THE EFFECT OF SEED DRESSING PESTICIDES ON THE SYMBIOTIC RELATIONSHIP BETWEEN *RHIZOBIUM LEGUMINOSARUM* BV *PHASEOLI* AND *PHASEOLUS VULGARIS* L.

A thesis submitted to University of Nairobi in part fulfillment for the Degree of Master of Science in Agriculture. 1988.

BY:

CHAGEMA JOHN KEDERA

DEDICATION

Dedicated to my departed grandparents. Daudi Mbarani and Rhoda Kalemera Seme.

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University to the best of my knowledge.

Signed ---------- Chagema Kedera

This thesis has been submitted for examination with the approval of the University Supervisors.

Signed ---------- Date: 6/7/88

1st Supervisor
Prof D.M. Mukunya

Singed ---------- Date: 15TH July, 1988

2nd Supervisor
Prof. S.O. Keya

Contents

Contents Cont'd Page

LIST OF TABLES

Contents Cont'd

LIST FIGURES

LIST OF PLATES

ACKNOWLEDGEMENTS

I wish to express appreciation to the Kenya National Council for Science and Technology (Kenya Government) for financing this investigation, to the Departments of Crop Science and Soil Science for providing the necessary equipment without charge, and to the MIRCEN Project for providing the Rhizobium leguminosarum bv phaseoli culture. Also a special appreciation is expressed to Prof. D.M. Mukunya and Prof. S.O. Keya for their invaluable advice, guidance and continuous encouragement. Special thanks go to the entire staff both academic and non-academic of the two departments, Crop and Soil Science, fellow students, MIRCEN Project staff and the casual labour force whose advice or practical effort helped in the completion of the project. I also thank my parents Mr. and Mrs. Thomas Kedera, aunts, uncles, brothers, sisters, cousins and friends for their continuous encouragement.

Thanks are also due to Mr. Milikau of N.A.L. for statistical analysis and Mrs. M. Musonye for typing the work.

ABSTRACT

Improvement of bean (Phaseolus vulgaris L.) production requires seed treatment with pesticides for control of pests. In order to maximize nitrogen fixation, inoculation of seed with effective Rhizobium is important. The pesticides may be incompatible with the survival of the inoculated Rhizobium on seed and hence affect symbiotic nitrogen fixation. The study looks at the influence of four chemical seed protectants namely; actellic or pirimophos methyl (O[2-diethylamino]-6-methyl-4-pyrimidinyl 0,0-dimethyl phosphorothioate); aldrin (1, 2, 3, 4, 10, 10-hexachloro -1, 4, 4a, 5, 8, 8a hexahydo-exo - 1, 4-endo- 5, 8-dimethanonaphthalene); Captan (N-(trichloro-methylthio)-4 - cylohexene-1, 2 dicarbomixide); and benomyl (Methyl-1-(butylcarbomyl) benzimidazol 2-yl carbamate) on Rhizobium leguminosarum bv phaseoli in pure culture, on seed and the subsequent nitrogen fixation in P. vulgaris L.

While captan at 100 ppm was toxic to the Rhizobium in pure culture, benomyl, actellic and aldrin allowed for growth even at 2000 ppm. Captan (25g per kg seed) was more toxic to the Rhizobium on seed leading to a faster decline in population. Benomyl (3.75g per kg seed) was less toxic compared to captan and the control. Aldrin (6.25g per kg seed) and Actellic (4.44g per kg seed) did not influence the survival of Rhizobium on seed. None of the pesticides had a significant effect on nodulation, shoot dry weight,

total nitrogen and grain yield under both greenhouse and field conditions. Thus in conclusion, under the experimental conditions the seed applied pesticides did not influence nodulation and nitrogen fixation in P. vulgaris L.

CHAPTER ONE

INTRODUCTION

1.1 Biological Nitrogen Fixation

It has been established that most crops can not do well if nitrogen in the utilisable form is not available in their growing environment. One of the major sources of nitrogen is artificial fertilizers whose production and consumption has ever been on the increase (Anon., 1985). Due to the high costs of these nitrogenous fertilizers many countries such as Kenya have been compelled to look for alternative sources of nitrogen. Kenya's consumption of the nitrogenous fertilizers was 35,961 metric tonnes for the period 1984/85 compared to 31,000 metric tonnes for the period 1983/84 (Anon., 1985). One utilisable source of nitrogen is Biological Nitrogen Fixation (BNF) (Anon., 1985; App and Eaglesham, 1982). This is achieved through free living micro-organisms such as Beijerinka, Klebsiela, Azotobacter and Clostridium and plant-microbial associations (Algae/Anabaena - Azola and legume-Rhizobium symbiosis) which are capable of converting atomospheric nitrogen into assimilable nitrogen (Keya, 1983; 1985). Postgate (1978), estimated that Biological Nitrogen Fixation provides about 175 million metric tonnes of nitrogen per year. The legume-Rhizobium symbiosis is the most

investigated (Keya, 1985) and it is estimated that nitrogen fixed in tropical legumes ranges between 40 and 450 kg/ha/year (Keya, 1983). Thus where crops respond to inoculation, the use of Rhizobium inoculants is a possible means of maximizing nitrogen fixation. Diatloff (1970) stated that seed inoculation with effective strains of Rhizobium can meet the nitrogen requirements of the legume to achieve increased yields. This phenomenon is of world-wide importance as it implies lesser dependence on expensive petroleum based nitrogenous fertilizer for legumes and could help in preventing possible environmental pollution from excessive use of mineral nitrogen fertilizers.

1.2 Beans

The dry beans (Phaseolus vulgaris L., sub family Papilionoideae; family, Leguminosae) constitutes an important food crop in Kenya. They are grown extensively (about 350,000 ha) seasonally either in pure stands or mixed with other crops such as maize (Keya, 1983). According to Graham (1980) bean is a crop of the small scale farmer commonly grown under conditions of low fertility and with minimal economic inputs. Beans are nodulated by Rhizobium leguminosarum biovar phaseoli, a culture of which is used to make the bean inoculants (Vincent, 1970; Somasegaran and Hoben, 1985).

The results of Keya et al (1981) indicate that inoculation could increase yield by between 7 and 17 percent. Keya (1983), further notes that inoculation has been found to be as good as applying 20 kg N/ha. The dry bean generally fixes about 44 kg N/ha/year (Keya, 1983). Beurnard and Michellon (1986) argue that despite this low level of nitrogen fixation, yield increases through inoculation remains modest as compared to the nitrogen fertilization. It should however be noted that Vincent (1974) reported that beans were generally weak in nitrogen fixation and show a variable response to inoculation (Graham, 1980), especially under field conditions with inoculation failure being a common problem. Graham (1980) noted three main factors as being instrumental in causing variability in nitrogen fixation in P. vulgaris L. These are, supply of carbohydrates to nodules, relative rates of nitrogen uptake from the soil and time of flowering. Other factors generally affecting nodulation and nitrogen fixation are the interactions of manganese, aluminium, molybdenum and calcium in soils; soil pH and temperature; soil water stress; competition for nodulation sites; use of chemical seed protectants; planting density; associated cropping and combined nitrogen (Dowson, 1970; Graham, 1980; Parker, 1985).

1.3 Diseases and Pests of Beans

In Kenya beans are reported to be attacked by about twenty pathogens and thirteen pests. However, only a small number of them cause economic damage (Stoetzer and Omunyin, 1983). The major diseases and causal agents are as follows: Halo blight (*Pseudomonas syringae* pv *phaseolicola* (Burk.) Dowson); common blight (*Xanthomonas campestris* pv *phaseoli* (E.F. Smith) Dowson); Anthracnose (*Colletotrichum lindemuthianum* (sacc and Magn) Bri. and Cav); Angular leaf spot (*Phaseoisariopsis griseola*. sacc.); Rust (*Uromyces appendiculatus* (Pers.) Lev.); Bean scab (*Elsinoe phaseoli* Jenkins.); Ashy stem blight (*Macrophomina phaseoli* (Maube) Ashby.); Root rot (*Pythium spp*. *Fusarium spp* and *Rhizoctonia spp*.) and the Bean common mosaic (Bean common mosaic virus). The bean fly (*Ophiomyia phaseoli*. Try) and the bean bruchid (*Acanthoscelides obtectus*. Say.) are the major pests in the field and stores respectively. Others are the black bean aphid (*Aphis fabae* Scop.); American bollworm (*Heliothis armigera*. Hb); Stripped bean weevel (*Sitonia linetus* L.) and the bean flower thrips.

1.4 Control of Bean Diseases and Pests

In order to control these bean diseases and pests, the methods adapted include: use of disease free seed, crop rotation, field sanitation, use of resistant varieties,

chemical sprays and seed dressing (Stoetzer and Omunyin, 1983; Agrios, 1982). The chemicals recommended are as follows: Baycor (B[(1,1-biphenyl)-4-Yloxy]-a-(1,1-dimethylethul)-1H-1,2,4 triazole-1-ethanol); Benomyl (N-1(butylcarbomoyl)-2-benzimidazole carbamate); Captan (N-trichloromethylthio)-3a,4,7a, tetrahydrophthalimide); Copper oxychloride; Cupric oxide; Captafol (CIS-N(1,1,3,2-tetrachloroethyl) thio-4-cyclonexene-1,2-dicarboximide); Dithane M45 (Zinc, ion plus manganese ethylene bisdithiocarbamate. Actellic (Pirimophos methyl); Bordeaux mixture (mixture of copper sulphate and calcium hydroxide forming basic copper sulphates); Thiodan (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6, 9-methano-2, 4, 3-benzodioxanthiapin-3 oxide); Diazinon (0,0-diethyl. 0-(2-isopropyl-6-methyl-4-pyrimidinyl) phosporothioate); Rogor E (0,0-dimethyl S-[2-methylamino)-2-oxoethyl] phosphoro-dithioate); Malathion (0,0-dimethyl phosphorodithioate of diethyl mercaptosuccinate); Aldrin (1, 2, 3, 4, 10, 10, hexachloro - 1, 4, 4a, 5, 8, 8a hexahydro - exo - 1, 4-endo - 5, 8 -dimethanonaphthalene); Thiram (Tetramethylthiuram disulfide); Fernasan D and Murtano (both a mixture of Lindane (1, 2, 3, 4, 5, 6-hexachlorocyclohexane) and thiram) (Stoetzer and Omunyin, 1983). Presently captan and thiram are widely used for seed treatment (McEwen and Stephenson 1979), the others being benomyl, aldrin, murtano and Fernasan D before planting. Actellic and malathion are storage pesticides.

1.5 Seed Dressing

Chemical seed treatment is now a common practice before planting, consequently the extent of seed germination and crop yields are increased (Odeyemi and Alexander, 1977). This is not only because the pesticides help prevent seed and seedling rots, damping off and other fungal diseases (Agrios, 1978; McEwen and Stephenson, 1979), but they also inhibit protozoa preying on rhizobia and possible competitors to the root nodule microsymbionts (Alexander, 1985). Inoculation of the seed or the planting furrows with the appropriate strain of Rhizobium ensures introduction of the highest number of bacteria into the soil closest to the roots leading to good nodulation (Smit. et al, 1983; Alexander, 1985). Problems arise when the legumes inoculants are to be used in conjunction with pesticide treatment of seed before planting. Keya (1970) noted that pesticides may drastically affect the symbiotic relationship. Keya (1974); Simon-Sylvestre and Fournier (1979) observed that the effects may be divided into two types of action which may be independent of each other - one on the bacterium itself and on its growth and the other on the host plant, its infestation, the phenomemon of root nodule formation and nitrogen fixation.

Alexander (1985) noted that both the pesticides and Rhizobium have very poor mobility in the soil. He stated that many chemicals do not move from their first introduction site into the soil. As for bacteria, rhizobial cells have

been found to be transferred for distances as short as 2.7 cm in soil (Madsen and Alexander, 1982 as cited by Alexander (1985). This implies that once the pesticide and the Rhizobium inoculants are applied on the seed and planted, they remain close enough in the soil.

The objectives of this study were therefore:

To study the influence of the Phaseolus vulgaris L. seed dressing pesticides on the growth and survival of Rhizobium leguminosarun bv phaseoli in culture and on seed respectively.

and

To determine whether the Phaseolus vulgaris L. seed dressing pesticides can be used together with Rhizobium leguminosarun bv phaseoli inoculants without interfering with the symbiotic relationship in soil.

CHAPTER TWO

LITERATURE REVIEW

2.1 Rhizobium - Pesticide Interaction

The exact influence of the pesticides on the Rhizobium and subsequent field nodulation has not been fully elucidated. Generally the effects have been studied by measuring growth in vitro either on agar or in liquid culture. The interaction of pesticide and Rhizobium in soil and sand has been measured by the degree of nodulation; nodule fresh and dry weight; shoot dry weight; leghaemoglobin content (or nitrogen fixing efficiency); nitrogenase activity; total nitrogen and possibly grain yield. From the results obtained attempts have been made to correlate pesticide treatment and nodulation or plant growth (Fisher, 1976).

2.1.1 Fungicide - Rhizobium Interaction

Many fungicides show some degree of toxicity towards rhizobia and or nodulation with toxicity being associated more with some strains of Rhizobium than with others (Anderson, 1978). Generally seed dressing chemicals containing mercury, copper and zinc have been found to be toxic to rhizobia (Ruhloff and Burton, 1951; Date, 1970; Pelmus, 1986). Kernkamp, (1948) reported that fungicides did

not influence nodulation. He found that application of Spergon (2, 3, 5, 6 - tetrachloro - 1, 4 - benzoquinone) with Rhizobium inoculants did not influence nodulation when the soybean seed was planted in soil that contained the bacteria. However, nodulation was significantly increased when the inoculant was applied without Spergon as compared to the inoculant with Spergon in any combination. Ruhloff and Burton (1951) found that when seed was chemically coated with Spergon, Phygon and Arasan (Tetramethylthiuram disulfide) followed by inoculation and then planted in moist soil, the number of Rhizobium cells able to survive could be sufficient to bring about nodulation hence nitrogen fixation. However, they cautioned that failure of nodulation may occur when planting conditions are unfavourable. This emphasised the importance of soil moisture at planting time.

According to Diatloff (1970) the in vitro toxicity of fungicides to rhizobia followed the order Ceresan (2-methoxy methyl mercuric chloride), thiram (Tetramethylthiuram disulfide) captan (N-trichloromethylthio) - 3a, 4, 7, 7a tetrahydo - phthalimide) chloranil (2, 3, 5, 6 - tetrachlor - 1, 4 - benzoquinone). Mikawi and Ghaffar (1970) as cited by Anderson (1978) reported that Ceresan, captan and benomyl (N-1(butylcarbomoyl) 2-benzimidazole carbamate) were bacteriocidal towards a number of Rhizobium strains. Investigations by Sud and Gupta as cited by Anderson (1978)

with thiram and its degradation product sodium dimethyl dithiocarbamate, showed that some rhizobia were sensitive but the degree depended on the Rhizobium spp. and the medium pH. Date (1970) reported that thiram could be used safely. Using some fungicides, Fisher (1970) found that the growth of Rhizobium in agar with 10, 50, 100, and 250 PPM each of captan, dodine (n-dodecylguanidine acetate) and oxycarboxin (5, 6, dihydro - 2 - methyl - 1, 4. oxathiin - 3 - carboxanilide - 4 - 4 dioxide) was markedly reduced with increasing concentration. Benomyl, carboxin (5, 6- dihydro -2 methyl - 4- oxathiin -3-carboxanilide), dimethirimol (5-n-butylk-2-dimethylamino-4-hydroxy-6-methyl pyrimidine), ethirimol (5-n-butyl-2-ethylamino)-6-methyl-4(1H) pyrimidinone), thiram, tridemorph (2, 6-dimethyl -4- tridecylmorpholine) and thiophanate methyl had little effect even at higher concentrations. Bandyopadhyay (1986) working with cowpea Rhizobium strains found that low concentrations (0.05 - 0.2%) of captan were non inhibitory while higher concentrations (0.3%) inhibited at least five strains. This had earlier been demonstrated by Wainwright and Pugh (1975) whose assay in vitro revealed the influence of increasing captan concentration on the amplitude of bacterial growth. Low concentrations of Dithane M45, thiram and captan were found to be non inhibitory to rhizobia by Poi and Gosh (1986).

Growth of R. phaseoli, R. leguminosarum and R. japonicum in a yeast extract-mannitol medium was found to be adversely affected by captan at 200 mg/ml by Banerjee and Banerjee (1987).

Fisher (1976), found that whereas thiram, oxycarboxin and ethylan CP, affected symbiotic nitrogen fixation, benomyl, captan, carbendazim, carboxin, dodine, ethirimol, dimethirimol, tridemorph, triforine and thiophanate methyl did not change the process in white clover grown in sterile sand and soil. He also showed that captan, ethylan CP, dodine and ethirimol lowered the respiratory activity of R. trifolii to a varying degree. However, the fungicide triadimefon (1-(4-chlorophenoxy)-3,3-dimethyl-1-(1,2,4-triazol-1-yl)butanone) which seriously inhibited growth of R. trifolii at high concentration barely decreased respiration showing little effect on overall metabolism (Fisher et al, 1979).

Van Fansen (1974) reported that addition of benomyl to liquid or agar medium inhibited the outgrowth in the media with high doses affecting the number of bacteria. However, addition of benomyl to soil was found not to cause changes in bacterial populations (Siegel, 1975). Leite (1982) working with soybeans found that benomyl did not affect nitrogen fixation but oxycarboxin and carboxin reduced the total plant nitrogen content. Further in soybeans, the fungicides,

captan, thiram and carbathiin at recommended rates were found by Rennie and Dubetz (1983) to have no effect on nodulation and nitrogen fixation under irrigation when granular inoculant was used. With the field bean cultivar lancer, application of captan and thiram on seed reduced nodulation with captan treatment resulting in significant yield reduction thus making it a potent inhibitor of nitrogen fixation (Rennie, 1986). Reddy et al. (1975) reported lack of effect of benomyl on nodulation contrary to the findings for aldicarp, oxamyl and carbofuran. They further reported that ethylene dibromide improved nodulation in soybean but mode of action was not established. Duczek and Buchanan (1981) reported that when the soil was populated with the appropriate strains of Rhizobium captan, did not have any effect on nodulation and seed yield in lentils (Lens esculenta). However, in soils where the rhizobia were absent, nodulation and seed yield were affected. Nodulation of pea and mung beans grown from surface sterilised inoculated seeds in captan treated soil, was significantly reduced with nodule numbers decreasing with increasing fungicide concentration (Banerjee and Banerjee, 1986). Bandyopadhyay (1986) notes that captan adapted strains of Rhizobium retain the ability to nodulate and fix nitrogen hence making it possible to use captan as a seed protectant and seed inoculation compatible.

In some cases, growth *in vitro* responses are very different from growth *in vivo* in the presence of the same fungicides. Thus whilst thiram was found to inhibit cultures of *R. leguminosarum* bv *vicae* on petri-dishes, it had no effect on the nodulation of vetch (*vicia fabae*) in sand or in various soils under greenhouse and light chamber conditions (Kecsks and Vincent, 1969 as cited by Anderson, 1978). Graham *et al* 1980 reported that contact of *R., leguminosarum* bv *phaseoli* with pentachloronitrobenzene (PCNB) thiram and captan on seed reduced their survival with captan being the most toxic. They further demonstrated that when chemically treated seeds were promptly planted, nodule numbers similar to inoculated but pesticide free plants were obtained. However, storage of such seeds for one week between inoculation and planting notably reduced nodulation. Curley and Burton (1975) found that 83% of *R. japonicum* cells applied to PCNB treated seeds were killed by the fungicide four hours after inoculation. Fuentes and Trejo as cited by Graham *et al* (1981) reported toxicity of PCNB to rhizobia, noting a drop from 400 x 10^3 to only 90 x 10^3 *R. leguminosarum* cells per seed after one hour exposure. However, Chamber *et al* (1982); Tesfai and Mallik (1986) reported that PCNB allowed for high nodulation in soybean and generally lacked in rhizobial toxicity. Chamber *et al* (1982) further found that TMTD (trichloromethylthiotetrahydrophthalimide) and benomyl interfered with nodule development besides killing *R. japonicum* nodulating *Glycine max*

(soybean). Captan and TMTD were found to greatly reduce the biomass if inoculation was done on seed as compared to inoculation in furrows (Chamber et al, 1982). In 1984, Singh and Srivastava, reported compatibility of thiram and Dithane M45 and Rhizobium in soybean with captan reducing nodulation. Lennox and Alexander (1981) found that the number and weight of pods and the weight and nitrogen content of the bean tops derived from seeds inoculated with a thiram resistant strain of R. phaseoli were increased if the seeds were treated with thiram before sowing in the soil. Curley and Burton (1975) found that captan at 0.8 g per kg seed significantly reduced the number of rhizobia after incubation while thiram at 0.6 gm per kg of seed had no effect on rhizobial numbers on seed but at 0.93 gm per kg of seed, it inhibited growth of R. japonicum in pure culture.

2.1.2 Insecticide - Rhizobium Interaction

Insecticides have a depressive action on Rhizobium with sensitivity varying with strains hence the negative effect on nodulation (Anderson, 1978). Kapusta and Rouwenhorst (1973) tested the interaction of ten insecticides with R. japonicum in pure culture and found that only disulfoton (O, O-diethyl s-(2-(ethylthio)ethyl) phosphorodithioate) at 7.5 PPM inhibited a mixture of the strains. They further showed that nodule fresh weight per plant was influenced by insecticides. This was in line with reports by Audus (1970) and Mendoza

(1973) as cited by Simon-Sylvester and Fournier (1979) indicating that insecticides have no effect on rhizobia and bacteria in general at normal doses. Medium and high levels of DDT (Dichloro diphenyl trichloroethane) were found by Urza et al (1986) to have a detrimental effect on growth dry matter production and nitrogen accumulation in clover. However, the effects on nodulation and nitrogenase activities were smaller. Diatloff (1970) working with five insecticides emulsions found the order of toxicity to rhizobia on wet beads was dimethoate [S-(N-methylcarbamoyl-methyl) phosphorodithioate) > lindane [1, 2, 3,4, 5, 6 hexachlorocyclohexane] >Isobenzan [1, 3, 4, 5, 6, 7, 8, 8 - octachloro - 1, 3, 3a, 4, 7, 7a hexahydro-4, 7, methanoisobenzafuran] > Endrin [1, 2, 3, 4, 10, 10, hexachloro-6, 7 epoxy, 1, 4, 4a, 5, 6, 7, 8, 8a octahydro-1, 4 - endo-endo, 5, 8 - dimethanonaphthaline]> Dieldrin [1, 2, 3, 4, 10, 10, hexachloro-6, 7-epoxy. 1, 4, 4a, 5, 6, 7, 8 - 8a octahydro 1, 4 endo-exo, 5, 8 dimethanonaphthaline]. However, Date (1970 reported that dieldrin when used at recommended rates for insect control was relatively safe.

Gowaal et al (1972) as cited by Anderson (1978) showed that under laboratory conditions lindane, DDT, hptachlor [1, 4, 5, 6, 7, 8, 8, hptachloro - 3a, 4, 7, 7a tetrahydro, 4, 7 methanoindine] and aldicarp [2-methyl -2- (methlthio) propion-aldehyde -x- (methyl-carbomoyl) oxime] significantly reduced whilst phorate [0.0 diethyl - -s-(ethylthio)methyl]

phosphorothioate) increased the number of nodules per plant of bean and clover. However, under field conditions, and at higher application rates, phorate, aldicarp and lindane did not affect nodulation of clover plants. Selim et al (1970) found that the normal rate of dieldrin and lindane showed no apparent harmful effects on the symbiotic nitrogen fixing bateria and growth of Vicia fabae (broad bean). High lindane concentration led to reduced nodule formation. Pareek and Gaur (1970) found that 5 PPM of DDT significantly enhanced nodulation in Phaseolus aureus while more than 100 PPM totally inhibited both root growth and nodulation. At low concentrations (10-100 PPM) malathion, aldrin and BHC were found to be non-inhibitory to rhizobia while at higher concentrations the response of the Rhizobium varied with species (Poi and Gosh, 1986). Damage to the inoculant by lindane, dimethoate, endrin and aldrin was reported by Brantwaite et al (1958) who also reported reduced crown nodulation of Centrosema pubescens. Bezbarua and Barua (1987) found dalapon to be toxic to rhizobia. In 1982, Tu reported that thiram alone or with lindane or diazinon significantly depressed the formation of ethylene from acetylene from 3 - 5 weeks but had no effect after 7 weeks, and that the treatment had a permanent deleterious effect on growth or nitrogen fixation in soybean.

Alexander (1985) noted that use of pesticides that are toxic to protozoa, should enhance colonisation by the Rhizobium. This has been established through studies (Ramirez and Alexander, 1980) using resistant strains of Rhizobium, to thiram and benomyl which are highly toxic to protozoa in beans and soybeans respectively. In both cases the seeds were coated with the pesticide and the inoculant.

In some cases the applied seed fungicide may fail to protect against the intended pathogen. Kataria et al (1985) found that 2 methoxyethyl - mercury chloride (MEMC) applied to cowpea seeds with Rhizobium provided little or no control of seedling rot caused by Rhizobium solani while a similar treatment but without rhizobia gave 40% control of the disease. They further showed that the fungicide quintozene was rendered ineffective against R. solani by rhizobia. In other experiments, simultaneous treatment with rhizobia considerably reduced the efficacy of captafol, chloroneb [1, 4 dichloro - 2, 5 -dimethoxybenzene], carbendazim and thiophanate methyl while that of carboxin, benomyl and thiabendazole remained the same.

CHAPTER THREE

MATERIALS AND METHOD

3.1 Baterial Culture

Rhizobium leguminosarum bv phaseoli. University of Nairobi MIRCEN Project (UNMP) strains number 13A, 107, 115, 445 and 446 were obtained from Nairobi MIRCEN, Departments of Soil Science and Botany, culture collection. They were grown in Yeast Extract-Mannitol (YEM) broth of composition: K_2HPO_4, 0.5g; $MgSO_4.7H_2O$, 0.2g; NaCl, 0.1g; $CaCO_3$, 3g; Mannitol, 10.0g and Yeast extract, 1.0g per litre of distilled water. the pH was adjusted to 6.8 (Vincent, 1970). For solid medium (Yeast extract-mannitol agar YEMA) 15.0 g per litre of agar-agar was added. The medium was sterilised by autoclaving at 15 lbs pressure, 121°C and for 30 minutes (Vincent, 1970; Somasegaran and Hoben, 1985).

In all situations the starter culture was obtained and a loopful inoculated into 250 mls of YEM broth contained in a 500 ml Erlenmeyer flask. This was then shaken on the rotary shaker at 20 rpm for 2 - 5 days at room temperature (Vincent, 1970).

The pesticides were incorporated in appropriate quantities to both Yeast extract-mannitol (YEM) broth and Yeast etract mannitol agar (YEMA) after autoclaving and cooling to 45°C (Fisher, 1976).

Congo red plates were prepared by adding to YEMA, 10 mls of a 1/400 aqueous solution of the congo red dye while the Bromothymol blue (BTB) plates were made by incorporating into YEMA, 5 mls of a 0.5% alcoholic solution of bromothymol blue per litre (Vincent, 1970; Somasegaran and Hoben, 1985).

3.2 Pesticides

The following pesticides were used: benomyl, captan, Actellic and Aldrin. Benomyl. (Methyl-1-(butylcarbomyl) benzimidazole 2-yl carbamate) and captan (N-(trichloromethylthio)-4 cyclohexene -1, 2 dicarbomixide) are systemic (eradicant and protectant) and protective fungicides respectively (McEwen and Stephenson, 1979). Captan loses fungitoxicity when autoclaved and is compatible with many other pesticides (Lukens, 1969). Benomyl in aqueous solution breaks down rapidly to benzimidazole carbamic acid methyl ester, a stable compound equally as toxic as benomyl (Clemons and Sisler, 1969).

Aldrin (1, 2, 3, 4, 10, 10 - hexachloro -1, 4, 4a, 5, 8, 8a hexahydro-exo-1, 4-endo-5, 8-dimethanonaphthalene) is a very stable, active contact persitant cydodiene (Cremlyn, 1979). Actellic or pirimophos methyl (O[2-diethylaniino)-6-methyl-4-pyrimidinyl O,O-dimethyl phosphorothioate) is a broad spectrum organophosphorus insecticide used mainly against storage pests (Martin and Woodcock, 1983). For

experiments under laboratory culture, low concentrations of the pesticides ranging between 10 and 2000 PPM were used while for field and greenhouse studies, the manufacturers recommended rates of application were used viz;

Aldrin, 500g per 80 kg seed; Captan 12.5g per 500 g seed; Actellic, 400 g per 90 kg seed and Benomyl 1.875g ai per kg seed. All these were in powder form.

3.3 Experimental Design

The completely randomised design (CRD) was used for laboratory and greehouse experiments while the randomised complete block design was used for the field trial experiment.

3.4 Culture of *R. leguminosarum* bv *phaseoli* in the Presence of the Pesticides

3.4.1 Point Inoculation

R. leguminosarum bv *phaseoli* was cultured in the presence of the pesticides using Fisher's (1976) method. Weighed amounts of the pesticides corresponding to 10, 50, 100, 250, 500, 1000 and 2000 parts per million (PPM) were incorporated into sterilised Yeast extract mannitol agar. This was contained in Erlenmeyer flasks at approximately 45°C

(Fisher, 1976) in the laminar flow hood. The flasks were then shaken for 40 seconds and the contents dispensed into petri dishes and allowed to set. Using one of the pointed ends of a drawing paper clip no. 3, three point inoculations, approximately equidistant from each other, with R. leguminosarum bv phaseoli strains 446, 445, and 13A were made per petri dish. The petri dishes were then inverted and incubated at 28°C for five days. The radial growth of the colonies was then measured in millimetres with the aid of the magnifying lens on the scientific colony counter.

Due to the apparent toxicity of captan, further experimentation was carried out using the concentrations 0, 10, 25, 50, 100, 250 and 500 parts per million in congo red plates. The strains of R. leguminosarum bv phaseoli used were 13A, 107, 115, 445 and 446. Radial growth was determined after 5 days.

3.4.2 Plate Count

For plate count, fourty eight hour old cultures of Rhizobium leguminosarum bv phaseoli were serial diluted as follows: one ml of the original culture was pipetted into 99 mls of diluent and thoroughly mixed, thus giving a 10^{-2} dilution. One ml of the resultant suspension was then pipetted into 99 ml of diluent and mixed giving a 10^{-4} dilution. One ml of the 10^{-4} dilution was then pipetted into

99 ml diluent and mixed thoroughly giving a 10^{-6} dilution. 0.1 mls of the 10^{-6} dilution was then plated (3 plates for each strain and for each concentration of each pesticide) onto Yeast extract mannitol agar in which the pesticides had already been incorporated. The concentrations for each pesticide were 10, 50, 100, 250 and 500 parts per million. There was a control (0) for each strain. Plate count was performed after incubation at 28°C for 72 hours.

Further experimentation was repeated for captan, as it appeared to be toxic to the rhizobia. Concentrations used were 0, 10, 25, 50, 100, 250 and 500 parts per million. A 0.1 ml aliquot of the 10^{-6} dilution of a 48 hour old culture of *R. leguminosarum* bv *phaseoli* strains 13A, 107, 115, 445 and 446 was plated (3 plates per strain per captan concentration) on congo red medium. These were incubated at 28 C and plate count performed after 72 hours.

For experiments in liquid culture (Yeast extract mannitol broth), weighed amounts of captan corresponding to 10, 50, 100, 250 and 500 PPM were incorporated into sterilised 299 ml aliquots of Yeast extract mannitol broth contained in a 500 ml Erlenmeyer flask constructed into a simple fermenter. A total of six simple fermenters (Figure 6) were used, one being for control (0 captan concentration).

Into each of the simple fermenters, 1.0 ml of a 48 hour old culture inoculum was introduced asceptically using a sterile syringe and needle. The cultures were incubated at room temperature. Sampling and observing for viability was performed at 0, 12, 24, 36, 60, 84 and 108 hours after inoculation during which 1.0 ml was removed asceptically using a sterile syringe and needle. These samples were serially diluted (Vincent, 1970) and 0.1 ml of the dilutions was plated on congo red plates which were then inverted and incubated at 28°C. Three plates were used per dilution per concentration of captan.

3.5 Survival of Rhizobia on Bean Seed

3.5.1 Inoculant Production

Legume-crop growing requires inoculation of plants with specific rhizobia. Thus to obtain satisfactory nodulation steps must be taken to ensure that the inoculum contains a certain number of rhizobia cells at the moment of use. Because of the above, inoculants were produced for experimentation.

The carrier used was filtermud (Anyango, 1984). This was ground to pass through the 250 mesh sieve, which makes carriers suitable for seed coating (Somasegaran and Hoben, 1985). Fifteen autoclavable bags were prepared and filled with 50.0 g of the sieved filtermud. Into each of

the bags, 5 ml of distilled water was added. The bags were then sealed and water incorporated into the filtermud by kneading. The carrier was then sterilised by autoclaving for 60 minutes at 121°C, after which they were allowed to cool in the autoclave (Somasegaran and Hoben, 1985).

Alcohol (70%) was used to swab one edge of the packet in preparation for injection. Using sterile one hundred ml syringe and needle, 45 ml of a 72 hour old culture of R. leguminosarum bv phaseoli strain 445, were injected asceptically into each of the cooled filtermud packets. The injection hole was then sealed and the packet kneaded until the inoculum was uniformly absorbed by the filtermud. The packets were then incubated for three days at 25°C for maturation (Vincent, 1970). They were then subjected to quality test, by which one gram of the inoculant was found to contain an average of 6.95×10^9 Rhizobium cells.

3.5.2 Preparation of Adhesive

Fourty percent solution of gum arabic (Acacia) was used as a sticker (Vincent, 1970; Somasegaran and Hoben, 1985). Powdered acacia (40.0g) was weighed out and poured into 100 ml of distilled water heated to near boiling and stirred until uniformly mixed. 2.5 g of calcium carbonate was then added and thoroughly dispersed. It was then stored in the refrigerator (Somasegaran and Hoben, 1985).

3.5.3 Seed Sterilization

Undamaged clean seeds of Phaseolus vulgaris L. variety Canadian Wonder (GLP-24) were obtained from the Bean Improvement Project of the Department of Crop Science. From these uniform size seeds were selected. These were surface sterilised by immersing in concentrated (98%) sulphuric acid for eight minutes and rinsing through 10 changes of sterile distilled water and finally with 2% calcium carbonate in water (Vincent, 1970; Somasegaran and Hoben, 1985). They were then air dried and kept in a sterile environment.

3.5.4 Seed Treatment

The pesticides were used at the recommended rates of manufacturers (Rennie and Dubtz, 1983) for 100.0g of the bean seeds. These were as follows: Aldrin, 0.625g ; Actellic, 0.444g; Captan, 2.5g; and Benomyl 0.375g.

There were a total of 15 treatments and a control as follows: 1. Actellic; 2. Aldrin; 3. Captan; 4. Benomyl; 5. Actellic + Aldrin; 6. Actelling + Captan 7. Actellic + Benomyl; 8. Aldrin + Captan; 9. Aldrin + Benomyl 10. Captan + Benomyl; 11. Actellic + Aldrin +

Captan; 12. Actellic + Aldrin + Benomyl; 13. Actellic + Captan + Benomyl; 14. Aldrin + Captan + Benomyl; 15. Actellic + Aldrin + Captan + Benomyl and 16 Control (pesticide free).

Sixteen batches of 100g of surface sterilised seed for each treatment were kept in separate sterile polythene bags and labelled corresponding to the above treatments. The pesticides were then added to the seeds, followed by 2.5 ml of 40% gum arabic solution and 8.0g of the Rhizobium inoculant. The contents in the bags were agitated for 60 seconds to coat the seeds evenly (Somasegaran and Hoben, 1985) after each addition.

3.5.5 Viable Rhizobia Count

The treated seeds were emptied into labelled sterile petri dishes in the laminar flow hood, and allowed to air dry and incubated for a period ranging from 0 to 11 days. The survival of Rhizobium on treated seed was evaluated by serial dilution and plate count.

From each of the 16 petri dishes, 5 seeds were removed asceptically and placed into 10.0 ml of sterile diluent in universal bottles. This was then shaken for 60 seconds to wash the inoculant off the seeds. Thus 2.0 ml of the resultant suspension contained rhizobia derived from one

seed. This was then serial diluted and 0.1 ml of each dilution plated (3 plates for each dilution) on congo red and bromothymol blue agar.

The survival of rhizobia was evaluated by determining the number of colonies derived on plates seeded with the suspensions from seeds incubated at different time lengths.

3.6 Influence of Seed Dressing Pesticides on Nodulation and Nitrogen Fixation

This experiment was aimed at determining whether the seed dressing pesticides can be used in conjunction with the Rhizobium inoculants at planting time. The study was undertaken using modified Leonard jar assemblies and plastic pots with Vermiculite and nitosol type of soil as the rooting medium respectively under glasshouse conditions. In addition there was a field trial carried out at the University farm at Kabete.

3.6.1 Modified Leonard Jar Assembly

The modified Leonard jar assemblies were prepared from plastic cups with a diameter of 7.5 cm at the top and 5 cm at the base and a depth of 9.5 cm. A slit was made along the diameter at the base of the cup. Two sponge pieces about 5 cm wide were then placed in the cup such that 10 cm extended from the base. Between the sponge pieces about 30 cm long

wick material of cotton rope was placed. While holding, the wick in a central position (between the two sponges) the cup was filled with coarse vermiculite previously soaked overnight in water, drained and thoroughly washed to a pH of 7.0 (Vincent, 1970; Somasegaran and Hoben, 1985). The packing was so done to minimise air spaces. The packed cup was then placed in position on the reservoir, a plastic jar to which it firmly fitted. The Vermiculite was then moistened by adding 200 ml of nitrogen-free nutrient solution. Excess solution was allowed to drain into the reservoir. The reservoir was then filled with 300 ml of N-free nutrient solution. The assembly (Fig. 1) was then placed in brown paper bags, a total of 64 assemblies were prepared. These were then sterilised by autoclaving for two hours at 121°C. They were then allowed to cool in the autoclave for 12 hours prior to removing (Somasegaran and Hoben, 1985).

The surface sterilised bean seeds were treated as earlier described (15 treatments and one control). Each treatment was replicated four times. Three treated seeds were directly planted per jar and kept in the laboratory until germination. These jars were then transferred to the glasshouse and randomly arranged. After 10 days, a thinning was made to two uniform plants per jar. The reservoir was filled with N-free nutrient solution twice a week and the

Figure 1: The Modified Leonard Jar Assembly

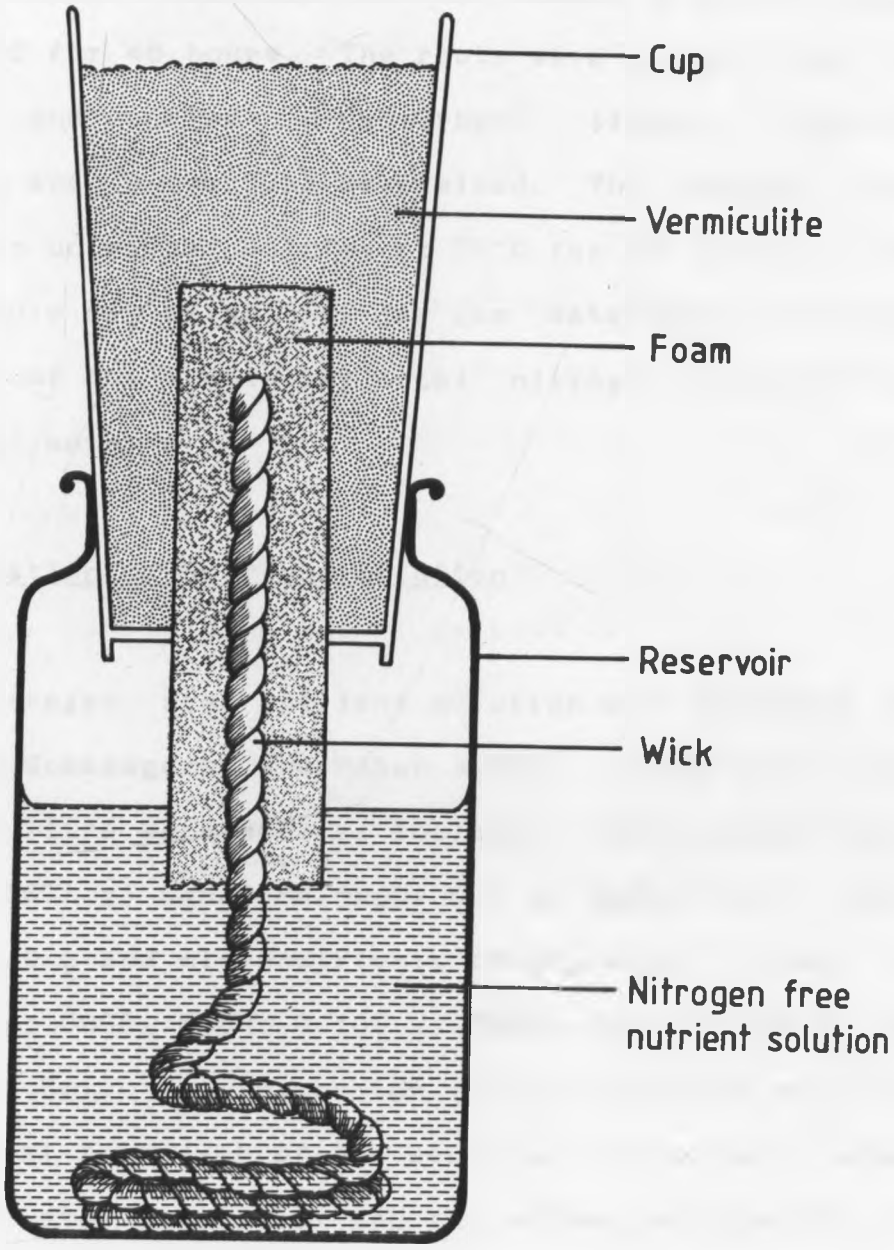


Fig. 1.

plants were observed for vigour and colour. The plants were harvested after 30 days of growth. The stems were cut at the point of cotyledon attachment and the shoots dried in paper bags at 70°C for 48 hours. The roots were washed free of Vermiculite and dried in absorbent tissue. Nodule distribution and number was determined. The nodules were then dried in universal bottles at 70°C for 48 hours. The shoot and nodule dry weight per jar was determined followed by grinding of the shoots for total nitrogen analysis by micro-Kjeldahl method.

3.6.2 Preparation of Nutrient Solution

The nitrogen free nutrient solution was prepared as described by Somasegaran and Hoben (1985). First six stock solutions per litre were made as follows:- $CaCl_2.2H_2O$, 249.1 g; KH_2PO_4, 136.1 g; $C_6H_5O_7Fe.5H_2O$. 6.7 g; $MgSO_4.7H_2O$, 123.3 g; K_2SO_4, 87.0 g and micronutrients ($MnSO_4.4H_2O$, 0.4462 g; H_3BO_3, 0.247 g; $CuSO_4.5H_2O$, 0.100 g; $CoSO_4.7H_2O$, 0.056 g; $NaMoO_4.2H_2O$, 0.048g). To prepare ten litres of N-free nutrient solution. 5 ml from each of the stock solutions were added to distilled water in a flask and the volume adjusted to 10 litres. The nutrient solution was sterilised by autoclaving, at 121°C for 15 minutes.

3.6.3 Preparation of Kabete Soil

The reddish brown soil at Kabete described as a humic nitosol was used in this experiment. This is a medium acid soil with moderate amounts of available calcium and magnesium, organic carbon, adequate potassium but is low in available phosphorus and total nitrogen.

The soil was collected from the upper 30 cm, using a spade, in unused polythene bags from the the University farm, Kabete. It was air dried in the shade and thoroughly mixed. The dried soil was then mixed with small stone pebbles in a ratio of 2:1 by volume and sterilised by autoclaving. This was then left to stablise for 30 days in the green house. This soil-stone pebble mixture was then transferred to plastic pots with a diameter of 15.5 cm at the top and 10.5 cm at the base and a depth of 14.5 cm. These pots had two slits at the base. The soil was then moistened to field capacity using distilled water.

3.6.4 Effect of the Pesticides on Bean Plants

The Canadian Wonder (GLP 24) seeds were surface sterilised and treated with pesticide, gum arabica and *Rhizobium* inoculant in a parallel experiment to that described in section 3:6:1. Six seeds for each treatment were directly sown per pot using a sterile forcep. Each

treatment was replicated four times and their arrangement randomised in the greenhouse. The seedlings were thinned to five uniform plants per pot after 10 days of growth. The soil was kept moist by using sterile water twice a week. The plants were observed for vigour and leaf colour. They were harvested after four weeks of growth and the following determined:- nodule distribution, number, and weight; shoot dry weight; and the total nitrogen by micro-Kjeldahl method.

3.6.5 Field Trail Experiment

The field trial experiment was carried out at the University farm, Kabete. The soil here was the humic nitosol defined as indicated under section 3:6:3. The trial was carried out as a pure stand of Phaseolus vulgaris L. and was blocked over time and site, with each block having four replicates.

The area was cleared, ploughed, harrowed, demarcated and marked. It measured 588 m^2 and sub-divided into four replication plots measuring 132m^2 (24m x 5.5m). In each replication plot there were 16 subplots, one for each treatment measuring 1.5 m x 5.5 m (8.25 m^2). In each sub-plot furrows were made 0.5m apart and 5 cm deep.

The surface sterilised seeds were treated with pesticide, gum arabica and inoculant as described under section 3:5:4. They were then planted in moist soil 10 cm apart along the furrows. The treatments were randomised within each replication plot.

The first experiment (Block I) was carried out under irrigation hence the soil was water soaked for three hours the day before planting. The second (Block II) was carried out during the short rains and the seeds were sown following a heavy downpour. In both trials the seeds were sown immediately after treatment and the furrows covered. The plants were frequently monitored for general vigour, leaf colour, diseases and pest damage. Weeding was carried out when necessary.

An early harvest of ten plants per treatment was undertaken when more than 50 percent of the plants had flowered (Somasegaran and Hoben, 1985). This was after 55 and 48 days for the 1st and 2nd trial respectively. The plants were removed from the ground carefully using a 3-pronged fork. The excess soil was discarded but care being taken not to lose nodules. The harvested plants were placed in paper bags and transferred to the laboratory, where the roots were separated from the shoots. The roots were carefully washed and the nodules removed.

The following were then determined as stated under section 3:6:1:- nodule number and dry weight; shoot dry weight; and total nitrogen by micro-Kjeldahl method. The yield harvest was later determined from 1m x 3m ($3m^2$) of the sub-plot. The pods were removed from the stalks and the seeds later removed. The seeds were then dried to about 11.5 percent moisture content and the weight determined. Yield was then expressed as Kg per ha.

CHAPTER FOUR

RESULTS

4.1 Effect of the Pesticides on the Growth of *Rhizobium leguminosarum* bv *phaseoli* in vitro

The results obtained using any of the methods demonstrated the toxicity of captan to the *Rhizobium* compared to benomyl, aldrin and actellic.

4.1.1 Point Inoculation

The experiment carried out on Yeast extract mannitol agar (YEMA) using the point inoculation technique indicated decreasing radial growth with increasing pesticide concentration (Table 1). The radial growth was determined after five days and compared with that of the control culture. Captan caused a marked reduction in radial growth causing more than 50 percent inhibition at 100 ppm on average for the three *R. leguminosarum* bv *phaseoli* strains 445, 446 and 13A. Benomyl, Actellic and Aldrin were less toxic as compared to captan, causing less than 50 percent inhibition of radial growth at 2000 ppm on average for the three strains (Fig. 2). There was no significant difference in the response of the *Rhizobium* strains to the various pesticides.

At 1000 ppm, and above of benomyl, actellic and aldrin the growth produced was an even conical shape as compared to a dome shape observed at lower concentration.

Table 1: Effect of pesticides on the growth of *Rhizobium leguminosarum* bv *phaseoli* on agar. Radial growth expressed as a percentage of control

		Concentration in Agar (Parts per Million)						
Pesticide	Strain	10	50	100	250	500	1000	2000
Benomyl	445	97.37	97.37	93.89	89.47	83.37	61.37	54.37
	446	97.74	78.95	80.23	67.67	61.43	60.15	55.01
	13A	100.00	83.74	86.04	84.93	93.02	53.52	50.01
Captan	445	85.05	77.16	33.37	14.00	1.79	0	0
	446	105.26	68.95	44.51	22.83	8.76	0	0
	13A	93.2	88.42	54.64	44.17	26.73	0	0
Aldrin	445	91.26	91.26	91.26	93.89	94.74	89.47	79.79
	446	93.98	85.19	78.95	69.95	65.19	55.79	53.76
	13A	96.51	94.21	86.04	90.72	79.69	70.97	61.64
Actellic	445	91.26	84.21	81.58	78.11	67.58	61.37	54.39
	446	97.74	83.98	75.19	72.71	72.71	52.63	45.01
	13A	91.91	86.04	80.25	74.46	69.78	57.01	53.50

Coefficient of variation = 20.08%

Further experimentation revealed decreasing radial growth of the R. leguminosarum bv phaseoli strains 13A, 107, 115, 445 and 446 following point inoculation with increasing concentration of captan (Table 2, Fig. 3 and 4). The coefficient of variation was 0.847. This indicated a negative linear relationship between captan concentration and the radial growth at the 5 percent level of significance.

During the course of experimentation, radial growth was not obvious at 50 ppm and above of captan after 2 days as compared to the observations for control, 10 ppm and 25 ppm. Growth only become evident at 50 ppm on the 3rd day. This indicated some level of toxicity of captan to the Rhizobium. The slow growth could mean that some of the Rhizobium cells seeded following point inoculation were killed. Since there is decreasing radial growth with concentration of captan, then the number of Rhizobium cells killed at the point of inoculation increases with increasing concentration until complete kill apparently occurs at 1000 ppm, the point at which no growth was observed.

Figure 2a: Radial growth of Rhizobium leguminosarum biovar phaseoli as influenced by benomyl on Yeast Extract-Mannitol Agar

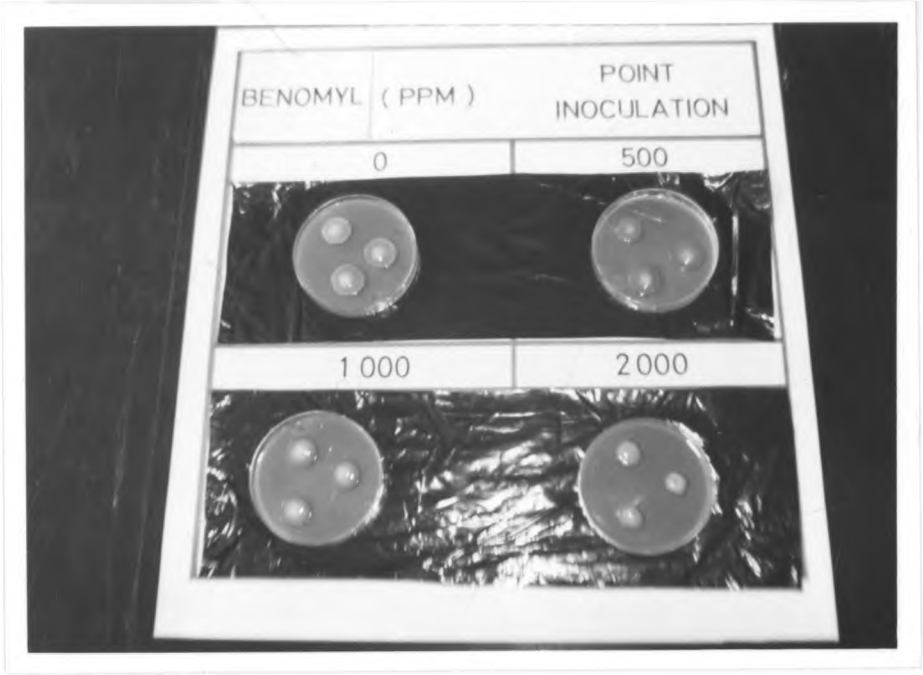


Figure 2b: Radial growth of R. leguminosarum bv phaseoli as influenced by actellic (Pirimophos methyl) on Yeast Extract-Mannitol Agar

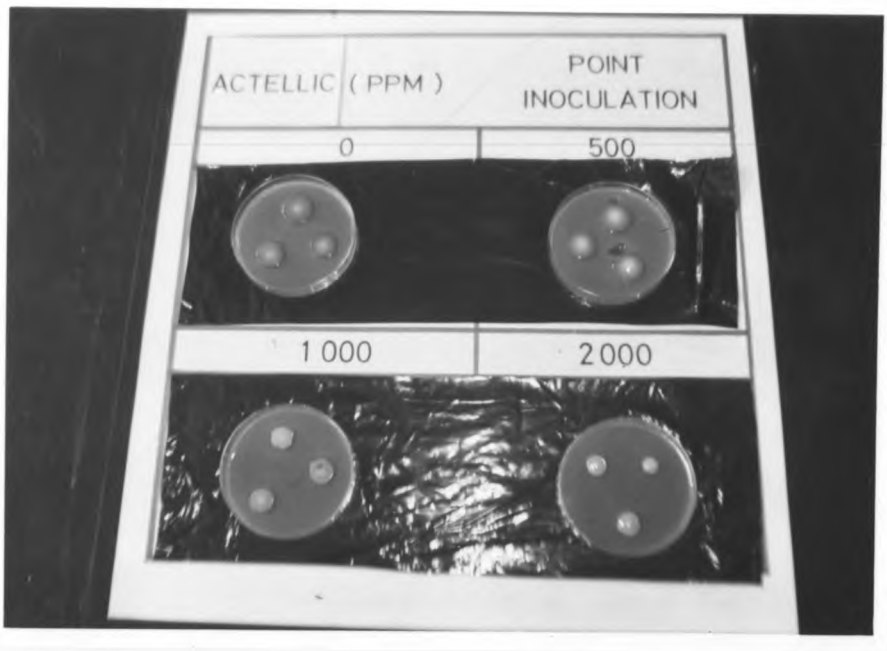


Figure 2c: Radial growth of R. leguminosarum bv phaseoli as influenced by Aldrin on Yeast Extract-Mannitol Agar.

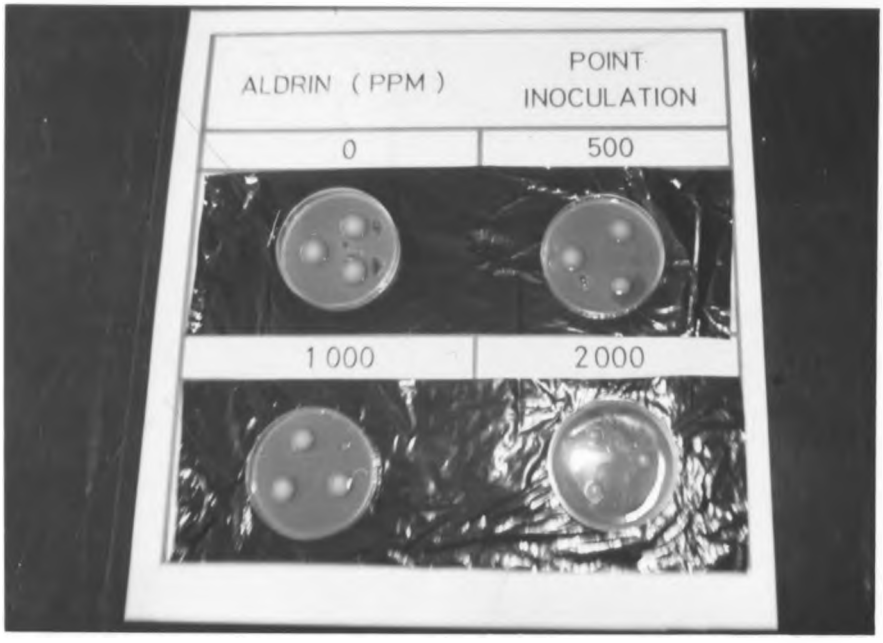


Table 2: Effect of Captan on radial growth (mm) of *Rhizobium leguminosarum* bv *phaseoli* on congo red agar using a point inoculation technique

	Captan Concentration in Parts per Million						
Strain	0	10	25	50	100	250	500
445	15.33	14.17	13.17	10.33	5.67	4.33	2.33
446	16.83	15.0	13.5	10.00	7.67	5.67	2.67
107	20.83	19.67	19.33	13.83	10.0	5.33	3.17
13A	21.00	19.00	16.83	14.00	9.67	5.00	2.50
115	20.83	19.67	17.83	14.33	8.50	3.83	2.00
Average	18.964	17.502	16.132	12.498	8.302	4.832	2.534

Coefficient of variation = 11.51%

Each figure is an average of six point inoculations

Figure 3: Effect of captan on radial growth of Rhizobium leguminosarum bv phaseoli

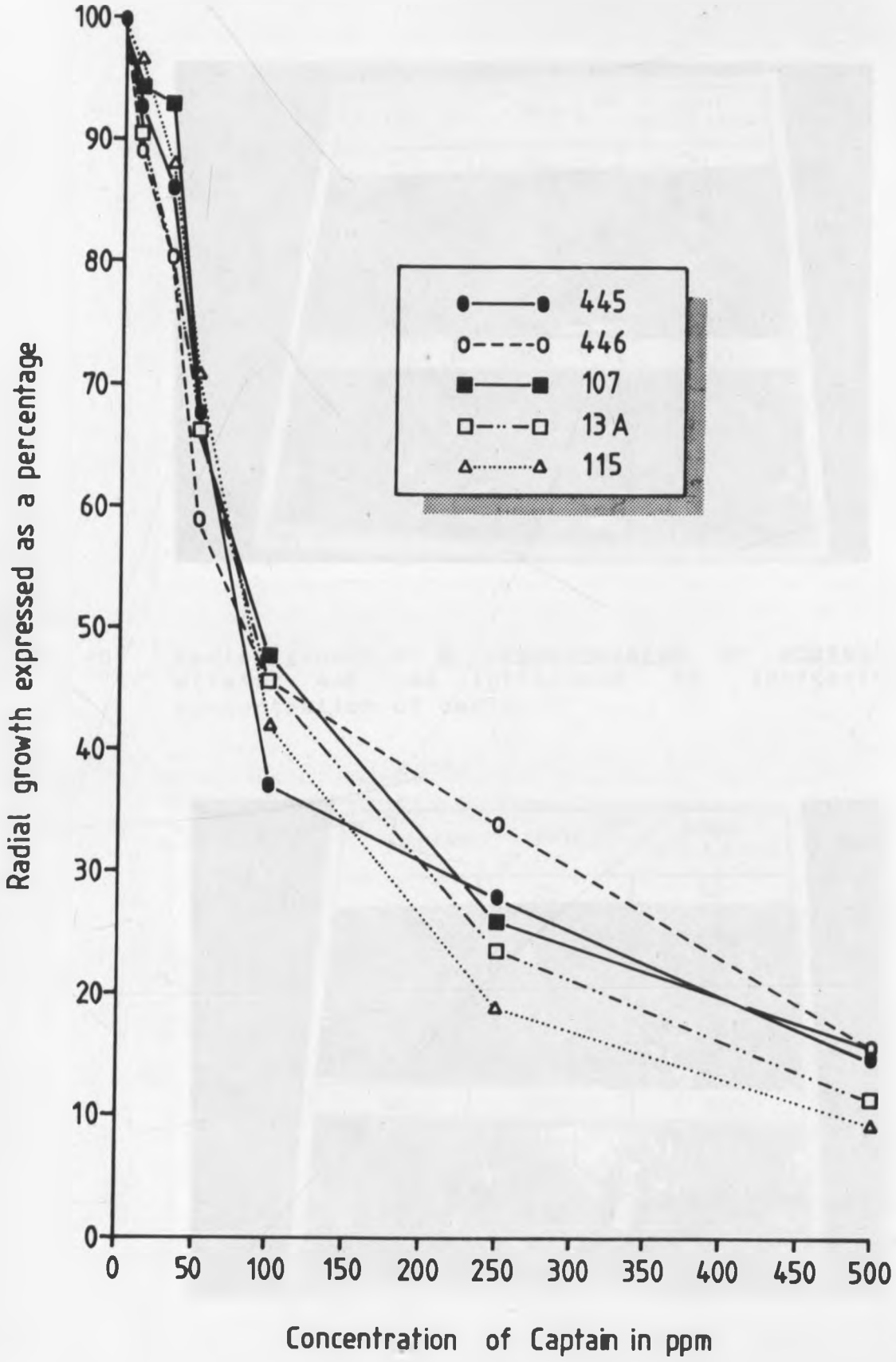


Fig. 3.

Figure 4a: Effect of increasing captan concentration on the radial growth of R. leguminosarum bv phaseoli strain 13A

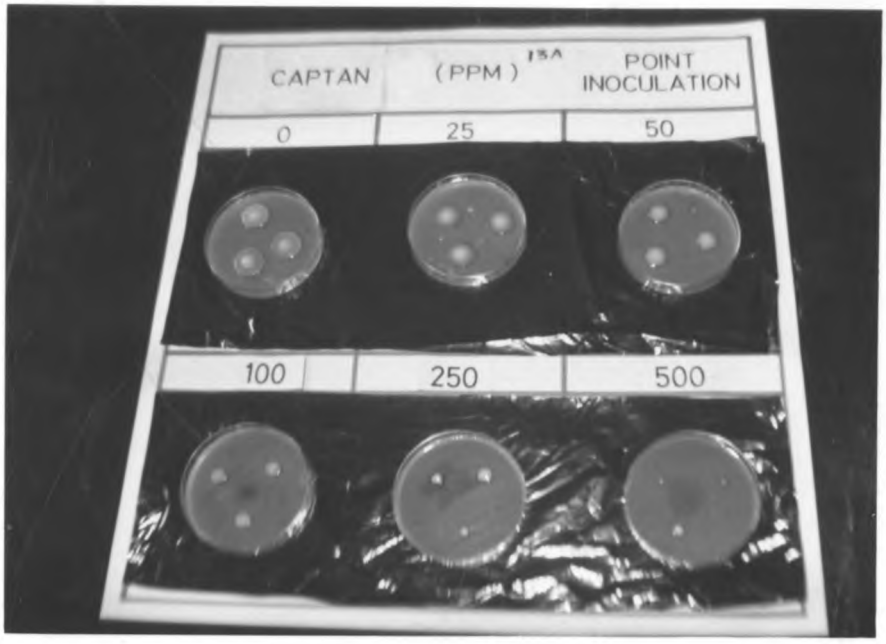
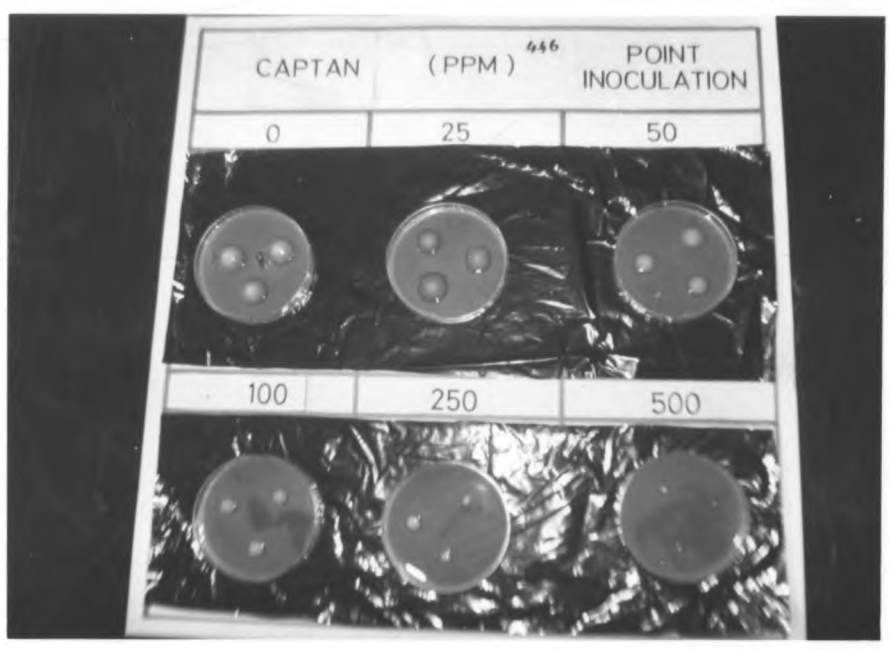


Figure 4b: Radial growth of R. leguminosarum bv phaseoli strain 446 as influenced by increasing concentration of captan



4.1.2 Plate Count Technique

Using the plate count technique after incorporation of the pesticides into the agar, growth of *Rhizobium* colonies was recorded for Benomyl, Actellic and Aldrin at 500 ppm (Table 3). The colonies were comparable to the control in size and shape (dome shaped). The number of colonies and hence survival, decreased with increasing pesticide concentration though not appreciably. The decline in numbers for *R. leguminosarum* bv *phaseoli* strain 445 and 446 on agar into which benomyl at 500 ppm was incorporated was greatest with just less than 68 percent survival recorded. However, for strain 13A, at the same concentration, over 80 percent of the cell still survived. Thus strain 445 and 446 were more sensitive as compared to strain 13A to 500 ppm of benomyl. The response was however nearly uniform at 250 ppm of benomyl with an average of 86 percent survival recorded for the three strains.

With Aldrin, at 500 ppm, the survival was 100 percent for all three strains. Actellic allowed over 80 percent survival at 500 ppm and over 93 percent survival at 250 ppm. Therefore the sensitivity of *Rhizobium leguminosarum* bv *phaseoli* was higher for benomyl as compared to aldrin and actellic.

The sensitivity to benomyl, aldrin and actellic was however not comparable to that expressed toward captan. There was no growth at 100 ppm and above of captan and at 50 ppm less than 15% survival was recorded (Tables 3 and 4). On control and at 10 ppm of captan the Rhizobium colonies began appearing after 48 hours, while at 50 ppm captan, only a few appeared after 72 hours. The colonies appeared much later for R. leguminosarum bv phaseoli strain 445 and 446 (92 hours) in contrast to strain 13A (Fig.5). There was no pesticide strain-interaction in the experiment at the 99% level of significance.

Table 3: The Effect of Pesticides on the Growth of *R. leguminosarum* bv *phaseoli* on Agar Using the Plate Count Technique (Average No. of Colonies per plate)

		Concentration in YEMA, Parts Per Million					
Pesticide	Strain	Control(0)	10	50	100	250	500
Benomyl	445	76	67	78	66	65	51
	446	91	101	75	81	79	53
	13A	225	209	201	211	197	182
Captan	445	76	65	8	0	0	0
	446	91	93	10	0	0	0
	13A	225	195	30	0	0	0
Aldrin	445	76	76	79	72	72	77
	446	91	93	92	90	82	89
	13A	225	209	214	174	189	196
Actellic	445	76	74	75	68	73	69
	446	91	89	92	82	89	92
	13A	225	233	195	201	210	181

Coefficient of variation = 25.89%

average of 3 plates

Figure 5: Plate count of Rhizobium leguminosarum bv phaseoli as influenced by captan. The colonies appeared at 50 ppm of captan earlier for strain 13A

Figure: 6: Simple fermenters. Growth recorded at control (no captan) 10 and 50 ppm of captan. Observe clear YEM at 250 and 500 ppm of captan

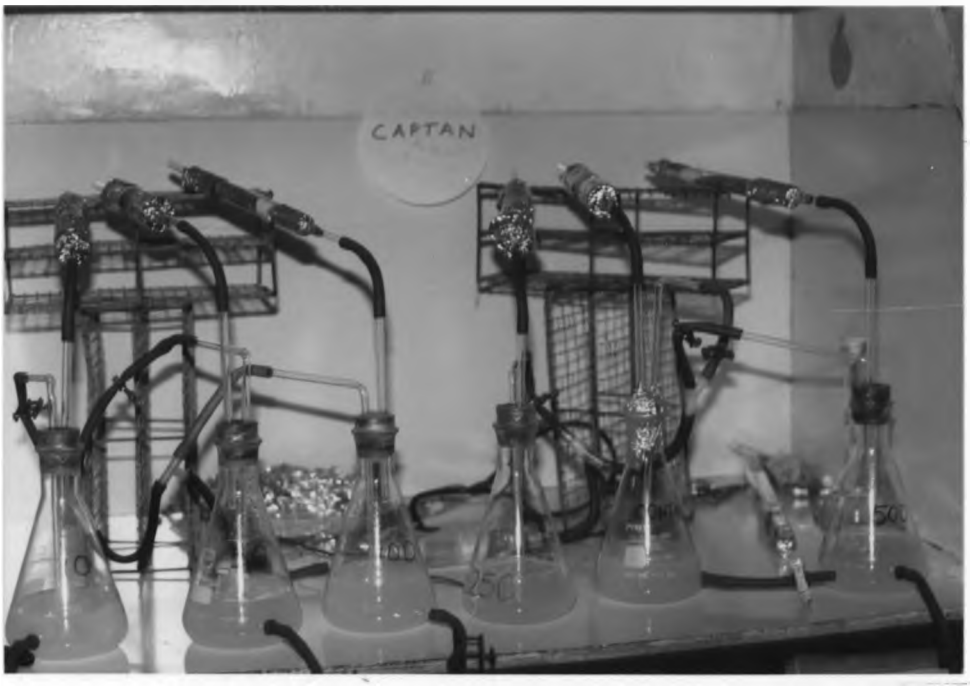


Table 4: Effect of Captan on the Growth of *R. leguminosarum* bv *phaseoli* Strains on Agar Using the Plate Count Technique

	Captan Concentration in Parts per Million			
Strain	(0)	10	25	50
107	201.33 ± 3.38	196.33 ± 3.71	135.33 ± 5.24	27.33 ± 1.45
115	165.67 ± 7.51	151.33 ± 11.72	75.67 ± 2.85	12 ± 0
13A	186.33 ± 7.42	157.67 ± 5.17	121 ± 3.61	23 ± 2
445	136 ± 7.02	129.33 ± 4.98	90 ± 5.29	6.67 ± 0.67
446	98.67 ± 4.98	91.33 ± 3.48	64.67 ± 3.48	5.33 ± 0.88

4.1.3 Growth of *Rhizobium leguminosarum* bv *phaseoli* in Simple Fermenters in Presence of Captan

The simple fermenters were constructed as indicated in figure 6. Growth of *R. leguminosarum* bv *phaseoli* was recorded for control (no captan) 10 ppm and 50 ppm of captan (Table 5). Captan at 50 ppm caused a lag phase as compared to control and 10 ppm captan (Fig. 7). This lag phase could be attributed to possible killing of most of the *Rhizobium* cells in the 1.0 ml inoculum as indicated in tables 3 and 4 where the population was very low as compared to the control. In addition, on Yeast extract mannitol agar, the *Rhizobium* took a longer time to establish itself at 50 ppm as compared to lower concentrations of captan. Moreover, the exponential phase occured after 36 hours in contrast to the control and 10 ppm where it occured much earlier (Fig. 7).

At 100 ppm, there was a sharp decline in the number of viable *Rhizobium* cells with time. No viable count was recorded after 36 hours (Table 5). At 250 ppm and 500 ppm no viable *Rhizobium* were recorded after 12 hours. This indicated complete toxicity of captan to *Rhizobium* at high concentration. The correlation coefficient (r) was +0.973, +0.976 and +0.902 for 0, 10 and 50 ppm of captan respectively. Thus there was a linear relationship between time and *Rhizobium* population upto 50 ppm of captan at the 1 percent level of significance.

Table 5: Growth of *Rhizobium leguminosarum* bv *phaseoli* in the Presence of Captan in Broth

Time in Hours	Strain	Concentration of YEM Broth, Parts per Million			
		Control (0)	10	50	100
12	445	0.058	0.042	0.00008	0.0000145
	13A	0.245	0.224	0.00339	0.000174
	107	0.261	0.028	0.0032	0.000143
24	445	0.303	0.148	0.000105	0.0000029
	13A	0.560	0.468	0.00606	0.0000073
	107	0.564	0.337	0.00576	0.0000073
36	445	1.270	0.757	0.000145	0
	13A	1.533	1.347	0.0148	0
	107	1.627	1.067	0.0133	0
60	445	2.38	2.16	0.0822	0
	13A	2.590	2.533	0.245	0
	107	2.55	1.37	0.217	0
84	445	2.75	2.46	1.217	0
	13A	3.487	3.443	1.380	0
	107	3.32	1.86	0.980	0
108	445	2.85	2.84	1.823	0
	13A	3.610	3.543	2.490	0
	107	3.53	2.00	1.060	0
Correlation coefficient (r)		0.973	0.976	0.902	

Figures are of population expressed as $x10^9$ cells per ml.
No growth recorded at 250 and 500 ppm

Figure 7a: Culture of Rhizobium leguminosarum bv phaseoli strain 445 in the presence of captan

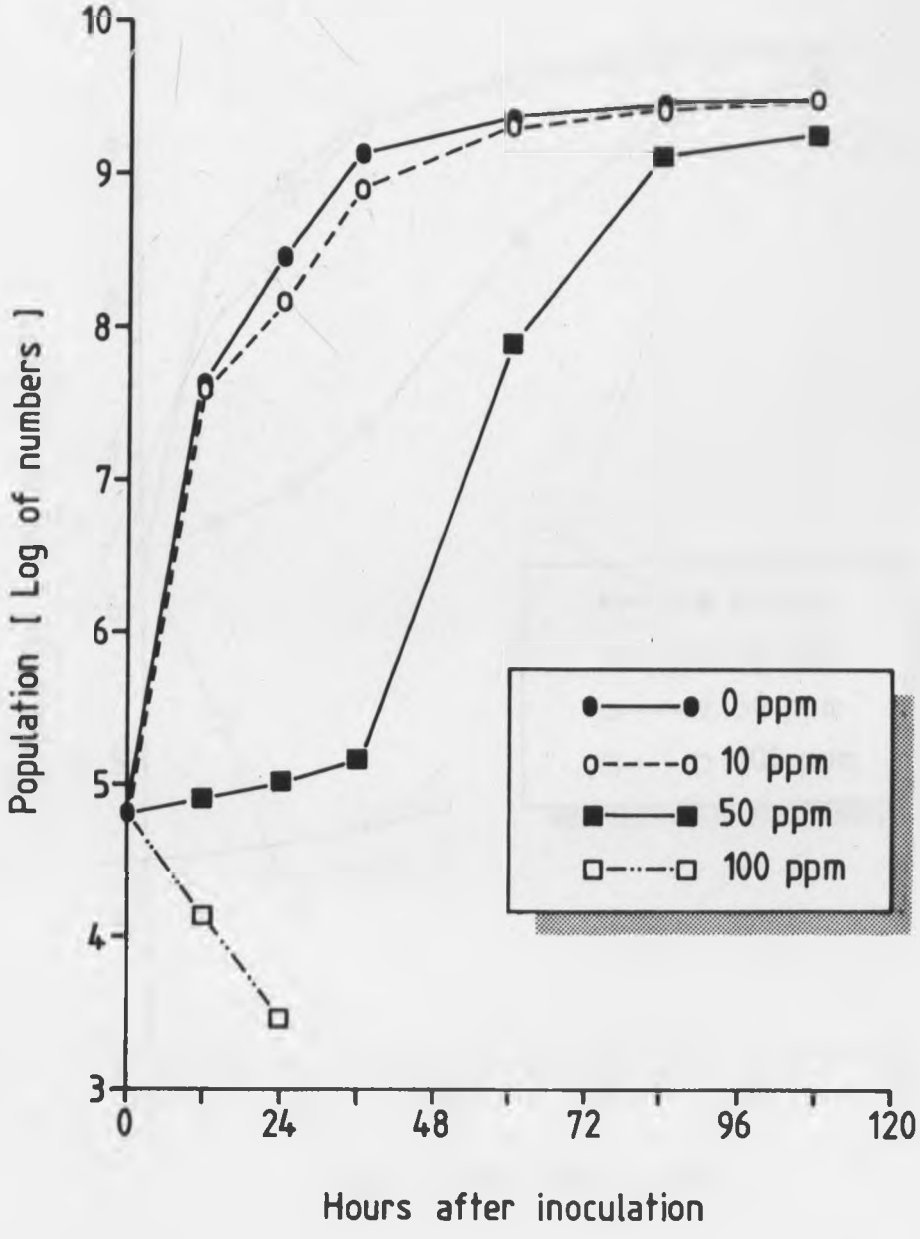


Fig. 7 [a]

Figure 7b: Culture of Rhizobium leguminosarum bv phaseoli strain 13A in the presence of captan

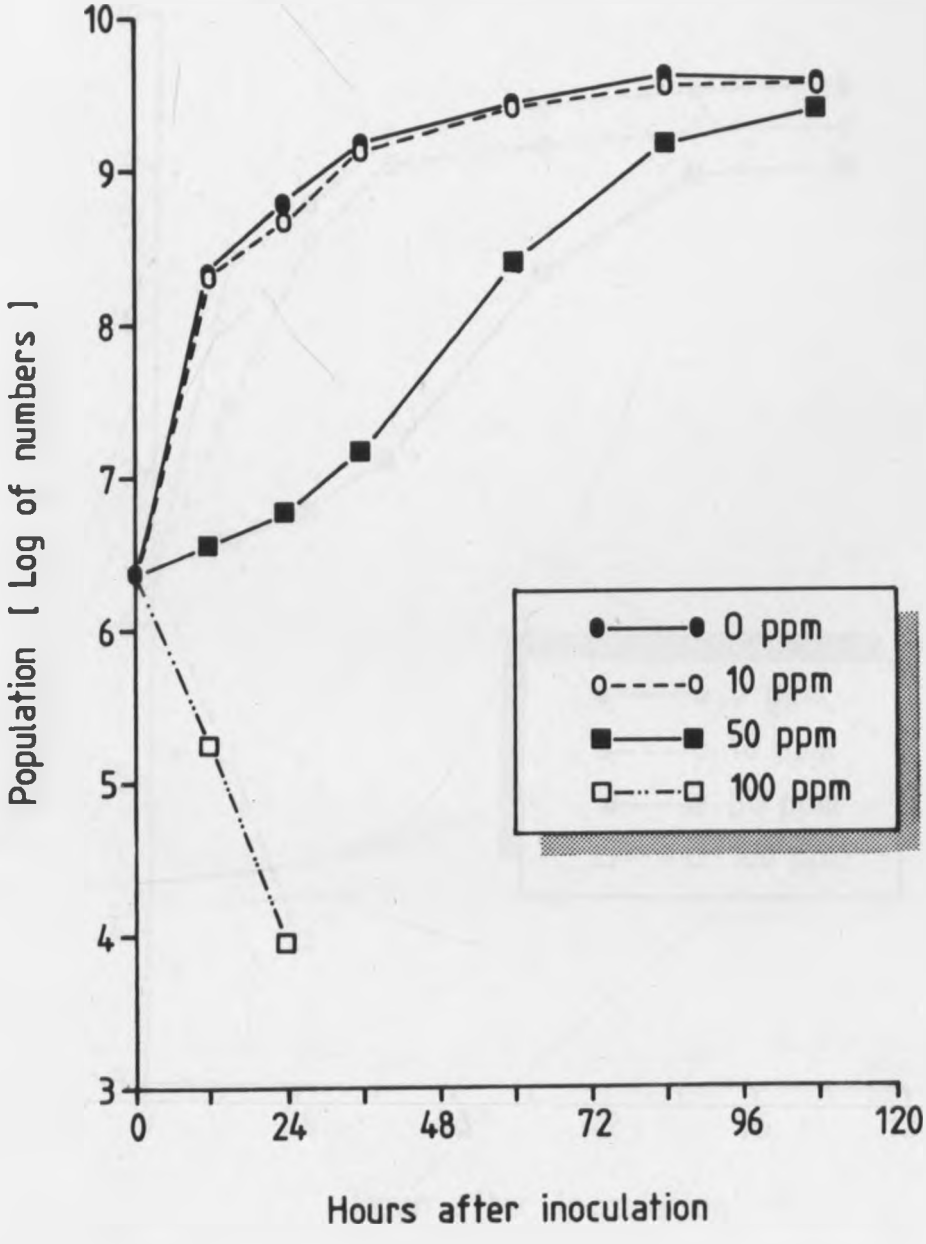


Fig. 7[b].

Figure 7c: Culture of Rhizobium leguminosarum bv phaseoli strain 107 in the presence of captan

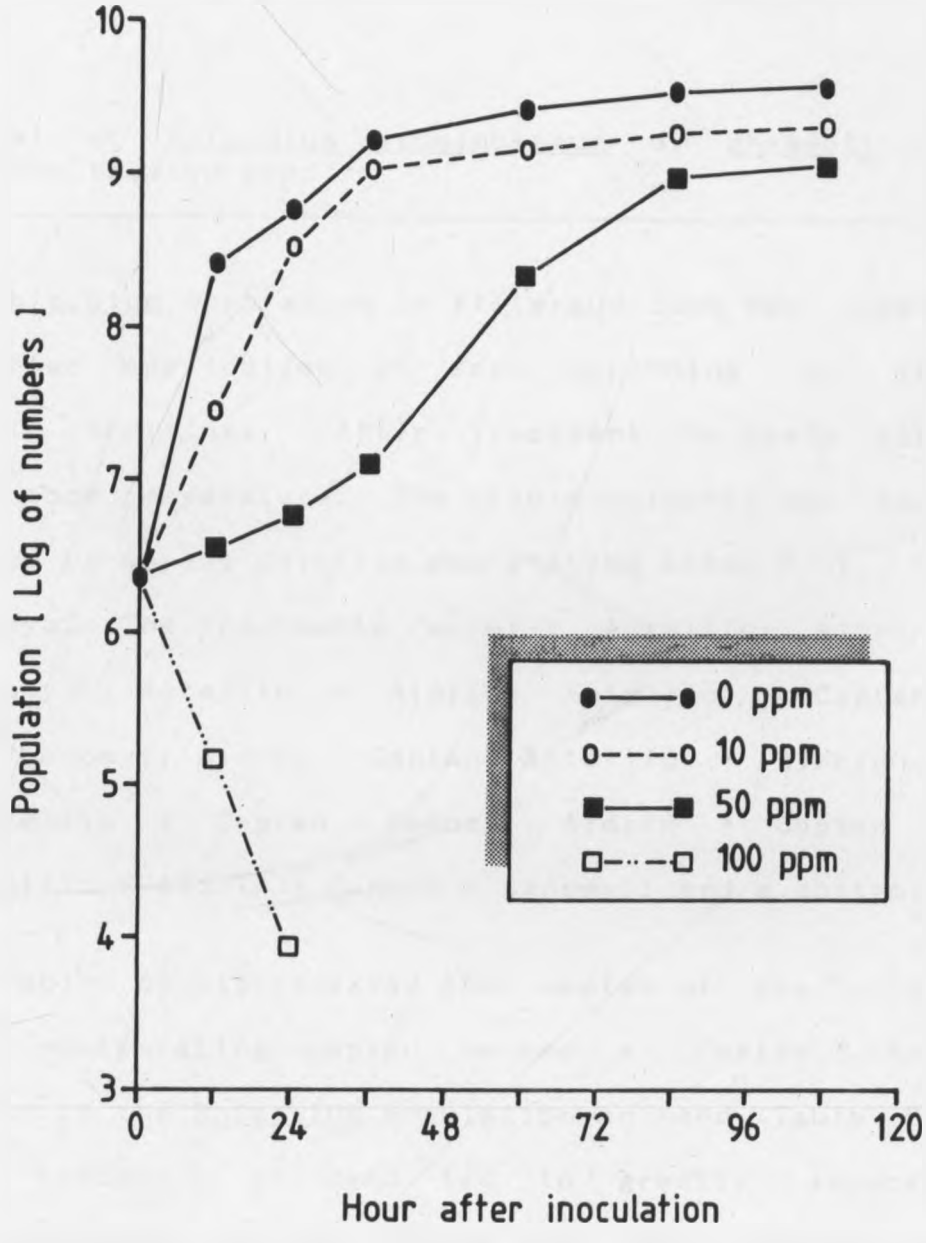


Fig. 7 [c]

The R. leguminosarum bv phaseoli strains response was similar except for the numbers due to the differences in growth rates.

4.2 Survival of Rhizobium leguminosarum bv phaseoli on pesticide treated seed

The Rhizobium population in filtermud does not remain constant after application on seed depending on the environmental conditions. After treatment the seeds were incubated at room temperature. The viable rhizobia per seed was determined by serial dilution and plating after 0, 1, 3, 5 and 11 days. The treatments were:- Actellic; Aldrin; Captan; Benomyl; Actellic + Aldrin; Actellic + Captan; Actellic + Benomyl; Aldrin + Captan; Actellic + Aldrin + Benomyl; Actellic + Captan + Benomyl; Aldrin + Captan + Benomyl; Actellic + Aldrin + Captan + Benomyl; and a control.

The viable counts revealed that captan or any other treatments incorporating captan caused a faster than normal decline in the Rhizobium population on seed (Table 6). Thus captan treatment of seed led to greatly reduced survival of Rhizobium. By the 5th day less than 4 percent of the applied Rhizobium cells were surviving and on the 11th day less than 0.1 percent were viable following captan dressing of seed. Survival of the Rhizobium on seeds treated with benomyl, aldrin and actellic and in their combination was more than 6.5 percent by the 5th day. The survival of

the rhizobia on untreated seed (control) on the 11th day was 7.0 percent which was higher than that of aldrin (6.89 percent) and benomyl (4.05 percent) but less than that of actellic (7.72 percent). This indicated that actellic and aldrin were not toxic to the Rhizobium on seed. Benomyl was less toxic to the Rhizobium on seed as compared to captan (Fig. 8).

Table 6: Survival of R. leguminosarum bv phaseoli on pesticide treated seed

Treatments	Days After Inoculation 0	1	3	5	11
Act	16.2 ± 1.57	6.24 ± 0.92	3.45 ± 0.18	1.78 ± 0.12	1.25 ± 0.05
Ald	18.00 ± 1.07	7.02 ± 0.46	3.77 ± 0.18	1.87 ± 0.17	1.24 ± 0.02
Cap	14.47 ± 1.34	4.00 ± 0.06	0.33 ± 0.06	0.13 ± 0.007	0.007 ± 0.001
Ben	22.20 ± 1.48	6.16 ± 0.12	3.82 ± 0.13	2.06 ± 0.14	0.90 ± 0.006
Act + Ald	17.20 ± 0.90	6.31 ± 0.05	3.46 ± 0.08	2.11 ± 0.35	1.66 ± 0.04
Act + Cap	13.0 ± 0.50	2.82 ± 0.05	0.80 ± 0.00	0.30 ± 0.06	0.006 ± 0.00
Act + Ben	23.6 ± 1.56	10.76 ± 0.52	3.79 ± 0.25	1.57 ± 0.16	0.35 ± 0.03
Ald + Cap	12.20 ± 0.31	1.74 ± 0.46	0.98 ± 0.12	0.16 ± 0.02	0.009 ± 0.001
Ald + Ben	26.0 ± 2.00	7.15 ± 0.03	3.73 ± 0.02	2.06 ± 0.21	1.01 ± 0.007
Cap + Ben	11.40 ± 0.20	1.53 ± 0.09	1.21 ± 0.06	0.37 ± 0.11	0.003 ± 0.001
Act + Ald + Cap	10.60 ± 1.00	1.24 ± 0.02	0.43 ± 0.02	0.11 ± 0.01	0.004 ± 0.00
Act + Ald + Ben	21.80 ± 0.58	5.04 ± 0.20	3.19 ± 0.37	1.73 ± 0.19	0.24 ± 0.02
Act + Ald + Ben	13.40 ± 0.35	1.87 ± 0.07	2.81 ± 0.26	0.47 ± 0.03	0.11 ± 0.05
Ald + Cap + Ben	10.53 ± 0.71	1.65 ± 0.27	0.87 ± 0.03	0.22 ± 0.04	0.004 ± 0.00
Act + Cap + Ben	12.80 ± 2.21	1.26 ± 0.06	1.08 ± 0.11	0.10 ± 0.00	0.005 ± 0.001
Control	18.87 ± 1.65	5.35 ± 0.45	3.67 ± 0.05	2.29 ± 0.24	1.32 ± 0.12

Act - Actellic; Ald - Aldrin; Cap - Captan; Ben - Benomyl;

Figures are of population expressed as $x10^5$ cells per seed

Figure 8: Survival of Rhizobium leguminosarum bv phaseoli on pesticide treated seed

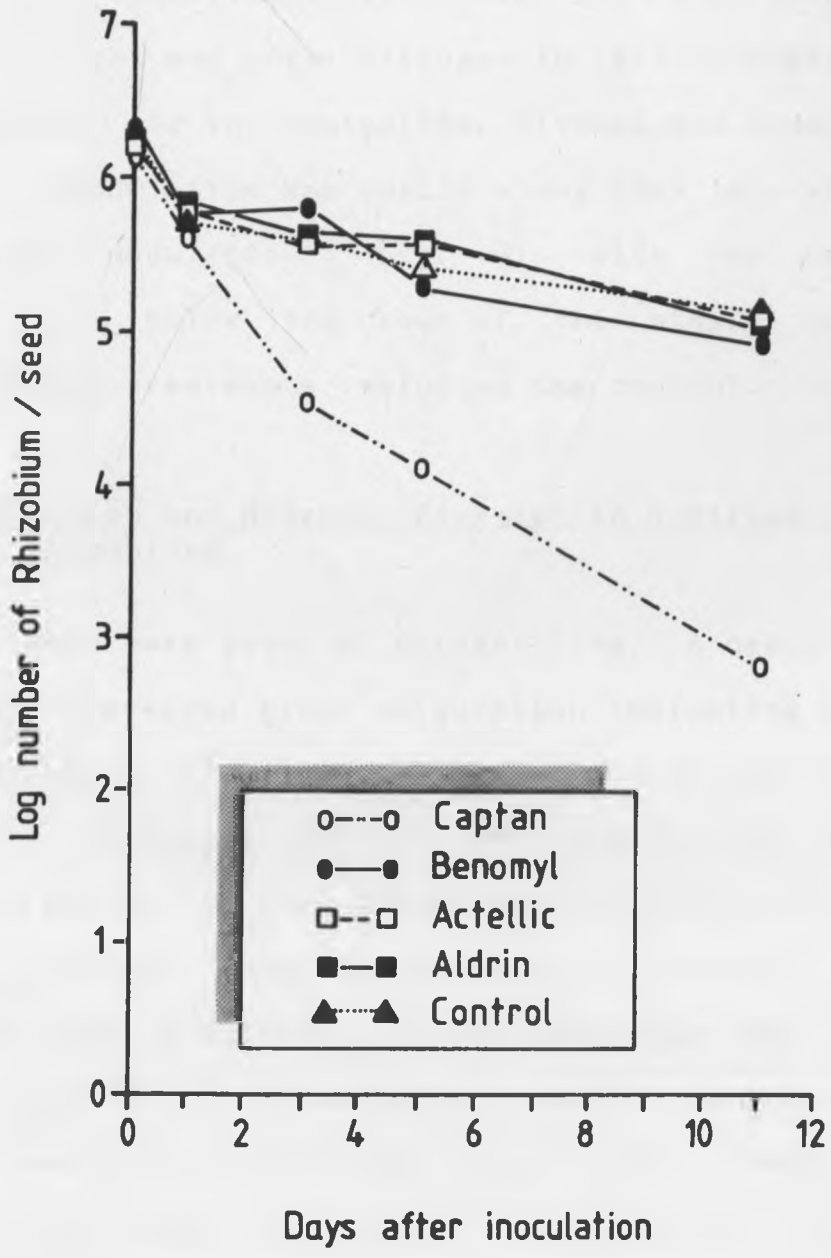


Fig. 8.

4.3 Effects of Pesticide on Nodulation, Plant Dry Weight and Total Nitrogen and Grain Yield

The pesticides, Actellic, Aldrin, Captan and Benomyl, did not cause significant differences in the nodulation, plant dry weight and total nitrogen in all situations of experimentation viz in Vermiculite, nitosol and under field conditions. Nodulation was mostly along the lateral roots (lateral root nodulation) (Fig. 9), with few cases of nodulation just below the crown of the stem. This was observed for all treatments including the control.

4.3.1 Nodulation and Nitrogen Fixation in Modified Leonard Jar Assemblies

The plants were green at harvest time. A cross-section of the nodules revealed brown colouration indicating that the nodules had been effective. Although nodulation was not significantly different between the treatments, it was greatest on average in the control (168.75 nodules per jar) with the treatment involving Actellic + Aldrin + Captan allowing for least nodulation (32.00 nodules per jar). (Table 7). This gave a wide range of 136.75 nodules. The nodulation was along the lateral roots of the plants. The mean nodule dry weight was highest in the control (382.5mg) at least in Actellic + Aldrin + Captan treatment. However, nodules formed in the treatment involving captan alone were heavier than the others, the mean weight was 320.00 mg for only 78.25 nodules. The mean shoot dry weight was highest

Figure 9a: Nodulation along the roots for treatment 15(Actellic + Aldrin + Benomyl + Captan)

Figure 9b: Nodulation along the roots for treatment 16 (Control)

for treatment involving Aldrin + Captan (1.77 gm) and least for treatment involving Actellic (0.91 gm). The mean total nitrogen content was highest in control and in treatment of Actellic + Benomyl (2.23 percent) and least in treatment involving Actellic + Aldrin and Captan (1.78 percent). Thus the nodulation, plant dry weight and total nitrogen may have been slightly influenced following the treatment of the seeds with the pesticides before inoculation although there were no significant differences of the treatments from the control.

Table 7: Influence of Seed Dressing Pesticides on Nodulation and Nitrogen Fixation in P. vulgaris L. (Leonard Jar Assemblies)

Treatment	Nodule Number	Nodule Dry Weight (Mg)	Shoot Dry Weight (Gm)	Total Nitrogen
Act	40.75	92.50	0.91	1.88
Ald	97.25	102.50	1.35	2.08
Cap	78.25	320.00	1.47	2.07
Ben	111.00	374.00	1.08	2.21
Act + Ald	88.00	187.50	1.32	1.87
Act + Cap	60.75	92.50	1.21	1.90
Act + Ben	132.25	202.50	1.49	2.23
Ald + Cap	193.50	317.30	1.77	2.13
Ald + Ben	76.75	95.00	1.28	1.86
Cap + Ben	46.75	145.00	1.19	1.82
Act + Ald + Cap	32.00	75.00	1.23	1.78
Act + Ald + Ben	56.00	122.50	1.09	1.91
Act + Cap + Ben	90.50	235.00	1.23	2.11
Ald + Cap + Ben	107.25	212.50	1.09	2.11
Act + Ald + Cap + Ben	92.00	190.00	1.38	2.02
Control	168.75	382.50	1.39	2.23

r = 0.774

Act - Actellic; Ald - Aldrin; Cap - Captan; Ben - Benomyl.

Each treatment was replicated four times (Figures are expressed per jar)

Figure 10a: Relationship between nodule dry matter weight and total nitrogen in Phaseolus vulgaris L. grown in modified Leonard jar assembly

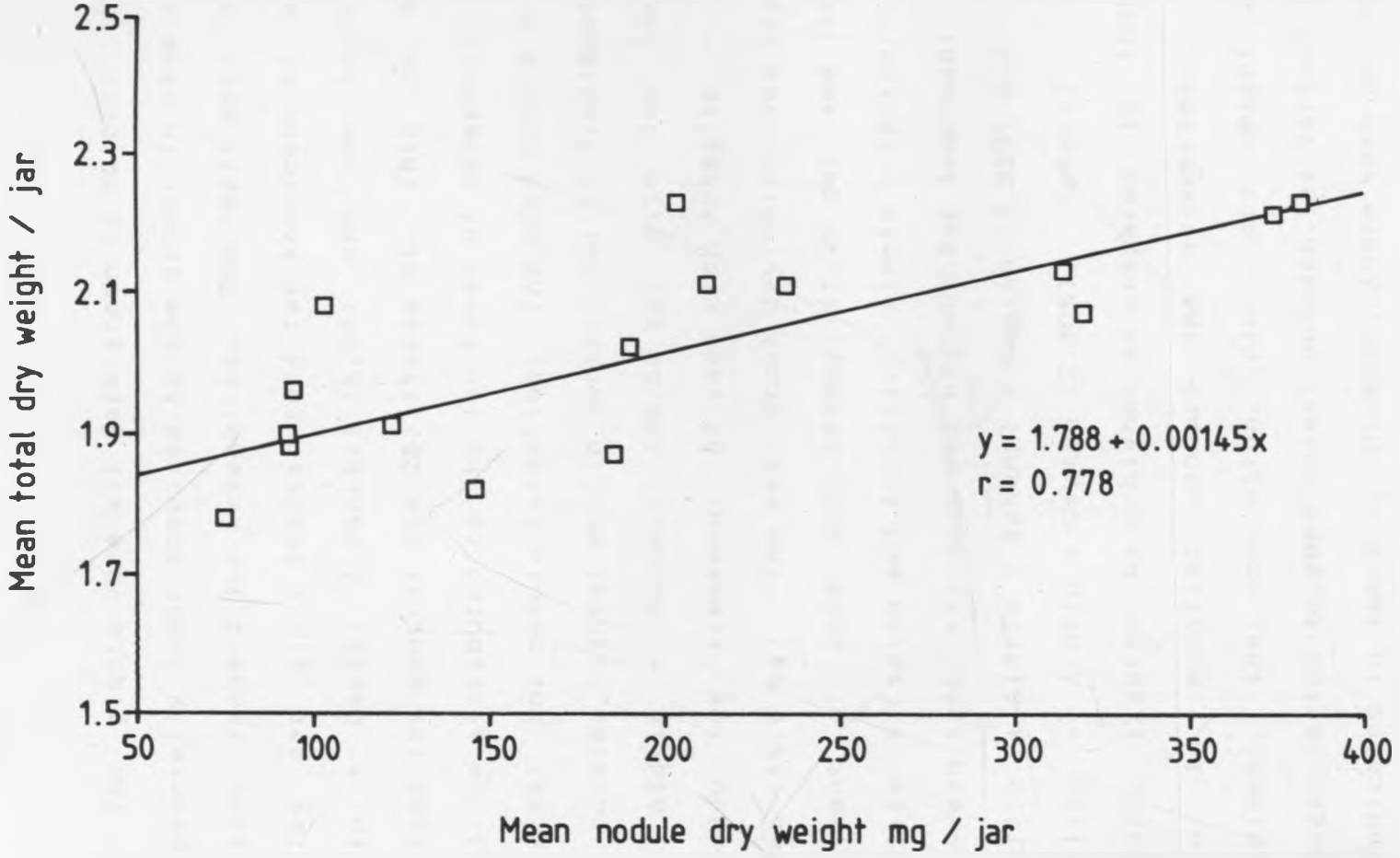


Fig. 10 [a]

4.3.2 Nodulation and Nitrogen Fixation in Soil in Plastic Pots

The plants were all dark green in colour. Nodulation was generally lower compared to the growth in vermiculite in modified leonard jar assemblies. The least mean number of nodules per plant was recorded for treatment of seed with Aldrin + Captan + Benomyl (5.95) and the highest being observed for control (14.25) (Table 8). This low nodulation could be attributed to the low level of phosphorus which is essential for nodule formation. The mean nodule dry weight was however highest not in control but in treatment of seed with Aldrin + Benomyl (46.50 mg) with the least being observed for treatment of seed with Actellic + Aldrin + Captan (15.0 mg). The mean shoot dry weight was highest for treatment of seed with benomyl (1.29 gm) and lowest for Actellic + Captan and Actellic + Aldrin + Captan (1.03 gm). The mean total nitrogen was highest for treatment involving Actellic + Aldrin + Benomyl + Captan (3.25%) and least for Actellic + Aldrin + Captan (2.80%). Generally the total nitrogen figures were higher as compared to those plants grown in modified leonard jar assemblies. In this experiment, the nodulation, plant dry weight and total nitrogen varied to some extent between the various treatments as indicated in table 8. However, there were no significant differences between the treatments.

Table 8: Influence of Seed Dressing Pesticides on Nodulation and Nitrogen Fixation in P. vulgaris L. (Potted Plants)

Treatment	Nodule Number	Nodule Dry Weight (Mg)	Shoot Dry Weight (Gm)	Total Nitrogen
Act	7.75	34.43	1.08	3.02
Ald	10.30	24.63	1.19	3.01
Cap	8.20	30.70	1.19	3.03
Ben	12.80	37.08	1.29	3.20
Act + Ald	9.75	18.45	1.09	2.89
Act + Cap	8.50	24.80	1.03	3.11
Act + Ben	8.40	34.20	1.28	3.30
Ald + Cap	9.95	26.80	1.19	3.05
Ald + Ben	8.80	46.50	1.26	3.18
Cap + Ben	8.15	23.58	1.17	2.93
Act + Ald + Cap	6.50	15.00	1.03	2.89
Act + Ald + Ben	9.10	20.55	1.11	2.80
Act + Cap + Ben	8.40	32.95	1.08	2.89
Ald + Cap + Ben	5.95	17.73	1.17	2.93
Act+Ald+Cap+Ben	11.95	31.65	1.15	3.25
Control	14.25	39.50	1.15	3.02
Coefficient of variation	18.67	1.47	12.69	10.35

Act - Actellic; Ald - Aldrin; Cap - Captan; Ben - Benomyl

Each treatment was replicated 4 times (Each figure is per plant

Figure 10b: Relationship between nodule dry weight and total nitrogen in Phaseolus vulgaris L. grown in soil in plastic pots

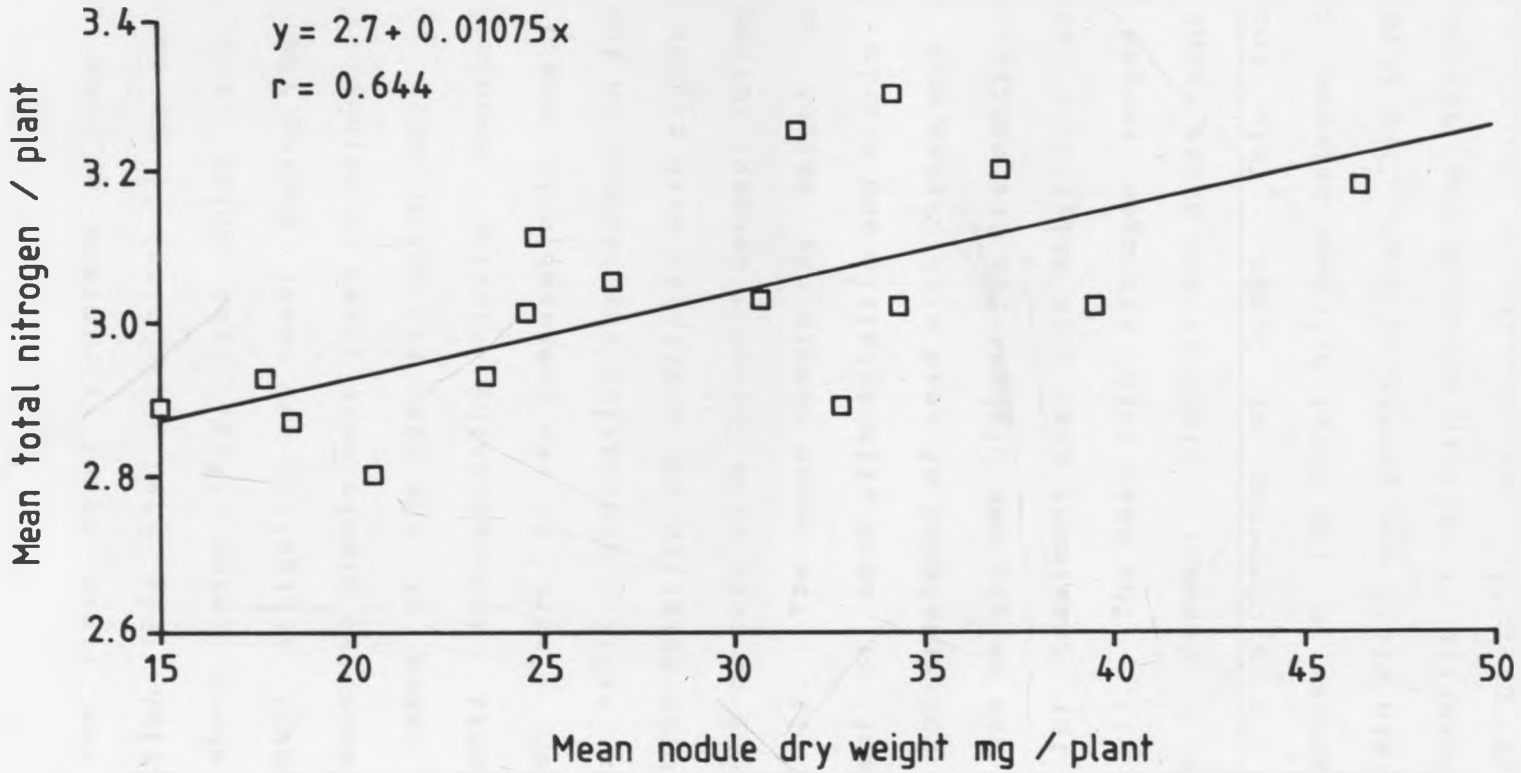


Fig. 10 [b].

4.3.3 Nodulation Nitrogen Fixation under Field Conditions

The field trial 1 (block I) was conducted under irrigation (Fig. 11a) while block II was carried out during the short rains (Fig. 11b) which were erratic hence supplement irrigation was used. During the course of the experiment the plants were green in colour. There were very minor cases of the bean fly which were not observed in treatments incorporating Aldrin. Nodulation and total nitrogen (Table 9) was comparable to that observed under section 4:3:2. Nodulation was highest in the treatment of seed with Actellic and Aldrin in both blocks and least for treatment of seed with Captan + Benomyl (block II) and cntrol (block I). The mean nodule dry weight was highest for treatment of seed with Actellic and Aldrin (block I) and least for treatment of seed with Captan and Benomyl. Mean shoot dry weight was highest for the control (block II) and least for treatment seed with Actellic + Aldrin + Captan (block I). The mean total nitrogen ranged between 2.60% (Captan + Benomyl - block I) and 3.14% (control block II) giving a difference of 0.46%. This indicated slight differences in the total nitrogen between the treatments. The grain yield was generally high. The treatment of seed with Actellic + Aldrin produced the highest grain yield (2476.67 kg/ha^{-1}), while treatment with Actellic + Aldrin + Captan produced the least (1717.67 kg/ha^{-1}).

Figure 11a: Field trial experiment under irrigation (August - October)

Figure 11b: Field trial experiment conducted during the short rains (November - January)

In general there were no significant differences between the treaments. In addition there was no interaction between the location (blocks) and the treatments for nodule number, nodule dry weight, shoot dry weight total nitrogen and grain yield.

The correlation coefficients between nodule dry weight and total nitrogen, for the glass house experiments, were 0.778 (Fig. 10a) and 0.644 (Fig. 10b) for growth in modified leonard jar assemblies and plastic pots respectively. This indicated a linear relationship between nodule dry weight and total nitrogen. Thus the results demonstrated the efficiency of the Rhizobium leguminosarum bv phaseoli following pesticide treatment in establishing an effective symbiotic relationship with P. vulgaris L.

However, under field conditions the correlation coefficient between nodule dry weight and total nitrogen wass 0.024 and 0.026 for blocks I and II respectively. Hence there was no linear relationship between nodule dry weight and total nitrogen.

Table 9: Influence of Seed Dressing Pesticides on Nodulation and Nitrogen Fixation in *P. vulgaris* L. (Field Plots)

Treatment	Nodule Number		Nodule Dry Weight(Mg)		Shoot Dry Weight(Gm)		Total Nitrogen		Grain Yield $KG\ HA^{-1}$	
	I	II	I	II	I	II	I	II	I	II
Act	8.80	7.95	19.7	36.10	5.96	5.87	2.97	3.04	2110.00	2022.02
Ald	13.55	12.60	28.2	34.75	6.58	7.05	2.78	3.03	2064.64	1970.02
Cap	16.40	14.35	31.0	40.13	6.98	6.06	2.81	2.87	2310.00	2083.36
Ben	11.20	14.85	28.4	38.45	5.83	6.46	3.84	2.80	2275.00	2166.23
Act + Ald	16.95	18.50	53.3	41.40	6.97	6.80	2.57	2.97	2476.67	2190.75
Act + Cap	7.95	10.95	33.6	23.30	7.29	6.08	2.96	3.08	2343.33	1921.78
Act + Ben	11.00	10.70	38.2	19.48	6.25	6.55	2.64	2.88	2093.34	2012.37
Ald + Cap	9.85	9.75	29.2	17.98	6.86	7.23	3.03	2.83	2153.34	2071.27
Ald + Ben	11.45	18.00	20.8	49.47	7.31	6.23	2.72	2.80	2232.50	2412.71
Cap + Ben	8.40	7.00	12.0	24.53	6.87	6.04	2.60	2.95	1803.34	2078.46
Act + Ald + Cap	8.85	15.25	20.4	50.55	5.71	7.56	2.65	2.92	2217.50	1717.67
Act + Ald + Ben	8.25	14.10	30.9	48.48	6.92	6.61	2.63	2.80	2202.50	2168.48
Act + Cap + Ben	14.65	14.60	49.2	22.10	6.62	6.93	2.81	2.80	2435.00	1720.79
Ald + Cap + Ben	13.75	15.70	29.2	51.18	6.46	6.75	2.81	3.09	2100.00	2233.79
Act + Ald + Cap + Ben	10.40	9.05	29.7	27.75	6.13	5.83	2.76	3.04	2110.03	2061.75
Control	7.40	14.05	20.8	33.40	5.86	8.142	2.68	3.14	2448.33	2058.12
Coefficient of variation	2.58		1.94		18.84		10.42		19.12	

Act - Actellic; Ald - Aldrin; Cap - Captan; Ben - Benomyl.
I and II - Blocks. Each treatment was replicated four times (Figures are for means only).

CHAPTER FIVE

DISCUSSION

The importance of pesticides and the Rhizobum-legume symbiotic association in modern agriculture has been stated . Compatibility of the Rhizobium and the pesticides can thus not be overlooked. The seed dressing pesticides captan, benomyl, aldrin and actellic are increasingly being used against Phaseolus vulgaris L. pathogens and pests. Their effects on Rhizobium leguminosarum biovar phaseoli. nodulation and nitrogen fixation in beans would, therefore, be of great consequence.

Growth of R. leguminosarum bv phaseoli in vitro was inhibited by relatively low concentrations of captan as compared to benomyl, aldrin and actellic. The inhibition is hardly surprising because Fisher (1976) had reported captan's bacteriocidal activity. The reduction of Rhizobium growth in agar with increasing captan concentration had also been reported (Diatloff, 1970; Fisher, 1976; Bandyopadhyay, 1986; Rennie, 1986; Poi and Goshi, 1986; Banerjee and Banerjee, 1987).

The survival of Rhizobium was adversely affected on seeds treated with Captan or any other pesticide containing captan with less than 4.0% surviving after 5 days. This confirmed early reports (Graham et al., 1980; Rennie, 1986)

of reduced survival of Rhizobium on captan treated seed. This severe reduction caused by captan reflects the drastic decrease in growth on agar, indicating possibility of the action being bacteriocidal rather than bacteriostatic.

The other pesticides tested did not have an appreciable effect on the growth of the Rhizobium on agar, with inhibition of more 60% growth at 500 parts per million compared to less than 30% growth of captan. Incorporation of benomyl in agar led to decreasing radial growth of the Rhizobium with increasing doses as had been reported by Van Fansen (1974) and Fisher (1976). Growth at 2000 ppm validated reports by Fisher (1976) that benomyl had little effect at high concentrations.

Survival of the Rhizobium on benomyl treated seed was comparable to the control (pesticide free). This result was as reported by Siegel (1975) that benomyl lacked in bacterial toxicity in soil. However, it was in contrast to reports by Mikawi and Ghafar (1970) as cited by Anderson (1978) that benomyl was bacteriocidal towards a number of Rhizobium spp. The lack of toxicity to the Rhizobium on benomyl treated seed was well reflected by the growth on agar.

The two insecticides, aldrin and actellic did not appreciably affect the survival of Rhizobium on seed thus confirming early reports that insecticides have no effect on

bacteria in general at normal doses (Audus, 1970; Mendoza, 1973 as cited by Simon-Sylvestre and Fournier, 1979). Survival on pesticide treated seed was well reflected by the non toxic effect observed on agar as well reported for aldrin by Kapusta and Rouwenborst (1973).

All the tested pesticides i.e. captan, benomyl, aldrin and actellic singly and in their combinations did not greatly influence the process of nodulation and nitrogen fixation in Phaseolus vulgaris L. This had been established for two other legumes namely Clover (Fisher, 1976) and soybean (Rennie and Dubetz, 1983). It should be noted that the seeds were planted immediately after pesticide treatment and inoculation in very moist medium (Vermiculite and soil). Milthorpe (1945) was cited by Diatloff (1970); Ruhloff and Burton (1951) have suggested the importance of soil moisture which could mitigate the potentially toxic effects of pesticides by dilution. The pesticides lack in movement from introduction sites into the soil (Alexander, 1985). Bacterial movement has been reportedto be enhanced by higher moisture levels (Alexander, 1985). This is supported by observation that in most cases there was lateral nodulation. Similar observation was made by Diatloff (1970). Birchfield (1959) as cited by McEwen and Stephenson (1979); Lukens (1969) reported that captan is rapidly broken down in moist soil to non toxic, compounds with a half life of 3-4 days and that decomposition in moist soil is more rapid than in dry soil.

Thus captan which had proved a potent inhibitor of the Rhizobium on agar and on seed did not affect nodulation and nitrogen fixation at the manufacturers recommended rate of application.

Lateral root nodulation was observed for all treatments including the control. This was contrary to the reported inhibitory effects of captan (Rennie, 1986) to nodulation. The inhibition effects of captan could be attributed to, intimate seed application of pesticide and inoculant; higher doses of pesticide; absence of moisture at planting time and a long time lapse between seed treatment and placement into the rooting medium. Ruhloff and Burton (1951); Graham (1980) reported that immediate planting of pesticide treated seeds led to nodulation comparable to that of pesticide free seeds.

Benomyl did not influence nodulation and nitrogen fixation thus corroborating early reports by Fisher (1976) and Leite (1982) working with clovers and lentils respectively, that symbiotic nitrogen fixation was not affected.

The two insecticides, aldrin and actellic, as with benomyl at the manufacturers recommended rates did not influence the survival of Rhizobium on seed, nodulation and nitrogen fixation. Damage to inoculants by aldrin was reported by Brantwaite et al (1958). That this was not observed could possibly be attributed to the varied response

of Rhizobium species to high concentrations of aldrin as found by Poi and Gosh (1986). In addition the level used was may be low enough not cause detrimental effects.

None of the pesticides used in this study significantly influenced the bean seed yield. This was expected since the pesticides had not significantly affected the nodulation shoot dry weight and the total nitrogen.

CHAPTER SIX

CONCLUSION

Only captan proved toxic to the Rhizobium at low concentrations unlike the others which allowed for growth even at 2000 ppm in pure culture. Captan was also more antagonistic to the Rhizobium on seed leading to a faster decline in population. The use of captan as a seed dressing pesticide has rapidly increased in recent years. This then calls for screening and selection of Rhizobium for captan tolerance. However, such strains with captan tolerance should have nitrogen fixing abilities similar to those of effective strains in the absence of captan.

The pesticides, captan, benomyl, aldrin and actellic did not significantly influence the process of nodulation, shoot dry weight and total nitrogen following the sowing of the seeds immediately after treatment into moist environment. Thus for captan which had significantly affected survival of the Rhizobium on seed, toxic effects must have been mitigated in the rooting medium. Benomyl, aldrin and actellic allowed for Rhizobium growth at high concentrations. In addition they did not significantly influence the survival of Rhizobium on seed as compared to control. This would call for investigations into the possibilities of these pesticides being broken down or utilised by the Rhizobium. There is also need to determine the effect of the Rhizobium on the

pesticides tenacity in controlling the pests and diseases. Additional investigations are required to determine if nitrogen fixation can be enhanced by use of the pesticides. A study into all these would then complete the compatibility question between the Rhizobium and pesticides.

The study results indicate that when seeds are treated with pesticides inoculated and immediately planted in moist soil, the number of Rhizobia able to survive may be sufficient to bring about effective symbiotic nitrogen fixation.

CHAPTER SEVEN

LITERATURE CITED

Agrios, G.N. (1982): Plant Pathology 2nd Edition. Academic Press. New York. San Francisco. London 699 PP.

Alexander, M. (1985): Enhancing Nitrogen Fixation by Use of Pesticides. A Review. Advances in Agronomy 38: 267 - 282

Anderson, J.R. (1978): Pesticides Effects on Non Target Soil Microorganisms: In "Pesticide Microbiology". Eds. Hill, I.R. and Wright, S.J.L. Academic Press. New York. PP. 313 - 533.

Anon. (1985): "Fertilizer Year Book". Vol. 35. Statistics Series No. 71 - P. 76. (F.A.O)

Anon. (1986): Environment and Energy Paper 6. (F.A.O)

Anyango, B. (1984): Assessment of Filtermud as a Carrier for Legume Seed Inoculants. M.Sc. Thesis. University of Nairobi.

App, A. and A. Eagleshan (1982): Biological Nitrogen Fixation: Problems and Potential. In "Biological Nitrogen Fixation - Technology for Tropical Agriculture". Eds. Graham, P.H. and Harris.

Bandyopadhyay, S. (1986): Induction of Fungicide Resistance to Rhizobia. Environmental Ecology 4: 730 - 733.

Bandyopadhyay, S. (1986): Growth and in vitro Sensitivity to Captan of Different Cowpea Rhizobium Strains. Environmental Ecology 4: 639 - 641.

Banerjee, A. and A.K. Banerjee (1987): Influence of Captan on Some Micro-organisms and Microbial Process related to the Nitrogen Cycle. Plant and Soil. 102: 239 - 246.

Beunard, P. and R. Michellon (1986): Effects of Bean Inoculation with Two Rhizobium Strains. Bacteriological Abstracts Vol. 22. No. 6: 5762.

Bezbarua, B. and G. Barua (1987): Dinitrofication and Nitrogen Fixation as Influenced by Pesticide Presence in Live Soils and Pure Cultures from Acid Tea Soils. Soils and Fertilizers Abstracts Vol: 50. No. 7 - 3926.

Brantwaite, B.M.; J.E. Annette and F.G. Swain (1958): Effects of Insecticides on Sod Sown Subclover. Journal of the Australian Institute of Agricultural Science. 24: 155.

Chamber, M.A. and F.J. Monthers (1982): Effects of Some Seed Disinfectants and Methods of Rhizobia Inoculation on Soybean (Glycine max.). Plant and Soil. 66: 353 - 360.

Clemons, G.P. and H.D. Sisler (1969): Formation of a Fungitoxic Derivative of Benlate. Phytopathology 59: 705 - 706.

Cremlyn, R. (1979): Pesticides: Preparation and Mode of Action. John Wiley and Sons. Chichester.

Curley, R.L. and J.C. Burton (1975): Compatibility of Rhizobium japonicum with Chemical Seed Protectants. Agronomy Journal. 67 : 807 - 808.

Date, R.A. (1970): Microbiological Problems in the Inoculation and Nodulation of Legumes. Plant and Soil 32: 703 - 725.

Diatloff, A. (1970): Effects of Some Pesticides on Root Nodule Bacteria and Subsequent Nodulation. Australian Journal of Experimental Agriculture and Animal Husbandry 10: 562 - 566.

Dowson, R.C. (1970): Potential for Increasing Protein Production by Legume Inoculation. Plant and Soil 32: 655 - 673.

Duczek, L.J. and J.A. Buchan (1981): The Effect of Captan Seed Treatment on Emergence, Nodulation, Seed Yield and Seed Protein Content of Lentils. Canadian Journal of Plant Science. 61 : 727 - 730.

Fisher, D.J. (1976): Effects of Some Fungicides on Rhizobium trifolii and its Symbiotic Relationships with White Clover. Pesticide Science. 7 : 10 - 18.

Fisher, D.J.; J.A. Pickard and C.M. McKenzie (1979): Uptake of the Systematic Fungicide Triadimefon by Clover and its Effect on Symbiotic Nitrogen Fixation. Pesticide Science. 10: 75 - 82.

Graham, P.H.; G. Ocampo; L.D. Ruiz and A. Duque (1980): Survival of Rhizobium phaseoli in Contact with Chemical Seed Protectants. Agronomy Journal. 72: 625 - 627.

Graham, P.H. (1981): Some Problems of Nodulation and Symbiotic Nitrogen Fixation in Phaeolus vulgaris L. Field Crops Research 4 : 93 - 112.

Kapusta, G. and D.L. Rowenhorst (1973): Interaction of Selected Pesticides and Rhizobium japonicum. Agronomy Journal. 65: 112 - 115.

Kataria, H.R.; J.S. Yadar; F.C. Gorg and R. Grover (1985): Inactivation of Seed Treatment Fungicides by Rhizobium. Pesticide Science. 16: 337 - 340.

Kernkamp, M.F. (1948): Chemical Treatment of Soybean Seed in Relation to Nodulation by Nodule Bacteria. Phytopathology. 38: 955 - 959.

Keya, S.O. (1970): Factors Affecting Rhizobium - Legume Specificity in Rhizobium trifoli. M.Sc. Thesis. Cornell University.

Keya, S.O. (1974): The Effect of Parasitic Bdellovibrio and Predatory Protozoa on Survival of Cowpea Rhizobium in Culture and in Laboratory Soil. Ph.D. Thesis. Cornell University

Keya, S.O.; H. Ssali and V.R. Balasundaram (1981): Potential and Constraints for Inoculation of Grain Legumes in Kenya. In "Current Perspectives in Nitrogen Fixation". Proceedings of the Fourth International Symposium in Nitrogen Fixation. Eds. A.H. Gibson and W.E. Newton. PP. 52. Australian Academy of Science. Canbera.

Keya, S.O. (1983): The Role of Biological Nitrogen Fixation in Crop Production. In FAO (1983). "More Food From Better Technology". Eds. Homes J.C. and Tahir, W.W. 418 - 426.

Keya, S.O. (1985): State of Art on Biological Nitrogen Fixation in Africa, in "Biological Nitrogen Fixation, in Africa". Proceedings of the First Conference of the African Association for Biological Nitrogen Fixation (AABNF) Ed. Ssali, H. and Keya, S.O.

Leite, L.C. (1982): Effects of Systematic Fungicides on Nodulation and Nitrogen Fixation in Soybean (Glycine max. (L) Merrill). Soybean Abstracts Vol. 5 No. 7. 1342.

Lennox, L.B. and M. Alexander (1981): Fungicide Enhancement of Nitrogen Fixation and Colonization of Phaseolus vulgaris by Rhizobium phaseoli. Applied and Environmental Microbiology. 41: 404 - 411.

Lukens, R.J. (1969): Heterocyclic Nitrogen Compounds. In "Fungicides on Advanced Treatise". Vol. II 395 - 445. Eds. Torgeson, D.C. Academic Press. New York. London.

Martin, H. and D. Woodcock, (1983): The Scientific Principles of Crop Protection. Edward Arnold. 1

McEwen, F.L. and G.R. Stephenson (1979): The Use and Significance of Pesticides in the Environment. John Wiley and Sons New York, Chichester, Brisbane. Toronto.

Odeyemi, O. and M. Alexander (1977): Use of Fungicide Resistant Rhizobia for Legume Inoculation. Soil Biol. 9 : 247 - 251.

Parker, C.A. (1985): Nitrogen Fixation for Developing Countries with Special Reference to Africa. In "Biological Nitrogen Fixation in Africa". Proceedings of the First Conference of the African Association for Biological Nitrogen Fixation. eds. Ssali, H. and S.O. Keya.

Pelmus, A. (1986): Aspects of the Compatibility of Some Fungicides Used in Soybean Seed Treatments and Bacterial Inoculation. Soils and Fertilizer Abstracts Vol. 49 No. 10 - 9568.

Pareek, R.P. and A.C. Gaur (1970): Effect of DDT on Symbiosis of Rhizobium Spp. with Phaseolus aureus. Plant and Soil 33: 297 - 304.

Poi, S.C. and G. Gosh (1986): Effects of Some Fungicides and Insecticides on Rhizobium and Azotobacter. Environmental Ecology 4: 503 - 506.

Postgate, J.R. (1978): Biological Nitrogen Fixation. In "Companion to Microbiology, Selected Topics for Further Study" PP 343 - 361. Longmans, London and New York.

Ramirez, C. and M. Alexander (1980): Evidence Suggesting Protozoan Predation on Rhizobium Associated with Germinated Seeds and in the Rhizosphere of Beans (Phaseolus vulgaris L.). Applied and Environmental Microbiology. 40: 492 - 499.

Rennie, R.J. (1986): Selection for Captan Tolerance in the Rhizobium phaseoli - Phaseoli vulgaris L. N_2-Fixing Symbiosis. Canadian Journal of Soil Science. 66: 143 - 150.

Rennie, R.J. and S. Dubetz (1983): Effect of Fungicides and Herbicides on Nodulation and N_2 Fixation in Soybean Fields Lacking Indigenous Rhizobium japonisum. Agronomy Journal 76; 451 - 454.

Reddy, D.D.R.; J.V. Rao and D.K. Kumar (1975): Effect of Selected Non Volatile Nematicides and Benomyl on Nodulation, Root Knot Nematode Control and Yield of Soybean. Plant Disease Reporter 59: 592 - 595.

Ruhloff, M. and J.C. Burton (1951): Compatibility of Rhizobia with Seed Protectants. Soil Science 72: 283 - 290.

Selim, K.G.; A.Z.S. Mahmoud; and M.T. Mokedi (1970): Effects of Dieldrin and Lindane on the Growth and Nodulation of Vicia faba (broad beans). Plant and Soil

Siegel, M.R. (1975): Benomyl - Soil Microbial Interactions. Phytopathology. 65: 219 - 220.

Singh, S.N.; S.K. Srivastava (1984): Interaction of Various Fungicides and Rhizobium Culture in Relation to Nodulation and Yield of Soybean. Agricultural Science Digest, India. 4: 191 - 192

Simon-Sylvestre, G. and J.C. Fournier (1979): Effects of Pesticides on the Soil Microflora. Advances in Agronomy: 31:- 1 - 92.

Smit, J.J.; A.O. Okongo; and J. Floor (1983). Dry Bean Agronomy. Kenya Farmer, August, 18 - 21.

Somasegeran, and H. Hoben (1985): Methods in Legume-Rhizobium Technology. Niftal. United States Agency for International Development. Contract No. DAN-0613-C-00-2064-00.

Stoetzer, H.A.I. and M.E. Omunyin (1983): Controlling Bean Pests and Diseases. Kenya Farmer. August 22 - 24.

Tesfai, K. and M.A.B. Mallik (1986): Effects of Fungicide Application on Soybean - Rhizobia Symbiosis and Isolation of Fungicide Resistant Strains of R. japonicum Environment Contamination and Toxicology 36: 819 - 826.

Tu, C.M. (1982): Influence of Pesticide Seed Treatment on Rhizobium japonicum and Symbiotically Grown Soybean in Soil Under Laboratory Conditions. Soybean Abstract Vol. 5: No. 7 - 1343.

Urza, H.; J. Romerot and V.M. Ruiz (1986): Effects of P.P., DDT on Nitrogen Fixation of White Clover in Volcanic Soils of Chile. MIRCEN Journal 2: 365 - 372.

Van Fansen, H.G. (1974): Effect of Fungicide Benomyl on Some Metabolic Processes and on Numbers of Bacteria and Actinomycetes in the Soil. Soil Biol. Biochemistry 6: 131 - 133.

Vincent, J.M. (1970): International Biological Programme (IBP) Manual - Root Nodule Bacteria. Blackwell Scientific Publications. Oxford and Edinburgh.

Wainwright, M. and G.J.F. Pugh (1975): Effects of Fungicides on the Numbers of Micro-organisms and Frequency of Cullulytic Fungi in Soils. Plant and Soil. 43: 561 - 572.17