively.

Extractable P level in filtermud from various countries has been determined. Mutanda (1978) observed 0.77%, Alexander (1971,1972) recorded contrasting values of 0.18% and 0.92% while Wood (1981) obtained 0.93%; Prasad (1974) gave 0.45%, Abu-Idris *et al.* (1979) reported 0.92% and Cooper & Abu-Idris (1980) recorded 0.01%. The values of extractable P in peat were given as 0.35%, Burton (1967), 0.10% and 0.20% for peat from Putfontein and Barrydale respectively (Strijdom & Deschodt, 1976).

The presence of exchangeable K in filtermud has been established. Mutanda (1978) reported 0.36%. Prasad (1974) obtained 0.48%, Abu-Idris *et al.* (1979) reported 0.51%, Cooper & Abu-Idris (1980) observed 0.47%. Records from S. Africa were 0.05% and 0.20% (Alexander, 1971, 1972) and 0.29% (Wood, 1981). Burton (1967) observed 5.21% exchangeable calcium in peat.

The level of exchangeable magnesium in filtermud has been reported. Prasad (1974) reported 0.74%, Abu-Idris *et al.* (1979) obtained 0.34% while Cooper & Abu Idris (1980) observed 0.26%. In South Africa Alexander (1971, 1972) recorded 0.09% and 0.41% respectively and Wood (1981) observed 0.47%. The Mg level in peat from Milwaukee, Wisconsin, U.S.A. was given as 1.14% (Burton, 1967).

2.2. Carriers

Peat-based inoculants have almost completely replaced the agar and liquid cultures which were very common a few decades ago (Burton, 1967). This is because of the superiority of peat-cultures compared to liquid cultures. Liquid cultures seem to lack the protection usually afforded to the rhizobia by peat (Date, 1970). Like liquid cultures, lyophilized cultures have also been shown to lack post-inoculation survival of rhizobia on seed (Date, 1968).

Much of the work on peat-based inoculants is attributed to the invaluable research conducted by Roughley & Vincent (1967), Roughley (1968) and Burton (1967). Their findings have contributed significantly not only to the knowledge on peat-based inoculants but also to the study of other potential carriers. Peat has remained the only universally accepted carrier material in many countries. This is because it satisfies most of the requirements of a good carrier. Despite the fact that peat is the best carrier identified to date, its suitability varies depending on the source and it is not easily available in many parts of the world. This has led to a worldwide search for potential carriers of uniform composition and several such materials have been reported.

Fraser (1966, 1975) devised an inoculant consisting of calcium sulphate granules impregnated with rhizobia, but this is not widely used. Polyacrylamide-entrapped *Rhizobium* (PER) as an inoculant is another synthetic carrier that has been reported from Senegal (Dommergues *et al.*, 1979). Survival experiments showed that PER wet or dry, compared well to laboratory made peat-based inoculants stored at 4°C. At 30°C, the protective effect of PER was conspicuous numbers dropping from 8.4×10^7 to 6.1×10^7 viable cells/ml while in peat-based inoculants the population dropped from 2.4×10^9 to 1×10^6 cells/0.8g. Pot experiments showed that *Rhizobium japonicum* cells entrapped in a polyacrylamide gel could be used as an inoculant for 'Chipewa' soybeans and compared favourably to laboratory made peat-based inoculant containing the same *Rhizobium* strain. Polyacrylamide entrapped *Rhizobium* is not commonly used either possibly because it may not be easily available in many countries at moderate cost.

A commercial mixture of talc and lyophilised cultures were also tested in the U.S.A. but this not being used either (Burton, 1967). Despite the advantages that a suitable synthetic carrier may have over natural products, none of the above is in general use.

The suitability of soil to support survival of

rhizobia has been advocated by many scientists (Fred *et al.*, 1932; Jensen, 1961; Vincent, 1965). This has directed most efforts to obtain carriers superior to peat, around a neutralised soil peat-base enriched with nutrients (Strijdom & Deschodt, 1976). Soil, coirdust and soybean meal mixture have been reported by various scientists (John, 1966 and Iswaran, 1972).

In Mauritius, Venkatasamy Peerally (1981) observed good survival of *Rhizobium* in soil bagasse mixture. Viable counts of up to 3.89×10^8 rhizobia/g of inoculant were obtained after 42 days of incubation at 28°C. Van Schreven (1970) had success with a soil-peat mixture.

Tilak & Subba Rao (1978) investigated indigenously available materials as carriers for legume inoculants in India. These were Indian peat, Indian peat + charcoal (1:1); well decomposed farm yard manure (FYM), FYM + charcoal (1:1); compost, compost + charcoal (1:1); filterpressmud, filterpressmud + charcoal (1:1); clay (vermiculite), teak leaf meal (TLM), clay + TLM (1:1); coconut shell powder (CSP), CSP + lignite and soil. Combinations of Indian peat, farm yard manure, compost or filterpress mud with charcoal (1:1) gave higher rhizobial count than individual carrier alone.

Various materials other than soil mixtures have also received attention as potential carriers of *Rhizobium* inoculants (Fred *et al.*, 1932 cited by Vincent, 1965). These include vermiculite, decomposed sawdust, perlite and rice husk compost. Sepiolite (clay) had low numbers of viable rhizobia after 112 days of incubation (Urio & Chowdhury, 1979).

In Zimbabwe two potential carriers have been reported, bagassilo (Ryder & Grant; Pers. communications) and cob-earth compost (Corby, 1976). Cob-earth is currently being used for inoculant production. The material is autoclaved before aseptic inoculation with *Rhizobium* broth culture. This inoculant contains at least 1×10^9 viable rhizobia/g of dry carrier. The method seems better suited to the fast growing strains of *Rhizobium* than slow growers. The number of viable cells/g of inoculant is comparable with the standard set by the Australian quality control, which is 1×10^9 rhizobia/g of inoculant (Date, 1976).

Filtermud, a waste product from white sugar factories has also shown a lot of promise (Philpotts, 1976; Tilak & Subba Rao, 1978; Urio & Chowdhury, 1979). Philpotts (1976) reported that autoclaved or irradiated filtermud suppressed the growth of the clover strain more than the cowpea strain whose numbers increased in the inoculant after holding period of 36 weeks.

Unsterilized filtermud on the other hand supported the survival of the clover strain for the same period of time whereas the cowpea strain numbers declined between 12 and 36 weeks.

Washed or unwashed sterilized filter mud supported high numbers of both *R. phaseoli* and *R. japonicum* after 112 days of incubation (Urio & Chowdhury, 1979). Another material that has received recent attention as a potential carrier is coal. Kandasamy & Prasad (1971, quoted by Paczkowski & Berryhill, 1979) showed that a peanut *Rhizobium* strain multiplied well in Indian lignite but their experiments lasted only 21 days.

Carriers consisting of 40% coal, 40% bentonite and 20% lucerne meal (CBL carriers) have been investigated by Strijdom & Deschodt (1976) in South Africa. The CBL carriers prepared with anthracite from two different sources of low grade coal were inoculated with cultures of *R. meliloti* or cow pea *Rhizobium*. After 140 days incubation, rhizobial survival of CBL carriers was comparable to that of peat. Paczkowski & Berryhill (1979) also reported the suitability of coal as an alternative carrier. Anthracite, bituminous coal, sub-bituminous coal and lignite with pH range of 4.7 to 7.5 were tested. All except an Illinois bituminous coal (pH 5.0) and a Texas lignite,

supported the growth and survival of three strains of *R. phaseoli* used. The coal-based inoculants in which rhizobial viability was maintained exceeded the quality standard for peat-based inoculants ($> 1 \times 10^6$ rhizobia/g) for at least 7 months. Sharma & Verma (1973) reported that lignite neutralized the calcium carbonate and supplemented with 10% lucerne meal worked as well as American peat. Sharma & Tilak (1974) investigated the comparative efficiency of lignite, sand, farm yard manure, soil and peat with respect to soybean inoculants and observed promising results with lignite. Halliday & Graham (1978) also reported good success with coal and it compared well to peat cultures. In a comparative study on survival of *R. trifolii* in three carriers, namely coal, peat and cachaza (filter mud broth), it was found that rhizobia did not survive in cachaza possibly due to its high acidity. Survival measured for 120 days at 28°C on coal was slightly better than on peat (Munevar & Graham, 1977).

Despite the fact that coal based carriers have recently been reported to perform as well as or even better than lignite carrier, the majority of the Indian inoculant manufacturers are using lignite as carrier for commercial production (Sahni, 1976).

This literature review leaves a firm impression that peat has not been challenged as a carrier and that it is relatively easy to devise a substrate that would support satisfactory growth and survival of rhizobia. The current problem is that these materials have not been studied sufficiently to be used in commercial inoculant production.

2.3. Some factors affecting growth and survival of *Rhizobium* in carriers

2.3.1. pH

Although *Rhizobium* strains may differ somewhat with respect to optimum pH in culture (Jensen, 1942), most strains grow well at pH 6.0 to 7.0. Consequently peat carriers are usually adjusted to pH 6.5 to 7.0 (Burton, 1964 and Roughley, 1970). Calcium carbonate is the neutralising agent most frequently used, although magnesium carbonate may also be used with certain peats. However sodium and potassium carbonates are unfavourable for rhizobial survival (Roughley & Vincent, 1967). Van Schreven (1970) confirmed the superiority of calcium carbonate over calcium carbonate with di-potassium hydrogen phosphate; ammonium hydroxide was found to be extremely harmful for rhizobia in peat.

2.3.2. Temperature

The maintenance of the initially large population of rhizobia obtained soon after inoculation of peat is affected by several factors like storage temperature and the moisture content of the inoculant. The survival of *R. trifolii* in cultures of unsterile peat stored at 25°C was poorer than in those stored at 5°C (Vincent, 1958). The survival of rhizobia at 26°C was improved in sterile peat (Roughley & Vincent, 1967). Continuous storage of rhizobia at 4°C in sterilized peat restricted initial multiplication and maximum numbers were not sustained until after 26 weeks. One week's pre-storage at 26°C before transfer to 4°C was sufficient to allow maximum numbers to be reached after four weeks (Roughley, 1968). If inoculants stored at temperatures of 4°C are to be used soon after production, incubation for at least one week at 26°C is recommended before use (Roughley, 1970).

Van Schreven (1970) reported the effect of three temperature regimes, namely: 2°C, 10°C and 28°C on survival of *R. leguminosarum*, *R. trifolii* and *R. meliloti*. Storage of cultures at low temperature had favourable effect on survival. Storage at 2°C reduced the effect of desiccation. Soil-peat cultures of *R. leguminosarum* stored for 4 years at 2°C still contained 1.5×10^8 viable

rhizobia/g moist material whereas after storing at 10°C to 20°C only 2.5×10^6 rhizobia/g were obtained.

Bajpai, Gupta & Baliram (1978) investigated the optimum survival temperature of *R. leguminosarum* using low grade Indian peat and a mixture of farm yard manure and tank silt (1:1 w/w) as carriers. The different temperature regimes were 20°C, 30°C, 40°C and 50°C. The overall optimum temperature for survival of the cells was found to be 30°C. There was an increase in viable cells when the temperature was raised from 20°C to 30°C. However, there was successive decline in the viable counts when the temperature was further raised from 30°C to 40°C or 50°C. Bowen & Kennedy (1959) working on both tropical and temperate legumes observed that temperate strain of *R. meliloti* were more tolerant to heat (35.5 to 42.5°C) than *R. leguminosarum* and *R. trifolii*. Brockwell (1963) noted that upto 40°C there was no serious rhizobial mortality but beyond this temperature the mortality was high.

Whilst it is impossible to set general expiry dates for cultures because of differing environments, sowing seasons and characteristics of the peat cultures, experience in Australia has shown that inoculants prepared with unsterilised peat and stored for three months at 4°C followed by six months under

retail conditions have proved generally satisfactory. The storage periods may be extended for pure cultures made in sterilized peat to six months at 4°C followed by upto nine months under retail conditions (Roughley, 1970).

2.3.3. Moisture content

The final moisture content of the inoculants has a marked effect on numbers of rhizobia (Van Screven *et al.*, 1954; Roughley & Vincent, 1967) and the optimum level differs for cultures prepared in sterilised compared to non-sterilised peat (Roughley, 1968). Not only is the initial moisture level critical but there is a marked relationship between death rate of rhizobia and the rate of water loss during storage of inoculant (Vincent, 1958). In non-sterile peat, a moisture content of 40 to 50% proved optimal for the three strains used. These were clover, lucerne and cowpea strains. Clover and lucerne strains survived poorly at 60% moisture content. At 30% moisture content, all strains died rapidly and the proportion of contaminant microorganisms increased. In sterilised peat, the rhizobia used were much more tolerant to higher levels of moisture and growth was optimal in the range of 40 to 60%. Numbers of all three strains of rhizobia examined were restricted by 30% moisture after storage

for only one week at 26°C (Roughley, 1968). In the U.S.A., Burton (1967) reported that the percent moisture content of the finished peat-based inoculant is between 35 to 40% on wet weight basis.

Paczkowski & Berryhill (1979) on their investigations with a coal-based carrier, achieved a final moisture content of between 60 to 70% of the carrier's water holding capacity. Corby (1976) observed that on completion, the cob-earth inoculant contained about 40% dry matter and 60% water by weight.

Based on evidence available in literature, it is clear that most of the values reported conform with the recommendations of Vincent (1958), Vincent & Roughley (1968). Studies on peat-based inoculants have shown that good survival of *Rhizobium* is achieved with moisture content ranging from 35 to 70% of the total wet weight of the finished product (Burton, 1967). This big range could be due to a factor such as organic matter content which could have a marked effect on the optimum moisture content of a particular peat carrier (Strijdom & Deschodt, 1976). The expression of optimum moisture status in terms of pF (water potential) values would therefore be more acceptable for comparative purposes (Roughley, 1976).

2.3.4. Sterilization of Carrier

Sterilization of carriers is important as it renders them suitable as media for rhizobia. Indeed, this is very necessary when a carrier like peat harbours micro-organisms which might interfere with multiplication of rhizobia. Sterilization is especially important when carriers are enriched with bacterial nutrients as practised in Europe, because contaminative micro-organisms soon dominate (Burton, 1967). The choice of method of sterilization depends not only on the type of container in which the carrier is packed but also on the facilities available. The methods employed include flash drying, autoclaving, gamma irradiation, and use of chemical sterilants. Flash-drying at 650°C for a few seconds is used in the U.S.A. and depends on quickly reducing the number of contaminants to a very low level and the survivors do not interfere with the growth and survival of rhizobia (Burton, 1967). There are no difficulties that have been experienced with this method in the U.S.A. and autoclaving flash dried peat continuously for 4 hours at 121°C or for 1 hour on three successive days brings about no improvement over the non-autoclaved peat (Burton, 1967). The advantage of autoclaving is that it is the only method which allows absolutely pure cultures to be prepared.

It however requires glass containers or bags of autoclavable film (Roughley, 1968). In Holland, the medium used for inoculant production is autoclaved for 3 hours at 125°C (Van Schreven, 1970). Longer sterilization times upto 5 hours had no long term deleterious effects to the survival of *Rhizobium* (Van Schreven, 1970).

In Australia, the most convenient system is to package dry (10 to 15% moisture), milled peat into polythene bags (0.05 mm thickness) and sterilized by gamma irradiation at 5 Krad. Sterilization by gamma irradiation is generally superior in promoting growth of rhizobia to autoclaving for 4 hours at 121°C (Roughley & Vincent, 1967).

Ethylene oxide has been used commercially in Australia to sterilize peat, problems associated with adequate penetration of gas into all of the peat and the subsequent removal of this gas necessitated treating the peat in bulk before packaging in an air tight container which would withstand vacuum. The numbers of rhizobia developed in gas treated peat showed a lot of variation from 1×10^9 /g to nothing at all. This variation was attributed to the problem in removing all the traces of ethylene oxide and the ability of this gas to form toxic complexes with organic matter (Roberts *et al*, 1943). Since the peat was treated in bulk, it proved difficult to package and inoculate without introducing

contaminants which often multiplied to numbers in excess of 1×10^9 /g peat (Date, 1970). Results with ethylene oxide showed that this treatment requires special equipment, sterilization is incomplete and that the treatment renders peat less favourable for rhizobia than steam sterilisation (Strijdom & Deschodt, 1976).

2.4. Methods of Seed Inoculation and Their Effects of *Rhizobium* Survival

2.4.1. Forms of inocula

Cultures used for inoculating seeds can take various forms. Cultures grown on agar or liquid broth may be simply suspended in water; freeze dried and peat-based cultures may be used as well. Survival of the suspended bacteria on the seed will however, be materially improved by the use of various stickers or adhesives.

Several such materials have been reported by different scientists, gum arabic an exudate from *Acacia* spp., has proved very beneficial in increasing longevity of rhizobia in combination with a peat-based inoculum (Brockwell, 1962). This however had no beneficial effect on *R. japonicum* applied to soybean seed in broth culture (Burton, 1976). Early methods of inoculation sometimes entailed use of molasses or sugar for sticking the inoculant to

the seed (Dobson & Lovvorn, 1949; Smith, Blaser & Thornton, 1945, cited by Burton, 1976). Further research has shown that stickers served other important functions (Vincent, 1958). Rhizobia die quickly on freshly inoculated seed, particularly with broth inocula, but sugars dissolved in the aqueous inoculum before application to seed substantially reduce this loss. Disaccharides are more effective than monosaccharides. Sucrose and maltose are superior to mannitol and sorbitol, alcohol sugars often used in growing rhizobia (Burton, 1976). Skimmed milk has also been reported to work well as a wetting agent (Date, 1970). Vincent (1970) has recommended substituted celluloses like 5% methyl ethyl cellulose (Cellofas A), methyl hydropropyl cellulose (Methofas) or carboxy methyl cellulose.

Carrier cultures like peat are almost always used as a suspended slurry either in plain water or with addition of 10% sucrose or 40 to 45% gum arabic as adhesives. Alternatively, the seeds could be wetted properly with the adhesive then moist, well mixed inoculum of peat can be added and mixed well (Vincent, 1970).

2.4.2. Dosage of applied inoculum

The number of rhizobia needed per seed varies with the species of the legume as well as soil condi-

tions. Working with tropical soils, Cloonan (1966) found that *Dolichos lablab* required higher levels of inoculum than did *Vigna sinensis*. Approximately 1×10^6 rhizobia/seed were required to give 50% or better crown nodulation in *Dolichos lablab*.

A sensible question which one would ask in connection with the size of inoculum is how many cells of viable rhizobia/seed would be required to bring about effective nodulation under field conditions. There is no definite answer to this question since the literature reveals a range from 50 to several thousand cells/seed (Fred, Baldwin & McCoy, 1932). The reason for this big range is because of all the variables encompassed by soil and weather conditions (Burton, 1967). Nonetheless, there is evidence that there is far greater numbers required than were formerly suspected. Certainly, thousands of rhizobia seed give a better assurance of success. Highly effective nodulation of soybeans is seldom obtained with fewer than 1000 cells/seed even under moderately favourable conditions (Burton, 1967).

Early evaluation of these factors indicated that a minimum of 100 rhizobia/seed was sufficient, however, this was increased to 300 or preferably 1000/seed as evidence of establishment and nodulation difficulties were recorded where the numbers were less (Date, 1970).

In Australia a standard of 300 rhizobia/seed at sowing has been found a safer minimum to give prompt nodulation under normal field conditions. Today, the Australian inoculant industry has fixed standards for the inoculant quality for the various legume seeds. This inoculant should provide a minimum of 3000 rhizobia/seed and at the end of its useful life would provide 300 rhizobia/seed. The average inoculant at the time of seed inoculation by farmer, is approximately 30 X the minimum standard and provides about 10,000 rhizobia/seed (Date, 1976). In Southern Australia, proportions of nodulated plants, increased number of nodules and greater yields were obtained when rhizobia on the seed were increased from 4×10^3 to 4×10^6 /seed (White, 1967). With more than 3×10^4 rhizobia/seed, one might expect excellent nodulation. However, Burton & Curley (1965), showed that 2×10^5 rhizobia/seed was needed for good nodulation of soybeans planted under optimal moisture conditions in Central Wisconsin, U.S.A. Alfalfa or clover seed planted under similar conditions was nodulated effectively by 5×10^2 rhizobia/seed.

Since such wide variations in number of rhizobia/seed can occur, it is clear that inoculant quality is an important factor in determining whether inoculum applied to the seed will promptly form effective nodules on the host roots.

2.4.3. Survival of *Rhizobium* on non-pelleted seeds

The recommended method of seed inoculation is ⁱⁿ the form of peat-slurry prepared with water or any form of adhesives (Roughley & Vincent, 1967; Burton, 1979). These adhesives have been developed to improve the stickability of the inoculum to the seeds, however there are several reports of the superiority of gum arabic to methyl ethyl cellulose (Brockwell, 1962). In a comparative study of peat slurries made from different wetting agents on subterranean clover inoculated with *R. trifolii*, a water slurry of peat culture gave 25 rhizobia/seed, gum arabic slurry of peat culture had 13000 rhizobia/seed while methyl cellulose of peat culture on the other hand had 4270 rhizobia/seed after 7 days (Date, 1970).

Vincent (1965) also reported the superiority of 40% gum arabic to other forms of stickers like 10% sucrose, 9% maltose or plain water. Using three forms of cultures namely, peat, broth and freeze dried, he found that peat based cultures survived better after 14 days when combined with gum arabic, giving 600 cells/seed. The other treatments had less than 90 cells/seed. Vincent's results were supported by Burton (1976), who reported 80 x as many viable rhizobia on peat-culture inoculated seeds as was found in broth inoculated ones after four weeks.

2.4.4. Survival of *Rhizobium* on Pelleted Seed

The method of inoculation using peat slurry still leaves the organisms exposed to the deleterious effects of acid fertilizers (Roughley, 1970), as well as dry and acid soils (Anderson & Spencer, 1948). Protecting the inoculant with a pellet of lime results in longer survival under these conditions. (Loneragan *et al.*, 1955.).

Refinements in this technique has led to its widespread use in aerial sowing, broadcasting and sowing under adverse soil and weather conditions (Roughley, 1970; Date, 1970).

The quality of a lime pellet on the seed and its ability to provide protection for the bacteria is determined by the concentration, type of adhesive and the form and particle size of the pelleting material (Roughley, 1970). Brockwell (1962) demonstrated the superiority of 45% gum arabic (W:V) in water in promoting survival of rhizobia over 5% methyl ethyl cellulose. This was confirmed by Date *et al.*, (1965) who claimed it was also superior to 5% substituted celluloses. A concentration of 40% was found superior to 10% and 20% gum arabic for both pellet formation and for survival of rhizobia

Roughley *et al.*, (1966) found that calcium carbonate which was passed through a 300 mesh sieve was necessary for optimal quality of the pellet. Shipton &

Parker (1967) studied the effect of lime pelleting on survival of rhizobia applied to yellow Lupin (*Lupinus luteus* L) and serradella (*Ornithopus compressus* L.) as water suspension washed from agar and as peat inoculum. Lime coating greatly reduced nodulation where the rhizobia were applied as a suspension but had no adverse effect with the peat inoculum.

When lime pellet inoculation spread to areas without acid soils, various other coating materials were substituted for limestone. Bentonite clay mixed with various organic supplements proved successful on subterranean clover (Bergersen, Brockwell & Thompson, 1958). While fine limestone is beneficial in adversely acidic conditions, precipitated calcium carbonate as a coating on inoculated seed can be detrimental.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Root-nodule Bacteria

Rhizobium strains used in this study were selected from cultures held by the University of Nairobi's MIRCEN Project Laboratory. These were *Rhizobium phaseoli* strain NUM 406 and *R. japonicum* strain NUM 504 which have been used for experimental inoculant production. The two strains were originally supplied by NifTAL project of the University of Hawaii, U.S.A., as TAL 662 and TAL 102, respectively.

3.2. Carriers and study sites

Fresh and decomposed filtermud were sampled from five preselected sugar cane factories in Kenya, namely, Ramisi Sugar Factory, officially known as Associated Sugar Company Limited, P.O. Box 90134, Mombasa; Miwani Sugar Mills Limited, Private Bag, Miwani; Muhoroni Sugar Mills Limited, P.O. Box 2, Muhoroni; Mumias Sugar Company Limited, Private Bag Mumias and Nzoia Sugar Company Limited, P.O. Box 285, Bungoma.

Filtermud was sampled in the months of June and August, 1981. Fresh filtermud was collected

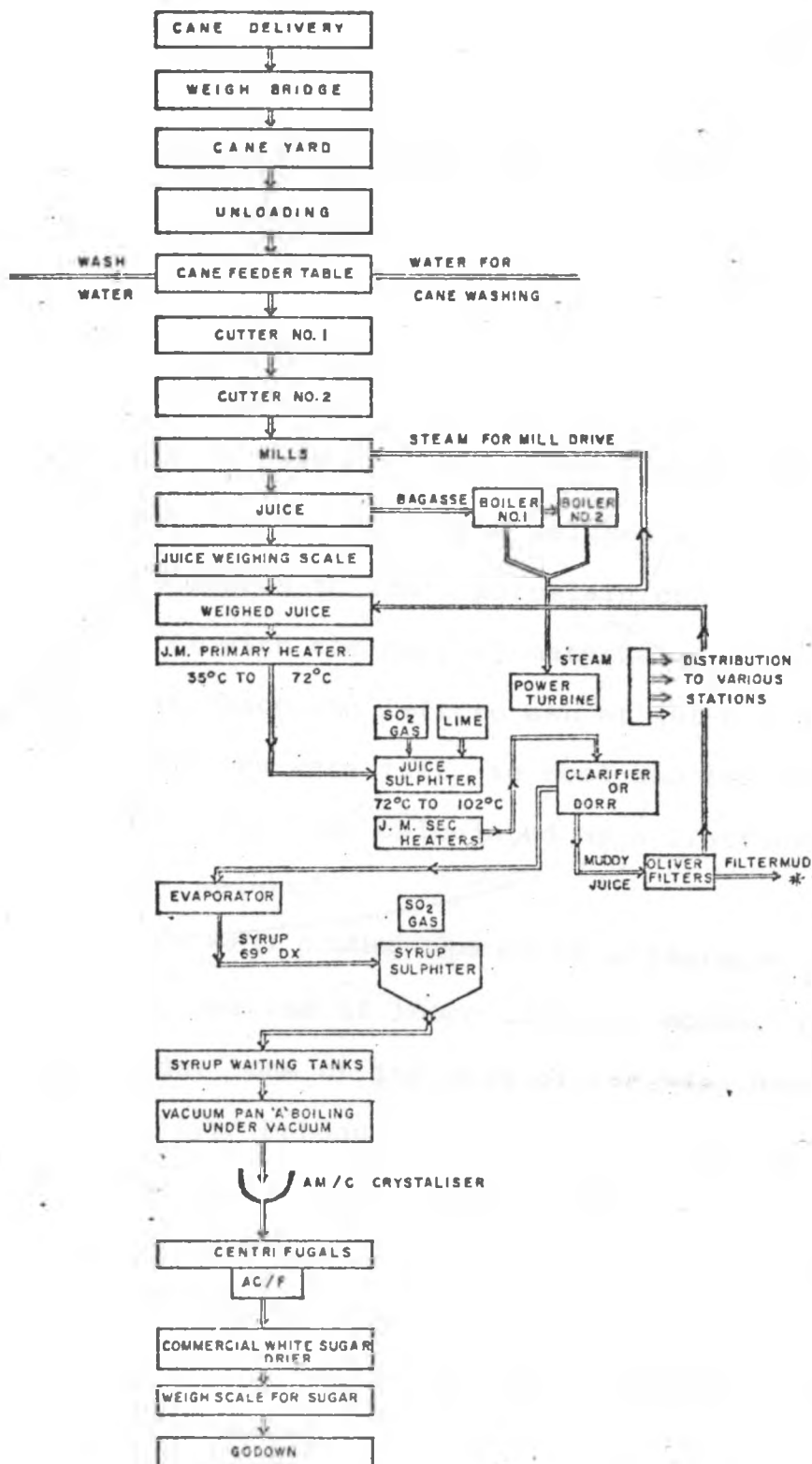
directly from the filters while decomposed filtermud was sampled from the factory dumping sites several months after disposal. Filtermud collected from Ramisi was sampled on the 26.6.81 at which time the decomposed filtermud was six months old. The samples of filtermud which were collected from Miwani and Muhoroni were sampled on the 12.8.81, decomposed filtermud from the two factories were three and six months old respectively. Filtermud from Mumias and Nzoia were collected on 13.8.81 while the decomposed materials were eight and three months old respectively. Sampling of filtermud was done during a fairly dry period. Fig. 1 shows a flow diagram of a raw sugar factory. Fresh filtermud is obtained at point* of the diagram.

Gamma irradiated peat, 5 Mrad, supplied by the Agricultural Laboratories, Sefton, N.S.W., Australia, was used in these investigations as a standard carrier. The peat was supplied as very fine powder ground to pass through a 200 mesh sieve.

3.2.1. Preparation of filtermud

Filtermud was air dried by spreading out on polythene sheets, ground into fine powder using a hammer mill (Sihra Engineering Works) and sieved to pass through a 2.0 and 0.6 mm-sieves for physico-chemical analysis while portions used for microbiological studies were passed through a 210 μm

FIG.1: FLOW DIAGRAM OF A RAW SUGAR FACTORY



Source:- Anonymous (1981)

sieve. The sieved filtermud was packed in polythene bags, sealed and stored at room temperature before use.

3.3. Determination of Physico-chemical Properties of Filtermud and Peat

3.3.1. Physical analysis

Ash content was determined by the furnace method. Filtermud ground and sieved to pass through a 2 mm sieve and peat were weighed in 1g portions and transferred to 25ml porcelain crucibles. The crucibles with the carrier materials were put in a muffle-furnace and left to ash at 400°C for 3 hours. The crucibles were left to cool and the percentage of ash content was calculated as a fraction of the original weight.

The water holding capacity of filtermud was determined by the use of 100cc circular moisture cans with open ends. One of the ends of can was covered with a four layer bandage and tied with rubber bands. The cans were filled with filtermud and levelled with a microscope glass slide. The filled moisture cans were put in a water trough submerged at the bottom and left until properly saturated with water through the process of capillary. Due to water absorption there was a tendency of swelling of filtermud after

saturation, a sharp knife was used to level off the swollen portion. The cans were weighed and then oven dried at 80°C to constant weights.

The water holding capacity of peat could not be determined using this method because of its water repellent nature.

Colours of dry and moist filtermud and peat were determined using a Munsell soil colour chart. Moist carriers, 1:2 (w/v) ratio were prepared by wetting 1g of carrier with 1ml of water. The soil colours from respective study sites were obtained from the soil survey section of the National Agricultural Laboratories, Nairobi, Kenya.

3.3.2. Chemical analysis

Determination of pH was achieved using a pH meter E350 B Metrohm Herisau. This meter was used for all pH determinations. The pH readings were taken in both distilled water and 0.01 M CaCl₂ at 1:5 (w/v) ratio. The method of Olsen & Dean (1965) was adopted for determination of extractable phosphorus. This method employs 0.5M sodium bicarbonate solution adjusted with sodium hydroxide to a pH of 8.5 as the extractant. Carriers were weighed out in 5.0g samples then shaken with 100 ml of extractant together with one teaspoonful of P-free activated charcoal on a MK V orbital shaker (L.H. Engineering Co. Ltd. Stoke Poges England),

at speed 7 for 30 minutes. The suspension was, filtered through a Whatman No. 42 filter paper.

For colour development, 10 ml of ammonium molybdate-hydrochloric acid solution was added to 10 ml of filtrate in a 100 ml volumetric flask. Then 1 ml of 0.025M SnCl_2 solution was added, shaken and made to 100 ml mark. A standard curve was prepared using standard P-solutions containing 0.1 to 5 ppm P. Measurements were taken on a Pye Unicam Spectrophotometer, SP 500, Series 2 at 882nm.

The Walkley & Black (1947) method was used for determination of organic matter. This method was however modified to suit the high organic matter of the carriers. Therefore, only 0.1g of peat or filtermud was used. The percentage of organic matter in each carrier was obtained by multiplying the values of organic carbon by 1.724, a conversion factor normally used in soil analysis (Ahn, 1972).

The level of total Nitrogen in the carriers was determined by the Kjeldahl method described by Bremner (1965). Carriers were weighed out in 1 g quantities and transferred to 300 ml Kjeldahl flasks. To this 10 g of mixed catalyst (a mixture of Selenium powder, 3g; Copper Sulphate, 10 g & Potassium Sulphate, 160 g) washed down with 7 ml distilled water was added. To this, 20 ml 36 N Sulphuric acid was added and left to stand for 20 minutes. The flasks were gently heated on digestion stands in fume

chambers. Bumping was reduced by adding five 2mm glass beads per flask. When the digest cleared, the process was continued for a further 2½ hours. After cooling, 100ml of distilled water was added and shaken to mix. The liquid was transferred to a 250 ml volumetric flask and the volume made up to 250 ml. Distillation for ammonium in the digest was done by adding 25 ml of 10N sodium hydroxide and 20 ml of 2% boric acid. The ammonium-N was determined by titrating with 0.01N sulphuric acid. The colour change at the end point is from green to grey to pink.

The levels of exchangeable bases were obtained using the ammonium-acetate method. The ammonium-acetate leaching procedure adopted in this investigation was described by Ahn (1972). A small plug of cotton wool was placed at the bottom of a leaching tube followed by a layer of 1 cm of acid washed quartz sand. Carrier materials were weighed in 5 g portions and mixed with 10 ml of the acid washed sand. The carrier sand mixture was transferred into the leaching tube using a funnel. A plug of cotton wool was placed on top of the mixture. The leaching tubes were put in the rack and clean 100ml volumetric flasks were placed below. Using a measuring cylinder 25 ml of ammonium acetate was added. The clip on the rubber tubing beneath the leaching tube was adjusted to give a leaching rate

of a drop every two seconds. When the first 25 mls of ammonium acetate had passed through, a second 25 ml aliquot was added. The procedure was repeated for the third and fourth aliquot. Each aliquot was allowed to pass through the soil samples before adding the next. When leaching had ceased the contents were made upto 100 ml mark with N-ammonium acetate, pH 7.0 and flasks were covered with stoppers. This leachate was stored at 4°C and was used for the determination of the bases below.

Calcium, calcium and magnesium was analysed using the versenate titration method. In the determination of calcium, 10 ml of 1N-ammonium acetate leachate was pipetted into a conical flask before 10 ml of 10% potassium hydroxide was added to raise the pH to about 12.0. To this, 1 ml of triethanolamine and three drops of 10% w/v KCN were added in order to chelate and therefore suppress interfering metallic ions. Five drops of solochrome dark blue indicator were added. The solution was titrated from red to blue with a standardised EDTA solution (ethylenediamine-tetra acetic acid or versenate solution).

The determination of calcium and magnesium was done by adding 5 ml ammonium buffer solution and 1 ml triethanolamine to 10 ml of 1N ammonium acetate leachate. To this three drops of eriochrome black indicator were added. This was titrated from red

to blue with standardised EDTA solution.

Potassium and sodium were determined by the flame photometer method. The leachate used for Ca and Mg determinations was used here. The operation of the EEL flame photometer was followed as outlined in technical paper No. 1 (Ahn, 1972).

3.4. Preparation of inoculants in the laboratory

3.4.1. Culturing of rhizobia

Cultures of rhizobia were maintained on agar slants in screw-capped test tubes and stored at 4°C. These cultures were sub-cultured on freshly prepared slants monthly. Agar slants were prepared from the routine complex medium of yeast extract mannitol agar (YEMA) without Congo red.

3.4.2. Preparation of medium

The routine complex medium, YEMA, had the following constituents, Mannitol, 10.0g; K_2HPO_4 , 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.1 g; yeast extract, 1.0 g and agar, 16.0 g. The first five chemicals were dissolved one by one in distilled water, the volume made to a litre and pH adjusted to 6.8 by adding a few drops of 1 N hydrochloric acid. Agar was added and dissolved by heating to boiling. The medium was dispensed in 5 ml aliquots

to test tubes and autoclaved for 30 minutes at 121°C.

3.4.3. Preparation of actidione stock solution

Actidione, a fungicide, was prepared by dissolving 0.4 g in 100.0 ml of distilled water and the solution was sterilised by filtration using 0.2 μ sartorius filters in pre-sterilized filtration equipment. The sterile fungicide was stored in sterile bottles at 4°C and a known amount was added to cooled YEMA medium to give a concentration of 0.002%. Actidione was incorporated to the bacterial medium just prior to pouring in the petri dishes. The fungicide was used in this study to suppress fungal contaminants.

3.4.4. Selection of carriers for inoculant production

Carriers with a pH range between 6.5 to 7.5 were used for inoculant production but those carriers with pH below or above the given limits were adjusted using 5% calcium carbonate and 5% calcium sulphate (Balasundaram, Personal Communications) respectively. The correct addition was achieved after predetermination of neutralization curves of these chemicals.

3.4.5. Packaging

Ten g portions of finely ground filter mud and peat were each packaged into autoclavable high density polythene bags (150 gauge). Ten percent yeast extract mannitol (YEM) broth (v/w) was added to the carriers and the bags were sealed using an Audion Elektro sealer. The carriers were mixed manually and autoclaved for two hours at 121°C on two consecutive days (Burton, 1967).

3.4.6. Preparation of inoculants

Inoculants were prepared by injecting the sterile carriers with *Rhizobium* broth cultures prepared as shown below. The YEM broth has the same chemical composition as YEMA except that the former lacks agar. The YEM broth was dispensed in 5 ml and 95 ml quantities into test tubes and 250 ml Erlenmeyer flasks respectively. The test tubes were capped and the flasks plugged with cotton wool, covered with aluminium foil and all were autoclaved for 30 minutes at 121°C. The stock culture slants were washed with 2ml sterile YEM broth and a loopful of the resulting broth suspension was transferred aseptically to 5ml sterile broth. The test tubes inoculated with *R. phaseoli* culture were incubated at 28°C for three days since it is

a fast grower. A slow grower like *R. japonicum* takes longer time and therefore broth cultures inoculated with it was incubated for five days at 28°C.

The 5 ml broth culture is known as starter culture in inoculant technology and is a means of detecting contaminated cultures. This is done by checking visible contamination within the cultures before transferring such cultures to larger flasks. The starter culture was transferred to the 95 ml broth medium in the Erlenmeyer flasks and put on a Gallenkamp Orbital Shaker at 100 revolutions/minute for five and seven days for *R. phaseoli* and *R. japonicum* respectively.

3.4.7. Determination of contaminants in the broth culture

The broth cultures from the shaker (shaker culture) were screened for contamination by fast growing contaminants. This was done by streaking a loopful of culture onto glucose-peptone agar slants 24 hours before inoculation of the carrier with the culture material. This helps to eliminate contaminants with a faster growth rate than rhizobia.

Glucose-peptone agar had the following constituents: glucose, 5.0 g; peptone, 10.0 g; agar, 15.0 g; and bromo cresol purple, 10.0 ml, all made to one litre. The pH was adjusted to 7.0 using 1N sodium hydroxide before the addition of agar. The medium was dispensed in 5.0 ml aliquots into test tubes and autoclaved for 30 minutes after which slants were prepared by cooling the medium whilst placing the test tubes at 45 degrees.

3.4.8. Inoculation of broth cultures into carriers

Sterile sealed carriers were checked for leakages; their contents were mixed well. The surface of the packet to be injected with broth culture was sterilized with 95% alcohol. Nine ml of broth culture was aseptically injected into the carriers by the use of a 10 ml disposable sterile hypodermic syringe and a 21 gauge needle. The punctured area was sterilized again with 95% alcohol, left to dry and sealed with adhesive tape. The injected packets were carefully mixed manually and stored at 28°C for six months. Some packets were used to determine the initial rhizobia numbers in broth culture. Nine ml broth culture was added to the carrier to give moisture content of about 50% of the total wet weight of the inoculant (Roughley, 1968). Plate 1 shows some of the inoculant packets after inoculation.

plate 1



Packets of peat-based and filtermud-based
inoculants , 20 g.

3.4.9. Preparation of medium

The medium used for this study was YEMA-Congo red. Clean glass petri dishes sterilised at 160°C for 2 hours were used. Sterile YEMA-Congo red medium cooled to 40°C was poured in the petri-dishes and left to set. The medium was incubated at 28°C in order to remove excess moisture and to allow for propagation of fast growing contaminants. Contaminated plates were discarded.

3.4.10. Preparation of water blanks

The water blanks were prepared before inoculation for plating purposes. Flat bottles of 300 ml capacity were encircled at 100 ml mark with a marker pen, a few drops of distilled water were added and they were closed for sterilisation. Distilled water was measured in 9 ml aliquots and dispensed into test tubes and covered with stoppers, and 250 ml of water was also dispensed into another lot of 300 ml flat bottles for dilution purposes. Clean glass beads, 4g of 2 mm size, were placed in screw capped test tubes with a few drops of water. These were all sterilised at 121°C for 30 minutes.

3.4.11. Serial dilution

The carrier-based inoculants were weighed in 10g quantities and transferred aseptically to empty

sterile 300 ml flat bottles. Sterile distilled water was added upto 100 ml mark before adding glass beads whose major function was to facilitate agitation. The bottles were covered and handshaken for 2 minutes. Tenfold serial dilution was carried out from 10^{-2} to 10^{-8} where 1 ml was pipetted from the 100 ml suspension to 9ml water blank tube, shaken and the procedure was repeated upto 10^{-8} dilution level. Plating was done at predetermined intervals from 10^{-5} to 10^{-8} during the first three months of incubation and 10^{-4} to 10^{-6} dilution levels were used for the last three months of the inoculation storage period. From each dilution, 0.2 ml suspension was plated in triplicates using the spread plate method. A sterile glass spreader was used to spread the suspension evenly on the YEMA Congo red plates (Brown *et al.*, 1962 and Vincent, 1970).

3.4.12. Enumeration of rhizobia

The cultured plates were incubated for 7 days at 28°C and the rhizobial colony counts were taken on a colony counter. Periodical plating of all the inoculants used for this investigation was done for a maximum period of six months. For the first three months, the inoculants were plated after every fortnight, then for the rest of the incubation period, plating was done at monthly intervals.

3.4.13. Determination of moisture content of inoculant

Moisture content of inoculant was measured in order to establish the moisture changes of the inoculant at each plating time. The percent moisture content was achieved by oven drying 1 g of inoculant at 80°C to a constant weight (Gardner, 1965).

3.5. Effect of Temperature on Growth and Survival of Rhizobia

In order to determine the effect of various temperature regimes on the growth and survival of rhizobia, the inoculants were stored at three different temperatures. These were 4°C, 28°C and 40°C. Inoculants were prepared by injecting broth cultures of *R. phaseoli* and *R. japonicum* into sterile carriers. Decomposed filtermud from Muhoroni and peat were the only carriers used for this study.

3.6. Survival of *Rhizobium* Cultures on Legume Seeds

The study was carried out to examine the effect of two adhesives on survival of rhizobia on seed. One month old inoculants were used for this purpose and both *R. phaseoli* and *R. japonicum* described in the earlier sections were used in this study as well. Inoculants prepared for the investigation in 3.4. were used.

3.6.1. Preparation of adhesives

Preparation of 10% (w/v) sucrose solution was achieved by dissolving 10 g of analytical grade sucrose solution in 100 ml sterile distilled water in a flask. The flask was plugged with cotton wool and covered with aluminium foil and stored at 4°C before use.

The method used for preparing 40% gum arabic was as described by Vincent (1970). Gum arabic was prepared by dissolving 100 g into 230 ml of sterile distilled water to make about 280 ml gum solution. The gum solution was neutralised with 4% fine calcium carbonate and stored at 4°C before use.

3.6.2. Sterilization of legume seed

In order to reduce contamination by other rhizobia or other microorganisms which might be on the seed surface, clean healthy seeds of *P. vulgaris* and *G. max* were sterilised as follows. The seeds were rinsed with 95% ethanol then surface-sterilised by treatment with 0.2% acidified HgCl₂ for 3 minutes. The seeds were rinsed ten times with sterile distilled water and dried with sterile chromatographic paper in an oven at 40°C until dry (Balasundaram, personal communications).

3.6.3. Preparation of water blanks

Ten ml of distilled water were pipetted into McCartney's bottles before adding ten 2 mm glass beads whose function was to facilitate agitation during serial dilution. Nine ml of distilled water were dispensed in test tubes and the blanks were sterilised for 30 minutes at 121°C.

3.6.4. Seed inoculation

Two different methods of seed inoculation were employed in this study. The sprinkler and slurry method were used to inoculate bean and soybean seeds respectively. The inoculation rates employed in the MIRCEN laboratory of the University of Nairobi were adopted. With the sprinkler method, the seeds were wetted with a known volume of adhesive and mixed with moist inoculant. A packet of 100 g inoculates 15 kg of large seeded legumes like beans, soybeans, cowpea and pigeon pea. Therefore for this study 0.67 g of moist inoculant aseptically mixed with 100.50 g seed previously wetted with 2 ml of adhesive was used. A few seeds were used for serial dilutions to determine the initial numbers of rhizobia at inoculation time while the remainder was stored at 25°C.

In the slurry method, 0.67 g of inoculant was mixed with 2 ml of the adhesive to form a slurry: this was used to inoculate 100.50 g of seed.

3.6.5. Serial dilution and planting

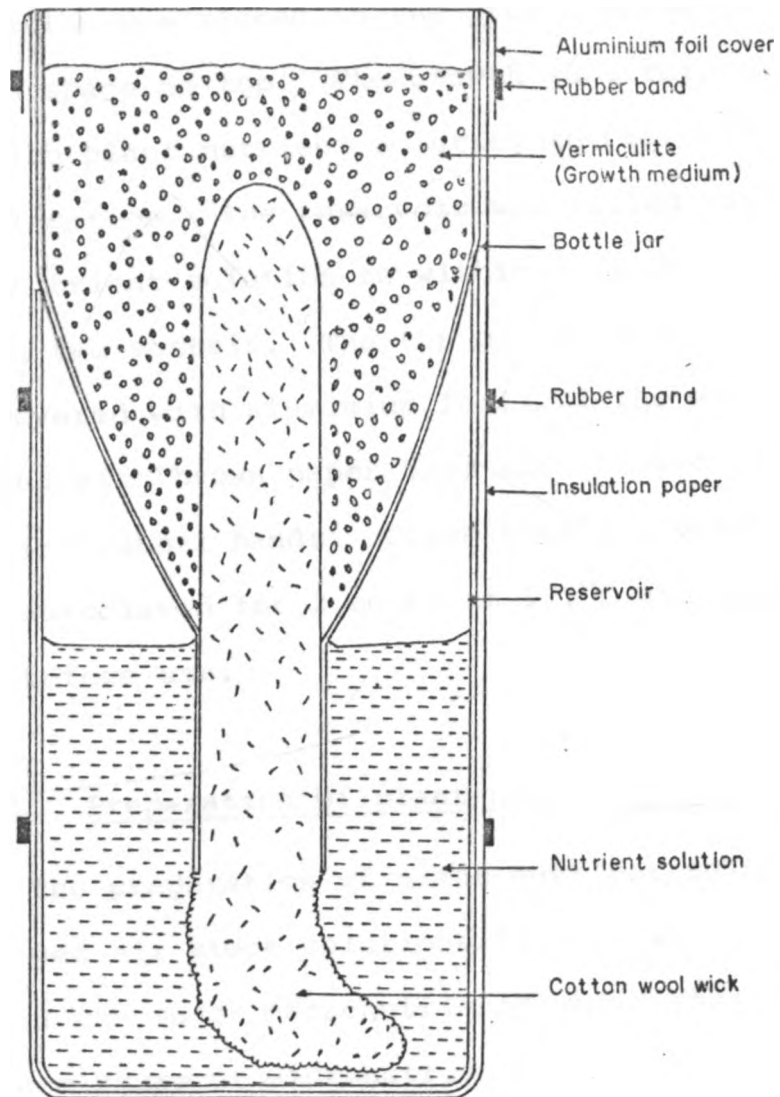
Using sterile forceps 10 seeds were transferred to sterile water blanks and hand shaken for 2 minutes. From this suspension 1 ml was serially diluted in tenfold dilutions from 10^{-1} to 10^{-4} using 9 ml water blanks. The spread plate method was employed to plate 0.1 ml of each dilution in triplicate. Planting was done at zero time and 1-6 days. The plates were incubated at 28°C for 7 to 14 days for bean and soybean rhizobia respectively. Viable counts of bacteria were enumerated using a colony counter.

3.7. Test for nodulation and nitrogen fixation in bottle assemblies

In order to establish the effectiveness of the rhizobial strains used for inoculant production, an experiment was carried out to assess the degree of nodulation and nitrogen fixation. This investigation was done in a green house using modified Leonard jars prepared as follows:

Flat bottomed wine bottles of 0.96 litre capacity were cut as described by Vincent (1970). The diagram of modified Leonard jar assembly is presented in Figure 2. The jars were washed and rinsed with distilled water, a cotton wick was provided to help

FIG. 2: MODIFIED LEONARD BOTTLE ASSEMBLY



Modified from Vincent (1970)

the capillary rise of moisture from the reservoir to the top of the growth vessel. Vermiculite, pH 6.3, previously washed and tested for effective nodulation was packed in the growth vessels leaving a 2 cm space on top. The growth substrate was wetted with plant nutrient solution to facilitate proper sterilisation. The reservoir was filled with the same nutrient solution to within 2 cm of the junction of the two vessels. The top of the growth vessel was covered with aluminium foil and the whole unit covered with brown paper bags and secured with elastic rubber bands. These bottle assemblies were autoclaved for 2 hours at 121°C and kept intact before use.

* 3.7.1. Preparation of plant nutrient solution

The preparation of plant nutrient solution involved six stock solutions five of which contained the major macronutrients while the sixth was a mixture of micronutrients. Solution 1 was calcium supplied as calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), 249.1 g/litre; solution 2 was phosphorus, KH_2PO_4 ; 136.1 g/litre; solution 3 was iron, $\text{C}_6\text{H}_5\text{O}_7\text{Fe} \cdot 5\text{H}_2\text{O}$, 6.7 g/litre; solution 4 was magnesium supplied as $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 123.3 g/litre and solution 5, potassium as K_2SO_4 , 87 g/litre. The constituents of solution 6 were $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$, 0.4462 g; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$,

0.288 g; H_3BO_3 , 0.247 g; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.100 g; $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$, 0.056 g; $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, 0.048 g. The salts were dissolved one at a time and the solution made to one litre. For each 10 litres of distilled water, 5 ml of each of solution 1 to 6 were added. The nutrients were sterilised at 121°C for 1 hour. For N-control treatment, 5 g of KNO_3 was dissolved in 10 litres of distilled water to give a concentration of 70 ppm of N.

This method was adopted from Vincent (1970) and NifTAL Project Training Course Manual 1979, University of Hawaii, U.S.A.

3.7.2. Sterilisation and pre-germination of seeds

In order to maintain sterile conditions, the seeds were surface sterilised and in the case of three treatments, pre-germinated seedlings were used. There were five treatments for this investigation, these included non-inoculated control, N-applied, broth culture inoculated, filtermud and peatbased culture inoculated.

Clean, healthy seeds were sterilised with concentrated sulphuric acid for 8 minutes, the acid was drained off and seeds rinsed with sterile 2% calcium carbonate in water. The calcium carbonate suspension was drained off and seeds pre-germinated on 0.8% (v/w) water agar before planting. Pre-germinated

seeds were planted in the sterile bottle assemblies for the following treatments: non-inoculated control, N-applied treatment and broth culture inoculated. In the remaining two treatments, sterilised seeds were inoculated with carrier-based inoculum before planting.

3.7.3. Planting of seedlings

The seeds were pre-germinated before planting to reduce the risk of germination failure in the Leonard jars. Pre-germinated seeds with a radicle of 2 cm long were planted in the bottle jars, three seeds in each jar. Three slots were made in a triangular pattern at equidistance and the seedlings were put in and the slots covered using sterile forceps.

3.7.4. Inoculation of seedlings and seeds

A small slot was made in the vermiculite at the seedling root with sterile forceps. 0.30 ml of the broth culture was pipetted at the root base and the slot covered. The remaining broth culture was diluted in a tenfold dilution from 10^{-1} to 10^{-8} with sterile yeast extract mannitol broth. Plating was done on 10^{-5} to 10^{-8} dilution levels on YEMA-Congo red medium.

The slurry method of seed inoculation was employed in this study. Carrier-based inoculants (6.67 g) were mixed with 25 ml sterile quarter strength YEM broth to form a slurry. A portion of the slurry (2.5 ml), was used to inoculate 100.50 g of seeds which were planted immediately in the bottle assemblies. All the planted Leonard jars were taken to a green house which had been previously cleaned and sprayed with disinfectant (20% lysol v/v).

3.7.5. Watering and harvesting of experimental plants

Plants were watered once a week with sterile $\frac{1}{10}$ strength nutrient solution. The plants were harvested after 5 and 8 weeks for beans and soybeans, respectively. Harvesting of shoots and root nodules was done in the laboratory. Shoots were cut about 2 cm from the first internode. They were packed in labelled pre-weighed paper bags and oven dried at 60°C to a constant weight. Shoot dry weights were taken per bottle assembly. Root-nodules were carefully removed, counted and oven dried at 60°C.

Oven dried shoots were ground with a grinder and small portions were used for the determination of total nitrogen.

3.8. Experimental design and statistical analysis

The design of all the experiments were randomized complete block. Experiments for physico-chemical analysis were done in triplicates. In survival studies there were two replications for each carrier used while in green house studies, five replicates were set for each treatment.

Analysis of variance was employed on experimental data using LSD to compare the treatment means. The data was analysed by a mini computer Olivetti P6040.

CHAPTER FOUR

RESULTS

4.1. Physico-chemical analysis

The physico-chemical properties of filtermud were determined in order to assess its ability to support the growth and survival of rhizobia.

4.1.1. Physical

The major physical properties of filtermud that were examined included colours of dry and moist filtermud, ash content and water holding capacity.

4.1.1.1. Determination of the colours of filtermud

In order to establish the colours of the carriers the Munsell colour chart (1973) was employed. The colours of soils, filtermud and Australian peat are presented in Table 1a and 1b, respectively. The colours of the soils in Table 1a were compiled from information obtained from the Kenya Soil Survey, National Agricultural Laboratories, Nairobi, Kenya (Siderius & Muchena, 1977). The colours of soil from the Ramisi area was similar to that of soil obtained from Nzoia area. These two soils had the same hue. Another section of Ramisi area exhibited

Table 1a. Munsell Colour Notations of Soils From
Sampling Areas of Filtermud

Area	Munsell notation	Colour names
RAMISI	5 YR 3/1	Very dark grey
	5 YR 3/2 to	Reddish brown
	10 YR 3/2	Very dark greyish brown
MIWANI	10 YR 3/1 to	Very dark grey
	10 YR 2/1	Black
MUHORONI	10 YR 5/3 to	Brown to
	10 YR 3/4	Dark yellowish brown
MUMIAS	N.A.	N.A.
NZOIA	5 YR 4/6	Yellowish red

N.A. = not available

Table 1 b. Colours of Filtermud and Peat

Sample		Dry samples		Moist samples 1:2 (w/v)		
Source	Type	Munsell notation	Colour names	Type	Munsell notation	Colour names
RAMISI	F	10 YR 4/1	Dark grey	F	10 YR 2/1	Black
	D	10 YR 4/1	Dark grey	D	10 YR 2/1	Black
MIWANI	F	10 YR 5/1	Grey	F	10 YR 3/1	Very dark grey
	D	10 YR 4/2	Dark greying brown	D	10 YR 2/1	Black
MUHORONI	F	10 YR 4/1	Dark grey	F	10 YR 2/1	Black
	D	10 YR 3/1	Very dark grey	D	10 YR 2/1	Black
MUMIAS	F	10 YR 5/2	Greyish brown	F	10 YR 4/2	Dark greyish brown
	D	10 YR 3/2	Very dark greyish brown	D	10 YR 2/1	Black
NZOIA	F	10 YR 5/2	Greyish brown	F	10 YR 3/1	Very dark grey
	D	10 YR 4/2	Dark greyish brown	D	10 YR 3/1	Very dark grey
AUSTRALIAN PEAT		10 YR 3/2	Very dark greyish brown		10 YR 2/1	Black

F = Fresh filtermud; D = Decomposed filtermud

soil colour similar to those of soils from near Miwani and Muhoroni although there were slight differences in their value and chroma. Fresh and decomposed filtermud showed very little variation in colour notation both in dry and moist forms. Most of the filtermud samples which had 10 YR 3/2 notation changed to 10 YR 2/1 when moistened. Fresh filtermud generally had colours which were lighter than those of decomposed filtermud. Hue designation for soils from Miwani, Muhoroni and part of Ramisi was the same as that of the filtermud samples from these sites. There was however no relationship between colours of soil and filtermud from a part of Ramisi area and Nzoia. Since the colours of filtermud from all the factories were similar, the colour of the soils in Sampling areas do not seem to have any significant influence on the colour of filtermud.

4.1.1.2. Ash content

The ash content of filtermud was determined in order to establish the amount lost on ignition. Fresh filtermud had an ash content ranging between 54.20% and 71.84% with a mean of 64.33%. Decomposed filtermud had fairly low values of ash compared to fresh filtermud, the lowest value of ash being 43.49% and the highest value being 60.08% with a mean of 49.59%. Peat had a high level of ash, 71.66%. There

was however very little variation in ash content between the type of filtermud and their respective sources. The ash content of peat was comparable to that of fresh filtermud.

4.1.1.3. Water holding capacity

The water holding capacity of filtermud revealed that the mean value for fresh filtermud was 669.09% while the value for decomposed materials was 249.53% (Table 2). It was not possible to measure the water holding capacity of peat due to its water repellent tendency.

Fresh filtermud exhibited higher water holding capacity than decomposed filtermud. The high amount of fibrous material in fresh filtermud could have contributed to its high affinity for water. This was not the case in decomposed samples which were collected while in the process of decomposition.

4.1.2. Chemical

The chemical properties of filtermud were determined so that the major nutrients available could be established. The results are presented in Table 3a and 3b with pH, extractable phosphorus, total nitrogen, organic carbon and exchangeable bases as the major parameters.

Table 2. Physical characteristics of filtermud from
factories in Kenya

Source of filtermud and peat		Ash content % d.w.	Water holding capacity % d.w.
RAMISI	F	66.74	650.65
	D	60.08	342.91
MIWANI	F	71.84	542.15
	D	43.49	281.49
MUHORONI	F	68.56	777.88
	D	44.09	236.01
MUMIAS	F	60.30	524.18
	D	55.60	227.80
NZOIA	F	54.20	890.60
	D	44.70	364.47
AUSTRALIAN PEAT		71.60	N.D.

F = Fresh filtermud D = Decomposed filtermud

N.D. = Not Determined.

4.1.2.1. pH

When the pH of filtermud and peat measured in water and 0.01 M CaCl₂ were compared, decomposed filtermud and peat had higher pH values than fresh filtermud. Decomposed filtermud showed a range between 6.70 and 7.90. The pH of filtermud in water ranged between 5.20 and 7.00. The average pH of peat in water was 6.70 which was comparable to the pH of decomposed filtermud.

4.1.2.2. Phosphorus

Extractable phosphorus measured in fresh filtermud ranged from 8.35 ppm to 14.45 ppm while the mean was 10.67 ppm. Decomposed filtermud exhibited a range between 4.92 ppm and 9.19 ppm with a mean value of 6.92 ppm P. The phosphorus level in peat was much lower than that observed in the two types of filtermud. The concentration of P in fresh and decomposed filtermud was significantly different ($P = 0.05$). The level of extractable phosphorus measured in filtermud from Kenyan factories is much lower compared to the published data from other countries (Alexander, 1972; Wood, 1981 and Abu-Idris *et al.*, 1979). The P level in peat was very low, 1.02 ppm, compared to the values of fresh and decomposed filtermud.

Table 3a.

Chemical Characteristics of Filtermud from Factories in Kenya

Source of filtermud and peat		pH		Extrac- table P ppm	Total N %	Organic C %	C/N Ratio	Organic matter %	Exchangeable bases - me/100g			
		1:5 H ₂ O	1:5 CaCl ₂ 0.01M						K	Na	Ca	Mg
RAMISI	F	7.00	6.50	8.35	1.54	34.67	22.58	59.94	6.15	1.28	198.70	5.30
	D	7.40	7.30	5.42	1.66	34.54	20.85	59.72	9.75	1.52	166.70	4.63
MIWANI	F	5.20	4.90	14.45	0.83	35.56	42.72	61.48	12.42	1.04	151.00	11.00
	D	7.90	7.60	9.19	1.15	22.95	21.02	39.68	10.42	1.04	127.00	5.00
MUHORONI	F	5.60	5.40	11.62	1.06	37.92	35.35	65.55	10.17	0.87	142.00	11.00
	D	7.30	7.20	6.95	1.38	20.46	14.80	35.38	14.65	0.71	126.00	4.00
MUMIAS	F	7.00	6.80	10.12	0.96	32.18	33.67	55.65	4.95	0.71	104.00	14.00
	D	6.70	6.40	8.09	1.86	24.29	13.08	41.99	2.22	0.78	107.00	5.60
NZOIA	F	5.30	4.90	8.82	0.88	31.07	31.14	47.03	7.75	0.70	106.00	17.00
	D	7.00	6.90	4.92	1.17	21.99	18.90	38.02	5.77	0.70	64.00	11.00
AUSTRALIAN PEAT		6.70	6.60	1.02	1.57	36.14	23.07	62.49	11.25	3.05	80.00	7.00

F = Fresh filtermud; D = Decomposed filtermud.

Table 3b.

Chemical Properties of Fresh and Decomposed Filtermud from Kenya

Carrier	Extractable	Total	Organic	C/N	Organic	Exchangeable bases - me/100 g			
	P ppm	N %	C %	Ratio	matter %	K	Na	Ca	Mg
Fresh filtermud	10.67	1.06	33.54	33.29	57.98	8.25	0.92	140.33	11.67
Decomposed filtermud	6.92	1.44	24.85	17.73	42.96	8.56	0.95	118.13	6.05
Australian peat	1.02	1.57	36.14	23.07	62.49	11.18	3.05	80.00	7.00
L.S.D. between types (P = 0.05)	0.16	0.07	0.30	1.81	0.52	0.72	0.19	4.24	0.74
C.V. %	2.62	7.31	1.32	9.39	1.32	10.88	21.91	4.46	11.21

The values are given as means for fresh and decomposed filtermud samples and peat.

4.1.2.3. Total Nitrogen

Total N in fresh filtermud averaged 1.06% with Miwani Sugar Factory showing the lowest value of 0.83% and Ramisi Factory the highest, 1.54%. Decomposed filtermud showed fairly high total N compared to fresh filtermud. Like with fresh filtermud, Miwani factory showed the lowest concentration of total N (1.15%) in decomposed filtermud while the highest value was obtained from Mumias Factory (1.86%). The mean of total N in decomposed filtermud was 1.44%. The difference between sources and types of filtermud was significant at $P = 0.05$.

Total N in peat was within the range found in decomposed filtermud samples, an average value of 1.57% was recorded. Total N values obtained from filtermud from Kenya was comparable with published data from different parts of the world (Alexander, 1972; Prasad, 1974; Abu-Idris *et al.*, 1979) except for one report by Alexander (1971) which gave a very low value of 0.43%. Similarly, total N concentration in peat agrees with earlier reports from other researchers (Burton, 1967; Strijdom & Deschodt, 1976).

4.2.2.4. Organic carbon

Organic carbon determined in filtermud showed that fresh filtermud contained more organic carbon

than decomposed filtermud. Mean values of 33.53% and 24.85% were observed for fresh and decomposed filtermud respectively (Table 3b).

The sources and types of filtermud significantly ($P = 0.05$) influenced the organic carbon level. The value of organic carbon measured in Australian peat (36.14%) was higher than the means for the two types of filtermud examined.

Generally, the values of fresh and decomposed filtermud from Kenyan factories are slightly lower than published data from other countries. Alexander (1972) recorded 37.10%; Abu-Idris *et al.* (1979) gave 40.00% and Wood (1981) obtained 39.50% organic carbon in filtermud from S. Africa. Organic matter values were calculated from the values of organic carbon by multiplying the latter by 1.724, a factor normally used in soil analysis. The trend of organic matter level in these carrier materials was therefore similar to what has been seen in organic carbon (Tables 3a and 3b) contents.

4.1.2.5. C/N Ratio

The C/N ratio was calculated for peat, fresh and decomposed filtermud. Fresh filtermud had a mean of 33.29 while the mean C/N ratio in decomposed filtermud was 24.85. In peat, the C/N ratio (23.07) observed was lower than values of fresh filtermud

and slightly higher than the levels established for decomposed filtermud. There was no significant difference between the C/N ratio of peat and filtermud samples.

4.1.2.6. Potassium

Exchangeable potassium determined in fresh filtermud gave a range between 4.95 m.e./100g to 12.42 m.e/100g with a mean of 8.25 m.e/100g. For decomposed filtermud, a lowest value of 2.22 m.e and a highest of 14.45 m.e. with a mean of 8.56 m.e. were observed. The level of exchangeable K in filtermud from different sampling areas varied significantly at $P = 0.05$. Both fresh and decomposed filtermud samples from Mumias factory showed very low exchangeable K (Table 3a). Australian peat exhibited significantly higher amount of exchangeable K (11.25 m.e) than fresh and decomposed filtermud.

4.1.2.7. Sodium

When the amount of exchangeable sodium was determined in filtermud and peat, it was observed that both fresh and decomposed filtermud exhibited much lower levels than observed in peat (3.05 m.e). An average of 0.92 m.e.Na was obtained for fresh filtermud whereas filtermud from Nzoia Factory gave the lowest value of 0.02 m.e.Na and Ramisi Factory the highest value of 0.3 m.e Na. Similarly, decomposed

filtermud showed very low concentration of exchangeable sodium (Table 3a). Sodium is the element whose level varied most with the highest coefficient of variation of 21.91%. The type of filtermud, fresh or decomposed, had no influence on the amount of exchangeable Na. Interactions between type and source of filtermud was not significant. The source of filtermud, however, had a significant influence on the level of exchangeable Na in filtermud ($P = 0.05$).

4.1.2.8. Calcium

The exchangeable calcium concentration determined in fresh filtermud ranged between 104.00 m.e. and 198.70 m.e. Ca and had an average of 140.33 m.e. Ca. Decomposed filtermud had a mean exchangeable Ca level of 118.33 m.e. Ca. The difference in the amount of Ca in fresh and decomposed filtermud was significant at $P = 0.05$. The amount of exchangeable calcium obtained in peat was within the range for decomposed filtermud (Table 3a).

4.1.2.9. Magnesium

The amount of exchangeable magnesium observed in fresh filtermud was fairly high compared to decomposed filtermud. In fresh filtermud an average of 11.67 m.e. Mg and a range of 5.30 m.e. to 17.00 m.e. Mg were obtained. Decomposed filtermud gave a range

of 4.00 m.e. to 11.00 m.e. Mg with a mean of 6.05 m.e. Mg. The exchangeable Mg concentration in peat was 7.00 m.e. This value was within the range of values observed in filtermud.

4.2. Influence of Filtermud and Peat on Survival of *Rhizobium phaseoli*

In order to assess the growth and survival of *R. phaseoli* in filtermud, inoculants were prepared using fresh and decomposed filtermud. Australian peat was used as a control carrier. Results of the survival of *R. phaseoli* in the carrier materials are presented in Table 4. Except for fresh filtermud from Miwani and Nzoia all the other filtermud samples and peat supported growth and survival of the test *Rhizobium* for at least five months.

Studies with fresh filtermud from the two factories mentioned above were discontinued after eight weeks as a result of contamination. The major cause of contamination could not be established although the presence of sugars in filtermud could have contributed to it. Periodical fluctuation in rhizobial counts, between 10^8 and 10^9 rhizobia/g of carrier, was observed in most carriers. Rhizobial population in all inoculants dropped between 1.65×10^8 and 7.59×10^8 rhizobia/g of carrier by the end of the incubation period (Table 4). The growth and survival

Table 4 Survival of *R. phaseoli* in fresh and decomposed filtermud

Log₁₀ viable cells/g of carrier

Source of carrier	Type	Incubation time in weeks at 28°C									
		0	2	4	6	8	10	12	16	20	24
RAMISI	F	9.22	10.23	9.55	9.75	9.35	N.D.	10.06	9.63	9.10	N.D.
	D	9.15	9.66	9.45	9.55	9.98	N.D.	9.55	9.40	9.51	8.88
MIWANI	F	8.96	0.00	0.00	8.11	8.23	N.D.	N.D.	N.D.	N.D.	N.D.
	D	8.92	9.47	9.64	9.89	9.84	N.D.	9.55	9.07	9.24	8.43
MUHORONI	F	9.07	0.00	8.88	9.44	8.48	N.D.	9.66	9.70	9.60	N.D.
	D	8.83	9.74	9.62	8.45	9.50	N.D.	8.95	8.97	9.01	8.22
MUMIAS	F	9.01	0.00	0.00	9.31	9.46	N.D.	9.31	8.43	9.41	N.D.
	D	9.10	8.97	9.71	9.66	9.72	N.D.	8.98	9.27	9.38	8.34
NZOIA	F	9.21	9.68	0.00	0.00	0.00	N.D.	N.D.	N.D.	N.D.	N.D.
	D	8.37	8.94	9.76	9.75	8.67	N.D.	9.27	9.50	9.28	8.37
AUSTRALIAN PEAT		9.21 ^a	0.00	9.72	9.46	9.65	N.D.	9.70	9.23	9.49	N.D.
		8.32 ^b	9.79	9.66	9.88	9.92	N.D.	9.97	9.44	9.04	8.65

70

F = Fresh filtermud D = Decomposed filtermud N.D. = Not Determined 0.00 = not recovered

a = Control for fresh filtermud-based inoculants b = Control for decomposed filtermud-based inoculants

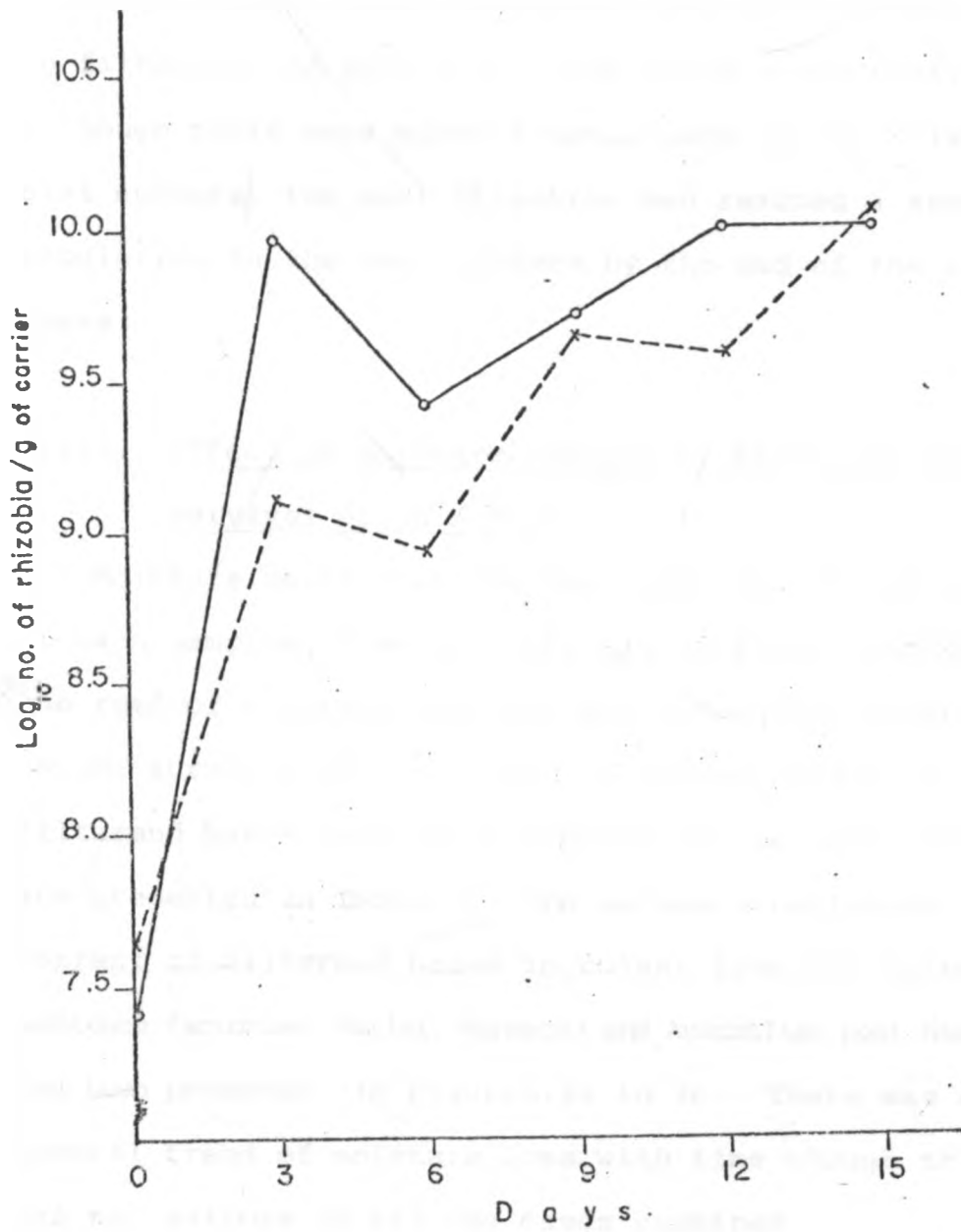
of *R. phaseoli* was influenced by the type and source of filtermud and the incubation period ($P = 0.01$).

On the average, decomposed filtermud proved to be a better carrier than fresh filtermud and the overall rhizobial survival pattern was comparable to that of peat. Among the sources, filtermud from Ramisi performed best followed by peat and decomposed filtermud from Muhoroni.

These results support the earlier findings of Philpotts (1976); Urio & Chowdhury (1979) who reported promising results with filtermud as a carrier of *Rhizobium* inoculants.

4.2.1. Growth of *R. phaseoli* in decomposed filtermud from Muhoroni

Decomposed filtermud from Muhoroni was selected for further studies. Although filtermud from Ramisi Sugar factory gave the best characteristics, it was not used for further studies since the factory does not operate continuously. Besides the factory has a fairly low output and is far from Nairobi. Peat was used as a control carrier in this study. The growth of *R. phaseoli* in filtermud and peat is presented in Figure 3. Initial rhizobial population was adjusted to between 2.5×10^7 and 4.0×10^7 rhizobia/g of carrier (Fig. 3). It was observed that after three days incubation at

FIG. 3: GROWTH AND SURVIVAL OF R. PHASEOLI IN CARRIERS

Australian Peat.....○—○

Filtermud from Muhoroni...x—x

Incubation temperature... 28°C

28°C, the counts were between 10^9 and 10^{10} rhizobia/g in filtermud and peat based inoculants respectively. Although there were minor fluctuations in the rhizobial numbers, the test *Rhizobium* had reached a steady population in the two carriers by the end of the two weeks.

4.2.2. Effect of moisture content of inoculant on survival of *Rhizobium*

Moisture content of the inoculant was determined at each sampling time with the aim of establishing the rate of moisture loss and its subsequent effects on the survival of *Rhizobium*. Moisture levels of filtermud based inoculants sampled for survival studies are presented in Table 5. The values of moisture content of filtermud based inoculant from two selected sugarcane factories, Ramisi, Mohoroni and Australian peat have also been presented in Figures 4a to 4c. There was a general trend of moisture loss with time though this was not uniform in all the cases examined.

Fluctuations in moisture level was common in all inoculants throughout the storage period. Low moisture level was not deleterious to rhizobial survival in most of the observed cases. For instance, inoculants made with fresh filtermud from Ramisi exhibited a very low moisture content, 11%, after five months. In the corresponding

Table 5.

Moisture levels (%) of carrier at various sampling times

Filtermud and peat samples		0 Time	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks	4 months	5 months	6 months
RAMISI	F	51.25	49.00	38.75	37.00	48.00	--	33.00	12.00	11.00	--
	D	51.75	52.00	51.50	49.00	50.00	--	37.00	42.00	35.00	35.00
MIWANI	F	53.50	50.00	45.00	45.00	46.50	--	--	--	--	--
	D	52.50	46.50	49.00	45.00	47.00	--	41.00	41.00	38.00	25.00
MUHORONI	F	51.75	49.00	43.25	39.00	49.00	--	26.00	28.00	33.00	--
	D	51.50	52.00	48.25	41.00	46.00	--	39.00	38.00	42.00	36.00
MUMIAS	F	53.00	53.00	49.75	42.00	45.75	--	38.00	40.00	--	--
	D	52.00	52.50	49.50	45.00	49.00	--	43.00	33.00	43.00	35.00
NZOIA	F	53.00	50.75	47.50	45.00	--	--	--	--	--	--
	D	53.75	50.75	49.00	45.00	44.00	--	39.00	42.00	39.00	31.00
AUSTRALIAN PEAT		54.50	50.00	42.30	42.00	44.00	--	39.00	39.00	45.00	--
		54.00	45.50	46.50	44.00	17.00	--	44.00	43.00	41.00	28.00

F = Fresh filtermud; D = Decomposed filtermud; -- = Not determined

FIG. 4a: MOISTURE LEVELS OF FILTERMUD-BASED INOCULANTS FROM RAMISI

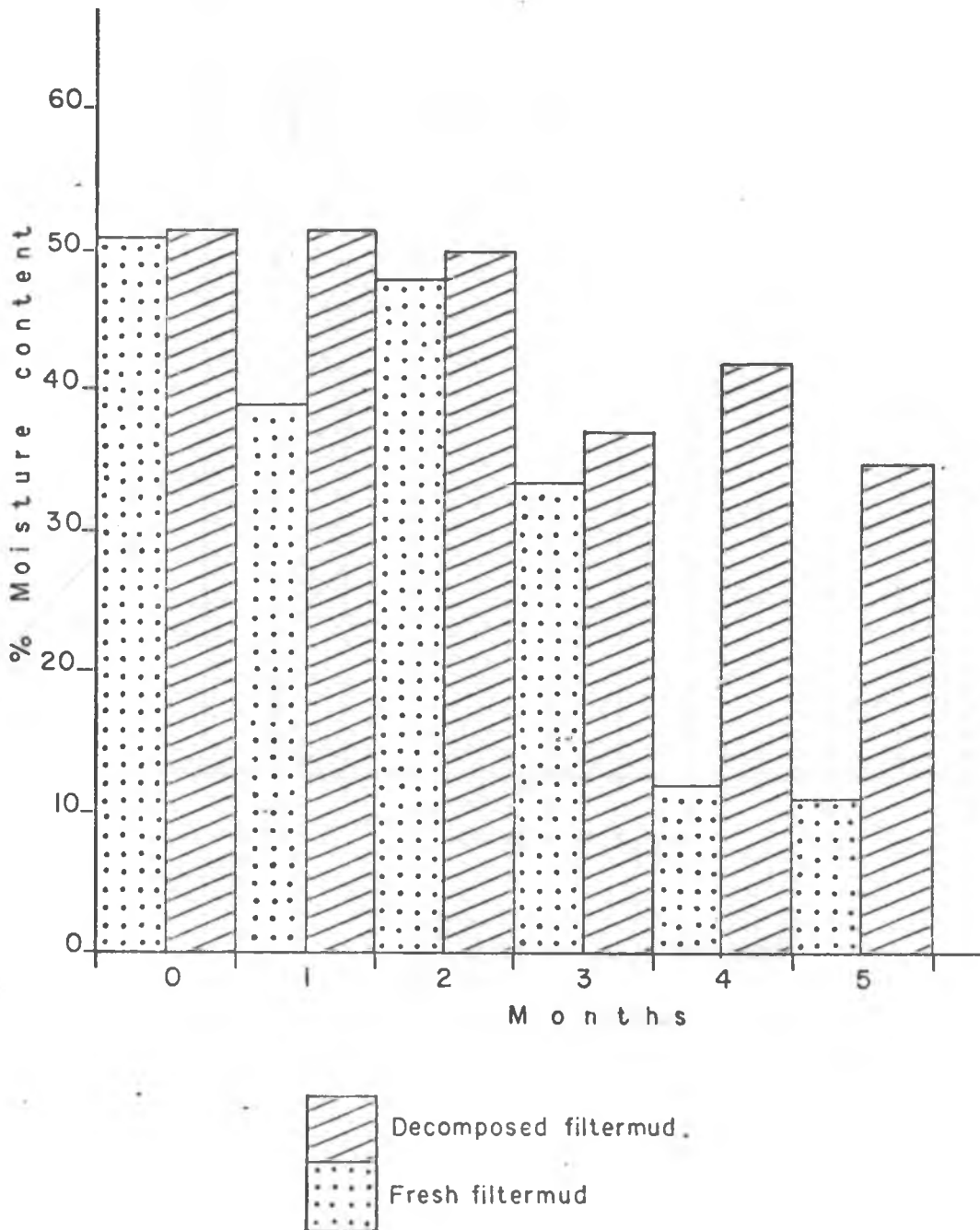


FIG. 4b: MOISTURE CONTENT OF FILTERMUD-BASED INOCULANTS FROM MUHORONI

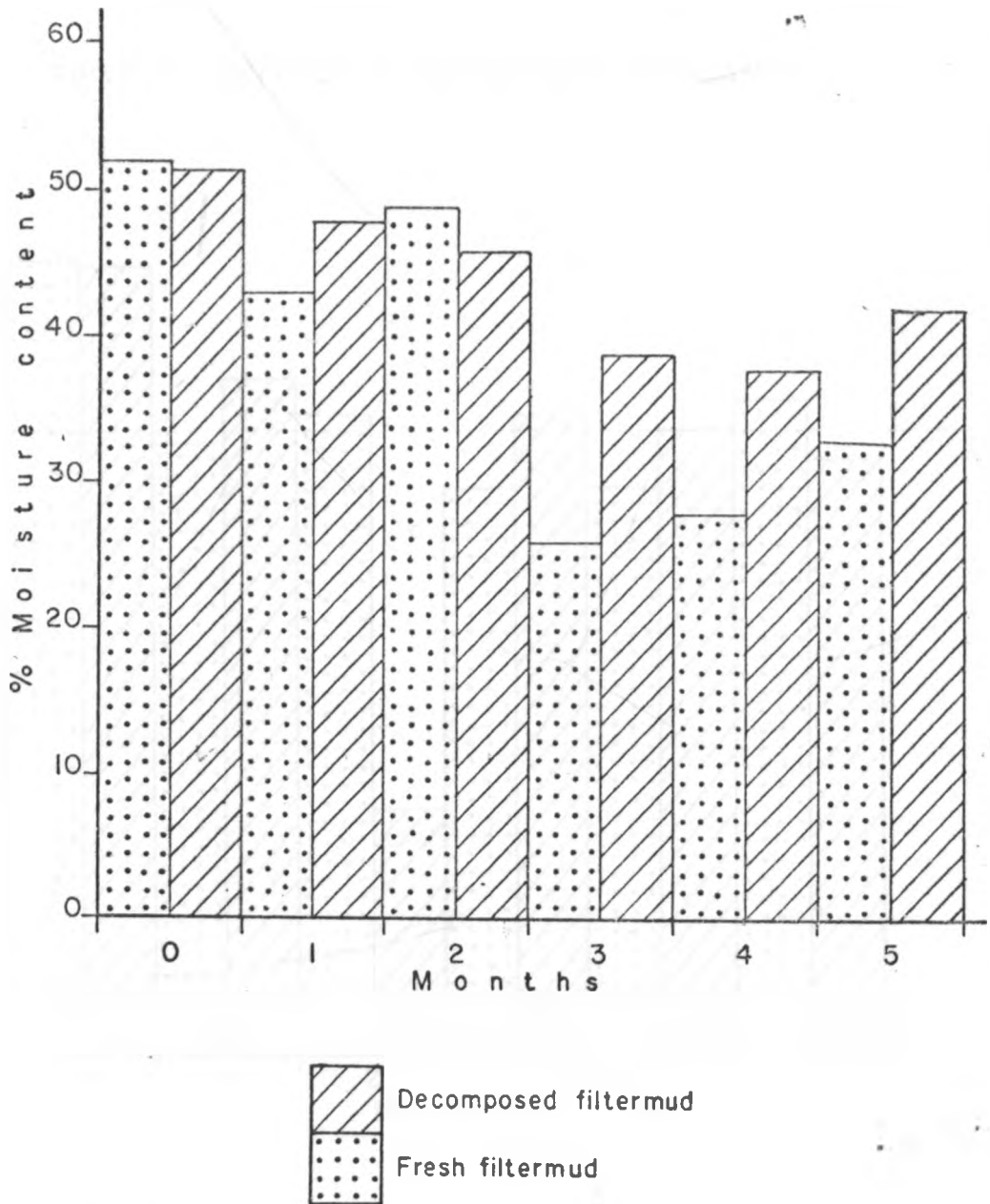
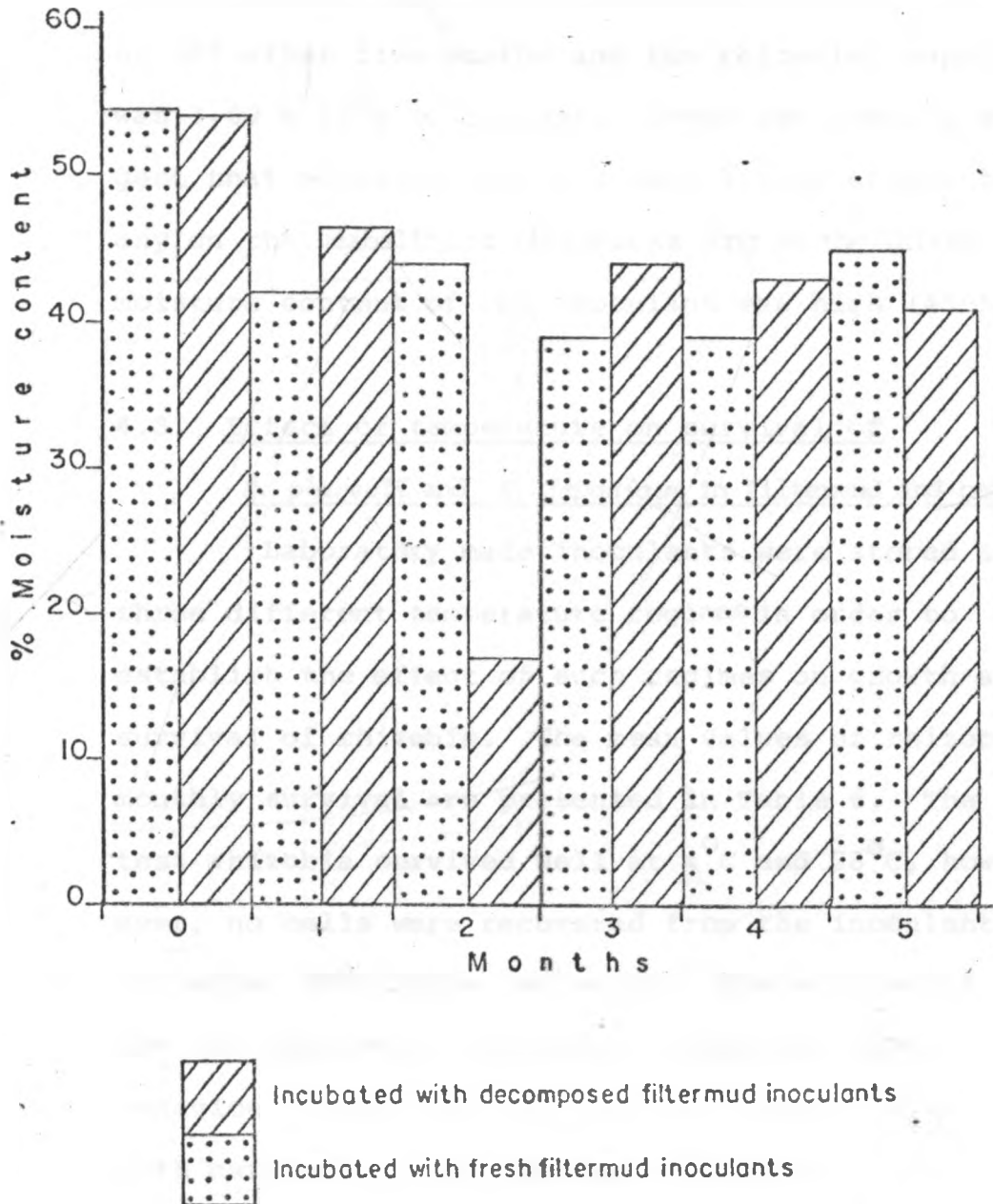


FIG. 4c: MOISTURE CONTENT OF PEAT-BASED INOCULANTS



survival study of the same inoculant the number of viable rhizobia was 1.26×10^9 /g of carrier. Inoculants prepared with peat exhibited moisture content of 45% after five months and the rhizobial population was 3.09×10^9 /g of carrier. These two results suggest that moisture loss has very little effect if any on the viability of rhizobia as long as the initial moisture content of the inoculant was high (45-50%).

4.3. Effect of temperature on survival of

R. phaseoli and *R. japonicum* in filtermud and peat

Laboratory made inoculants were stored in three different temperature regimes in order to establish the effect of such regimes on growth and survival of rhizobia. The mean values of rhizobial monthly survival are presented in Table 6. The test rhizobia survived well at 4°C and 28°C, however, no cells were recovered from the inoculants stored at 40°C after one month. The strains of the two species of *Rhizobium* showed the same behaviour under the three storage conditions in both carriers. Cold storage (4°C) restricted cell multiplication of rhizobia for the first two months, Table 6. Optimal storage temperature was 28°C for at this temperature regime, high cell counts of 1×10^9 rhizobia/g of carrier were achieved. These high rhizobial populations were

Table 6. Survival of *R. Phaseoli* and *R. japonicum* in filtermud and peat at various temperature regimes

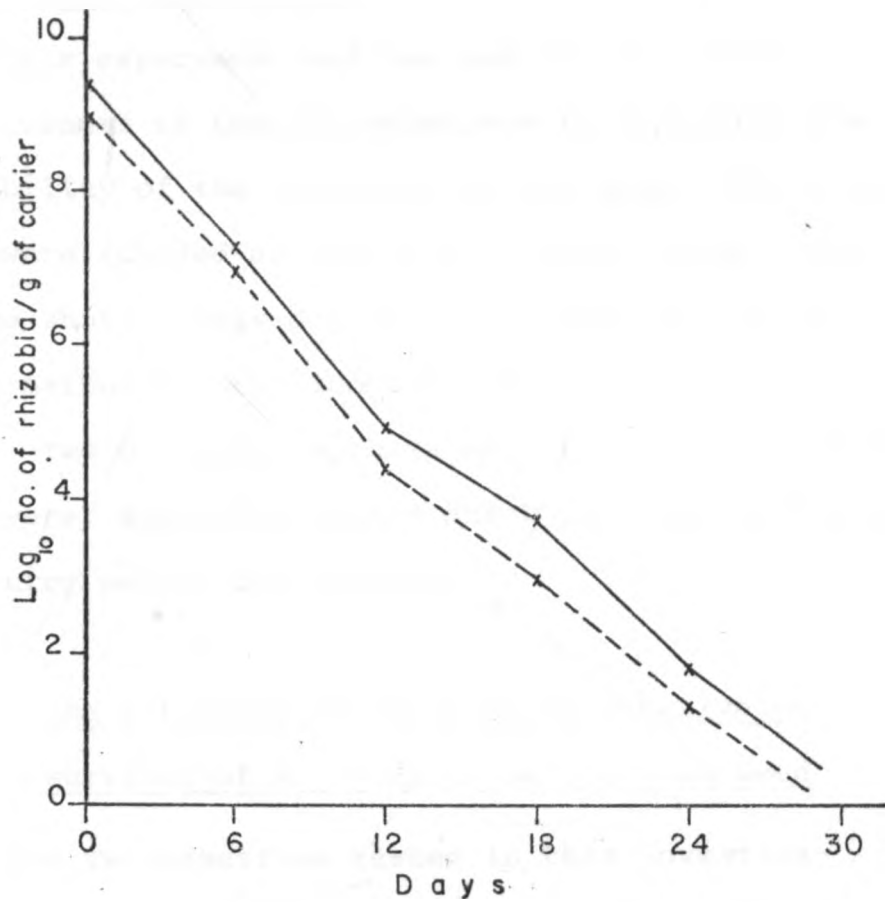
Carrier source and type	<i>Rhizobium</i> spp.	Initial count	Storage temperature °C	Log ₁₀ No. rhizobia/g of carrier		
				Months		
				1	2	3
Decomposed Muhoroni filtermud	<i>R. phaseoli</i>	9.30	4	9.00	8.25	9.14
			28	10.11	9.39	9.67
			40	0.00	0.00	0.00
	<i>R. japonicum</i>	9.14	4	8.46	8.47	9.07
			28	9.95	9.19	9.31
			40	0.00	0.00	0.00
Australian peat	<i>R. phaseoli</i>	9.57	4	9.17	8.65	8.97
			28	9.97	9.66	9.30
			40	0.00	0.00	0.00
	<i>R. japonicum</i>	9.80	4	9.35	9.26	9.58
			28	9.56	9.98	9.85
			40	0.00	0.00	0.00

observed with both species and carrier materials tested. High mortality rates were observed in inoculants stored at 40°C. This was an indication that none of the strains used was resistant to high temperatures. Although storage at 28°C proved optimal, 4°C is the recommended temperature for storage for longer periods of time. This is because at low temperatures the growth rate of microorganisms is minimal therefore even the population of any contaminant is under control. A more detailed investigation of mortality rate of *R. phaseoli* in inoculants stored at 40°C is given in the next section. Like in most of the sections where peat has been used, it served as a control carrier for this investigation as well.

4.3.1. Survival of *R. phaseoli* in filtermud and peat-based inoculants at 40°C

In a separate investigation, the performance of *R. phaseoli* in the two carriers was monitored at 40°C, the results are presented in figure 5. By the 24th day, the numbers of rhizobia had dropped below 1×10^2 cells/g of carrier. No viable cells were recovered after one month.

FIG. 5: EFFECT OF TEMPERATURE ON GROWTH AND SURVIVAL OF R. PHASEOLI IN FILTERMUD AND PEAT (40°C)



Filtermud-based inoculant x-----x
Peat-based inoculant x-----x

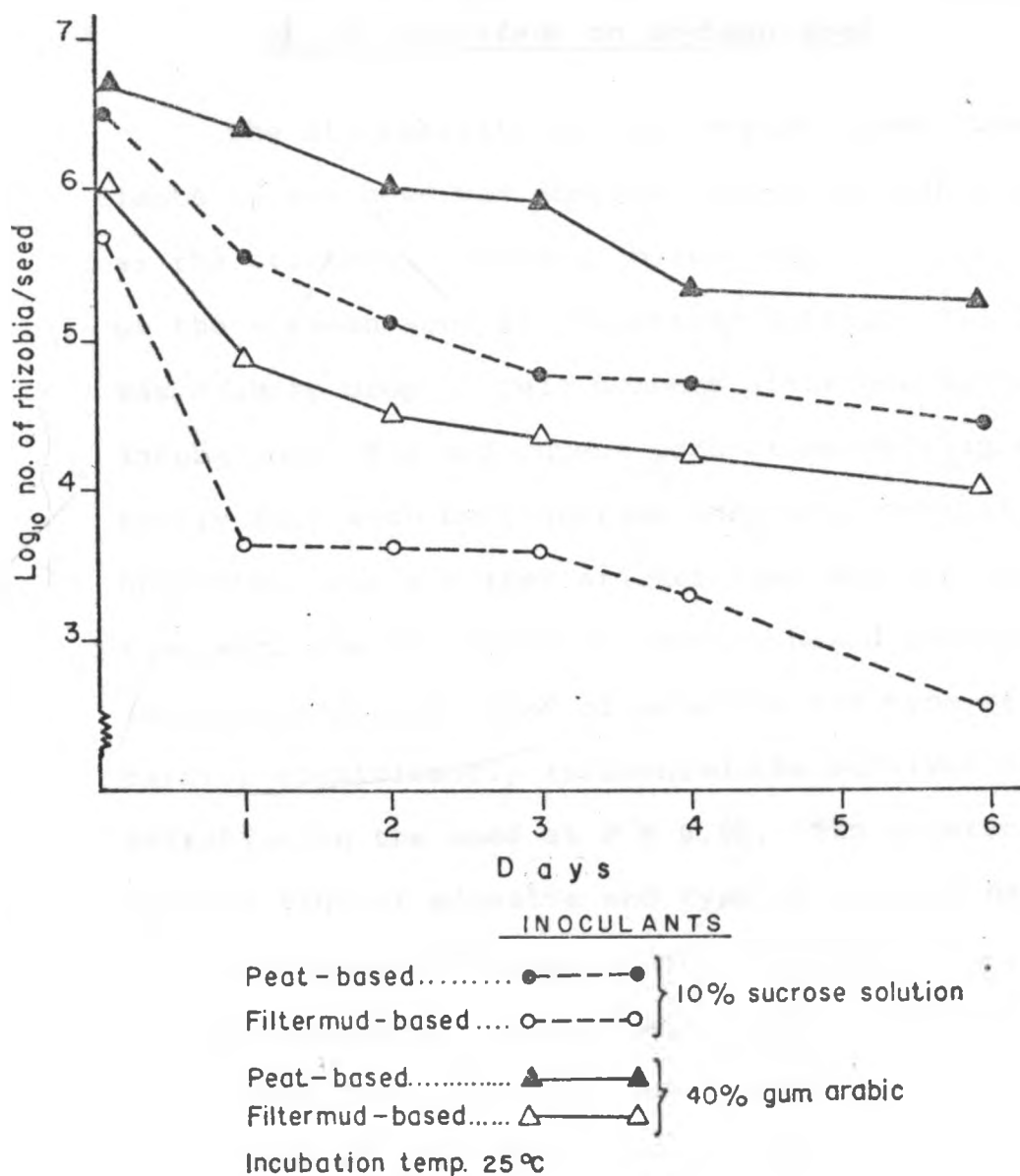
4.4. Survival of some *Rhizobium* species on the seed using two adhesives

This experiment was carried out to assess the effectiveness of the two adhesives in improving the stickability of the inoculant on the seed. The inoculants were applied as peat and filtermud base. Due to some shortcomings during this investigation, a common method of seed inoculation could not be employed. Two different methods were therefore adopted. These were, sprinkler method which was used on beans and slurry method for soybeans.

4.4.1. The influence of the type of adhesive on survival of *R. phaseoli* on the bean seed

The two adhesives tested in this investigation were 40% gum arabic and 10% sucrose solution. The results are presented in Figure 6. The stickability of the inoculant and hence survival of rhizobia on seed was improved with 40% gum arabic. This was true with both the carriers used for inoculant preparation. The pattern in peat-based inoculants with either of the adhesives was similar except that lower cell counts were observed with 10% sucrose solution. The same was true with filtermud-based inoculants. Peat inoculants showed a slightly better stickability to the seed than filtermud inoculants with both adhesives. Incubation period, source of carrier and

FIG. 6: INFLUENCE OF TYPE OF ADHESIVE ON SURVIVAL OF R. PHASEOLI ON BEAN SEED



the type of adhesive significantly influenced the survival of rhizobia on the seed at $P = 0.05$.

4.4.2. Influence of the type of adhesive on survival of *R. japonicum* on soybean seed

The stickability of the carrier based inoculants to the seed was compared using two adhesives as the stickers. Survival pattern of *R. japonicum* on the soybean seed is presented in Figure 7. There was a sharp drop of cell numbers after one day's incubation. The subsequent population decline was fairly slow with both carrier-adhesive combinations. Gum arabic was a better sticker than sucrose solution with the two types of carrier based inoculants. Incubation period, kind of adhesive and type of carrier significantly influenced the survival of *Rhizobium* on the seed at $P = 0.05$. The interaction between kind of adhesive and type of carrier had no significant influence on the survival of rhizobia on the seed. Fairly high numbers of viable cells were recovered from seeds after six days of incubation at room temperature with all carrier adhesive combinations.

The physical appearance of seeds inoculated with peat and filtermud slurry of 40% gum arabic was compared on plate 2. The two carriers compared well in their ability to stick to the seed with the help of a sticker.

FIG. 7: INFLUENCE OF TYPE OF ADHESIVE ON SURVIVAL OF R. JAPONICUM ON SOYBEAN SEED

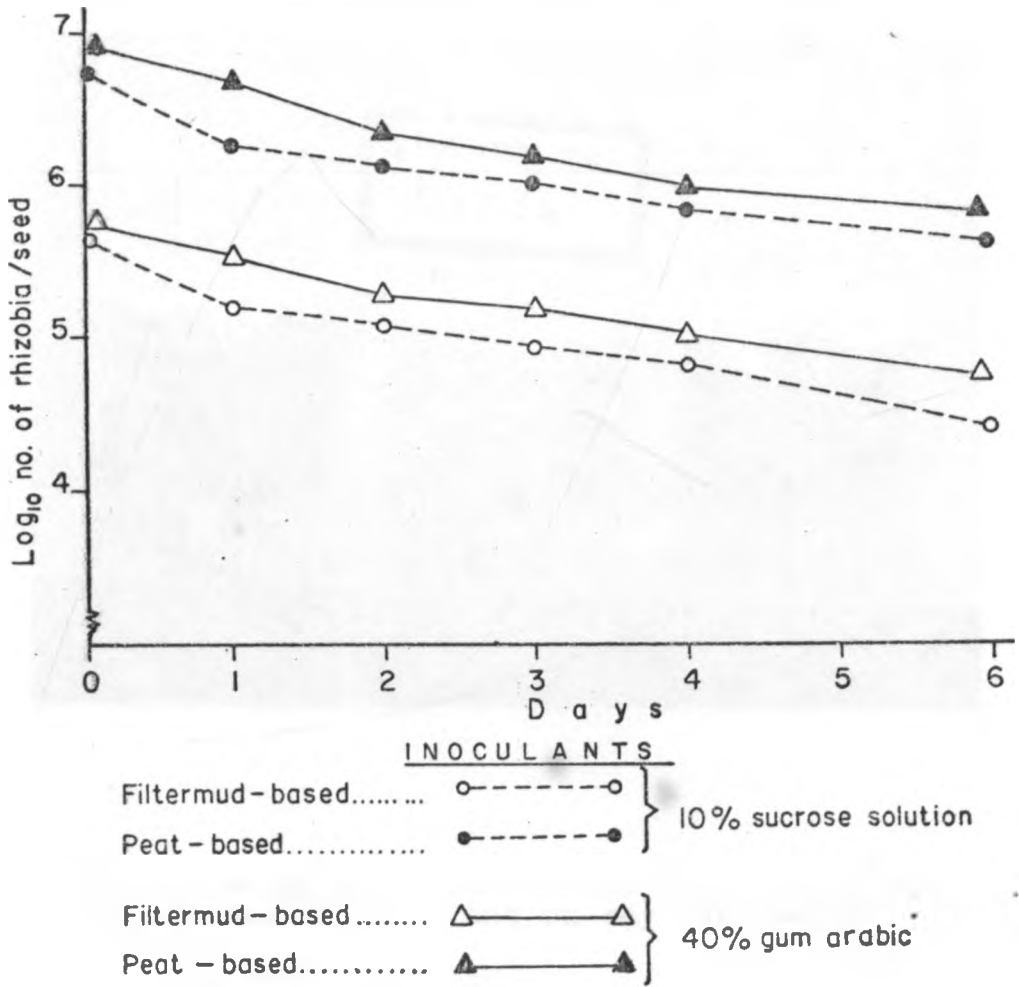


Plate 2



Comparison of the stickability of filtermud-based and peat-based inoculant on soy bean seeds

- 1) Peat based inoculum
- 2) Non-inoculated
- 3) Filtermud based inoculum

4.5. Response of *P. vulgaris* and *G. max* to inoculation

This experiment was conducted in order to assess the effectiveness of the test rhizobia strains in nitrogen fixation. *Phaseolus vulgaris* cv 'Canadian Wonder' and *Glycine max* cv 'Bossier' were used as test cultivars under green house conditions. Due to poor growth and yellowing noticed on beans during the early stages of the experiment, the duration of growth had to be reduced from 64 to 40 days in order to recover viable nodules. Inoculation studies with soybeans on the other hand lasted for 64 days.

4.5.1. Response of *P. vulgaris* to inoculation

The effectiveness of *Rhizobium* in nitrogen fixation was assessed in this experiment. There were four treatments and one control as shown in Table 7.

Plants in all treatments showed vigorous growth upto the trifoliate leaf stage. Later, yellowing was noticed in all treatments and the trifoliate leaves expressed it more than the first two leaves. This yellowing was accompanied by retarded plant growth in the broth, peat and filtermud based inoculated treatments.

Table 7. Effect of different forms of inoculant on nodule development and growth of *Glycine max* and *Phaseolus vulgaris*

Treatments	<i>G. max</i>				<i>P. vulgaris</i>			
	Shoot		Nodule		Shoot		Nodule	
	dry weight	N content	dry weight	Number	dry weight	N content	dry weight	Number
Non-inoculated control	2.386	0.922	0	0	1.832	1.612	0	0
5% applied nitrogen	7.042	3.788	0	0	3.390	2.312	0	0
Broth inoculum	5.312	2.376	0.468	143	1.472	1.414	0.128	260
Filtermud-based inoculum	6.076	3.100	0.420	92	1.684	1.596	0.080	216
Peat-based inoculum	6.824	3.426	0.458	91	1.350	1.136	0.058	189
Coefficient of variation	9.745	8.899	10.450	N.D.	13.160	8.899	27.799	N.D.
L.S.D. P = 0.05	0.722	0.248	0.068	N.D.	0.343	0.248	0.036	N.D.
P = 0.01	0.995	0.332	0.100	N.D.	0.473	0.332	0.052	N.D.

N.D. = Not Determined

Among the treatments, applied nitrogen expressed better growth than *Rhizobium* inoculated treatments. Reduced growth vigour and yellowing observed in most of the treatments were typical nitrogen deficiency symptoms (plate 3).

Results of the green house studies are shown in table 7. Treatment of broth inoculum gave the highest number of nodules while peat-based inoculum treatment gave the least number of nodules. Broth inoculum has been shown to increase the number of nodules more than carrier based inoculum (Balasundaram, personal communication). No nodules were recovered from the non-inoculated control and nitrogen applied treatment suggesting lack of contamination by *Rhizobium*. Inoculation with broth culture significantly influenced the nodule dry matter of experimental plants more than carrier-based cultures ($P = 0.01$). The high C.V. (22.78%) in nodule dry matter yield indicated a high variation in nodulation. There was however no statistical difference in nodulation with the two carrier based inocula used.

Applied nitrogen treatment significantly increased dry matter yield compared to *Rhizobium* inoculated treatment ($P > 0.001$). There was no significant difference between control and *Rhizobium* inoculated treatments in dry matter yield. Among inoculated treatments themselves, there was no

Plate 3



Response of bean (*P. vulgaris*) to inoculation and N-application

- 1) Non-inoculated
- 2) Broth inoculum
- 3) Peat-based inoculum
- 4) Filtermud based inoculum
- 5) Applied Nitrogen

statistical difference in dry matter yield. Broth culture inoculum and filtermud-based inoculum had a significant influence on the plant total N at $P = 0.05$ and $P > 0.01$ respectively.

4.5.2. Response of soybean to inoculation

This experiment was conducted in order to examine the effectiveness of *R. japonicum* in nitrogen fixation in soybeans. Results presented in Table 7 show the various treatments. Plants in all treatments showed vigorous growth except those which were neither inoculated nor supplied with nitrogen. These non-treated plants showed yellow leaves and retarded growth after the emergence of trifoliate leaves. The best growth was achieved with applied nitrogen treatment followed by peat culture inoculated treatment. Plants were harvested 64 days after planting just at the onset of the flowering stage. Broth inoculum increased nodule numbers although there was no significant difference in nodule dry weight among the three *Rhizobium* inoculated treatments.

The difference between the four treatment means and the control mean was significant at $P = 0.001$. The type of carrier used as the inoculant base had no significant effect on dry matter yield and total nitrogen ($P = 0.01$). This was shown by the similarity of results obtained from the two treatments which were inoculated with carrier based cultures. These results have indicated the high response

Plate 4



Response of soybean (*G. max*) to inoculation and N-application.

- 1) Non-inoculated
- 2) Broth inoculum
- 3) Peat-based inoculum
- 4) Filtermud-based inoculum
- 5) Applied Nitrogen

response of *G. max* to inoculation with *R. japonicum*.

4.5.3. Comparison of plant total N in *P. vulgaris* and *G. max* as influenced by inoculation

Nitrogen determination on part of the shoot dry matter was carried out in order to establish the level of fixed nitrogen as a result of inoculation. Total N levels of the two legumes used are presented in Figure 8 . The total nitrogen in *P. vulgaris* was highest in applied nitrogen treatment. The control gave higher total N than inoculated treatments. Although there was nodulation, the results suggest that the strain used was not effective in nitrogen fixation. *G. max* however responded well to inoculation, which was an indication that the strain of *R. japonicum* used was effective. Peat-culture gave the highest total N followed by filtermud culture inoculated treatment among the *Rhizobium* inoculated treatments. Among inoculated treatments peat or filtermud cultures did not show any statistical difference in nitrogen fixation in the two legumes used. This is an indication that filtermud inoculants are as good as peat-based type.

FIG. 8 : INFLUENCE OF INOCULATION AND APPLICATION OF NITROGEN ON PLANT TOTAL NITROGEN

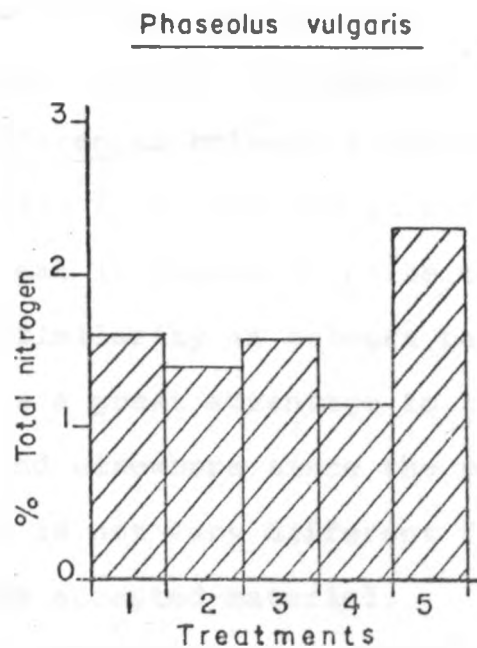
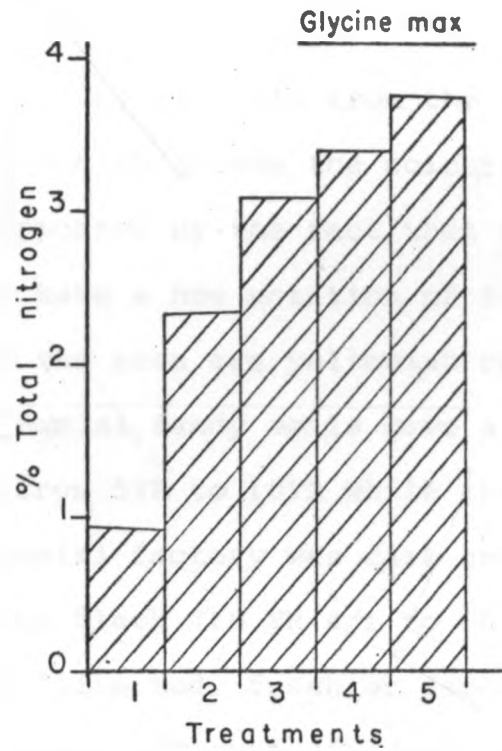


FIG. 8:(1) Non-inoculated (2) Broth inoculum
 (3) Filtermud-based inoculum (4) Peat-based inoculum
 (5) Applied nitrogen

CHAPTER FIVE

DISCUSSION

The colours of soils from the sampling areas described did not influence the colours of filtermud. This is supported by the fact that filtermud samples from Nzoia have a hue notation of 10YR while the soils from the area are yellowish red (5 YR 4/6). Similarly Ramisi sandy soils gave a range of hue notations from 5YR to 10YR while the colour of filtermud from Ramisi factory was dark grey but when moistened became black (10 YR 4/1 to 10 YR 2/1).

Moist filtermud, fresh or decomposed, exhibited a colour similar to that of moist peat. The colour of inoculant is an important factor which is likely to influence inoculant acceptance among farmers. Colour differences between a newly developed carrier material like filtermud and an already established one like peat is likely to raise controversy. Therefore, the similarity in colours between filtermud and peat is a great advantage to the inoculant industry in Kenya and elsewhere since the product being introduced is not very different from the existing and already accepted material.

Fresh filtermud and peat exhibited higher amounts of ash than decomposed filtermud. This is due to the fact that the fresh filtermud and peat had higher amounts of organic matter than decomposed filtermud.

Decomposed materials were obtained at various stages of decomposition and therefore part of their organic component had been mineralised. This finding conforms with reports from Philpotts (1976) and is further supported by the work of Abu Idris *et al.* (1979). The above workers observed fairly high ash content suggesting that they could have examined fresh filtermud.

Fresh filtermud showed higher water holding capacity than decomposed filtermud. This could have been due to the high amount of organic matter and also fibrous material found in fresh filtermud. The fibrous material acted as "sponge" in absorbing a lot of water. This is in agreement with earlier research which had shown that filtermud has a high water holding capacity (Philpotts, 1976). Apart from physical descriptions, published information on the physical properties of filtermud is scarce. The present study has contributed by analysing and reporting additional physical parameters of filtermud.

The chemical properties of filtermud observed concur with the findings of various investigators (Alexander, 1972; Abu Idris *et al.*, 1979; Wood, 1981). Decomposed filtermud had higher pH values than fresh filtermud. The pH value of decomposed filtermud also compared well with that of peat.

A number of factors are likely to have affected the pH levels of fresh filtermud. During the filtration and clarification processes of crushed cane, certain chemicals are added to the juice. These include sulphur, phosphoric acid and lime. The first two chemicals are for juice clarification while the third one is mainly used for pH adjustment. The presence of any of these additives in excess is likely to affect the pH of the juice and this effect would be more pronounced in fresh than decomposed filtermud.

The level of extractable phosphorus in juice in most factories ranged from 100 to 300 ppm (Mutanda, personal communications). This could have contributed to the marked difference in the amount of P obtained in fresh and decomposed filtermud. The low C.V. = 2.62% however indicated that there is very little variation with regard to P between the types of filtermud examined in this study. The extractable phosphorus level of filtermud from Kenya was low compared to those of other countries (Prasad, 1974; Alexander, 1972; Abu Idris *et al.*, 1979; Wood, 1981). The high amount of P in other countries may reflect fertilizer practice. For instance, in S. Africa, 50 kg P (114 kg P_2O_5) per ha per crop is applied (Alexander, 1972). In Kenya, the application rates are low, 21 kg P (50 kg P_2O_5) per crop (Mutanda, 1978). The phosphorus content of peat was

lower than the values obtained in filtermud.

The total N level observed in fresh filtermud was lower than that of decomposed filtermud which conforms with reports by Abu Idris *et al.*, (1979). They obtained 230% increase in total N after stock piling filtermud for twelve months. The mean total N values of the two types of filtermud were 1.06% and 1.44% for fresh and decomposed samples, respectively. These are fairly low compared to most of the reported values. Alexander (1972) reported 1.93% while Prasad (1974) obtained 1.71% total N in filtermud. Another high total N level was reported by Wood (1981), 1.52%. Some of these high values could be due to nitrogenous fertilizer used during cane growth.

Total N in Australian peat (1.57%) compared well with that reported by Burton (1967), which was 1.53%. Reports from S. Africa have given a fairly high value of 1.90% (Strijdom & Deschodt, 1976). Peat is, however, known to vary in both physical and chemical properties even from one deposit (Roughley, 1967). The total N level of peat was higher than those obtained from fresh and decomposed filtermud. During decomposition, organic matter is mineralised to nitrogenous and other compounds. Therefore, samples with high total N will have reduced organic carbon level. This explains why fresh filtermud samples had low total N and high organic carbon content.

The mean value of organic carbon (33.54%) for fresh filtermud compares well with that (37.10%) reported by Alexander (1972). Likewise, Abu Idris *et al.* (1979) and Wood (1981), observed fairly high values of 40.00% and 39.50% organic carbon, respectively. Peat exhibited had a higher amount of organic carbon than these both types of filtermud.

Fresh filtermud which had high organic carbon and low total N levels, exhibited higher C/N ratios than decomposed filtermud. The difference between the C/N ratios of filtermud and peat was not significant. This conforms with the findings of Abu Idris *et al.* (1979) who observed that decomposition of organic matter increases N mineralization. They obtained an increase from 1.13% to 2.26% total N after twelve months. This range corresponds to C/N ratio of 32 and 10 of the same filtermud samples. Similarly Wood (1981) reported a value of 29.99, close to the range obtained in this study. The value of C/N ratio of peat was comparable to the values obtained from some decomposed filtermud samples.

Unlike the elements that have been discussed, the type of filtermud had very little influence on the level of exchangeable bases apart from Calcium and Magnesium. The concentration of K in fresh and decomposed filtermud and peat showed no significant

difference. The source of filtermud had no influence on the level of K. The lowest K values obtained from fresh and decomposed filtermud from Mumias was remarkably low. The values of K were 4.95 and 2.22 m.e./100g in fresh and decomposed filtermud respectively. This could be as a result of low K status of Mumias soils. The mean values of K obtained in this study are within the range of figures reported by various authors. Alexander (1972) obtained 5.12 m.e. while Prasad (1974) found 12.28 m.e./100g; Abu Idris *et al.* (1979) gave 13.04 m.e. and Cooper & Abu Idris (1980) observed 12.02 m.e./100 g. Australian peat had K concentration of 11.25 m.e./100 g, this compares well with some of the values of fresh and decomposed filtermud. The level of K in peat agrees well with earlier reports of Strijdom & Deschodt (1976).

Both fresh and decomposed filtermud exhibited low amounts of exchangeable Na. This is the element whose level varied most with the highest C.V. of 21.91%. The type of filtermud had no influence on the level of Na. The sources of filtermud influenced the amount of Na in filtermud ($P = 0.05$). Compared to filtermud samples, peat had an extremely high amount of Na (3.05 m.e./100g). This value is slightly lower than that reported by Strijdom & Deschodt (1976) which was 4.0 m.e./100 g.

Among exchangeable bases, the magnitude of Ca in fresh filtermud was high compared to the values reported by other workers. The means were 140.33 m.e./100g and 118.13 m.e./100g for fresh and decomposed filtermud, respectively. Alexander (1972) obtained 37.75 m.e. Ca while Abu Idris *et al.* reported 58 m.e. Ca and Wood (1981) found 55.75 m.e. Ca. The addition of lime to cane juice could have influenced the amount of Ca especially in fresh filtermud. The Ca level in decomposed filtermud was fairly low compared to that of fresh filtermud since the former was collected months after disposal. The exposure to environmental factors like natural precipitation leading to leaching might have influenced the magnitude of some of these elements in decomposed filtermud. Despite this observed variation between the the two types of filtermud with respect to Ca content, the C.V. is not high (C.V. = 4.46).

The concentration of Mg in filtermud was lower than the published data. The mean values for fresh and decomposed filtermud were 11.67 m.e. and 6.05 m.e., respectively. In South Africa, Alexander (1972) obtained 16.00 m.e./Mg while Wood (1981) found 19.34 m.e./Mg. Abu Idris *et al.*, (1979) reported 15.22 m.e./Mg while Cooper & Abu Idris (1980) found 10.69 m.e. /Mg in Trinidad . The lower Mg level established in this study could possibly

reflect low Mg status of soils in the filtermud sampling sites. These findings have left a firm impression that degree of decomposition had a considerable influence on the physico-chemical properties of filtermud. This however, affected the level of exchangeable bases to a lesser extent.

Rhizobium phaseoli survived in both fresh and decomposed filtermud as well as it did in the peat. The cell counts obtained after five and six months were 1×10^9 and 1×10^8 rhizobia/g of carrier, respectively. This range was comparable to the standard set by the Australian Inoculant and Control service for commercial inoculants which is greater than 1×10^6 rhizobia/g of peat (Roughley, 1970; Date, 1970; Burton, 1976). The finding conforms with the survival values reported by Philpotts (1976). She obtained 1.78×10^8 rhizobia/g of inoculant with the clover strain TAI and 1.05×10^9 rhizobia/g of inoculant with the cowpea strain CB 756 in air dried autoclaved filtermud after 12 weeks.

This report is further supported by Urio & Chowdhury (1979) who found high counts with filtermud based inoculants. Cell densities as high as 1×10^8 rhizobia/g of carrier were sustained for *R. phaseoli* and *R. japonicum* in filtermud based inoculants previously autoclaved, washed or unwashed.

Tilak & Subba Rao (1978) however reported that a mixture of filtermud and charcoal 1:1 (w/w) increased rhizobial numbers more than individual carriers. The reports have given a clear indication that sterilisation is essential for *Rhizobium* carriers. This is because under such conditions rhizobial multiplication is not inhibited by other micro-organisms which may be harboured in non-sterile carriers.

In a separate investigation, growth of *R. phaseoli* in decomposed filtermud from Muhoroni and peat was examined. The test *Rhizobium* showed a similar growth pattern in both carriers. Maximum cell densities of between 1×10^9 to 1×10^{10} cells/g of carrier were obtained after three days. Although there were minor fluctuations in the cell counts, the *Rhizobium* reached a steady population after fifteen days in the two carriers.

There was a general decrease in moisture level of inoculants with time. The drop in moisture content was however not uniform in all inoculant packets sampled. The fluctuation in moisture content of the inoculants could have been due to variations in porosity of polythene bags. Loss of moisture, (moisture content of 11%) had no drastic effect on the cell densities (1×10^9 rhizobia/g; of carrier). By the end of six months incubation period most culture had lost moisture from an

average of 50% to below 30%. Loss of moisture from the inoculants did not show any drastic effect on rhizobial population. This result could be due to the fact that the initial moisture content of these inoculants was high. This finding is in agreement with earlier reports of Roughley & Vincent (1967) who observed that the final moisture content of the inoculants has a marked effect on numbers of rhizobia. But the finding contradicts claims of Vincent (1958) that there is a marked relationship between death rate of rhizobia and the rate of water loss during storage of inoculants.

Storage temperature influenced the survival of both *R. phaseoli* and *R. japonicum*. Storage at 4°C for three months did not significantly increase numbers of rhizobia. Maximum viable counts of 1×10^9 and 2.46×10^9 rhizobia/g of carrier was obtained in filtermud and peat-based inoculants stored at 28°C for three months. Storage at 40°C had drastic effects on rhizobial survival, no viable cells were recovered after one month from both carrier based inoculants. These results agree with the findings of Roughley (1968) who noted that continuous storage at 4°C restricted rhizobial multiplication but pre-storage at 26°C before transfer to cool conditions was recommended.

Similarly, Bajpai *et al.*, (1978) observed successive decline in the cell counts when the temperature was raised from 30°C to 40°C or 50°C in his studies with *R. leguminosarum*. Iswaran, *et al.* (1970) reports corroborate with earlier findings. He found that counts of *R. japonicum* at temperatures ranging from 28°C to 35°C were appreciable but at 40°C, there was a rapid decline in rhizobial population.

The death rate of *R. phaseoli* in filtermud and peat was examined at 40°C. The results showed a rapid mortality rate of rhizobia. By the end of the 24 day period, cell counts had dropped from 1×10^9 to $<1 \times 10^2$ rhizobia/g of carrier in both carrier materials. Bowen and Kennedy (1959) reported slightly different behaviour. They observed that temperate strains of *R. meliloti* were more resistant to heat than *R. leguminosarum* and *R. trifolii* at temperature ranges between 35.5°C to 42.5°C.

Gum arabic 40% and 10% sucrose solution were used as adhesives (stickers). The superiority of 40% gum arabic observed in this investigation concurs with reports of Brockwell (1962) and Vincent (1965). Brockwell indicated that gum arabic was superior to methyl ethyl cellulose. Similarly observations reported by Vincent showed

gum arabic was superior to 10% sucrose solution, 9% maltose solution and plain water. The treatment of seed with a filtermud slurry in 10% sucrose solution gave the least rhizobial counts after six days. The numbers of cells recovered were however greater than the value recommended by the Australian Inoculant Control Service of more than 300 rhizobia/g seed (Date, 1970). This number, though acceptable in Australia cannot be considered as a standard for all regions because of all the variables encompassed by soil and weather conditions.

Research has shown that far greater numbers are required than were formerly suspected (Burton, 1979). Certainly, thousands instead of hundreds of rhizobia per seed give better assurance of success. High mortality rate was observed with the sprinkle method of seed inoculation.

The slurry method of seed inoculation was adopted with soy bean seeds. A gradual cell decline was observed and by the end of the six days incubation period cell densities as high as 10^4 rhizobia/seed was obtained. Although the two methods of seed inoculation were not compared, research has shown that survival of soy bean rhizobia on seed differ less with the method of seed inoculation under laboratory conditions (Balasundaram, 1980). Despite the superiority of gum arabic over

sucrose solution, the findings of this investigation have shown that even a 10% sucrose solution slurry of filtermud culture can give satisfactory results. This is supported by reports from Burton (1976) who indicated that the use of sugar solution as a sticker reduced mortality rate on inoculated seed. Burton also observed that disaccharides were more effective than monosaccharides. The protective action of sucrose improved with increased sugar concentration upto 25% and differences between treatments increased with incubation period. Burton further indicated that inoculation methods effective on one kind of seed are not necessarily effective on another.

Species and strains of rhizobia may also differ in their ability to survive on seed and in the soil (Brockwell & Phillips, 1965). High rhizobial numbers on seed are definitely necessary for good nodulation. Burton (1967) maintained that effective nodulation of soybean is seldom obtained with fewer than 100 rhizobia/seed even under moderately favourable conditions. Date (1970) recovered 13000 rhizobia/seed from subterranean clover seed inoculated with a gum arabic slurry of peat, after seven days. Date also noted that some host species are less compatible than others for the survival of rhizobia.

Beans showed a very poor response to inoculation. There was no significant difference in dry matter yield between control and treatment means of the green house studies. There was no appreciable difference among three *Rhizobium* inoculated treatments in nodulation and dry matter yield. The poor performance of the three types of inocula tested could have been due to a number of factors. Possibly the *R. phaseoli* strain tested had lost the ability to form effective symbiosis with the tested plants. The other possibility could be that "Canadian Wonder", the bean cultivar tested, was not compatible with the particular *R. phaseoli* strain used. These two suggestions are supported by the poor nodulation and low amount of plant total nitrogen observed in this experiment. *Rhizobium* strains have been shown to have some specificity with legume cultivars, therefore, effective symbiosis would be achieved where the right host/strain combinations prevail. Based on this fact the inoculant production technology recommends the use of multi-strain inoculants as this would allow application to a wide spectrum of legume cultivars (Balasundaram, personal communication).

Despite the depressing results obtained in this particular investigation, several cultivars of beans have been shown to respond satisfactorily to inoculation with effective rhizobia under green

house conditions. In strain testing, Burton *et al.*, (1954) cited by Graham & Halliday, 1977) observed an increase in total N from 15.4 mg. to 49.3 mg/plant. Similarly, Venkatasamy & Peerally (1981) using soil bagasse carrier, found good response of bean to inoculation in pot experiments.

The University of Nairobi *Rhizobium* MIRCEN is currently producing and supplying filtermud-based legume inoculants to farmers. Many farmers who have used the filtermud based inoculant for beans have reported very high yields. Two of such reports have been published in *Rhizobium* MIRCEN Newsletters. One of the farmers reported a yield of 6 bags from a plot planted with 50 kg inoculated seeds over 2 bags from similar sized plot and same quantity of non-inoculated seeds (University of Nairobi *Rhizobium* MIRCEN Newsletter, 1981). In another report a farmer got 5 bags/acre from inoculated plot over 3 bags from a plot planted with non-inoculated seeds (University of Nairobi *Rhizobium* MIRCEN Newsletter, 1983).

Soy beans, however, showed good response to inoculation. There was a significant difference between the control and the three inoculated means ($P = 0.001$). The dry matter yield of plants inoculated with *Rhizobium* as peat or filtermud based compared favourably well. Although broth inoculum showed the overall best crown nodulation, it had no significant influence on nodule dry matter over the other two treatments. The carrier-based

cultures gave higher dry matter yield and total N than broth inoculum.

Published data on response of soy bean to rhizobial inoculation under green house conditions is scarce. But results from this investigation have shown that it responds well to inoculation with an effective strain of *R. japonicum* judged from dry matter yield and plant total N estimates.

The amount of N_2 fixed by *Rhizobium* spp. in the three inoculated treatments with soybean and beans indicated that *R. japonicum* strain tested was more effective than the *R. phaseoli* strain. Effectiveness of *Rhizobium* cultures applied as broth, peat-based and filtermud based were also compared. There was no statistical difference between filtermud and peat cultures in increasing nitrogen fixation in the two legumes. Among the three types of inocula tested, broth type gave the best crown nodulation and the poorest results in terms of dry matter yield and N_2 -fixation. Broth or liquid cultures however, have been found more effective in leonard jars since there is no competition nor harsh environmental hazards to which they are exposed which may promote cell decline (Balasundaram, personal communication). The results of this study agree with the above fact in respect of good nodulation but this did not increase nitrogen fixation in test plants more than other treatments.

CHAPTER SIX

CONCLUSION

The search for alternative carriers has often been prompted by lack of suitable peat in many parts of the world especially the tropics. Based on the studies of filtermud from Kenya, results have shown that fresh or decomposed filtermud, can be used for inoculant production. Decomposed filtermud would be preferable to the fresh materials.

The degree of decomposition of filtermud influenced the major physico-chemical properties of the material. One of these properties was the pH which was closer to the neutral value for most decomposed filtermud samples. Decomposed filtermud exhibited lower water holding capacity than fresh samples. Extractable phosphorus, organic carbon, C/N ratio, calcium and magnesium had values which were significantly higher ($P = 0.05$) in fresh filtermud than decomposed materials. The total N level in decomposed filtermud was higher than in fresh samples. There was however no significant difference between the levels of K and Na in the two types of filtermud. Compared to filtermud, peat showed very low P concentration but the magnitude of total N, organic carbon, K and Na

were the highest while C/N ratio, Ca and Mg values were within the ranges of fresh and decomposed filtermud.

The main objective of this study was to investigate the growth and survival of *Rhizobium* spp. in filtermud in relation to the physico-chemical properties of the material. All filtermud samples tested supported the growth and survival of *R. phaseoli* irrespective of the degree of decomposition. The cell densities of 2.34×10^9 /g of fresh filtermud and 3.09×10^9 /g of peat were obtained after an incubation period of five months. In decomposed filtermud inoculants viable counts of 2.82×10^8 /g of carrier were obtained compared to 4.46×10^8 /g in peat-based inoculant after six months. The difference between the viable counts in survival studies in the two carriers was not significant showing that filtermud is comparable to peat as a carrier. The viable counts were above the minimum value of more than 1×10^6 rhizobia/g of carrier, recommended by Australian Inoculant and Control Service.

Although the survival of *R. phaseoli* in the two carriers did not differ much in terms of rhizobial numbers, decomposed filtermud would be preferred for inoculant production. This is because the problem of contamination was not as prevalent

as in the inoculants prepared with fresh filtermud. Secondly, fresh filtermud samples had pH values which were either acidic or alkaline (5.2 - 5.6, 7.9) while most of the decomposed filtermud samples had pH values near neutral (6.5 - 7.5). Thirdly, fresh filtermud discharges an offensive smell while decomposing.

Freshly prepared inoculants need to be incubated at 28°C for at least two weeks in order to attain maximum cell counts of about 1×10^9 rhizobia/g of carrier before transfer to cold storage at 4°C. The *Rhizobium* spp. tested were not resistant to high temperatures such as 40°C. Reports from earlier research have also shown that high temperatures accelerated dehydration thereby increasing the mortality rate of the bacteria. Therefore storage of rhizobia at high temperatures should be avoided.

Gum arabic improved the stickability of the inoculant and the survival of rhizobia on the seed more than 10% sucrose solution. The superiority of gum arabic over sucrose solution was observed with both methods of seed inoculation adopted. The sucrose solution slurry of filtermud gave fairly high cell densities of 3.2×10^3 rhizobia/seed after 6 days incubation period. This value was above the recommended Australian minimum number of

300 rhizobia/seed. Therefore 10% sucrose solution could safely be used as an adhesive in cases where gum arabic is not available. As some host species are less compatible than others for rhizobial survival and the effectiveness of inoculation method also varies from one seed to another, further research on the effect of different methods of inoculation on survival on the seed of various legumes under green house and field conditions is desirable. Effect of sucrose concentrations higher than 10% on the stickability of filtermud based inoculum on the legume seeds could also be investigated.

The effectiveness of the rhizobia used was tested with appropriate legumes under green house conditions. Results indicated that with the use of effective rhizobia, filtermud based inoculants could increase nodulation and nitrogen fixation in legumes that respond to inoculation as much as peat based inoculant.

The findings of this study have led to the conclusion that filtermud can serve as a suitable carrier for legume inoculants provided it is air dried and sterilised. Based on these conclusions, the Nairobi Rhizobium MIRCEN is presently producing and selling filtermud-based inoculants to over 1500 Kenyan farmers per year.

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