ANTIBIOTIC ACTIVITY OF SOME PLANT EXTRACTS ON *PSEUDOMONAS SYRINGAE* PV. *PHASEOLICOLA* AND *XANTHOMONAS CAMPESTRIS* PV. *PHASEOLI.*

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as University supervisors.

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DEDICATION

To my parents,

Mr. Stephen Ngaruiya

and

Ruth Watiri

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a.i. = Active ingradient

NA = Nutrient agar

ABSTRACT

Several plant extracts were screened for antibacterial activity against *Pseudomonas syringae* pv. *phaseolicola* and *Xanthomonas campestris* pv. *phaseoli in vitro*. Extracts from *Eucalyptus citriodora* Hook, *Nothoscordum inodorum* L., *Cupressus lucistanica* Mill, *Tagetes minuta* L. and *Santolina chamaecyparissus* L. were found to be active on the two phytobacterial pathogens. The volatile oil of *E.citriodora* produced a significantly (P = 0.05) wider zones of growth inhibition on *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli* when compared to the other plant extracts. The growth inhibition zones produced by the volatile oil of *E. citriodora* measured 32.83 mm and 24.50 mm on *X. campestris* pv. *phaseoli* and *P. syringae* pv. *phaseolicola* respectively. There was an interaction between the test pathogens and the extract type. This was demonstrated by the significantly (P = 0.05) high sensitivity of *X. campestris* pv. *phaseoli* to the different plant extracts when compared to *P. syringae* pv. *phaseolicola*.

Further investigations were carried out to determine the minimum inhibitory concentration of the volatile oils from *E. citriodora* and *T. minuta* on *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli*. *T. minuta* extract caused total growth inhibition on the two phytobacterial pathogens at 0.20% concentration while *E. citriodora* extract caused complete growth inhibition on *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli* at

0.78% and 0.39% concentrations respectively. Fractionation of the volatile oils from *E. citriodora, T. minuta, C. lucistanica* and *S. chamaecyparissus* was carried out and the resultant fractions tested for antibacterial activity against *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli*. The crude extracts of *E. citriodora* and *T. minuta* had a significantly (P = 0.05) higher activity than the hexane and ether fractions on *P. syringae* pv. *phaseolicola*. The test for minimum inhibitory concentration of the ether fraction of *E. citriodora* indicated that it lies between 1.56% and 3.13% concentrations. The ether fraction of *T. minuta* did not produce a zone of growth inhibition when diluted beyond 6.25% concentration. Generally, the activity of ether fraction of *E. citriodora* with respect to its activity against *X. campestris* pv. *phaseoli* and *P. syringae* pv. *phaseolicola* was significantly (P = 0.05) higher than that of *T. minuta*. A test was carried out to establish the stability of the active compounds in *T. minuta* and *E. citriodora* crude extracts when subjected to various temperatures. All the temperature regimes viz., $0^{\circ}C$ $10^{\circ}C$, $20^{\circ}C$ $30^{\circ}C$ $40^{\circ}C$ $50^{\circ}C$ $60^{\circ}C$ $70^{\circ}C$ $80^{\circ}C$ $90^{\circ}C$ $100^{\circ}C$ and $121^{\circ}C$ tested did not affect the activity of the two plant extracts under study with respect to their activities against *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli*. The active fractions were not denatured by temperatures as high as $121^{\circ}C$. For instance the activity of *E. citriodora* extract subjected to $121^{\circ}C$ on *P. syringae*

pv. *phaseolicola* was not significantly (P=0.05) lower than that subjected to $0^{o}C$

In vivo test for antibacterial activity on *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli* indicated that streptomycin sulphate and copper oxychloride reduced halo blight infection significantly (P = 0.05) when compared to bean seedlings receiving foliar spray of *E. citriodora* or *T. minuta* extract under green house conditions. No significant (P = 0.05) difference was observed in halo blight and common blight infection among bean plants sprayed with streptomycin sulphate or copper oxychloride and those seed dressed with 0.59% concentration of crude extract of *E. citriodora* or 0.15% concentration of crude extract of *T. minuta.*

Phytotoxicity tests for different concentrations of ether fractions and crude extracts of *T. minuta* and *E. citriodora* were conducted using fourteen day old bean plants of cv. Rosecoco-GLP-2 in the greenhouse. Plants which were sprayed with 6.25%, 9.38% and 12.5% concentrations of ether fraction of *T. minuta* extract showed defoliation two weeks after spraying. Bean plants sprayed with 3.13% concentration of ether fraction of *E. citriodora* and 0.20% concentration crude extract of *T. minuta* showed some brown spots on the leaves. No signs of phytotoxicity were observed on plants sprayed with 0.78%, 0.59% and 0.39% concentrations of crude extract of *E. citriodora.*

Field trials for the control of halo blight and common blight of beans was carried out using 0.59% concentration crude extract and 1.17% concentration ether fraction of *E. citriodora*. A significantly (P = 0.01) higher halo blight infection was observed among treatments which had artificially inoculated seeds when compared to those obtained from small scale farmers receiving similar treatments. Bean plants sprayed twice with 0.59% concentration of crude extract of *E. citriodora* showed a significantly (P=0.05)lower halo blight severity when compared to the untreated control. Dressing bean seeds and later spraying bean plants with *E. citriodora* volatile oil at seedling stage and at pod filling stage reduced halo blight severity when compared to bean plants which were treated (dressed or sprayed) with the ether fraction from the same plant extract. Halo blight incidence in dressed inoculated seeds and foliar sprayed seedlings was not significantly (P = 0.05) different from that of the untreated control. Copper oxychloride and streptomycin sulphate reduced infection by common blight significantly (P = 0.05) compared to *E. citriodora* extracts. The ether fraction and crude extracts from *E. citriodora* did not significantly (P0.05) lower common blight infection (applied as seed treatment or foliar spray) when compared to the untreated control. Yield assessment indicated that bean seeds which were dressed with the volatile oil from *E. citriodora* produced a high yield. For instance, there was a significantly (P=0.05) higher yield/hectare from plots

planted with dressed inoculated seeds when compared to those from plots planted with similar seeds which were foliar sprayed only.

Although the activity of these plant extracts was lower than that of streptomycin sulphate, they can be exploited as natural bactericides for controlling halo and common bacterial blights of beans.

INTRODUCTION

1.1 Bean production in Kenya.

Phaseolus vulgaris L. (common bean) is the most important pulse crop in Kenya (Acland, 1971). It is grown over an area of approximately 300,000 hectares mostly in mixed stands with other crops such as maize, sorghum, millet, potatoes, cassava and cotton. For the 1974/75 cropping season, 93.7% of the hectarage under beans in Kenya was in mixed stands (Anon, 1979). The crop is best suited to the medium altitude areas from 900-2000m, although it is often found growing at altitudes as high as 2700m in some parts of Kenya (Acland, 1971). The Central and Eastern provinces are currently the greatest bean producers in Kenya (Anon., 1987).

The main bean cultivars grown in Kenya include cv Rosecoco-GLP-2 which is well suited to the growing conditions in the wet parts of Kenya, cv. Canadian Wonder-GLP-24 for the high to medium rainfall areas; cv. Mwezi Moja-GLP-1004 for the dry, marginal areas; cv. Mwitemania (GLP-X.92) with wide adaptation to production environment; cv. Zebra (GLP-806) for semi-arid areas and cv. Haricot (GLP-585). Other types include cvs. Rosecoco (GLP-288 and GLP-77) and cv. Mwezi Moja (GLP-X.1127A). Yields vary greatly according to climate, soil conditions, the level of crop management, the purity of seeds used and the efficiency of pest and disease control. In 1990, yields per hectare for Africa was 672 kg/ha (Anon, 1991). In

East Africa, the average yield varies from 225-670 kg/ha although with better crop management and more efficient crop protection, a yield of 1120 kg/ha is attainable. Cv. `Canadian Wonder' is reported to have given a yield of 3140 kg/ha experimentally (Kay, 1979). In Kenya bean yields are generally low with a national average below 500 kg/ha (Mukunya and Keya, 1975).

1.2 Importance of beans:

Legumes such as beans are important in Kenyan agriculture for the supply of relatively inexpensive plant protein, for both rural and urban population (Smartt, 1976). They are used together with other crops such as maize, benanas, potatoes and other vegetables. Fresh leaves from young plants are sometimes boiled and eaten as vegetable with ugali. Analysis of dry mature beans gives 22% protein, 1.6% fat, 57.8% carbohydrates 4% fibre and other components e.g. 137 mg/100 mg calcium (Kay, 1979). Unlike animal proteins beans are safe to use since they do not contain cholesteral.

The whole plant while fresh may be ploughed under at around flowering stage and used as green manure or harvested and fed to livestock (Mukunya and Keya, 1975)but these practices are not common in Kenya. They also harbour rhizobium bacteria which fix free nitrogen from the atmosphere thus maintaining soil fertility.

1.3 Bean production problems

The major problems in bean production include:- uneven rainfall distribution, poor cultural practices and destruction by pests and diseases. Generally diseases are more important than insect damage (Anon, 1985). Beans are attacked by fungi, bacteria and viruses. Disease pressure is aggrevated by farmers planting contaminated seeds from the previous harvest. Bacterial diseases of beans are of considerable importance in many bean producing areas of the world (Mukunya and Keya, 1975; Smartt, 1976) and strenous efforts are meant to prevent outbreaks of these diseases. Three distinct bacterial pathogens have been isolated from beans but only two viz halo blight and common blight pathogens cause such concern. Losses as a result of halo blight (*Pseudomonas syringae* pv. *phaseolicola*) and common blight (*Xanthomonas campestris* pv. *phaseoli* have been reported to be as high as 43% and 40-60% respectively in U.S.A. (Smartt, 1976; Sherf and Macnab, 1986;). Bacterial diseases of beans have not been well studied in Kenya but have been reported to cause yield losses (Nattrass, 1961, Acland, 1971; Mukunya and Keya, 1975). Halo blight is common in the cooler and wetter areas while common blight is more important in warm conditions (Acland, 1971).

Several chemicals have been used to control bacterial blights (Mukunya and Keya, 1975; Weller and Saettler, 1976, Taylor and Dudley, 1977) but they have limitations.

1.4 Use of plant extracts

The systematic study of higher plants for the purpose of detecting antibiotics in their tissues is comparatively recent (Skinner, 1955). However, these investigations which have been inspired largely by the desire to find new substances toxic to pathogenic microorganisms have followed naturally from the old age practice of using plants and their extracts as drugs for the cure of human diseases. They have been used traditionally and are still being used here in Kenya medicinally for the control of certain human diseases.

Among the reasons which have made scientists turn to antibiotics of botanical origin is the hope of discovering a compound that will exhibit a minimum toxicity to plants, maximum toxicity against parasites, penetration of plant cells and high rate of accumulation thus becoming detrimental to the parasite (Ark and Alcorn, 1956).

High costs, environmental pollution aspects and sometimes inavailability of imported pesticides are major problems to contend with. Botanical pesticides would play a major role in integrated pest management programmes in developing countries as they are cheap and based on locally available resources. There is a reservoir of yet untapped bioactive natural products in tropical flora

which may be used as medicine and pesticides or which may act as useful models for medicinal and pesticidal compounds.

There is increasing accumulation of information on antimicrobial activity of plant extracts. However, most of the information is on antifungal activity and very little information is available on antibacterial activity, yet bacteria cause important crop diseases in the tropics. This prompted us to screen some plants for antibacterial activity. The local plants investigated in this work include *Tagetes minuta* L., *Aleurites molluccana* L. (Willd), *Eucalyptus citriodora* Hook, *Nothoscordum inodorum* L., *Cupressus lucistanica* Mill, *Schinus molle* L., *Santolina chamaecyparissus* L. and *Chrysanthemum cinerariaefolium* Trev. (Vis). The study was carried out with the following objectives:-

- To explore our local plant resources to establish renewable bactericides against *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli.*
- To separate hydrocarbons and oxygenated hydrocarbons from the active extracts and test for their antibacterial activity.
- To establish the minimum inhibitory concentration of the most active plant extracts *in vitro*,
- To determine the effect of temperature on the most promising plant extracts.
- To test the efficacy of the most promising plant extracts under greenhouse and field conditions.

2. LITERATURE REVIEW

2.1 Halo blight of beans

2.1.1 Geographical distribution and importance of the disease

Halo blight disease of beans and it's causal agent *Pseudomonas syringae* pv. *phaseolicola* was first recognized by Burkholder in 1926 in New York on dry bean (Sherf and Macnab, 1986). It has a wide distribution and is a serious pathogen in areas of cool moderate temperatures (Acland, 1971). In Kenya, the disease is widely distributed and occurs more frequently in cool, high rainfall areas (Kinyua and Mukunya, 1981). Origa (1991) found that out of 30 bean samples collected from Kisii, Nyeri and Meru districts, 12 bean samples had the pathogen on the surface only and 12 bean samples had the pathogen both on the surface and inside the seeds.

2.1.2 Symptomatology

Symptoms vary with the degree of infection, age of plant when infected, tissue involved and environmental conditions prevailing (Sherf and Macnab, 1986). On the leaves, at first, watersoaked spots resembling pin-pricks appear on the underside of the leaves 3-5 days after inoculation (Sherf and Macnab, 1986). These spots turn brown in a few days and the surrounding tissue gradually become yellow-green often ½ inch across. Leaves with systemic infection do not have halos but are characterized by yellow interveinal tissue interspersed between dark green tissue (Zaumayer, 1932, Sherf and Macnab,

1986). Severe infection can lead to defoliation , wilting and death of plants.

On the pods, symptoms first appear as small water-soaked pin-pricks. The spots gradually enlarge to form dark sunken spots of various sizes (Vock, 1978). Halos dc not develop around the pod lesions (Sherf and Macnab, 1986). Developing seed may be shrivelled or discoloured.

On the stem, if the infection is severe, dark lesions develop which produce a light cream coloured exudate which when dry forms shiny crust (Vock, 1978).

2.1.3 Host range

The bacterium has a wide host range which includes:- *P. vulgaris* (common bean), *Vigna sinensis*, *Vicia faba* (broad bean), *Glycine max* (soybean), *Vigna sesquipedalis* (asparagus bean), *Phaseolus lunatus, Phaseolus radiatus, Phaseolus coccineus, Pisum sativum* and *Trifolium pratense* (red clover). It also attacks *Dolichos lablab* and *G. javanica* (Mukunya and Keya, 1975; Sherf and Macnab, 1986).

1.14 Etiology

1.14.1 Nomeclature

Halo blight is caused by a bacterium known as *Pseudomonas syringae* pv. *phaseolicola* (Burk, 1926) Young *et al.*, 1978. Other synonyms include:- *Phytomonas medicarginis* var. *phaseolicola* (Burk), *Bacterium puerariae* Hedges, *Bacterium medicarginis* var. *phaseolicola* (Burk) Link and Hull, *Pseudomonas medicarginis* var. *phaseolicola* (Burk) Stapp and Kottle, and *Pseudomonas phaseolicola* (Burk) Dows.

2.1.4.2 Morphology and Physiology

The bacterium is a single celled straight rod (Palleroni, 1984). It is motile with 1-6 polar or bipolar flagella (Burkhorder and Starr, 1948). It is gram negative and strictly aerobic (Fahy and Llyod, 1983). It's optimum growth temperature lies between 20 °C and 30 °C and colony growth is usually white to cream in colour with bluish hues (Buchanan and Gibbons, 1974). It produces a blue-green flourescence on King's B medium when examined under ultra-violet light (Shert and Macnab, 1986; Lelliot and Stead, 1987). It shows levan production and a negative oxidase test (Fahy and Llyod, 1983). It does not produce hydrogen sulphide but shows a positive catalase reaction (Burkholder and Starr, 1948).

2.1.4.3 Infection by halo blight pathogen

The bacterium enters the plant through stomata and wounds (Walker and Patel, 1964; Scuster and Coyne, 1974). It produces extracellular polysaccharides which induce watersoaking in the host tissue (El Banoby and Rudolph, 1979). It also produces a toxin (phaseotoxin) which induces chlorosis (Hoitink, 1966). These toxins are apparently sensitive to temperature (Hubbeling, 1957). In growing plants, the bacterial cells may move into the stem and invade the xylem vessels (Sherf and Macnab, 1986). The bacteria may then move from the lower stem internally and pass through the pedicel into the pod and seed via the vascular bundles.

2.1.4.4. Survival and dissemination

Between cropping season the pathogen survives in plant debris on the soil, powdered plant material in store and in or on infected seeds (Scuster and Coyne, 1974; Origa, 1991). Other infected beans and leguminous plants such as *Dolichos lablab* and *G. javanica* can be sources of inoculum (Mukunya and Keya, 1975). The bacteria are very effectively and rapidly spread by wind-driven rain, machinery, irrigation equipments, insects, people, domestic and wild animals (Walker and Patel, 1964; Mukunya and Keya, 1975; Sherf and Macnab, 1986). Sprinkler irrigation favours the disease more than furrow irrigation and hail injury provides avenue of entry by the bacterium into the plant tissue (Mukunya and Keya, 1975). Infected seeds are important for long distance and local dissemination of the bacterium (Smartt, 1976; Origa, 1991).

2.2 Common blight of beans

2.2.1 Geographical distribution and importance of the disease.

The disease has been reported in several parts of the world (Saettler, 1971). In Africa, it has been reported in Kenya, Central Africa Republic, Egypt, Ethiopia, Malagasy, Malawi, Nigeria, Mozambique, Zimbambwe, Somalia, Uganda, Tanzania, S. Africa, Sudan, Zambia and Morocco (Anon, 1980). Common blight and fuscous blight of beans were found prevalent in all areas studied but reached epidemic proportions in a few areas such as Meru, Kitui, Machakos, Kakamega, Embu, Transzoia and Murang'a districts (Muthangya, 1982). Common and fuscous blight of beans were found to be prevalent at lower altitudes (1000m - 2000m). Mukunya and Keya (1975) found that *Xanthomonas sp.* had high incidence of 9-24% in farmers seeds sampled in Central and Eastern provinces in Kenya.

It is difficult to quantify losses due to common blight since they occur together with fuscous blight of beans and they exhibit common symptoms (Logan, 1960).

2.2.2 Symptomatology

The bacterium attacks leaves, pods, seeds and stem. On the leaves, it causes the formation of watersoaked lesions and as the disease advances irregular, brown spots surrounded by yellow margins are formed (Saetler, 1971). The spots may coalesce forming irregular patches and the disease may lead to

premature shedding of leaves (Sherf and Macnab, 1986). Wilting and flagging of the top foliage occurs quickly followed by top necrosis in severe infection (Saettler, 1971). On pods, watersoaked greasy spots are formed; pods shrivel and become watersoaked (Sherf and Macnab, 1986). Seeds from severely infected
pods are shrivelled (Sherf and Macnab, 1986) while the stems show longitudinal brown necrotic lesions.

2.2.3 Host range.

The bacterium has a limited host range. Buchanan and Gibbon (1974) defined the host range of the bacterium to include *D. lablab, Lupinus* polyphyllus and *P. vulgaris*.

2.2.4 Etiology

2.2.4.1. Nomenclature

Common blight is caused by a bacterium called *Xanthomonas campestris* pv. *phaseoli* (Smith) Dowson. Smith named the causal organism of the disease as *Bacillus phaseoli* (Buchanan and Gibbons, 1974) but after investigating the cultural characteristics in 1901 and 1905, he transfered it to genus *Pseudomonas* and *Bacterium* respectively; and later to *Phytomonas* Dowson placed it under the genus *Xanthomonas* (Buchanan and Gibbons, 1974). Synonyms include:- *Bacillus phaseoli* (Smith), *Pseudomonas phaseoli* (Smith), *Bacterium phaseoli* (Smith), *Phytomonas phaseoli* (Smith), *Xanthomonas phaseoli* (Smith) Dowson.

2.2.4.2 Morphology and physiology

The bacterium is a single celled straight rod (Bradbury, 1984). It is motile due to its pollar flagellum (Burkholder and Starr, 1948). It is gram negative and strictly aerobic (Lelliot and Stead, 1987). Growth on media is usually yellow (Burkholder and Starr, 1948). It shows a negative or weak oxidase reaction and a positive catalase reaction (Bradbury, 1984). Acid is produced from small amounts of many carbohydrates but not from rhamnose, inulin, adonitols, dulcitol, inositol or salicin and rarely from nitrates (Bradbury, 1984).

Hydrogen sulphide is produced from nutrient broth containing cystein (Burkholder and Starr, 1948). The optimum temperature for its growth lies between 25 °C and 27 °C (Ekpto and Saettler, 1976). At 20 °C and 75% relative humidity, the cells have a shorter viability due to depletion of reserve nutrients through metabolic activity (Sleesman and Laben, 1976). Some species exist as a mixture of mucoid and non-mucoid types and are easily separated by plating on yeast extract dextrose carbonate agar [YDCA] (Correy and Starr, 1957).

2.2.4.3 Infection by common blight pathogen

Penetration into the host tissue occurs through natural openings and wounds (Sherf and Macnab, 1986). The bacterium produces polysaccharide toxins which block waterflow in the

vascular system of the plant (Goodman *et al.*, 1967). The pathogen may also enter the sutures of the pods from the vascular system of the pedicel and then passes into the funiculus and to the seedcoat (Coyne and Schuster, 1974). Water is important for bacterial multiplication in plant tissue (Sherf and Macnab, 1986).

2.2.4.4. Survival and Dissemination

Between cropping seasons, common blight pathogen survives in diseased seeds or in the soil in association with crop residues (Sherf and Macnab, 1986). The pathogen is also reported to survive on beddings for animals and on farm yard manure (Zaumeyer, 1930).

It is spread effectively and rapidly by wind-driven rains, machinery, irrigation equipments, insects, people, domestic and wild animals (Sherf and Macnab, 1986). According to a report by Kaiser and Vakili (1978), some leaf chewing insects (*Cerotoma ruficornis* Oliv, *Chalcodemus ebeninus* Boheman and *Diaprepes abbreviata* L.) and leaf sucking insects (*Empoasa* sp. and *Nezara viridura* L.) were found to transmit the bacterium.

2.3. Control of halo blight and common blight.

Bean blights are of economic importance in bean production in Kenya and controlling them is essential (Muthangya, 1982). Some of the recommended control measures include:- Use of disease free seeds, suitable crop rotation programmes, deep

ploughing of plant debris and development of resistant cultivars (Mukunya and Keya, 1975). Some chemicals have also been used for instance timely spray of copper oxychloride (Taylor, 1972; Mukunya and Keya, 1975). Copper hydroxide 56%, 40% potassium (hydroxy-methyl) and methyl dithiocarbamates gave good results against common bacterial blight on leaves (Weller and Saettler, 1976). Mercuric chloride can be used for seed treatment but it is discouraged due to its high mammalian toxicity and residues (Person and Ecgerton, 1939). It also causes reduction in germination of the treated seeds. Antibiotics such as streptomycin have been reported to offer reasonable control of the two bacterial blights (Zaumayer *et al.*, 1952; Taylor, 1972). However, dry beans are mainly grown by small scale farmers who are unable to afford these expensive chemicals. Inavailability of land to practice crop rotation and ability of the pathogens to survive in plant debris for a long time also limits the effectiveness of cultural methods.

2.4 Antimicrobial activities of plant extracts

The antimicrobial activity of plant extracts such as volatile oils has been reported by authors from different parts of the world. Michenkova *et al.* (1983) investigated some plant extracts for antibiotic action against *Pseudomonas syringae* pv. *phaseolicola*, *Xanthomonas campestris* pv. *phaseoli*, *Clavibacter michiganense* subsp. *michiganense*, *P. syringae* pv. *atrofascians* and *Erwinia carotovora* subsp. *carotovora* and found that the strongest inhibitors were in plants from families asteraceae

and lamiaceae. The volatile oils from *Calendula officinalis* and *Thymus serpyllum* gave the highest antibiotic activity *in vitro* with *C. michiganense* subsp. *michiganense* as the most sensitive test pathogen followed by *X. campestris* pv. *phaseoli.*

Expressed juice of acqueous and organic solvent extract of garlic cloves were found to have antimicrobial activity on some phytopathogenic bacteria and fungi. It offered bean plants protection against *P. syringae* pv. *phaseolicola* and *Colletotrichum lindemuthianum* (Ark and Thompson, 1959). Extracts from garlic, onions, *Malus sieboldii, Reynoutria japonica* and *Rheum coreanum* were found to inhibit the growth of *Phytopthora* species (Paik, 1989). Some work by Kishore *et al.* (1988) indicated that the volatile oils of *Anethum graveolens* Linn. and *Curcuma longa* Linn. effectively controlled damping off of *Vigna radiata (Phaseolus aureaus*) when applied as a seed treatment. Volatile oils from leaves of *Cinnamomum tamala* Nees and Eidam also exhibited fungicidal activity against *Aspergillus* species (Misra and Batra, 1987).

Some work by Hethelyi *et al.* (1987) shows that the volatile oil of *Tagetes minuta* inhibited the multiplication of *Pseudomonas syringae* pv. *pisi, P. syringae* pv. *tabaci, X. campestris* pv. *vesicolor* and other bacteria. According to Hanudin (1987), the extracts of *Tagetes erecta* and shallot suppressed bacterial wilt of tomatoes (*Pseudomonas solanacearum* E.F. Smith) in an *in vivo* test. Miah *et al.*, (1990)

reported that T erecta extracts could inhibit more than 50% normal fungal growth of *Monographella albescens* and *Rhizoctonia solani.*

Jacob *et al.* (1988) investigated the antimicrobial activity of *Eucalyptus* leaf extract and found that when used as a 30 minute seedsoak prior to planting, the extract was effective against *Pythium aphanidermatum* on *Solanum melongena.* In an experiment carried out on gram negative bacteria (*X. campestris* pv. *phaseoli, Pseudomonas syringae* pv. *syringae, Erwinia carotovora* subsp. *carotovora, Agrobacterium tumefascians)* and gram positive bacteria *(C. michiganense* subsp. *michiganense, Rhodococccus fasciens, Bacillus subtilis*) and some fungi, it was found that gram positive bacteria were more sensitive to an onion extract than gram negative bacteria (Dmitriev *et al.,* 1989). Gullivar (1949) as quoted by Korzybski *et al.* (1967) found that the growth of *P. syringae* pv. *syringae, Xanthomonas campestris* pv. *begoniae, X. campestris* pv. *malvacearum, Corynebacterium sepedonicum, Streptomyces scabies* and *Verticillium dahliae* could be inhibited by berberine from *Berberis vulgaris, Hydrastis canadensis,* and *Xanthoxylum clava herculis.*

Plant extracts have been used also for the control of animal pathogens. *Pseudomonas aeruginosa* and other animal pathogens were found to be sensitive to extracts from *Juniperus oxycedrus L.* sub sp. *oxycedrus, Spartium junceum* L.,

Helichrysum italicum subsp.*microphyllum, Inula viscosa* L. and *Asphodelus microcarpus* (Bonsignore *et al.*, 1990). The volatile oil of *Aframomum melagueta* and its constituents were found to inhibit the growth of *P. aeruginosa* and *Pseudomonas flourescence* among others
(Oloke *et al.*, 1987). *P. aeruginosa* could also be inhibited by essential oils from *Tanacetum cilicium* (Thomas, 1989).

Some antibacterial substances occur in inactive forms in plants and require enzymatic reactions to become active. For instance extracts from seeds and staiks of common radish (*Rhaphanus sativus*) contain an antibacterial principle which becomes active under the influence of enzyme myrosinase (Ivanovics and Horvaths, 1947).

2.5 Test plants used

(a) *Eucalyptus citriodora* Hook (Myrtaceae)

It is known as `lemon scented gum or spotted gum. It is an evergreen tree which is a native of Australia and Tasmania (Usher, 1984). The wood is light brown to grey-brown. The various oils of *Eucalpytus* sp. are used medicinally as expectorants, in treating colds, as a mild antiseptics and to reduce fever (Howes, 1974). The tree is grown for it's oil in Spain and Portugal (Usher, 1984). The oil is used in perfumery and is

also applied externally as a counter-irritant (Howes, 1974).

(b) *Tagetes minuta* L. (compositae)

It is a strongly scented garden weed up to 2m tall with yellowish flower heads. It exhibits antibacterial activity (Hethelyi *et al.*, 1987). A decoction of the leaves is used to treat stomach upsets, as a diuretic and to induce sweating (Usher, 1984). The essential oil from the leaves is used as an insect and vermin repellent (Uphof, 1968). It is also an effective larvicide used to kill maggots in wounds.

(c) *Santolina chamaecyparissus* L. (compositae)

It is an ornamental shrub with yellow flowers and greyish green leaves (UPhof, 1968). It is alsc known as Lavender cotton (Usher, 1984). The flowers are sometimes used to treat ringworms (Uphof, 1968 ; Usher, 1984) while stems are used to repel moths. The essential oil extracted from the leaves is used in perfumery (Usher, 1984).

(d) *Schinus molle* L. (Anacardiaceae)

It is also known as Brazilian pepper tree, California pepper tree (Usher, 1984). It is an evergreen tree, 20 feet cr more in height with

pendulous branches and produces red seeds which are used to adulterate pepper (Uphof, 1968). It is reported to have fungitoxic activity on storage and animal pathogenic fungi (Maffei and Chialva, 1990). The fruits are used to make a drink (Uphof, 1968). The gum from the trunk is chewed and the ground bark is used as a purgative for men and animals (Uphof, 1968; Usher, 1984).

(e) *Chrysanthemum cinerariaefolium* (Trev) Vis. (Compositae).

Pyrethrum is a well known perennial herb grown as a cash crop in Kenya for the production of insecticides (Uphof, 1968; Usher, 1984).

(f) *Aleurites molluccana* L. (Willd). (Euphorbiaceae).

It is commonly known as candlenut and oil tree (Usher, 1984). The seeds are a source of drying oils which is used as a wood preservative, for manufacture of soap, paints and varnishes (Uphof, 1968). The oil is used for painting boats to provide protection against marine boring worms (Uphof, 1968; Usher, 1984).

(g) *Cupressus lucistanica* Mill (cupressaceae)

It is an evergreen tree with a reddish brown bark. The wood is used for general

construction work. The bark is used as an astringent (Usher, 1984). The essential oil of a related plant (*Juniperus oxycedrus* L.) was found to inhibit the growth of *Pseudomonas aeruginosa* (Bonsignore *et al.*, 1989). *C. lucistanica* gives an oil used as adjuvant in soap, room sprays and deodorants (Carmo and Frazzo, 1989).

(i) *Nothoscordum inodorum* L. (Liliaceae)

It is a bulbous plant that closely resembles those in the genus *Allium*. `Nothos' means false and `Scordum' means garlic thus the plant is also known as false garlic (Bailey, 1961). Related species to this plant such as *Allium sativum* (garlic) are known to produce substances that inhibit microbial growth (Ark and Thompson, 1959)

3. MATERIALS AND METHODS

3.1 Pathogens:

3.1.1. Isolation of halo blight and common blight pathogens

Diseased leaves of beans were harvested from plants growing in the University of Nairobi farm, Kabete. Lesions and parts bordering them were cut and surface sterilized by submerging them in 2.5% (w/v) sodium hypochlorite for 2-3 minutes. They were rinsed several times with sterile distilled water and then macerated in a test tube containing sterile distilled water. The bacterial suspension was streaked onto nutrient agar [beef extract, 2g; peptone, 30g; bacto agar, 15g; distilled water, 1000 ml]. A replicate of 4 plates per isolate were made and incubated in precision gravity convection incubator for 2-3 days. The cultures of halo blight and common blight pathogens were purified by a series of single colony transfers.

3.1.2. Identification

(A) ***P. syringae*** **pv.** ***phaseolicola***

The following studies were carried out to verify the isolates.

(i) <u>General morphology</u>

Gram staining was carried out using 18 hr old cultures. This also allowed for the observation of

cell size, shape and arrangement. The method by Skerman (1957) was used.

(ii) Cell motility

Bacterial cells were placed in a drop of water on a cover slip as described by Kiraly *et al.* (1970). The cover slip was inverted over the cavity of a microscope slide and observed under oil using 100 x objective.

(iii) Potassium hydroxide solubility test

This was carried out as a confirmatory test to the gram-stain test. One drop of 3% potassium hydroxide (K0H) was placed on a glass slide. A few colonies from 18 hr old cultures were picked with an inoculating loop and stirred in KoH for 5 sec. The inoculating loop was then raised from the drop and the presence or absence of thread of slime following the loop for about 1.5 cm was recorded.

(iv) Flourescein pigment production

Two day old bacterial cultures were streaked onto King's B medium [proteose peptone, 10; K_2PH0_4 (anhydrous) 15g; Difco agar, 15g; glycerol, 15 ml; distilled water, 1000 ml] contained in a petri-dish (King *et al.*, 1954). After 3 days of incubation at 27°C in a precision gravity convection incubator, the plates were examined in a dark room

under ultraviolet light for a blue green flourescence. Three plates per isolate were used.

(v) Levan production

Levan production was determined in nutrient agar medium containing 5% (w/v) sucrose according to the method described by Lelliot and Stead (1987). Two day old bacterial cultures were streaked onto the medium and incubated at room temperature for 3 days. Observations were made for absence or presence of heavy mucoid, convex colonies.

(vi) Oxidase test

It was performed using Kovac's method (1965). A piece of Whatman No. 1 filter paper was soaked in 1% acqueous solution of tetramethyl-P-phenylenediamine dihydrochloride. With the use of a glass rod, 24-hr old bacterial culture grown on NA medium was smeared on the reagent impregnated filter papers and observed for any colour change. Three replicates per isolate were performed.

(vii) Arginine dihydrolase test

This was carried out according to Thornley's method (1966). About 5 ml of arginine medium (bacto-agar, 10g; Nacl, 5.0g; $K_2H\ PO_4$, 0.3g; phenol red, 0.01g; distilled water, 1000 ml) were dispensed into 10ml tubes. The medium was stab-inoculated by using a 24-hr old bacterial culture. After inoculation, the medium was then covered with a layer of sterile oil

to a depth of 60 mm and the test tube incubated at 27°C in a precision gravity convection incubator. Three replicates were made per isolate. Observations for any change in colour were made after 3 and 6 days of incubation.

(viii) Catalase reaction

Three to four drops of 3% hydrogen peroxide were added onto 24-hr old bacterial cultures (Skerman, 1959). Observations for the presence or absence of effervescence were made.

(ix) Production of hydrogen sulphide

Nutrient broth (Beef extract, 3.0g; peptone, 10g; distilled water 1000 ml) containing cystein in a screw cap test tube was inoculated using a 24-hr old culture according to the method described by Kiraly *et al.* (1970). Strips of filter paper, moistened with a 10% lead acetate were introduced such that they were held between the caps and test tubes and suspended over the broth without touching it. The tubes were incubated at 27°C and examined after 3, 7 and 14 days for any colour change of the paper.

(x) Utilization of sugars

The sugars used in this experiment were:- D-arabinose, sucrose, manitol, lactose, and glucose according to the method described by Kiraly *et al.* (1970). Nutrient broth was used as a basal medium

into which the above sugars were added at the rate of 0.5%. The pH was adjusted to 7.0 and an indicator bromothymol blue added before sterilization. Approximately, 0.1 ml of bacterial suspension at 5 x 10^7 CFu ml^{-1} concentration was added into each test tube. The experiment was carried out in 3 replicates and the test tubes incubated for 48 hrs at 27°C. Records of any colour change were taken daily.

3.1.2 (B) *X.campestris* pv. *phaseoli*

General morphology, cell motility, KoH solubility test, oxidase test, catalase test, levan production, production of hydrogen sulphide and tests on utilization of sugars were carried out as described in 3.12 (A).

Other studies that were carried out to verify the isolate included:-

(i) Tolerance on Triphenyl tetrazolium chloride

The cultures were tested for TTC tolerance on 2% NA containing 0.1% and 0.02% triphenyl tetrazolium chloride (TTC) and 5 spot inoculation per plate were made on TTC agar medium in 3 replicates according to the method described by Lelliot and Stead (1987). Observations were recorded after 3-5 days of incubation at 27 oC.

(ii) Sodium chloride tolerance

The method described by Burkholder and Starr (1948) was used with some modifications. The cultures were streaked on to NA oontaining 1, 3 and 5% (w/v) sodium chloride. Three replicates were made. The plates were incubated at 27°C and observations made 7 anc 14 days after inoculation.

(iii) Gelatin liquefaction

Single streaks of the isolate were made on NA containing 0.4% gelatin and incubated at 27°C for 3 days (Lelliot and Stead, 1987). The surface of the medium was then flooded with 5 ml of acid mercuric chloride solution and presence or absence of a clear zone noted. The experiment was replicated 3 times.

(iv) Starch hydrolysis test

The isolate was streaked on NA medium containing 2% starch and then incubated at 25°C for 4 days (Leiliot and Stead, 1987). The surface of the medium was flooded with iodine solution and observations made for any clear zone. The experiment was replicated 3 times.

3.1.3 Maintanance of cultures

Isolates of *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli* were maintained in nutrient agar slants at 4°C .

3.1.4 Inocula standardization

An acqueous suspension of an isolate was prepared by washing bacterial cells from 48-hr old culture in a test tube with sterile distilled water. It was shaken and then made to 10ml. Serial dilutions were made until 10^{-10} dilution was obtained. Approximately 0.1ml of 10^{-5}, 10^{-6}... 10^{-10} were removed and plated on nutrient agar in petri dishes. Three transfers of each dilution were made and a bent rod used to spread the 0.1 ml of dilution evenly on the surface of the medium. The plates were incubated at room temperature for 2 days after which a colony counter was used to count colonies on the plates containing 300 or less colonies.

Spectrophotometer (WPA S105) was used to determine the absorbance of the original suspension and that of 10^{-1}, 10^{-2} 10^{-4} and 10^{-6} dilutions (starting with 10^{-6}). Sterile distilled water was used as a blank to zero the spectrophotometer set at a wavelength of 620nm. The number of bacterial cells/ml in the original sample was obtained by multiplying the number of colonies on the petridish by the dilution factor multiplied by 10. The number of bacterial cells per ml in 10^{0} 10^{-1}, 10^{-2}, 10^{-4} and 10^{-6} were calculated and the values used to obtain a standard population curve for the bacteria.

3.15: Pathogenicity test

3.1.5.1 Source of healthy certified seeds

The bean cultivar used in all the experiments was cv. Rosecoco-GLP-2. Six kilograms of healthy certified seeds were kindly supplied by Mr. Muigai of National Horticultural Research Station, Thika, Kenya.

3.1.5.2. Growth of bean plants in the greenhouse

Healthy certified seeds were planted in polythene bags containing steam sterilized soil whose composition was top soil : manure: sand: ballast in the ratio of 2:1:1:1.Three seeds were planted in every bag but thinning was done one week after emergency leaving one healthy seedling.

One week before planting, the greenhouse was thoroughly disinfected with copper oxychloride at the rate of 1:400 and 0.1% Dimethoate (5ml in 2l water) to control bacterial and fungal pathogens and insects respectively.

3.1.5.3. Leaf inoculation

The method described by Lelliot and Stead (1987) was used. Fourteen day old seedlings were used for pathogenicity test. The bacterial suspension was adjusted to $5x10^7$ colony forming units $(CFU)ml^{-1}$ concentration using a spectrophotometer (WPA S105) and sprayed onto bean seedlings using a quick-fit atomizer until the leaf surfaces became watersoaked. The inoculated bean plants were then covered with polythene bags

and incubated for 48 hours after which the polythene bags were removed. Control plants were sprayed with sterile distilled water. Three replicates per pathogen were done. These experiments were carried out to test for pathogenicity of *P. syringae pv. phaseolicola* and *X. campestris pv. phaseoli* isolates.

3.2 Screening of plant extracts

3.2.1. Source of plants extracts

The plant extracts used in this study were extracted from plants collected from Nairobi and it's environs. The plants included:- *Eucalyptus citriodora* Hook, *Tagetes minuta* L., *Santolina chamaecyparissus* L., *Schinus molle* L., *Aleurites molluccana* L. (Willd), *Cupressus lucistanica* Mill and *Nothoscordum inodorum* L. They were then authenticated in the herbarium in Botany Department, University of Nairobi. Pyrethrum marc was supplied by courtesy of Pyrethrum Board of Kenya - Nakuru.

3.2.2. Methods of extraction

The leaves of *E. citriodora*, and *S. molle*, aerial parts of *T. minuta*, aerial parts of *S. chamaecyparissus* and seed husks of *C. lucistanica* were steam distilled in a modified clevenger's apparatus. In each case, 1 kg of plant parts described above was put into the distillation apparatus and four litres of tap water added. Steam distillation was carried out for 8 hrs to obtain volatile oils. The oils were transfered into conical flasks and dried overnight by mixing 20ml of the oils with about 5 grams of anhydrous sodium sulphate. The oils were retained and thereafter tested for antibacterial activity on *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli*.

Pyrethrum marc was extracted using soxhlet apparatus. About 250g of the marc were placed in a porous thimble and the latter placed in the inner tube of the soxhlet apparatus. It was succesfully extracted with 250ml of chloroform and 250 ml of methanol subjected to heat at 55°c-60°c. One kilogram of crushed seed kernels of *A. molluccana* were also successively extracted with 250 ml of hexane, 250 ml of chloroform and 250 ml of methanol at room temperature for two days for each solvent. The solvents were then evaporated using a rotary evaporator at 60°C-65°C. All the extracts were then tested for their antibacterial activities against *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli*.

3.2.3 *In vitro* test for antibacterial activity of some plant extracts.

Plant extracts obtained from *E. citriodora, S. molle, T. minuta, S. chamaecyparissus, C. lucistanica, C. cinerariaefolium, N. inodorum* and *A. molluccana* in the forms

described in table 2 were tested for antibacterial activity using petri-dish zonal inhibition technique. *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli* were used as test pathogens. Ten ml of nutrient agar were dispensed into each petri-dish to provide a basal layer on top of which 5 ml of seeded medium with either *P. syringae* pv. *phaseolicola* or *X. Campestris* pv. *phaseoli* adjusted to a concentration of 10^7 $CFUml^{-1}$ were poured. Filter paper discs, diameter 11.5 mm (Whatman paper No. 1) were emmersed in the extracts and placed on the seeded media. Streptomycin sulphate at 1% concentration was used as a reference standard. All plates were incubated at room temperature for 48 - hrs after which presence or absence of growth inhibition zones was noted and the diameters measured using a clear glass ruler. A 2 x 7 factorial experiment was set with 14 treatment combinations. The experimental design used was completely randomized design with 3 replicates.

3.2.4 Determination of the active fraction

3.2.4.1 Method of extraction

The volatile oils from *E. citriodora, T. minuta, C. lucistanica* and *S. chamaecyperissus* were extracted as described above. They were then fractionated as ether and hexane fractions.

3.2.4.2. Fractionation of volatile oils

Volatile oils from *E. citriodora, T. minuta, C. lucistanica* and *S. chamaecyparissus* were fractionated by column chromatography. Fifty grams of silica gel were deactivated by mixing with distilled water to make a slurry and then transfered into a column (100cm long, 5cm in diameter). It was allowed to run until the water level reached that of silica gel. The remaining water was washed with 100 ml methanol, 100 ml acetone, and 100 ml ether consecutively. These were then washed using 250 ml hexane. Once the hexane run was over, 5g of a given oil were introduced into the column. Hexane was used to elute the non-oxygenated hydrocarbons after which di-ethylether was used to elute the oxygenated hydrocarbons. Hexane and ether were then evaporated from the two fractions using a rotary evaporator set at 60°C. The two fractions from each volatile oil were retained and later tested for antibacterial activity against *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli.*

3.2.4.3. Antibacterial activity test

The ether and hexane fractions obtained as described above from the volatile oils (extract) of each plant species under study viz., *E. citriodora, T. minuta, C. lucistanica* and *S. chamaecyparissus* were tested for antibacterial activity against *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli.* Petri-dish zonal inhibition technique described in 3.2.3 was used. Crude extracts (volatile oils) were included for

comparison. Hexane and di-ethylether were used as controls. This formed a 2 x 4 x 5 factorial experiment which was replicated 4 times. The treatment combinations were laid out in a completely randomized design. Comparison of zones of growth inhibition produced by the crude extracts and the fractions was made. Further

analysis was carried out to establish whether there was any interaction between the test pathogens and different plant extracts.

3.2.5 Determination of minimum inhibitory concentration (MIC) of plant extracts.

3.2.5.(a) Crude extracts of *E. citriodora* and *T. minuta.*

The minimum inhibitory concentrations of the two crude extracts prepared as described above were determined using tube dilution technique. Volumes of 2.5 ml of sterile nutrient broth were prepared and mixed with 2.5 ml of the crude extract. Two drops of tween 80 were added and shaken vigorously to mix the two phases, that is nutrient broth and volatile oil. About 2.5 ml of this solution was transfered into another tube containing 2.5 ml of sterile nutrient broth and shaken. The same process was progressively repeated to give eleven dilutions. Sterile nutrient broth (2.5 ml) was used as a control.

A bacterial suspension at a concentration of 2.5×10^4 colony forming units (CFU) ml^{-1} was prepared from 24 hr old cultures and 200 µl added into each of the diluted extracts (volatile oils). These were then incubated on Gallenkamp orbital shaker at 65 revolutions per minute for 24 hrs and thereafter 100 µl suspensions from each tube was plated on nutrient agar. Presence or absence of bacterial growth was recorded 48 hrs after incubation. A completely randomized design was used with 3 replicates.

3.2.5(b) Ether fractions of *E. citriodora* and *T. minuta* extracts.

Approximately 2.5 ml of sterile distilled water was mixed with each ether fraction of *E. citriodora and T. minuta* in the ratio of 1:1. Two drops of tween 80 were added and the solutions shaken vigorously. Then 2.5 ml of this solution was transfered into another screw cap bottle containing 2.5 ml of sterile distilled water. Serial dilution was carried out upto the 7th dilution. Petri dish-zonal inhibition technique described above was used to test each dilution for antibacterial activity against *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli*. Sterile distilled water containing 2 drops of tween 80 was used as a control. A 2 x 2 x 7 factorial experiment was set with 28 treatment combinations. The experimental design used was completely randomized design with 4 replicates.

3.2.6. Determination of the effect of temperature on the antibacterial activity of plant extracts.

The volatile oils of *E. citriodora* and *T. minuta* were used in this experiment. Quantities of 1 ml of each volatile oil were put in sterile screw cap bottles kept at the following temperatures for 15 minutes viz 0°C. 10°C, 20°C, 30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C, 100°C and 121°C. Low temperatures i.e. 0°C and 10°C were maintained using a refrigerator whereas high temperatures between 30°C-100°C were maintained using waterbaths.

Petri-dish zonal inhibition technique described above was used for testing for antibacterial activity against *P. syringae*

pv. *phaseolicola* and *X. campestris* pv. *phaseoli.* A 2 x 2 x 12 factorial experiment was set with 48 treatment combinations. The experimental design used was a completely randomized design with 4 replicates. The zones of growth inhibition were measured after 48 hrs and later, the data was subjected to statistical analysis to establish whether there was any temperature effects on the extracts and interactions between temperature and plant extracts.

3.2.7 Control of the bean halo and common bacterial blights *in vivo* using the plant extracts.

3.2.7.1 Source of seeds

The bean cultivar used in the *in vivo* test was cv. Rosecoco-GLP-2. Healthy certified seeds were obtained from Mr. Muigai of National Horticultural Research Station, Thika, Kenya.

3.2.7.2 Test for phytotoxity

The experiment was carried out to determine whether or not the different concentrations of volatile oils from *E. citriodora* and *T. minuta*; and their ether fractions had any phytotoxic effects in bean plants. The bean seedlings were separately grown in the greenhouse as described above. Fourteen day old seedlings were separately sprayed with 0.39%, 0.59% and 0.78% concentrations of volatile oil from *E.citriodora*; 1.56%,2.34% and 3.13% concentrations of ether fraction of volatile oil from *E. citriodora*; 0.10%, 0.15% and 0.20% concentrations of volatile

oils from *T. minuta*; and 6.25%, 9.38% and 12.50% concentrations of ether fraction of volatile oil from *T. minuta*. Di-ethylether, at 100%, 50%, 25% and 5% concentrations was sprayed on another set of seedlings and served as controls. Bean seeds were separately soaked for 8 hrs in 100%, 50%, 25% and 5% concentrations of di-ethylether; 0.59% concentration of volatile oil from *E. citriodora, 2.34%* concentration of ether fraction of volatile oil from *E. citriodora*, 0.15% concentration of volatile oil from *T. minuta* and *9.38%* concentration of ether fraction of volatile oil from *T. minuta*. The seeds were then dried for 3 days and thereafer planted in the greenhouse. All the treatments were carried out in triplicate. The plants were observed for 2 weeks and any signs of phytotoxicity recorded.

3.2.7.3 Inoculation of bean seeds and seedlings with bean bacterial blight pathogens.

About 250g of healthy certified seed were inoculated with *P. Syringae* pv. *phaseolicola* and *X.campestris* pv. *phaseoli*. This was done by soaking the seeds in a given bacterial suspension at a concentration of 5×10^7 CFU ml^{-1} for 12 hrs and then air drying at room temperature for 3 days (Muthangya, 1982). The inoculated bean seeds were treated as described below before potting. Another set of healthy certified bean seeds were potted in the greenhouse and treatments carried out on 9 day old seedlings as described below. All the plants were grown in the greenhouse as described above.

3.2.7.4 Treatments on beans in the greenhouse

Treatment 1: inoculated seeds were dressed with 0.59% concentration (MIC) of volatile oil from *E. citriodora*.

2: Inoculated seeds were dressed with 2.34% concentration (MIC) of the ether fraction of volatile oil from *E. citriodora*.

3: Inoculated seeds were dressed with 0.15% concentration (MIC) of volatile from *T. minuta*.

4: Inoculated seeds were dressed with 9.38% concentration (MIC) of the ether fraction of volatile oil from *T. minuta*.

5: Inoculated seeds were planted in the greenhouse to serve as a control.

For treatments 6-17, 9 day old healthy seedlings were inoculated with a bacterial suspension adjusted to a concentration of 10^7 CFU/ml and allowed to air dry.

Three plants per treament were sprayed with a specific concentration of a given extract as shown below and the treatments designated as 6, 7.....17. (Table 1).

Table 1: Concentrations of different plant extracts used to spray beans in the greenhouse.

Plant species	Plant extract	Concentration (%)*					
		Tt	Higher than MIC	Tt	MIC	Tt	Lower than MIC
E. citriodora	Volatile oil	6	0.78 0.39	7	0.59 0.29	8	0.39 0.20
	Ether fraction	9	3.13 3.13	10	2.34 2.34	11	1.56 1.56
T. minuta	Volatile oil	12	0.20 0.20	13	0.15 0.15	14	0.10 0.10
	Ether fraction	15	12.50 12.50	16	9.38 9.38	17	6.25 6.25

Tt = treatment

Note* The values in the first row of each plant extract shows the concentration of that plant extract that was sprayed to control halo blight of beans while concentration in the second row were used to spray bean plants to control common bacterial blight.

Treatment 18: Foliar inoculated seedlings were sprayed with 0.1% streptomycin sulphate.

Treatment 19: Foliar inoculated seedlings were sprayed with copper oxychloride 0.1% a.i:

Treatment 20: Foliar inoculated seedlings not treated.

Similar treatments were applied on seeds and seedlings inoculated with *X. campestris* pv. *phaseoli*. The inoculated bean seedlings were then covered with polythene bags for 24 hrs after treatment. They were then uncovered and placed on greenhouse benches until symptoms developed. The experimental design used was completely randomized design with 3 replicates.

3.2.7.5 Data collection and analysis

The severity of halo and common bacterial blights on all leaves of bean seedlings was recorded 3 weeks after treatments. A scoring system was developed based on the percent leaf infection. The mean grades were obtained as follows:-

$$\text{Mean grade} = \frac{\text{grade reading}}{\text{number of readings}}$$

Mean grades obtained in different treatments were subjected to statistical analysis to test for significance.

3.2.8 Determination of the efficacy of various spray rates and timing of *E. citriodora* extract on the bean bacterial blights under field conditions.

3.2.8.1 Source of bean seeds

Cultivar Rosecoco-GLP-2 was used in this study and healthy certified seeds were kindly supplied by Mr. Muigai of National Horticultural Research station, Thika, Kenya. Bean seeds cv. Rosecoco-GLP-2, from 12 small scale farmers in a given district viz., Kiambu, Embu and Kisii districts were bulked to give a 6 kg sample of uncertified seeds.

3.2.8.2 Seed inoculation

Artificial inoculation was carried out on healthy certified seeds following Muthangya's method (1982) using *P. syringae* pv. *phaseolicola*. Bean seeds were soaked in a bacterial suspension at 5×10^7 CFU ml^{-1} concentration for 12 hrs and then air dried at room temperature for 3 days.

3.2.8.3 Growth of plants in the field

The experiment was conducted on the University of Nairobi farm, field station at Kabete. The farm is located at an altitude 1800 m, latitude 1° 15' S and longitude 36° 44' E. The area receives a mean annual rainfall of about 1000 mm with a mean maximum temperature of 23°C and a mean minimum temperature of 12°C. The soil consists of a well

drained, deep, dark-reddish brown to dark red friable clay with acid humic top soil (humic nitosols) developed from Limuru Trachyte (Michieka, 1977).

Bean seeds treated as described below were planted in 2m x 3m plots during October 1991-January 1992 and March-June (1992) growing seasons. The spacing used was 40 cm between rows and 10 cm within rows. Approximately 60 kg/ha of P_2O_5 and 30 kg/ha of N were applied during planting. All plots were maintained weedfree through handweeding throughout the growing seasons.

3.2.8.4 Treatments for season I using halo blight bacterial pathogen (October 1991 - January 1992).

Treatment 1: Inoculated seeds were soaked for 8 hrs in 0.59% concentration of volatile oil from *E. citriodora*. The seeds were then dried for 3 days before planting.

Treatment 2: Seed treatment as in (1) followed by foliar spray at seedling stage with 0.59% concentration of the volatile oil of *E. citriodora*.

Treatment 3: Seed treatment as in (1), followed by foliar spray at seedling stage and 2 weeks later.

Treatment 4: Seed treatment as in (1) followed by foliar spray at seedling stage, 2 and 4 weeks later.

Treatment 5: Seed treatment as in (1) followed by foliar sprays and seedling stage, 2, 4 and 6 weeks later.

Treatment 6: Seedlings obtained from inoculated untreated seeds were sprayed at seedling stage.

Treatment 7: Treatment as in (6), followed by foliar spray 2 weeks later.

Treatment 8: Treatment as in (6) followed by foliar spray at 2 and 4 weeks later.

Treatment 9: Treatment as in (6) followed by foliar spray at 2, 4 and 6 weeks later.

Treatment 10: Inoculated seeds were planted in the field to serve as control.

Treatment 11: Uncertified seeds from farmers were soaked for 8 hrs in 0.59% concentration of volatile oil from *E. citriodora*. The seeds were then dried for 3 days before planting.

Treatment 12: Seed treatment as in (11) followed by foliar sprays at seedling stage with 0.59% concentration of the volatile oil of *E. citriodora*.

Treatment 13: Seed treatment as in (11) followed by foliar spray at seedling stage and 2 weeks later.

Treatment 14: Seed treatment as in (11) followed by foliar spray at seedling stage, 2 and 4 weeks later.

Treatment 15: Seed treatment as in (11) followed by foliar spray at seedling stage, 2, 4 and 6 weeks later

Treatment 16: Seedlings obtained from untreated, uncertified bean seeds from farmers were sprayed at seedling stage.

Treatment 17: Treatment as in (16), followed by foliar spray 2 weeks later.

Treatment 18: Treatment as in (16), followed by foliar spray 2 and 4 weeks later.

Treatment 19: Treatment as in 16, followed by foliar spray 2, 4 and 6 weeks later.

Treatment 20: Uncertified seeds from farmers which were not treated were planted in the field to serve as control.

All the plant extracts were suspended in 1000 litres of water and sprayed using a lever operated knapsack sprayer.

3.2.8.5 Treatments for Season II (March-June, 1992) against halo blight of beans.

(a) Seed inoculation

The healthy certified seeds of cv. Rosecoco-GLP-2 were inoculated with *P. syringae* pv. *phaseolicola* at a concentration of 5×10^7 CFU ml^{-1} following the procedure described above. They were then treated as follows:-

(b) Treatments

1: Inoculated seeds were soaked for 8 hrs in 0.59% concentration of the volatile oil from *E. citriodora.*

2: Seed treatment as in (1) followed by foliar spray with 0.59% concentration of the volatile oil from *E. citriodora* one month after planting.

3: Seed treatment as in (1) followed by foliar spray one month after planting, and later after pod formation.

4: Seedlings arising from untreated seeds were foliar sprayed with the volatile oil one month after planting.

5: Treatment as (4) but followed by foliar spray after pod formation.

6: Inoculated seeds were soaked for 8 hrs in 1.17% concentration of ether fraction from the volatile oil of *E. citriodora*. The seeds were then dried for 3 days before planting.

7: Seed treatment as in (6) then sprayed with 1.17% concentration of the ether fraction of *E. citriodora* one month after planting.

8. Treatment as in (6), followed by foliar spray one month after planting and after pod formation.

9. Seedlings arising from untreated seeds were foliar sprayed with the ether fraction of volatile oil of *E. citriodora* one month after planting.

10: Treatment as in (9) but followed by foliar spray after pod formation.

11: Inoculated seeds were planted in the field to serve as control.

12: Uncertified seeds from farmers soaked for 8 hrs in 0.59% concentration of the volatile oil of *E. citriodra*.

13: Seed treatment as in (12) followed by foliar spray one month after planting.

14: Seed treatment as in (12) followed by foliar spray one month after planting, and later after pod formation.

15: Seedlings arising from untreated farmers seeds were foliar sprayed with the volatile oil one month after planting.

16: Treatment as in (15) but followed by foliar spray after pod formation.

17: Uncertified seeds from farmers were soaked for 8 hrs in 1.74% concentration ether fraction of volatile oil from *E. citriodora*. The seeds were then dried for 3 days before planting.

18: Seed treatment as in (17) then sprayed with 1.74% concentration of the ether fraction of *E. citriodora* volatile oil one month after planting.

19: Treatment as in (17), followed by foliar spray one month after planting and later after pod formation.

20: Seedlings arising from untreated farmers seeds were foliar sprayed with the ether fraction of *E. citriodora* volatile one month after planting.

21: Treatment as in (20) but followed by foliar spray after pod formation.

22: Untreated farmers seeds were planted in the field to serve as control.

23: Inoculated seedlings were sprayed with 0.1% a.i. copper oxychloride 4 times, at 10 days interval (Taylor, 1972).

24: Inoculated seedlings were sprayed with 0.1% a.i. streptomycin sulphate 4 times, at 10 days interval (Taylor, 1972).

The experimental design used was randomized complete block design with 3 replicates. Twenty plants per plot were sampled randomly, tagged for later disease assessment.

3.2.8.6 Treatments, for season II (March-June, 1992) against common bacterial blight of beans.

The experiment was carried out as described in 3.2.7.5 above, but the healthy certified seeds were inoculated with common bacterial blight pathogen *X. campestris pv. phaseoli*. The experiment was laid out in a randomized complete block design with 3 replicates.

3.2.8.7 Data collection and analysis

The following parameters were recorded from the 20 bean plants tagged in each plot.

(a) Disease incidence

This was recorded as the number of bean plants showing symptoms of (i) halo blight and (ii) common blight expressed as a percent of the total number of bean plants assessed in each plot. The results were recorded one week after the last spray during the pod filling stage. The data was then subjected to statistical analysis.

(b) Disease severity

The severity of halo and common bacterial blights was recorded one week after the last spray. All the leaves on each of the 20 tagged plants were assessed. This was recorded as the percentage leaf area infected per plant. The scoring system developed earlier was used for disease assessment. Mean grades were obtained and the disease severity data (%) subjected to statistical analysis.

(c) Pod infection

All the pods in each of the twenty plants were assessed for infection two weeks after the last spray using a scale developed based on the size and number of lesions on the pods.

(d) Yield and yield components

Dry beans were harvested and the following parameters taken:-

- Yield/hectare
- Number of seeds from the 20 plants
- Number of pods from the 20 plants
- The number of seeds from 10 pods
- Hundred seed weight.

4.RESULTS

4.1. Identification of bean halo and common bacterial blights pathogens:

The following identification results cover the observations made from various identification experiments carried out on the bacterial isolates.

4.1 (A) *P. syringae* pv. *phaseolicola*

(i) Growth on nutrient agar

On nutrient agar, circular, raised cream white colonies with smooth margins were observed after 48 hrs of incubation.

(ii) Gram reaction test

Under the microscope, the rod shaped bacterial cultures were observed to retain a pinkish red colour. This was an indication that they were gram negative.

(iii) Cell motility

Using the hanging drop technique, the bacterial cells were observed to be actively motile indicating that they were flagellated.

(iv) Potassium hydroxide solubility test

This was carried out as a confirmatory test to the gram-stain test. A thread of slime measuring about 1.5 cm was observed following the loop indicating a positive result for the KoH solubility test.

(v) Flourescein pigment production

The cultures produced blue-green flourescent pigment on King's B medium after 3 days of incubation.

vi. Levan production

A heavy mucoid, convex growth of colonies was observed on nutrient agar with 5% sucrose indicating a positive levan production test.

vii. Oxidase test

There was no colour change on the filter papers impregnated with 1% tetramethyl-p-phenylenediamine dihydrochloride. This indicated a negative oxidase test.

viii. Arginine dihydrolase test

No colour change was observed in the arginine medium stab-inoculated with bacterial cultures indicating a negative arginine dehydrolase test.

ix. Catalase reaction

A positive catalase reaction was indicated by the presence of effervescence after adding hydrogen peroxide onto bacterial culture.

x. Production of hydrogen sulphide

There was no hydrogen sulphide produced by cultures inoculated into nutrient broth containing cystein.

This was indicated by lack of colour change on the strips of filter papers containing lead acetate.

xi. Utilization of sugars

Production of acid by the bacterial cultures was observed in all sugars except mannitol. A positive test was indicated by a change in medium colour to yellow. The colour of the medium containing mannitol remained bluish-green.

xii. Pathogenicity test

The bacterial cultures used in this test induced watersoaking spots at the site of inoculation 6 days after inoculation. The leaves of cv Rosecoco-GLP-2 showed characteristic brown spot surrounded by yellow-green halo (plate 1). These tests clearly indicate that the isolated bacterial cultures were of *Pseudomonas syringae* pv. *phaseolicola*.

4.1 (B) *X. campestris* pv. *phaseoli*

The following identification results cover the observations made from various identification experiments carried out on the bacterial isolates.

(i) Growth on nutrient agar

On the nutrient agar, circular raised yellow colonies with smooth margins were observed after 48 hrs.

Plate 1: Leaflet of cv. Rosecoco-GLP-2 plant showing halo blight symptom (hbs) after inoculation with *Pseudomonas syringae* pv. *phaseolicola*.

Key hbs: - halo blight symptom.

(ii) Gram reaction test

Under the microscope, the rod shaped bacterial cells were observed to retain a pinkish red colour. This was an indication that they were gram negative.

(iii) Cell motility

Using the hanging drop technique, the bacterial cells were observed to be actively motile, indicating that they were flagellated.

(iv) Potassium hydroxide solubility (KoH) test

This was carried out as a confirmatory test to the gram-stain test. A thread of slime measuring about 1.5 cm was observed following the loop indicating a positive result for the KoH solubility test.

(v) Oxidase test

There was no colour change on filter papers impregnated with 1% tetramethyl-p-phenylenediamine dihydrochloride. This indicated a negative oxidase test.

(vi) Catalase reaction

A positive catalase reaction was indicated by the presence of effervescence after adding hydrogen peroxidase onto the bacterial cultures.

(vii) Levan production

No heavy mucoid, convex growth of colonies was observed on nutrient agar containing 5% sucrose indicating a negative levan production test.

(viii) Production of hydrogen sulphide

A black colour was observed on the strips of filter papers containing lead acetate. This shows a positive test for production of hydrogen sulphide.

(ix) Utilization of sugars

A positive test for production of acid was observed in all sugars except mannitol. The medium colour change to yellow in positive tests and bluish green in negative tests.

(x) Tolerance on TTC

The growth of culture isolates stab-inoculated on media containing 0.1% TTC and 0.02% TTC was inhibited even after 1 week of incubation.

(xi) Sodium chloride tolerance

The bacterial cultures grew on media containing 1% concentration of sodium chloride but not on media containing 3% and 5% concentration of sodium chloride.

(xii) Gelatin liquefaction

A clear zone was formed on the surface of the medium after flooding with acid mercuric chloride solution. This indicated that the culture isolates could liquify gelatin.

(xiii) Starch hydrolysis test

A clear zone was observed on the medium after flooding it with iodine solution indicating that the culture isolates hydrolysed starch.

(xiv) Pathogenicity test

The bacterial cultures used in this study induced watersoaking spots at the site of inoculation 4-day after inoculation. The leaves of cv. Rosecoco-GLP-2 showed characteristic brown spots surrounded by yellow margins (Plate 2). These tests clearly indicate that the bacterial cultures were *Xanthomonas campestris* pv. *phaseoli.*

Plate 2: Leaves of cv.Rosecoc-GLP-2 showing common bacterial blight symptoms (cbs) after inoculation with *Xanthomonas campestris* pv. *phaseoli.*

Key; cbs - common bacterial blight

4.2 Screening of plant extracts for antibacterial activity

4.2.1 *In vitro* test for antibacterial activity of plant extracts against phytobacterial pathogens.

Plant extracts from *E. citriodora, S. molle,T.minuta, S. chamaecyparissus, C. lucistanica, N. inodorum, C. cinerariaefolium* and *A. molluccana* as given in table 2 were tested for antibacterial activity against *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli.* All the active extracts were volatile oils except that of *N. inodorum* which was an acqueous extract. Out of the 14 plant extracts tested, extracts from *E. citriodora, N. inodorum, C. lucistanica, T. minuta* and *S. chamaecyparissus* were found to be active against *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli.*

Table 3 shows differences in the potency of the plant extracts and the sensitivity of *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli.* The growth inhibition zones produced by *E. citrodora* extract was significantly (P = 0.01) wider than those induced by *S. chamaecyparissus, T. minuta, C. lucistanica* and *N. inodorum* extracts on *P. syringae* pv. *phaseolicola* (Plate 3). The antibacterial activities of extracts from *N. inodorum* and *C. lucistanica* against *P. syringae* pv. *phaseolicola* were significantly (P = 0.01) higher than

Table 2: Antibacterial activity of plant extracts against two phytobacterial pathogens

Plant Species	Plant part	Extract form	Test pathogen	
			PSP	XCP
E. citriodora	Leaves	Steam distillate	+	+
N. inodorum	Leaves	Acqueous extract	+	+
C. lucistanica	Fruits	Steam distillate	+	+
S. chamaecyparissus	Aerial part	Steam distillate	+	+
T. minuta	Whole	- do -	+	+
T. minuta	Hasks	Chloroform extract	-	-
T. minuta	Hasks	Methanol extract	-	-
S. molle	Leaves	Steam distillate	-	-
C. cinerariaefolium	Marc	Methanol extract	-	-
C. cinerariaefolium	-do-	Chloroform extract	-	-
A. molluccana	Nuts	Hexane extract	-	-
A. molluccana	-do-	Methanol extract	-	-
A. molluccana	-do-	Chloroform extract	-	-

Key:

PSP - *P. syringae* pv. *phaseolicola*

XCP - *X. campestris* Pv *phaseoli*

\+ = Zone observed

\- = No zone observed.

Table 3: Zones of growth inhibition produced by plant extracts and streptomycin sulphate on *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli* (disc diameter = 11.5 mm).

Plant Extracts	Diameter in mm excluding disc*		
	P. syringae pv. *phaseolicola*	*X. campestris* pv. *phaseoli*	Mean Extracts
E. citriodora	24.50	32.83	28.67
N. inodorum	12.50	14.17	13.34
C. lucistanica	14.58	10.17	12.38
T. minuta	6.67	8.30	7.49
S. chamaecyparissus	2.83	2.67	2.75
Streptomycin sulphate 1%	34.83	35.33	35.08
Sterile distilled water	0.00	0.00	0.00
Mean pathogen	13.70	14.78	

*The values are means of 3 replicates.

	0.05	0.01
LSD (extracts)	1.51	1.82
LSD (pathogens)	0.81	0.97
LSD (Extract x pathogen)	2.14	2.58

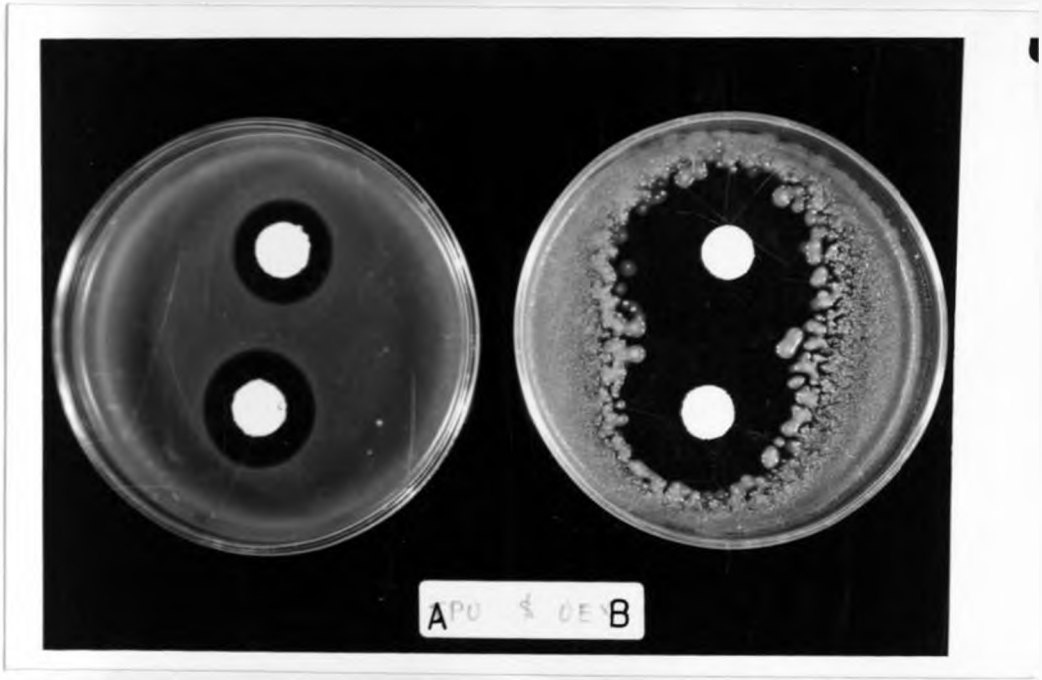


Plate 3: Growth inhibition zones produced by *Tagetes minuta* extract (A) and *Eucalyptus citriodora* extract (B) on *Pseudomonas syringae* pv. *phaseolicola* cultures on nutrient agar after 2 days of incubation.

those of extracts from *T. minuta* and *S. chamaecyparissus. T. minuta* extract showed significantly (P = 0.01) higher activity on the halo blight pathogen than the extract from *S. chamaecyparissus.* Although the extract from *S. chamaecyparissus* showed the lowest activity among the plant extracts tested, it had a significantly (P = 0.01) higher activity than the control (sterile distilled water). Streptomycin sulphate at 1% concentration showed significantly (P = 0.01) wider zones of growth inhibition on *P. syringae* pv. *phaseolicola* when compared to all the plant extracts tested (Plate 4).

The zones of growth inhibition produced by *E. citriodora* extract was significantly (P = 0.01) wider than those induced by *S. chamaecyparissus, T. minuta, C. lucistanica* and *N. inodorum* extracts on *X. campestris* pv. *phaseoli.* Extracts from *N. inodorum* showed a significantly (P = 0.01) higher antibacterial activity against *X. campestris* pv. *phaseoli* than those from *C. lucistanica, T. minuta* and *S. chamaecyparissus.* The zones of growth inhibition produced by extracts from *C. lucistanica* and *T. minuta* on *X. campestris* pv. *phaseoli* were not significantly (P = 0.05) different but they were significantly (P = 0.01) wider than those induced by *S. chamaecyparissus* extract. The antibacterial activity of *S.chamaecyparissus* extract was noted to be significantly

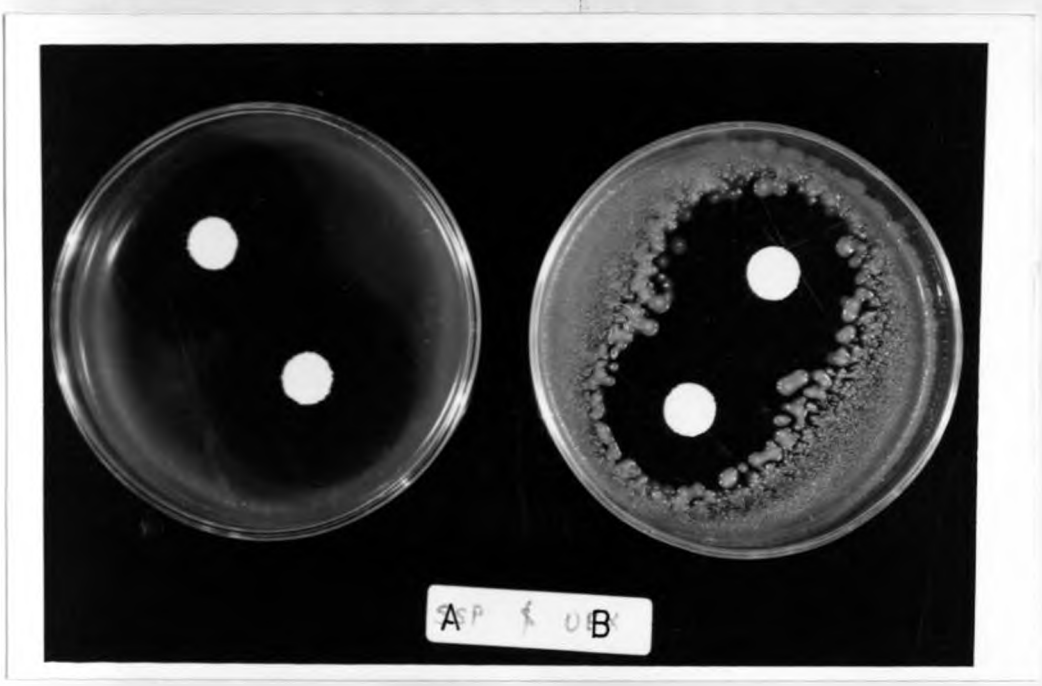


Plate 4: Growth inhibition zones produced by 1% streptomycin sulphate (A) and *Eucalyptus citriodora* extract (B) on *Pseudomonas syringae* pv. *phaseolicola* cultures on nutrient agar after 2 days of incubation.

(P = 0.01) higher when compared to control (sterile distilled water). Streptomycin sulphate at 1% concentration produced significantly (P = 0.01) wider zones of growth inhibition on *X. campestris* pv. *phaseoli* than all the plant extracts used in this study. There was an interaction between the test pathogens and the extract type indicating that the two test pathogens had a different response to a particular extract (Appendix 1). The sensitivity of *X campestris* pv. *phaseoli* to all the extracts was significantly (P = 0.01) higher than that of *P. syringae* pv. *phaseolicola*. Among the plant extracts which were found to be active, *E. citriodora* extract was noted to have a significantly (P = 0.01) higher activity against halo blight and common blight bacteria.

4.2.2. Antibacterial activity of separated fractions of plant extracts.

The crude extracts, ether and hexane fractions of *E. citriodora, T. minuta, C. lucistanica* and *S. chamaecyparissus* were tested for antibacterial activity against *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli*. All the different forms of plant extracts tested were active against *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli* except the hexane fraction of *S. chamaecyparissus*. Table 4 shows the growth inhibition zones produced by crude extracts, ether and

Table 4: The antibacterial activities of crude plant extracts and their ether and hexane fractions against halo and common bacterial blight pathogens.

Test pathogen (A)	Plant species (C)	Diameter* in mm excluding disc (11.5 mm) Plant extracts (B) CE	HF	EF	E	H	Means (A x C)	Mean (A)
P. syringae pv. *phaseolicola*	*E. citriodora*	24.50	6.00	8.38	0	0	7.78	
	T. minuta	6.63	3.50	5.30	0	0	3.09	4.52
	C. lucistanica	14.56	3.63	9.25	0	0	5.49	
	S. chamaecyparissus	2.88	0	5.58	0	0	1.70	
	Means (A x B)	12.14	3.28	7.13	0	0		
X. campestris pv. *phaseoli*	*E. citriodora*	32.88	10.00	10.75	0	0	10.73	
	T. minuta	8.50	3.75	8.50	0	0	4.15	
	C. lucistanica	10.13	6.50	13.00	0	0	5.93	5.64
	S. chamaecyparissus	2.63	0	6.00	0	0	1.73	
	Means (A x B)	13.54	5.06	9.56	0	0		
							Mean (C)	
Means (CxB)	*E. citriodora*	28.69	8.00	9.57	0	0	9.25	
	T. minuta	7.57	3.63	6.90	0	0	3.62	
	C. lucistanica	12.35	5.07	11.13	0	0	5.71	
	S. chamaecyprissus	2.76	0	5.79	0	0	1.71	
	Mean (B)	12.84	4.18	8.35	0	0		

* The values represent a mean of 4 replicates.

CE = crude extract

HF = Hexane fraction

EF = Ether fraction

E = Di-ethylether

H = hexane

	0.05	0.01
LSD(A)	0.19	0.25
LSD(B)	0.30	0.40
LSD(C)	0.27	0.35
LSD(AxB)	0.42	0.56
LSD (AxC)	0.38	0.50
LSD (BxC)	0.59	0.79
LSD (AxBxC)	0.84	1.11

hexane fractions of *E. citriodora, T. minuta, C. lucistanica* and *S. chamaecyparissus* on *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli.* The growth inhibition zones produced by the crude form of *E. citriodora* on *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli* were significantly (P = 0.01) wider than those produced by ether and hexane fractions from the same plant. The ether fraction of *E. citriodora* extract showed a significantly (P = 0.01) higher activity than the hexane fraction on *P. syringae* pv. *phaseolicola*, but no significant (P = 0.05) difference was observed between their activity on *X. campestris* pv. *phaseoli.* The crude extract of *T. minuta* produced a significantly (P = 0.01) wider zone of growth inhibition on *P. syringae* pv. *phaseolicola* than ether and hexane fractions but when tested against *X. campestris* pv. *phaseoli*, no significant (P = 0.05) difference was observed between the activity of the crude extract and ether fraction. The ether fraction was significantly (P = 0.01) more active than the hexane fraction (Plate 5) on *X. campestris* pv. *phaseoli.* The activity of the crude extract of *C. lucistanica* on *P. syringae* pv. *phaseolicola* was significantly (P = 0.01) higher than those of the ether and hexane fraction but when tested against *X. campestris* pv. *phaseoli,* the ether fraction was noted to have a significantly (P = 0.01) higher activity than the crude extract and hexane fraction. The growth inhibition zones produced by the

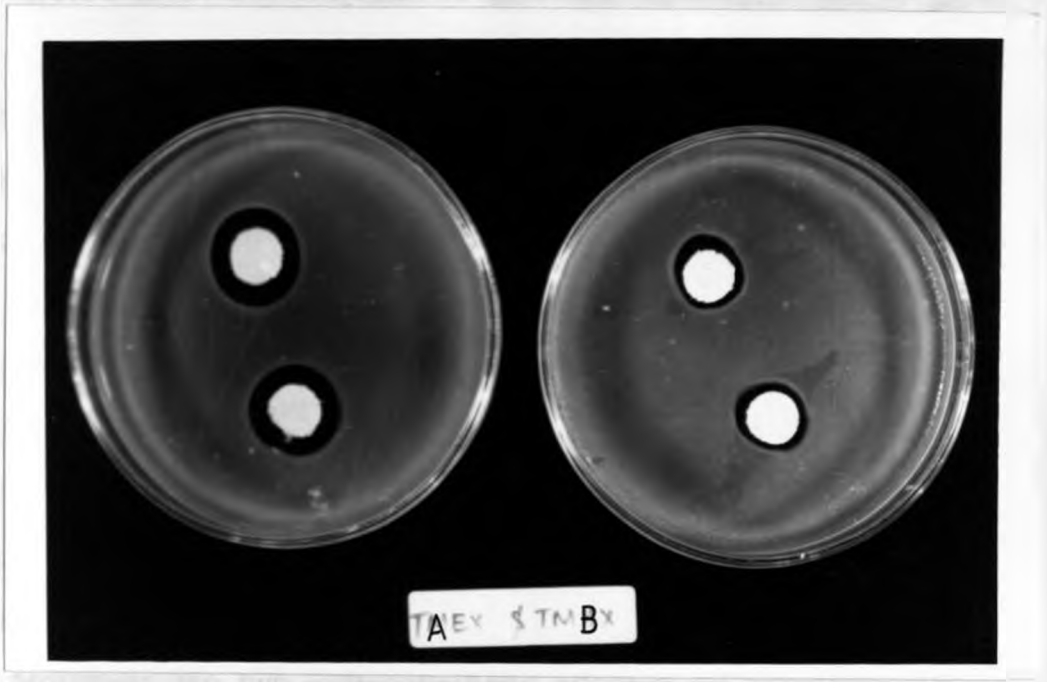


Plate 5: Growth inhibition zones produced by ether (A) and hexane (B) fractions of *Tagetes minuta* extract on *Pseudomonas syringae* pv. *phaseolicola* cultures on Nutrient agar.

ether fraction of *S. chamaecyparissus* on *P. syringae* pv. *phaseolicola* were significantly (P = 0.01) wider than those produced by the crude extract and hexane fraction. This was also noted when the extracts were tested against *X. campestris* pv. *phaseoli*. Generally, the extracts from *E. citriodora* had a significantly (P = 0.01) higher activity than those from *T. minuta, C. lucistanica* and *S. chamaecyparissus*. The crude forms of all the extracts were significantly (P = 0.01) more active on *X. campestris* pv.*phaseoli* and *P. syringae* pv. *phaseolicola* than hexane fraction. No zone of growth inhibition was produced by di-ethylether and hexane on *P. syringae* pv. *phaseolicola* and *X. campestris* pv. phaseoli. *X. campestris* pv. *phaseoli* was significantly (P = 0.01) more sensitive than *P. syringae* pv. *phaseolicola* to the plant extracts used in this study (Appendix 2). However, the two test pathogens showed no significant (P = 0.05) difference in response to *S. chamaecyparissus* extract.

4.2.3 Minimum inhibitory concetrations of plant extracts against *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli*

The minimum inhibitory concentrations of the crude extracts and ether fractions of *E. citriodora* and *T. minuta* were determined using *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli* as the test pathogens.

(a) Crude extract of *E. citriodora* and *T. minuta*

The minimum inhibitory conentrations of the crude extracts of *T. minuta* and *E. citriodora* were determined against *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli* using tube dilution technique. Table 5 shows the minimum inhibitory concentrations of the two extracts on *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli*. The tube dilution technique shows varying results from petri-dish zonal inhibition technique. *T. minuta* extract could cause total inhibition of growth of the two phytobacterial pathogens at 0.20% concentration whereas *E. citriodora* extract caused total growth inhibition of *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli* at 0.78% and 0.39% concentrations respectively. This indicates that *T. minuta* extract had a higher potency than *E. citriodora* extract on the two phytobacterial pathogens. The results indicate that the minimum inhibitory concentration of *T. minuta* extract on *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli* lies between 0.20% and 0.10% concentrations (plate 6a and 6b). The minimum inhibitory concentration of *E. citriodora* extract on *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli* lies between 0.78% and 0.39% concentration; and 0.39% and 0.20% concentrations respectively.

Table 5: **Minimum inhibitory concentration of *E. citriodora* and *T. minuta* extracts against *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli*.**

Concentration in %	E. citriodora extract		T. minuta extract	
	PSP	XCP	PSP	XCP
50	-	-	-	-
25	-	-	-	-
12.5	-	-	-	-
6.25	-	-	-	-
3.125	-	-	-	-
1.525	-	-	-	-
0.78125	-	-	-	-
0.390625	+	-	-	-
0.1953125	+	+	-	+
0.0976565	+	+	+	+
0.0488281	+	+	+	+

Key:

- No growth

+ growth

PSP = *P. syringae* pv. *phaseolicola*

XCP = *X. campestris* pv. *phaseoli*

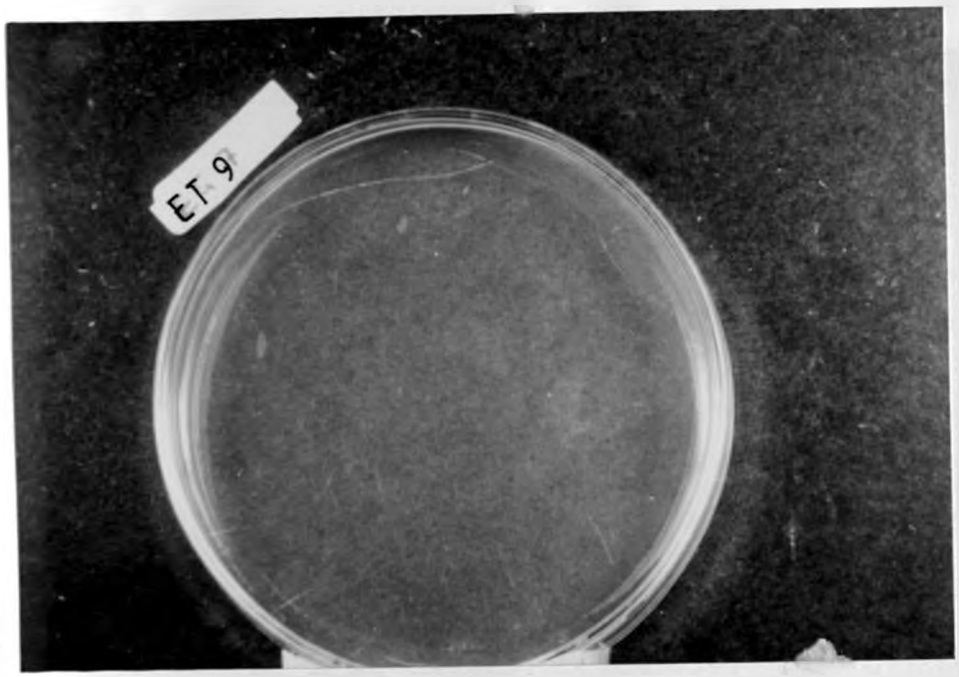


Plate 6a: Total inhibition of growth of *Xanthomonas campestris* pv. *phaseoli* by 0.20% concentration *Tagetes minuta* extract.

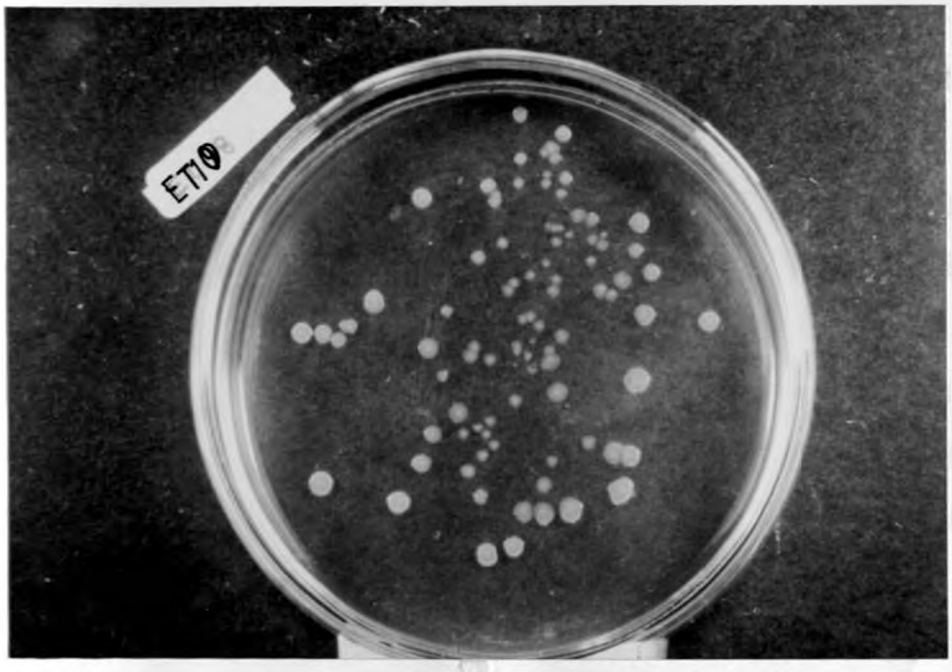


Plate 6b: Some colonies of *Xanthomonas campestris* pv. *phaseoli* that survived after treatment with 0.10% concentration *Tagetes minuta* extract on nutrient agar after 2 days of incubation.

(b) Ether fractions of *E. citriodora* and *T. minuta* extracts.

Table 6 shows the diameters of zone of growth inhibition produced by different concentrations of ether fractions of *E. citriodora* and *T. minuta* on *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli*. About 50% ether fraction of *E. citriodora* was observed to produce a wider zone of inhibition when compared to the undiluted form (Plate 7 and 8) on *X. campestris* pv. *phaseoli*. As the concentrations of the extracts decreased, the zones of inhibition were noted to decrease. Based on the petri-dish zonal inhibition technique, the minimum inhibitory concentration of *E. citriodora* ether fraction lies between 1.56% and 3.13% for the two test pathogens. No zones of growth inhibition were observed around discs impregnated with 6.25% concentration of *T. minuta* ether fraction. This suggests that the minimum inhibitory concentration of this extract on *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli* lies between concentrations of 6.25% and 12.5%. Generally, the activity of the ether fraction of *E. citriodora* was significantly ($P = 0.01$) higher than that of *T. minuta*. The sensitivity of the two test pathogens to the two extracts was not significantly different at $P = 0.05$. There was no interaction between the pathogens and extract concentration (Appendix 3).

Table 6: Zones of growth inhibition[a] produced by diluted ether fractions of the essential oils of *E. citriodora* and *T. minuta* (disc diameter = 11.5 mm).

Test pathogen (A)	Extract type (C)	Extract concentration % (B) 50	25	12.5	6.25	3.13	1.56	0.78	Means (AxC)	Mean (A)
P. syringae pv. *phaseolicola*	*E. citriodora* ether fraction	34.75	23.75	15.75	3.63	12.50	0	0	14.34	
	T. minuta ether fraction	17.5	14.63	12.50	0	0	0	0	6.38	10.36
	Mean (A x B)	26.13	19.19	14.13	6.82	6.25	0	0		
X. campestris pv. *phaseoli*	*E. citriodora* ether fraction	35.75	25.00	15.50	14.00	12.50	0	0	14.68	
	T. minuta ether fraction	16.25	14.00	12.50	0	0	0	0	6.11	10.39
	Means (A x B)	26.00	14.50	14.00	7.00	6.25	0	0		
Means (AxC)	*E. citriodora* ether fraction	35.25	24.38	15.63	13.82	12.50	0	0		Mean (C) 14.51
	T. minuta ether fraction	16.88	14.32	12.50	0	0	0	0		6.25
	Mean (B)	26.06	19.35	14.06	6.91	6.25	0	0		

[a]The values are averages of 4 replications.

	0.05	0.01
LSD(A)	0.15	0.19
LSD(B)	0.27	0.36
LSD(C)	0.15	0.19
LSD(AxB)	0.39	0.52
LSD (AxC)	0.21	0.28
LSD (BxC)	0.39	0.52
LSD (AxBxC)	0.55	0.73

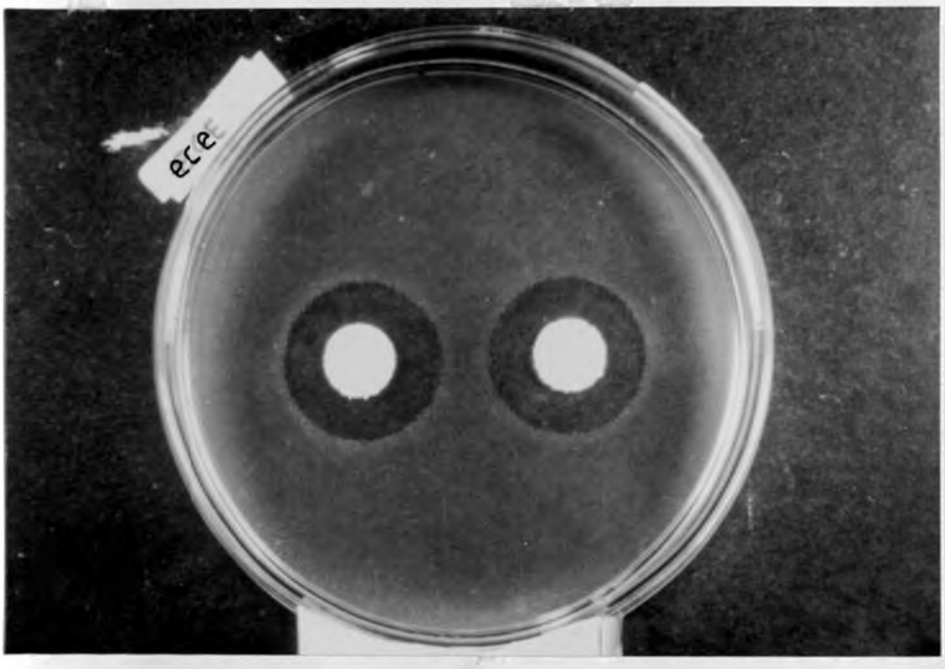


Plate 7: Zone of growth inhibition produced by undiluted ether fraction of *Eucalyptus citriodora* extract on *Xanthomonas campestris* pv. *phaseoli* cultures on nutrient agar after 2 days of incubation.

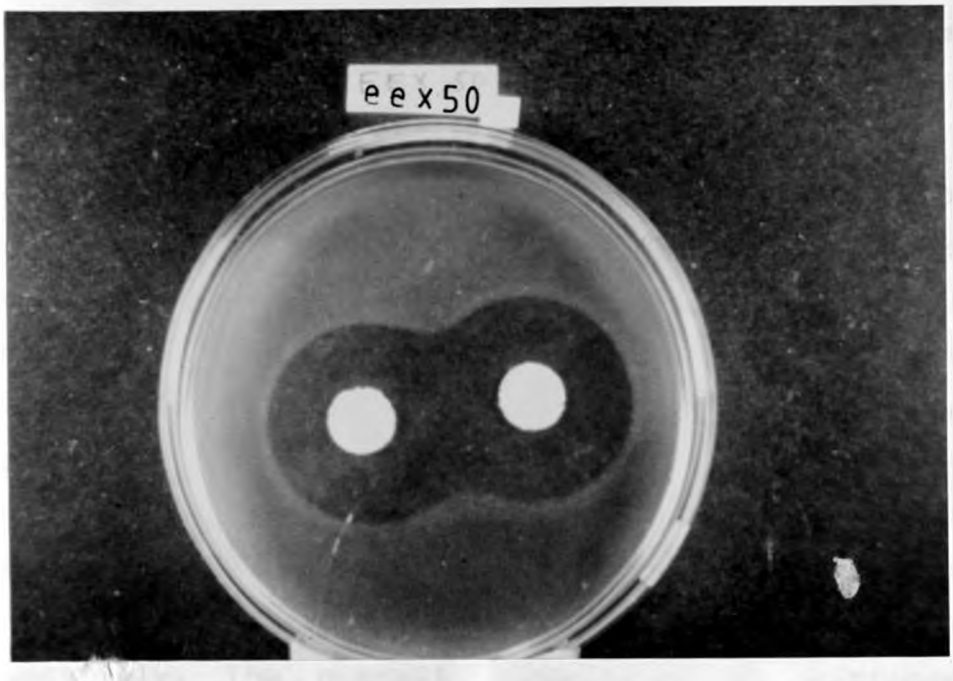


Plate 8: Zones of growth inhibition produced by 50% concentrations of ether fraction of *Eucalyptus citriodora* extract on *Xanthomonas campestris* pv. *phaseoli* cultures on nutrient agar after two days of incubation.

4.2.4 Effect of temperature on the antibacterial activity of plant extracts

The crude extracts (volatile oils) from *E. citriodora* and *T. minuta* were subjected to different temperatures viz., 0°C, 10°C, 20°C, 30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C, 100°C and 121°C and then tested for antibacterial activities against *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli*. Appendix 4a shows the growth inhibition zones produced by *E. citriodora* and *T. minuta* extracts on *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli* after the extracts were subjected to the various temperatures. No significant (P = 0.05)) difference was observed between the activity of plant extracts subjected to various temperatures (Appendix 4b). For instance, *E. citriodora* extracts subjected to 0°C and that subjected to 121°C produced zones of growth inhibition on *P. syringae* pv. *phaseolicola* measuring 31.00 mm and 33.75 mm respectively. The overall activity of *E. citriodora* extract against the two phytobacterial pathogens was significantly (P = 0.01) higher than that of *T. minuta* extract at all temperatures. Figs. 1 and 2 show the response of the antibacterial activity of the plant extracts subjected to different temperatures. *E. citriodora* volatile oils subjected to 10°C and 20°C produced the widest growth inhibition zones on *X. campestris pv. phaseoli*. The growth inhibition zones produced by *T. minuta* extract on *P. syringae* pv. *phaseolicola* after subjecting the extracts to 0°C and

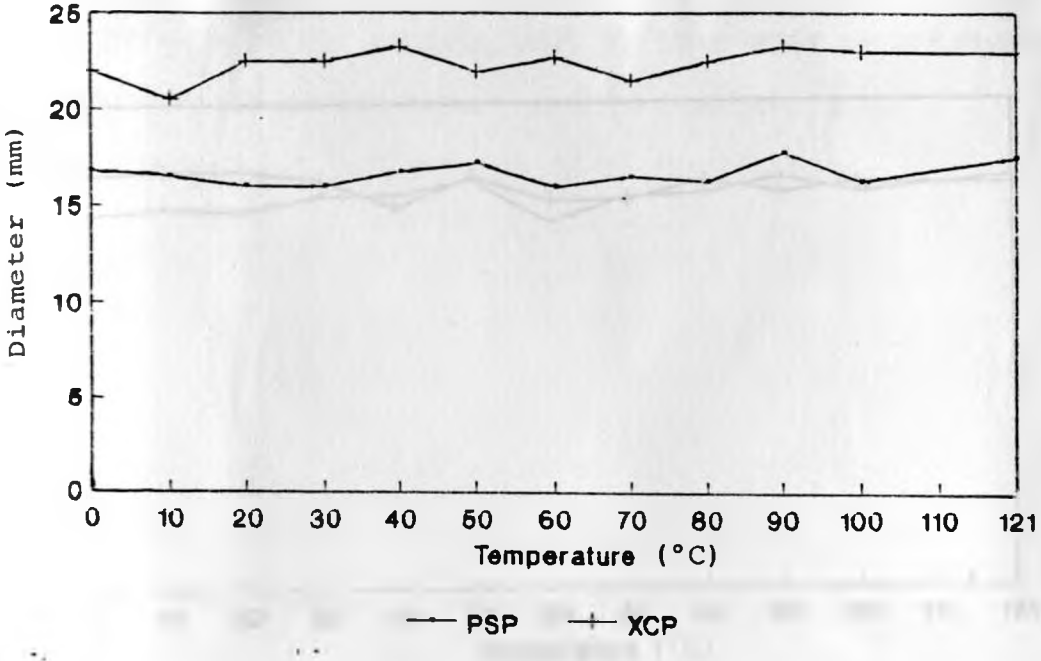
Table 7: **Effect of temperature on the activity of *T. minuta* and *E. citriodora* extracts[a] on *P. syringae* pv. *phaseolicola* and *X. Campestris* pv. *phaseoli*.**

Test pathogen (A)	Extract type (C)	Temperature °C (B) 0	10	20	30	40	50	60	70	80	90	100	121	Means AxC	Mean(A)
P. syringae pv. *phaseolicola*	*E. citriodora*	31.00	31.50	31.25	32.50	32.50	33.75	30.25	32.25	32.50	33.75	32.50	33.75	32.29	
	T. minuta	16.75	16.50	16.00	16.00	16.75	17.25	16.00	16.50	16.25	17.75	16.25	17.50	16.63	24.46
	Means (AxB)	23.88	24.00	23.63	24.25	24.63	25.50	23.13	24.38	24.38	25.75	24.38	25.63		
X. campestris pv. *phaseoli*	*E. citriodora*	34.25	34.50	34.50	33.75	31.50	34.00	32.00	32.00	33.50	32.50	33.25	33.00	33.23	
	T. minuta	22.00	20.50	22.50	22.50	23.25	22.00	22.75	21.50	22.50	23.25	23.00	23.00	22.40	27.82
	Means(AxB)	28.13	27.50	28.50	28.13	27.38	28.00	27.38	26.75	28.00	27.88	28.13	28.00		
															Mean(C)
Means (AxC)	*E. citriodora*	32.63	33.00	32.88	33.13	32.00	33.88	31.13	32.13	33.00	33.13	32.88	33.38		32.81
	T. minuta	19.38	18.50	19.25	19.25	20.00	19.63	19.38	19.00	19.38	20.15	19.63	20.25		19.51
	Mean (B)	26.01	25.75	26.07	26.19	26.00	26.76	25.26	25.57	26.19	26.82	26.26	26.82		

[a]Means of 4 replicates

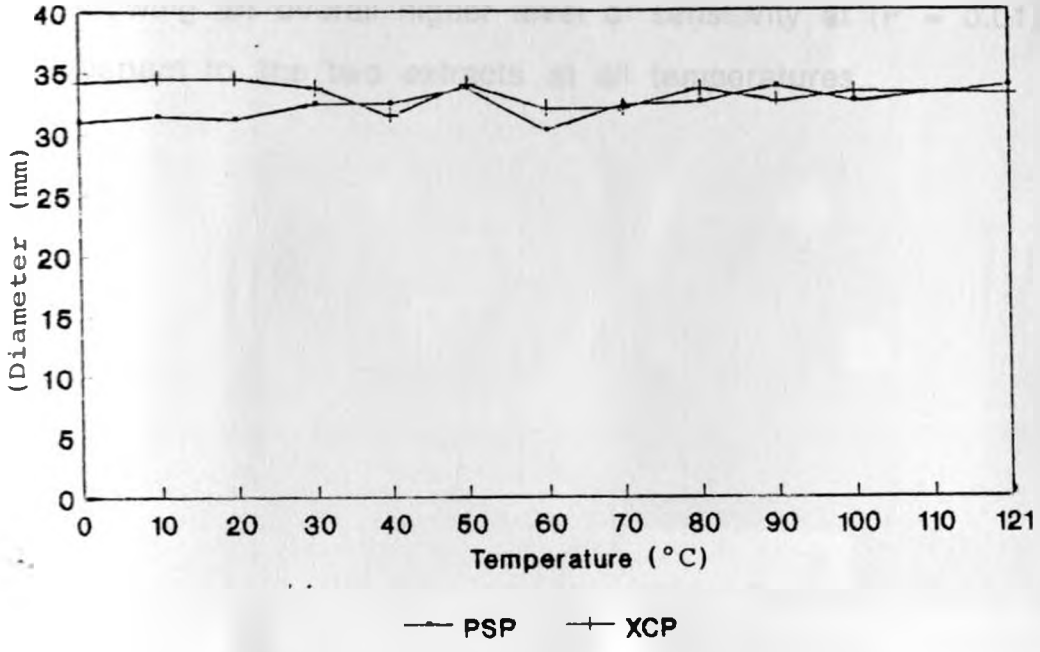
	0.05	0.01		0.05	0.01
LSD(A)	0.58	0.76	LSD(AC)	0.82	1.07
LSD(B)	1.42	1.86	LSD(BC)	2.00	2.63
LSD(C)	0.58	0.76	LSD(ABC)	2.83	3.72
LSD(AB)	2.00	2.63			

Fig. 1: Effect of temperature on the activity of *T. Minuta* extract against *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli*.



PSP • P.syringae pv. phaseolicola
XCP • X. campestris pv. phaseoli

Fig. 2: Effect of temperature on the activity of *E. citriodora* extract against *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli*



PSP = P. syringae pv. phaseolicola
XCP = X. campestris pv.phaseoli

121 °C for 15 minutes were not significantly (P = 0.05) different (Plate 9).

There was an interaction between the pathogens and extract type (Appendix 4). This shows that the bacteria responded differently to the extracts, with *X. campestris* pv. *phaseoli* showing an overall higher level of sensitivity at (P = 0.01) with respect to the two extracts at all temperatures.

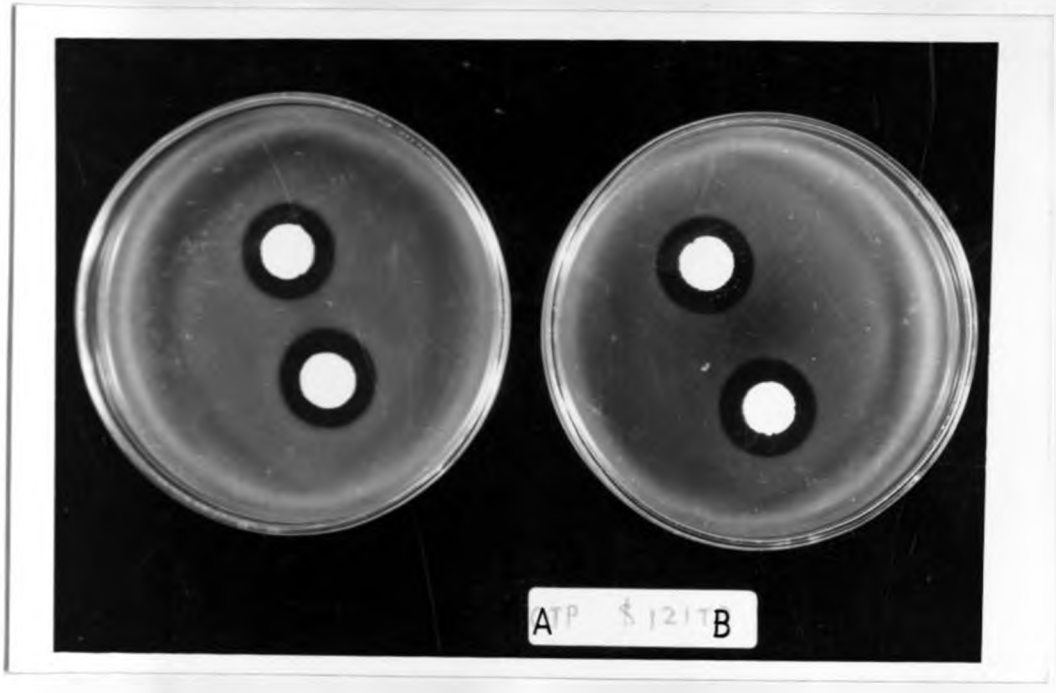


Plate 9: Comparison of the activity of the volatile oil of *Tagetes minuta* subjected to 0 °C (A) and 121 °C (B), against *Pseudomonas syringae* pv. *phaseolicola* in culture after 2 days of incubation.

4.3 Phytotoxicity test

Plant extracts from *E. citriodora* and *T. minuta* were tested for phytotoxicity on bean plants. The different concentrations used included:- 0.78%, 0.59%, and 0.39% concentrations of *E. citriodora* volatile oil; 3.13%, 2.34% and 1.56% concentrations of the ether fraction of *E. citriodora* volatile oil; 0.20%, 0.15%, 0.10% concentrations of *T. minuta* volatile oil; 12.50%, 9.38% and 6.25% concentrations of ether fraction of *T. minuta* volatile oil; and 100%, 50% and 25% concentrations of diethylether. All the bean plants sprayed with different concentrations of *E. citriodora* crude extract (volatile oil) showed no phytotoxicity effect. Bean plants sprayed with 3.13% concentration of ether fraction of volatile oil from *E. citriodora* showed some brown spots on leaves but other concentrations had no effect. Seeds treated with 2.34% concentration of ether fraction of volatile oil from *E. citriodora* did not germinate. Foliar spray with 12.50%, 9.38% and 6.25% concentrations of ether fraction of volatile oil from *T. minuta* resulted in defoliation (plate 10). Bean plants sprayed with the crude form (volatile oil) from *T. minuta* remained healthy except some brown spots which were noted on the leaves of bean plants sprayed with 0.20% concentration of the volatile oil. Seeds treated with 9.38% concentration of ether fraction of volatile oil from *T. minuta* did not germinate.

Plate 10: Defoliation of cv. Rosecoco-GLP-2 plants after spraying with 6.25% concentration of ether fraction of volatile oil from *Tagetes minuta* (A) compared to control plants sprayed with water (B).

Bean seeds which were soaked in 100%, 50% and 25% concentrations of di-ethylether did not germinate. The seedlings which were sprayed with 100%, 50% and 25% concentration of di-ethylether showed partial wilting on one side of the midrib of bean leaves. Those sprayed with 5% concentration of di-ethylether and sterile distilled water did not show any signs of phytotoxicity.

4.4. *In vivo* test for the control of the two bacterial blights of beans in the greenhouse.

Some bean seeds were inoculated with the phytobacterial pathogens and then treated with different concentrations of extracts from *E. citriodora* and *T. minuta*. Foliar inoculated seedlings were sprayed with the plant extracts and then assessed for infection. A scoring system, developed based on the percent leaf area infected was used for assessment (plate 11). The scoring system followed is outlined below .

Severity scale of halo blight and common blight of beans.

Scale:

1. No visible disease symptoms.
2. Approximately 5% of the leaf surface area covered with round lesions.
3. Approximately 10% of the leaf surface area covered with lesions of about 5mm in diameter. There is some limited systemic chlorosis.

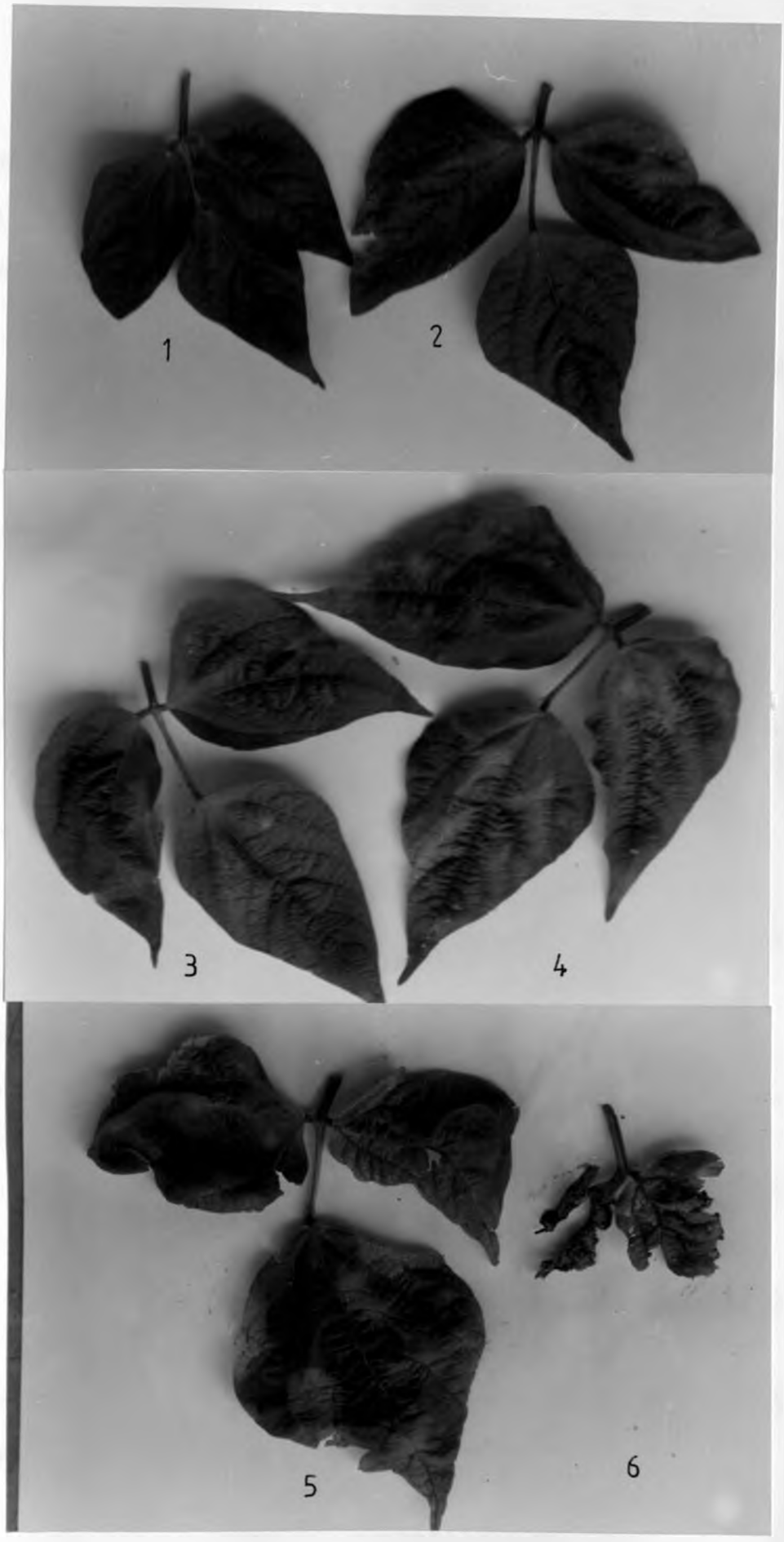


Plate 11: Disease severity scale used for assessment of bean infection by Pseudomonas syringe pv phaseolicola.

4. Approximately 25% of the leaf surface area affected by either large lesions or by resulting chlorosis and there is limited leaf distortion.
5. Approximately 50% of the leaf surface area affected by chlorosis and coaleascing lesions that measure 10 mm in diameter. The leaf appears distorted and the necrotic areas have fallen out, leaving some holes.
6. 75% or more of the leaf tissue affected by necrotic lesions and chlorosis. The leaf is distorted and some necrotic parts have fallen out, leaving the leaf tattered. Defoliation also occurs.

Table 8a shows the severity of halo blight on cv Rosecoco-GLP-2 plants treated with different plant extracts, using different methods of application. There was a significant (P = 0.01) difference among the treatments (Appendix 5a). Copper oxychloride (0.1% a.i) and streptomycin sulphate (0.1% a.i) used as standard treatments for halo blight control gave the best control of the disease compared with the other foliar sprays. Bean plants treated with copper oxychloride (0.1% a.i) and streptomycin sulphate (0.1% a.i) had significantly (P = 0.01) lower halo blight infection than those plants sprayed with plant extracts (Table 8a). There was no significant (P = 0.05) difference between halo blight infection of bean plants arising from seeds treated with 0.59% and 0.15% concentrations of *E. citriodora* and *T. minuta* extracts respectively and those plants sprayed with copper oxychloride (0.1% a.i) and streptomycin

sulphate (0.1% a.i). These two crude plant extracts reduced halo blight infection significantly (P = 0.01) when compared to untreated control (Plate 12). Halo blight infection on bean plants sprayed with 3.13% ether fraction of *E. citriodora* extract was significantly (P = 0.01) lower than that of the untreated control. There was no significant (P = 0.05) difference in halo blight infection of beans sprayed with 0.78%, 0.59% and 0.39% concentrations of *E. citriodora* volatile oil and 2.34% and 1.56% concentrations of ether fraction of volatile oil from *E. citriodora* and 0.20%, 0.15% and 0.10% concentrations of *T. minuta* volatile oil and the untreated control.

Table 8a. Severity of haloblight on cv. Rosecoco-GLP-2 plants which had received various treatments.

Treatments	*Haloblight severity (%) on leaves as on 3rd July 1992
1. Seed dressed with 0.59% *E. citriodora* extract	3.26
2. Seed dressed with 2.34% ether - *E. citriodora extract*	-
3. Seed dressed with 0.15% *T. minuta*	5.76
4. Seed dressed with 9.38% ether-*T. Minuta* extract	-
5. Seed inoculated (control)	14.60
6. Foliar inoculated + sprayed with 0.78% *E. citriodora* extract	15.20
7. Foliar inoculated + sprayed with 0.59% *E. citriodora* extract	14.17
8. Foliar inoculated + sprayed with 0.39% *E. citriodora* extract	14.59
9. Foliar inoculated + sprayed with 3.13% ether - *E. citriodora* extract	12.40
10. Foliar inoculated + sprayed with 2.34% ether - *E. citriodora* extract.	14.42
11. Foliar inoculated + sprayed with 1.56% ether - *E. citriodora* extract	14.17
12. Foliar inoculated + sprayed with 0.20% *T. minuta* extract	13.43
13. Foliar inoculated + sprayed with 0.15% *T. minuta* extract	13.19
14. Foliar inoculated + sprayed with 0.10% *T. minuta* extract	14.59
15. Foliar inoculated + sprayed with 12.50% Ether - *T. minuta* extract	-
16. Foliar inoculated + sprayed with 9.38% ether - *T. minuta* extract	-
17. Foliar inoculated + spray with 6.25% ether - *T. minuta* extract	-
18. Foliar inoculated + spray with 0.1% streptomycin sulphate	2.90
19. Foliar inoculated + spray with 0.1% Copper oxychioride	3.45
20. Foliar inoculated (control)	14.33
Mean	**11.36**

* The values represent a mean of 3 replicates

- No data was collected due to defoliation which occured after spray with the plant extracts.
- The extracts were all volatile oils

$LSD_{0.05} = 2.20$

$LSD_{0.01} = 2.96$

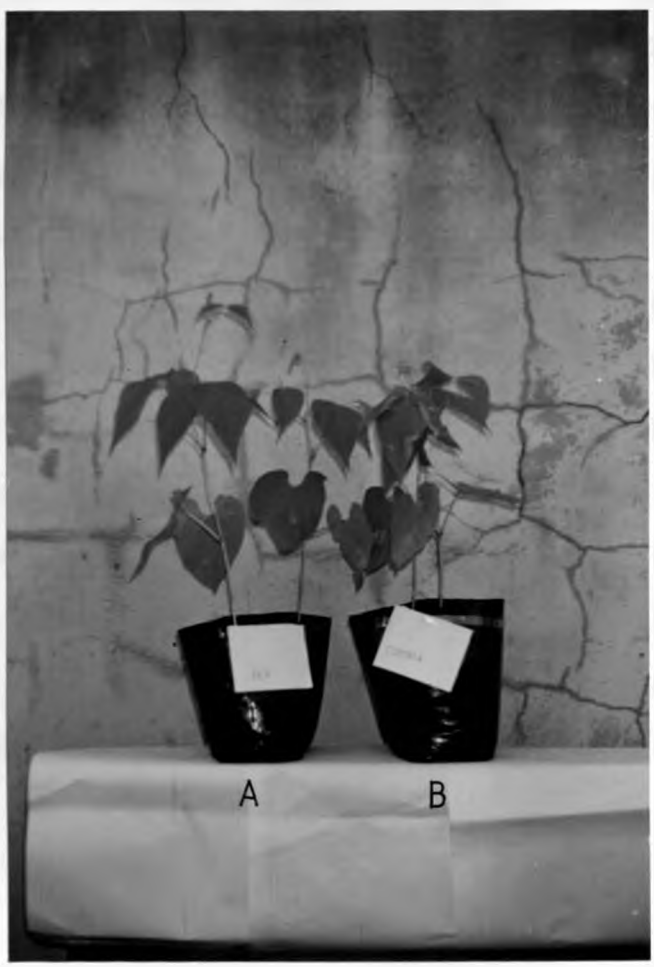


Plate 12: Cv. Rosecoco-GLP-2 plants treated with *Eucalyptus citriodora* volatile oil as a seed soak (A) against halo blight of beans and an unsprayed control (B).

Table 8b shows the severity of common blight on bean plants sprayed with different plant extract concentrations. There was no significant (P = 0.05) difference in common blight severity among bean plants from diseased seeds dressed with 0.59% and 0.15% concentrations of *E. citriodora* and *T. minuta* volatile oil respectively. There was no significant (P = 0.05) difference in common blight infection among bean plants which had received seed dressing treatments with *E. citriodora* and *T. minuta* volatile oils at 0.59% and 0.15% concentrations respectively and those foliar sprayed with streptomycin sulphate (0.1% a.i) and copper oxychloride (0.1% a.i) [Table 8b]. *E. citriodora* volatile oils (0.59%) and *T. minuta* volatile oils (0.15%) used for seed dressing; and copper oxychloride (0.1% a.i) and streptomycin sulphate (0.1% a.i) used as foliar sprays reduced disease severity when compared to common blight infection in unsprayed control (appendix 5b). Volatile oils from *E. citriodora* (0.39%) and ether fraction of the volatile oil from *E. citriodora* (3.13%) applied as foliar sprays significantly (P = 0.05) reduced the common bacterial blight infection when compared to the unsprayed control.

Table 8b. Severity of common blight on bean plants which had received various treatments.

Treatments	*Commonblight severity (%) on leaves as on 3rd July 1992
1. Seed dressed with 0.59% *E. citriodora* extract.	6.47
2. Seed dressed with 2.34% ether - *E. citriodora* extract	-
3. Seed dressed with 0.15% *T. minuta* extract	5.42
4. Seed dressed with 9.38% ether - *T. minuta* extract	-
5. Seed inoculated (control)	15.40
6. Foliar inoculated + sprayed with 0.39% *E. citriodora* extract	10.34
7. Foliar inoculated + sprayed with 0.29% *E. citriodora* extract	12.65
8. Foliar inoculated + sprayed with 0.20% *E. citriodora* extract.	15.07
9. Foliar inoculated + sprayed with 3.13% ether - *E. citriodora* extract	10.66
10. Foliar inoculated + sprayed with 2.34% ether - *E. citriodora* extract.	14.08
11. Foliar inoculated + sprayed with 1.56% ether - *E. citriodora* extract.	13.44
12. Foliar inoculated + sprayed with 0.20% *T. minuta* extract	12.99
13. Foliar inoculated + sprayed with 0.15% *T. minuta* extract	15.23
14. Foliar inoculated + sprayed with 0.10% *T. minuta* extract	14.58
15. Foliar inoculated + sprayed with 12.50% ether - *T. minuta* extract	-
16. Foliar inoculated + sprayed with 9.38% ether - *T. minuta* extract	-
17. Foliar inoculated + sprayed with 6.25% ether - *T. minuta* extract	-
18. Foliar inoculated + sprayed with 0.1% streptomycin sulphate	3.91
19. Foliar inoculated + sprayed with 0.1% copper oxychloride	4.05
20. Foliar inoculated control	14.33
Mean	**11.25**

• The values represent a mean of 3 replicates

- No data was collected due to defoliation which occured after spraying with the plant extracts.

- The extracts were all volatile oils

$LSD_{0.05} = 3.45$

$LSD_{0.01} = 4.65$

There was no significant (P = 0.05) difference in common bacterial blight infection on beans sprayed with 0.29%, 0.20% concentrations of *E. citriodora* volatile oils and 2.34%, 1.56% concentrations of ether fraction of *E. citriodora* volatile oil and 0.20%, 0.15%, 0.1% concentrations of *T. minuta* volatile oil and the untreated control.

The bean seeds which were soaked in 2.34% and 9.38% concentrations of the ether fractions of volatile oils from *E. citriodora* and *T. minuta* respectively failed to germinate. Defoliation occured from all the plants that were sprayed with 6.25%, 9.38% and 12.5% ether fraction of *T. minuta* volatile oil. No data was collected on these treatments and therefore they were excluded in the analysis of variance.

4.5 Efficacy of various rates of extracts from *E. citriodora* against *P. syringae* pv. *phaseolicola.*

4.5.1 Season 1, October 1991-January, 1992

The prevailing weather conditions at Kabete Campus during October 1991-January 1992 are given in Appendix 6. The low rainfall and high temperature did not favour bean halo blight infection. Bean plants treated with the volatile oil from *E. citriodora* at (0.59%) concentration were assessed for infection by halo blight. The severity of halo blight on beans, expressed as a percent infection of the total leaf area was assessed using the disease scale described earlier.

Disease incidence was recorded as the number of plants

infected out of the tagged 20 plants and expressed as a percent. Pod infection was assessed based on number of lesions and their sizes on pods (Plate 13). The following scoring system was used on pod infection:-

1. No visible disease symptoms on pods.
2. 1-2 watersoaked lesions on the pod measuring about 5 mm in diameter.
3. A few watersoaked lesions on the pod which had coalesced to measure 10 mm in diameter.
4. Several coalesced watersoaked lesions on the pod causing deformation.
5. Many coalesced watersoaked lesions all over the pod surface producing bacterial exudates. The pod appeared distorted or were empty.

Table 9 gives halo blight severity, expressed as a percent infection of the total leaf area, disease incidence expressed as a percent of the number of plants infected and pod infection. The severity of halo blight in plots planted with artificially inoculated seeds was significantly ($F = 0.01$) higher than that in plots sown with seeds obtained from small-scale farmers (Appendix 7a). Plots sown with artificially inoculated seeds which were seed dressed with 0.59% concentration of *E. citriodora* volatile oil had a significantly ($P = 0.01$)

Plate 13: Pod infection scale used for assessment of bean infection by *Pseudomonas syringae* pv. *phaseolicola*.

Table 9: **Halo blight severity and incidence on bean plants sprayed with volatile oils from *E. citriodora* against halo blight in 1991. *(Data recorded on 15th January 1991).**

Treatments	Halo blight severity on leaves	% halo blight incidence	Halo blight Infection
1. Inoculated + dressed seeds	4.68	86.67	4.50
2. Inoculated + dressed + 1 spray	3.23	83.33	4.25
3. Inoculated + dressed + 2 sprays	4.35	85.00	3.96
4. Inoculated + dressed + 3 sprays	3.85	88.33	4.01
5. Inoculated + dressed + 4 sprays	4.16	86.67	4.01
6. Inoculated + 1 spray	3.41	85.00	4.00
7. Inoculated + 2 sprays	3.89	91.67	4.41
8. Inoculated + 3 sprays	4.69	93.33	4.12
9. Inoculated + 4 sprays	4.66	86.67	4.19
10. Inoculated, no spray (control)	3.76	85.00	3.48
11. Farmers' dressed seeds	0.84	20.00	1.16
12. Farmers' dressed + 1 spray	0.84	23.30	1.38
13. Farmers' dressed + 2 sprays	1.33	21.67	1.32
14. Farmers' dressed + 3 sprays	0.96	20.00	1.41
15. Farmers' dressed + 4 sprays	0.89	23.30	1.30
16. Farmers' + 1 spray	1.11	21.67	1.06
17. Farmers' + 2 sprays	1.25	18.33	1.26
18. Farmers' + 3 sprays	0.82	20.00	1.41
19. Farmers' + 4 sprays	0.86	18.33	1.24
20. Farmers' + no spray (control)	1.12	18.33	1.38

	0.05	0.01
LSD (*disease severity*)	1.19	1.60
LSD (*disease incidence*)	25.04	33.67
LSD (*pod infection*)	0.68	0.91

* The values represent a mean of 3 replicates

higher halo blight severity when compared to the plots planted with farmers' seeds which were treated with the same plant extract. There was a significantly (P=0.05) higher halo blight severity in plots planted with artificially inoculated seeds which were dressed with 0.59% concentration of *E. citriodora* volatile oil when compared to those receiving similar treatments but followed by one foliar spray. No significant (P = 0.05) differences were observed in halo blight severity in plots sown with farmers' seeds which were dressed with 0.59% concentration volatile oil from *E. citriodora* and the untreated control. No significant (P=0.05) differences in halo blight severity were observed among plots planted with farmers seeds which were dressed with volatile oils from *E. citriodora* and those receiving similar treatments but followed by foliar sprays. Halo blight severity in all plots planted with seeds which were artificially inoculated, followed by treatment with 0.59% *E. citriodora* volatile oil (dressed or sprayed) was not significantly (P=0.05) lower than the untreated control.

The incidence of halo blight in plots planted with artificially inoculated seeds was significantly (P=0.01) higher than that of plots sown with seeds obtained from small scale farmers (Appendix 7b). Plots sown with artifically inoculated seeds which were dressed with 0.59% concentration of

E.citriodora volatile oil had a significantly (P=0.01) higher halo blight incidence when compared to plots sown with farmers' seeds which were treated with *E. citriodora* volatile oil irrespective of whether they were seed dressed or sprayed. There was no significant (P=0.05) difference in halo blight incidence in plots planted with artificially inoculated seeds which were dressed with volatile oil from *E. citriodora* and those plots sown with inoculated seeds with similar treatments but followed by foliar spray. No signficant (P=0.05) difference was observed in halo blight incidence in plots planted with farmers seeds which were dressed with *E. citriodora* volatile oil and the untreated control. No signficant (P=0.05) difference in halo blight incidence was observed in plots sown with farmers seeds which were dressed and those plots receiving similar treatment but followed by foliar spray. Halo blight incidences in all plots sown with seeds which were artificially inoculated, followed by treatments with 0.59% concentration of *E. citriodora* volatile oil (dressed or sprayed) were not significantly (P=0.05) lower than that of the untreated control.

Pod infection in plots sown with artificially inoculated seeds was found to be significantly (P = 0.01) higher than that on plants in plots sown with seeds obtained from small scale farmers (Appendix 7c). There was a significantly (P = 0.01) higher pod infection in plants in plots sown with artificially inoculated seeds dressed with 0.59% concentration of

E. citriodora volatile oil when compared to those in plots sown with farmers seeds which were treated with the volatile oil irrespective of whether they were seed or foliar treated. No significant (P = 0.05) difference was observed in pod infection among plots sown with artificially inoculated seeds which were seed dressed and those in plots with similar treatment followed by foliar spray. Pod infection in plots sown with farmers seeds which were dressed with the *E. citriodora* volatile oil was not significantly (P = 0.05) lower than that of the untreated control. No significant (P = 0.05) difference in pod infection was observed among plots sown with farmers seeds which were dressed and those in plots receiving similar treatments followed by foliar sprays. Pod infection was not significantly (P=0.05) lower in plots sown with artificially inoculated seeds which were treated with 0.59% concentrations of *E. citriodora* volatile oil (seed dresssed or foliar sprayed) when compared to the untreated control.

4.5.2 Effect of plant extracts on total yield and yield component in 1991/92.

The bean plants treated with 0.59% concentration of *E. citriodora* volatile oil to control halo blight were harvested and yield data recorded. The parameters recorded included:- yield per hectare, number of seeds per 20 plants, number of pods from the 20 plants, number of seeds per 10 pods taken randomly and 100 seed weight (Table 10). Plots planted with inoculated seeds which were dressed with *E. citriodora* volatile oil gave the highest yield/hectare whereas plots sown with inoculated seeds which were later sprayed twice with the same plant extract gave the lowest yield/hectare. The yield per hectare from plots sown with inoculated seeds which were seed dressed was significantly (P=0.05) higher than that of seeds obtained from plots sown with inoculated seeds which received foliar spray only (Appendix 8a, Table 10). A significantly (P=0.05) higher yield/hectare was observed in plots sown with dressed inoculated seeds as compared to plots sown with farmers' seeds which were dressed with the volatile oil. There was a significantly (P=0.05) higher yield/hectare from plots planted with dressed inoculated seeds when compared with those planted with farmers seeds which were foliar sprayed only. No significant (P=0.05) difference in yield/hectare was observed in plots sown with dressed inoculated seeds when compared to those receiving similar treatments but were later sprayed. Generally, a higher yield per hectare was realised from plots

Table 10: Yield Components of cv. Rosecoco-GLP-2 treated with *E. citriodora* volatile oil against *P. syringae* pv. *phaseolicola* in 1991/92

Treatments	Yield/hectare (kg)	No. of seeds per 20 plants	No. pods/ 20 plants	Seeds per 10 pods	100 seed weight (g)
1. Inoculated + dressed seeds	3140	924	296	51	50
2. Inoculated + dressed + 1 spray	2837.86	862	257	53	53
3. Inoculated + dressed + 2 spray	3080.59	940	278	54	53
4. Inoculated + dressed + 3 spray	2913.31	830	268	48	46
5. Inoculated + dressed + 4 sprays	2960.04	678	236	53	53
6. Inoculated + 1 sprays	2161.31	650	210	54	47
7. Inoculated + 2 sprays	1912.61	577	188	51	47
8. Inoculated + 3 sprays	2147.36	635	209	50	49
9. Inoculated + 4 sprays	1980.51	590	197	50	47
10. Inoculated no spray (control)	2030.45	605	195	51	45
11. Farmers' dressed seeds	2234.36	666	221	50	51
12. Farmers' dressed + 1 spray	2629.45	772	258	50	53
13. Farmers' dressed + 2 sprays	2546.29	665	239	49	51
14. Farmers' dressed + 3 sprays	2355.4	692	229	50	51
15. Farmers' dressed + 4 sprays	2165.21	646	216	50	48
16. Farmers' + 1 spray	2162.83	641	212	51	48
17. Farmers' + 2 sprays	2150.72	644	215	50	49
18. Farmers' + 3 sprays	2517.74	737	227	52	45
19. Farmers' + 4 sprays	2109.98	639	201	52	48
20. Farmers' + no sprays (control)	2155.32	636	198	52	50
Means	2409.58	712	229	51	49

+ The values are menas of 3 replicates

	0.05	0.01		0.05	0.01
L.S.D. no of seeds/20 plants	180.13	242.25	L.S.D. No. seeds/10 pods	27.79	37.10
L.S.D. Yield/ha	70.65	94.43	L.S.D. 100 weed weight	34.69	46.36
L.S.D. No. pods/20 plants	45.95	61.79			

planted with farmers' dressed seeds compared to those which received foliar spray only as well as the untreated control plots. The yield/hectare from plots planted with farmers' seeds which were later sprayed once with *E. citriodora* volatile oil was significantly (P=0.05) higher than that from other plots which received similar treatment but were sprayed more than once.

Plots planted with dressed inoculated seeds which were later sprayed twice with 0.59% concentration of volatile oil from *E. citriodora* gave the highest number of seeds per 20 plants whereas plots sown with inoculated seeds which received foliar sprays twice gave the lowest number of seeds per 20 plants. The number of seeds per 20 plants from plots sown with inoculated seeds which were dressed with 0.59% concentration of *E. citriodora* volatile oil was significantly (P = 0.05) higher than that from plots sown with inoculated seeds which received foliar sprays only (Table 10). Plots planted with dressed seeds also realised a significantly (P=0.05) higher number of seeds per 20 plants when compared to the untreated control plots (Appendix 8b). The number of seeds per 20 plants from plots sown with inoculated seeds which were dressed with the volatile oil from *E. citriodora* was significantly

(P=0.05) higher than that from plots sown with seeds collected from small scale farmers (dressed and non treated seeds). There was no significant (P=0.05) difference in the number of seeds per 20 plants from plots sown with inoculated seeds which were dressed with *E. citriodora* volatile oil and those from plots which received similar treatments but later sprayed. No significant (P=0.05) difference was observed between the number of seeds/20 plants from plots sown with farmers seeds which were dressed with *E. citriodora* volatile oil and that from plots sown with farmers' seed without seed treatment. The number of seeds per 20 plants from plots sown with farmers' seeds which were dressed only was not significantly (P=0.05) different from that from plots sown with farmers' seeds which received similar treatment but later sprayed. The number of seeds from 20 plants from plots sown with farmers seeds receiving various treatments was not significantly (P=0.05) different from that harvested from the untreated control plots.

Plots planted with inoculated seeds which were dressed with *E. citriodora* volatile oil gave the highest number of pods/20 plants whereas plots sown with inoculated seeds which were later sprayed twice produced the lowest number of pods/20 plants. The number of pods harvested from 20 plants from plots planted with dressed inoculated seeds was significantly (P^{1} = 0.05) higher than that harvested from plots sown with inoculated seeds which were not dressed (Appendix 8c, Table 10) with *E. citriodora* volatile oil. The number of pods

from plots sown with dressed inoculated seeds which were then sprayed once or four times was not significantly (P = 0.05) different from that obtained from plots planted with farmers' seeds which were dressed only or dressed and later sprayed with *E. citriodora* volatile oil. Bean plants from plots planted with inoculated seeds which were later dressed produced a significantly (P = 0.05) larger number of pods compared with those from farmers' seeds which were not dressed. No significant (P = 0.05) difference was observed among the number of pods from plots sown with inoculated seeds which received foliar treatments only and those from plots sown with farmers' seeds which received similar treatments. There was no significant (P = 0.05) difference in the number of pods from beans from plots planted with inoculated seeds which later received foliar treatments and the untreated control plots. A significantly (P = 0.05) higher number of pods was observed on bean plants from plots planted with farmers' dressed seeds that received foliar spray once when compared to those from bean plants from plots sown with farmers' seeds which were not dressed but were foliar sprayed four times. No significant (P = 0.05) difference was observed among the number of pods harvested from bean plants from plots sown with farmers' seeds that were seed dressed only when compared to those from plots with similar treatments but which were later sprayed. The number of pods harvested from plots planted with farmers' seeds receiving various treatments was not significantly (P = 0.05) higher than that of the untreated control plots except that

harvested from plots sown with farmers' dressed seeds which later were once foliar sprayed.

Bean plants from plots planted with inoculated seeds which later received one foliar spray of *E. citriodora* volatile oil gave the highest number of seeds/10 pods and those planted with farmers' dressed seeds which received two foliar sprays of the same plant extract gave the lowest number of seeds/10 pods. There was no significant (P = 0.05) difference among the number of seeds per 10 pods taken randomly from various treatments (Appendix 8d, table 10). Bean plants from the untreated control plots produced a lower number of seed per 10 pods (49) but was not signficantly lower when compared with those from plots receiving various treatments.

The 100-seed weight was highest in bean plants from plots planted with inoculated dressed seeds which later received one foliar spray of *E. citriodora* volatile oil and lowest in bean plants from plots planted with inoculated seed which were not treated with the plant extract. No significant (P = 0.05) difference was observed among 100-seed weights from various treatments (Appendix 8e, table 10). The untreated control plots gave 44.60g as 100-seed weight, which was not significantly (P = 0.05) lower than those of seeds from plots which received the other treatments.

4.5.3 Season II, March-June, 1992

4.5.3.1 Halo blight severity, incidence and pod infection

The efficacy of *E. citriodora* volatile oil to control halo blight was further evaluated in the long rains of 1992 and the ether fraction of *E. citriodora* was included in the treatments. Copper oxychloride 0.1% and Streptomycin Sulphate were included as standard treatments of halo blight.

Bean plants treated with the volatile oil (0.59%) and the ether fraction (1.17%) of *E. citriodora* were assessed for infection by halo blight. The severity of halo blight on leaves was assessed using the disease scale described earlier. Disease incidence was recorded as the number of plants infected out of the tagged 20 plants and expressed as a percent. Pod infection was assessed using the scoring system described earlier.

Table 11 shows halo blight severity, expressed as a percent infection of the total leaf area, disease incidence expressed as a percent of the number of plants infected and pod infection.

Plots sown with inoculated seeds which were later dressed with *E. citriodora* extract (volatile oil) showed a significantly ($P = 0.05$) higher halo blight severity when compared with that in plots sown with dressed inoculated seeds which were later sprayed twice using *E. citriodora* extract (table 11, appendix 9a). A significantly ($P = 0.05$) higher halo blight severity was

Table 11: Halo blight severity and incidence on bean plants sprayed with volatile oil from *E. citriodora* against halo blight in 1992. *(Data recorded on 19th June 1992).

Treatments	Halo blight severity on leaves	% halo blight incidence	Halo blight infection on pods
1. Inoculated + seed dressed (Cr.ext.)	6.28	95.00	1.11
2. Inoculated +seed dressed+1 spray (Cr.ext)	5.89	98.33	1.09
3. Inoculated, dressed + 2 sprays (Cr.ext)	3.69	83.33	1.06
4. Inoculated + 1 spray (Cr. ext)	4.76	96.67	1.06
5. Inoculated + 2 sprays (Cr. ext)	2.85	93.33	1.06
6. Inoculated + seed dressed (Ether Fr.)	5.15	95.00	1.09
7. Inoculated + dressed + 1 spray (Ether Fr.)	5.84	91.67	1.04
8. Inoculated + dressed + 2 sprays(Ether Fr.)	7.08	100.00	1.04
9. Inoculated + 1 spray (Ether Fr.)	4.18	91.67	1.09
10. Inoculated + 2 sprays (Ether Fr.)	4.94	98.33	1.10
11. Inoculated (control)	4.97	93.33	1.04
12. Farmers' dressed seeds (Cr. ext)	0.57	26.67	1.06
13. Farmers' dressed + 1 spray (Cr. ext)	0.50	35.00	1.06
14. Farmers' dressed + 2 sprays (Cr. ext)	1.91	60.00	1.03
15. Farmers', 1 spray (Cr. ext)	1.21	40.00	1.01
16. Farmers', 2 sprays (Cr. ext)	2.56	55.00	1.06
17. Farmers', dressed seed (Ether Fr.)	0.77	35.00	1.02
18. Farmers' dressed + 1 spray (Ether Fr.)	1.20	51.67	1.08
19. Farmers' dressed + 2 sprays (Ether Fr.)	1.36	48.33	1.03
20. Farmers', 1 spray (Ether Fr.)	1.50	55.00	1.04
21. Farmers', 2 sprays (Ether Fr.)	0.73	35.00	1.04
22. Farmers' (control)	1.09	53.33	1.05
23. Copper oxychloride (0.1% a.i)	1.72	60.00	1.05
24. Streptomycin sulphate (0.1% a.i.)	3.90	93.33	1.06

		0.05	0.01
* Means of 3 replicates			
(Cr. ext) = Crude extract	LSD (disease severity)	1.38%	1.84%
(Ether Fr.) = Ether fraction	LSD (disease incidence)	24.31%	33.49%
of *E. citriodora* volatile oil	LSD (pod infection)	0.07%	0.10%

Observed in plots planted with inoculated beans dressed with *E. citriodora* volatile oil when compared with that in plots planted with inoculated beans which were sprayed twice with the same plant extract. There were no significant ($P = 0.05$) differences in halo blight severity among plots planted with seeds which were dressed with *E. citriodora* crude extract (volatile oil) followed by one spray and those plots sown with seeds dressed with the ether fraction of the same plant extract. Plots sown with dressed seeds, followed by 2 sprays with *E. citriodora* volatile oil showed a significantly ($P = 0.05$) lower halo blight severity when compared to that in plots sown with seeds which were dressed with the ether fraction of the same plant extract *(E. citriodora* extract). Plots planted with farmers seeds which were dressed or sprayed with the two plant extracts had a significantly ($P = 0.05$) lower halo blight severity when compared to plots sown with inoculated seeds which were dressed or sprayed. There was a significantly ($P = 0.05$) lower halo blight severity in plots planted with inoculated seeds which were followed by 2 sprays of *E. citriodora* volatile oil when compared to plots sown with inoculated seeds which were dressed with ether fraction of *E. citriodora* volatile oil. Plots planted with inoculated seeds and later sprayed twice with the crude extract of *E. citriodora* volatile oil had a significantly ($P = 0.05$) lower halo blight severity than the untreated control. No

significant (P = 0.05) difference was observed in the severity of halo blight in plots sown with farmers' seeds which were dressed only or followed by foliar spray with *E. citriodora* volatile oil when compared to plots sown with farmers seeds receiving only one spray with the same plant extract. There was no significant (P = 0.05) difference in halo blight severity in plots sown with farmers' seeds which were dressed with volatile oil of *E. citriodora* when compared to plots planted with seed dressed with the ether fraction of the same plant extract. No significant (P = 0.05) difference in severity of halo blight was noted in plots planted with farmers' seeds which were dressed with the ether fraction of *E. citriodora* volatile oil when compared to plots which received foliar spray of the same plant extract. Foliar spray of copper oxychloride (0.1% a.i) reduced halo blight severity significantly (P = 0.05) when compared to foliar sprays of volatile oil and ether fraction of *E. citriodora*. No significant (P = 0.05) difference in halo blight severity was observed in plots receiving streptomycin sulphate (0.1% a.i) when compared to plots planted with dressed seeds followed by foliar sprays with *E. citriodora* volatile oil or inoculated seeds receiving foliar spray of the same plant extract.

The highest halo blight incidence was observed in plots sown with inoculated dressed seeds which were later sprayed twice with the ether fraction of *E. citriodora* volatile oil. The lowest disease incidence was recorded in plots planted with farmers' seeds which were dressed with *E. citriodora* volatile

oil. Halo blight incidence in plots sown with bean seeds which were dressed only or followed by 1 or 2 sprays of *E. citriodora* volatile oil was not significantly (P = 0.05) different from that in plots which were only sprayed with the plant extract (Appendix 9b). There was no significant (P = 0.05) difference in halo blight incidence among plots sown with bean seeds which were dressed with *E. citriodora* volatile oil and the untreated control. There was no significant (p=0.05) difference in halo blight incidence in plots sown with bean seeds which were dressed with *E. citriodora* volatile oil when compared to plots sown with seeds which where dressed with the ether fraction of the same plant extract. A significantly (P = 0.05) higher level of halo blight incidence was observed in plots planted with inoculated bean seeds which were dressed with the two forms of the extracts as compared to plots planted with farmers' seeds with similar treatments. There was no significant (P = 0.05) difference in halo blight incidence among plots planted with inoculated seeds which received foliar spray of *E. citriodora* volatile oil and those which received foliar spray of ether fraction of *E. citriodora* volatile oil. A significantly (P = 0.05) higher level of halo blight incidence was observed in plots sown with inoculated seeds which received foliar spray of either ether or crude form of *E. citriodora* when compared to plots planted with farmers' seeds which received similar treatments. There was no significant (P = 0.05) difference in halo blight incidence in plots planted with farmers' seeds which were seed dressed and those plots planted with seeds which

were not dressed with the volatile oil from *E. citriodora*. No significant (P = 0.05) difference in halo blight incidence was noted in plots sown with bean seeds which were dressed with the ether fraction of *E. citriodora* volatile oil and those planted with seeds which were not dressed. Plots planted with inoculated seeds which were later sprayed with copper oxychloride (0.1% a.i) showed a significantly (P = 0.05) lower disease incidence when compared to plots sown with inoculated seeds which were treated with either the ether fraction or volatile oil of *E. citriodora*. No significant (P = 0.05) difference was noted in halo blight incidence in plots which were treated with streptomycin sulphate (0.1% a.i) and those treated with the plant extracts (seed dressed and foliar sprayed).

Analysis of data on pod infection indicated that there was no significant (P = 0.05) difference in pod infection among bean plants treated differently using the plant extracts (Appendix 9c). Pod infection in plots planted with bean seeds which were treated with volatile oil from *E. citriodora* (seed dressed or foliar sprayed) was not significantly (P = 0.05) different from those treated with the ether fraction. No significant (P = 0.05) difference was noted in pod infection in plots sown with inoculated seeds which were dressed with the two forms of plant extracts when compared to plots which were foliar sprayed. Pod infection in the untreated control was not significantly (P = 0.05) higher than in plots receiving other treatments.

4.5.3.2 Effect of plant extracts on total yield and yield components from halo blight infected bean plants in 1992 long rains seasons.

The bean plants treated with crude extract (volatile oil) and ether fraction of *E. citriodora* were harvested and the following parameters recorded viz. yield per hectare,number of seeds per 20 plants, number of pods per 20 plants, number of seeds per 10 pods and 100-seed weight (Table 12).

The highest yield per hectare was observed in plots planted with farmers seeds which were later sprayed once with the ether fraction of *E. citriodora* volatile oil. The lowest yield/hectare was recorded in plots sown with dressed inoculated seeds which were later sprayed once with the volatile oil from *E. citriodora*. The yield per hectare from plots sown with inoculated seeds which were dressed with *E. citriodora* volatile oil was significantly (P = 0.05) higher than that from plots receiving similar treatments followed by foliar sprays as well as plots treated with the ether fraction of the same plant extract (seed dressed or foliar sprayed). No significant (P=C.05) difference was observed in yield per hectare in plots planted with inoculated seeds which were treated (seed dressed or foliar sprayed) with the ether fraction from *E. citriodora* when compared to the untreated control plots. The yield per hectare from plots sown with inoculated seeds which were dressed with *E. citriodora* volatile oil was significantly (P=0.05) higher than that from plots planted with

farmers' seeds receiving similar treatments. A significantly (P=0.05) higher yield/ha was observed in plots planted with farmers' seeds which received one foliar spray of the ether fraction of *E. citriodora* volatile oil when compared with that from plots treated with copper oxychloride or streptomycin sulphate.

Plots planted with farmers' seeds which were sprayed once with ether fraction of *E. citriodora* volatile oil produced the highest number of seeds per 20 plants. The lowest number of seeds per 20 plants was recorded in plots planted with inoculated dressed seeds which were later sprayed twice with the ether fraction of *E. citriodora* volatile oil.

The data on the number of seeds per 20 plants indicated that there was no significant (P = 0.05) difference in seed number among bean plants receiving different treatments

(Appendix 10b). The number of seeds per 20 plants harvested from plots planted with inoculated seeds which were dressed only or later sprayed with ether fraction or volatile oil from *E. citriodora* was not significantly (P = 0.05) higher than that from the untreated control plots. No significant (P = 0.05) difference was observed in the number of seeds harvested from plots planted with artificially inoculated seeds when compared to those from plots sown with farmers' seeds. The number of seeds per 20 plants from plots planted with farmers' seeds which were treated with volatile oil from *E. citriodora* was not significantly (P=0.05) different from that from plots treated with ether fraction. The untreated control plots did not show a significantly (P=0.05) lower number of seeds per 20 plants when compared to those from plots planted with farmers seeds treated differently.

The highest number of pods per 20 plants was recorded in plots planted with farmers' seeds which received two foliar sprays of ether fraction of *E. citriodora* volatile oil. Plots planted with inoculated seeds which received two foliar sprays of the ether fraction of *E. citriodora* volatile oil gave the lowest number of pods per 20 plants. The data on the number of pods per 20 plants indicated that there was no significant (P=0.05) difference in pod number among plants receiving different treatments (Appendix 10c). The number of pods per 20 plants from plots planted with inoculated seeds which were dressed only or later sprayed with ether fraction of *E. citriodora* or its volatile oil was signficantly (P=0.05) higher

than that from the untreated control plot. No signficant (P=0.05) difference was noted in the number of pods per 20 plants harvested from plots sown with artificially inoculated seeds when compared to those from plots sown with farmers' seeds. Volatile oils from *E. citriodora* applied in plots planted with farmers' seeds did not significantly (P = 0.05) increase the number of pods per 20 plants when compared to plots treated with ether fraction. The untreated control plots did not show a significantly (P = 0.05) lower number of pods per 20 plants, when compared to those in plots treated with copper oxychloride, streptomycin sulphate, ether fraction and volatile oil from *E. citriodora*.

The highest number of seeds per 10 pods was recorded in plots planted with farmers' dressed seeds which were later sprayed twice with the volatile oil from *E. citriodora*. The lowest number of seeds per 10 pods was observed in plots planted with farmers dressed seeds which received 2 foliar sprays of ether fraction of *E. citriodora* volatile oil.The number of seeds per 10 pods was not significantly (P = 0.05) different among bean plants receiving different treatments (Appendix 10d). The number of seeds per 10 pods from plots planted with dressed inoculated seeds was not significantly (P = 0.05) different from that harvested from plots which received foliar sprays, irrespective of the plant extract used. There was no significant (P = 0.05) difference in seed number per 10 pods among plots planted with farmers seeds which were either dressed or later foliar sprayed. No significant (P = 0.05)

difference was noted in seed number per 10 pods between bean plants obtained from the untreated control plots and those from plots treated differently.

Plots planted with farmers' dressed seeds which received two foliar sprays of the ether fraction of *E. citriodora* volatile oil produced the highest 100-seed weight. The lowest 100-seed weight was recorded in plots planted with dressed inoculated seeds which were sprayed twice with the ether fraction of *E. citriodora* volatile oil. The weight of 100 seeds taken randomly from each treatment was not significantly (P = 0.05) different from that of the untreated control (Appendix 10e). Plots sown with inoculated seeds which received either seed treatment or foliar treatment of *E. citriodora* volatile oil or its ether fraction gave 100-seed weight which was comparable to that obtained from plots sown with farmers' seeds receiving similar treatments. Plots planted with bean seeds which were later foliar sprayed with copper oxychloride or streptomycin sulphate did not give a significantly (P = 0.05) higher 100-seed weight compared to plots planted with seeds which were treated differently using the volatile oil from *E. citriodora* and the ether fraction.

4.6 Efficacy of various rates of extracts from *Eucalyptus citriodora* on common bacterial blight caused by *Xanthomonas campestris* pv. *phaseoli* (Season II, 1992).

Bean plants treated with crude extracts (volatile oil) and ether fractions from *E. citriodora* were assessed for infection by common bacterial blight. The severity of the disease on leaves was assessed using the disease scale described earlier. Disease incidence was recorded as the number of plants infected out of the tagged 20 plants expressed as a percent. Pod infection was assessed using the scoring system described earlier.

Table 13 shows common bacterial blight severity on leaves, disease incidence and pod infection of bean plants receiving various treatments. There was no significant (P = 0.05) difference in common bacterial blight severity in plots planted with inoculated bean seeds which were dressed with the volatile oil from *E. citriodora* and those plots which were foliar sprayed with the extract only. No significant (P = 0.05) difference in common bacterial blight severity was observed in plots planted with inoculated seeds which were dressed with the crude extract (volatile oil) and those plots sown with seeds dressed with the ether fraction of *E. citriodora* volatile oil. There was no significant (P = 0.05) difference in the severity of common bacterial blight in plots planted with inoculated seeds which

Table 13: Commonblight severity and incidence on bean plants sprayed with volatile oil from *E. citriodora* against bean common blight in 1992. * (Data recorded on 21st June 1992)

eatment	Common blight severity (%)	% common blight incidence	Common blight infection on pods
Inoculated + dressed (Cr. ext)	5.63	83.33	1.03
Inoculated, dressed + 1 spray (Cr.ext)	4.84	85.67	1.04
Inoculated, dressed + 2 sprays (Cr.ext)	5.01	95.67	1.10
Inoculated + 1 spray (Cr. ext)	4.48	89.33	1.06
Inoculated + 2 sprays (Cr. ext)	4.68	94.67	1.03
Inoculated + seed dressed (Ether Fr.)	5.83	96.33	1.04
Inoculated + dressed + 1 spray (Ether Fr.)	4.86	89.33	1.05
Inoculated + dressed + 2 sprays(Ether Fr.)	5.08	98.33	1.07
Inoculated + 1 spray (Ether Fr.)	4.85	85.33	1.06
Inoculated + 2 sprays (Ether Fr.)	4.31	96.67	1.08
Inoculated (control)	4.63	83.00	1.07
Farmers' dressed seeds (Cr. ext)	1.91	23.33	1.05
Farmers' dressed + 1 spray (Cr. ext)	1.44	29.00	1.09
Farmers' dressed + 2 sprays (Cr. ext)	1.33	27.33	1.05
Farmers', 1 spray (Cr. ext)	1.71	33.67	1.05
Farmers', 2 sprays (Cr. ext)	1.66	37.33	1.03
Farmers' dressed seeds (Ether Fr.)	1.41	33.67	1.05
Farmers' dressed + 1 spray (Ether Fr.)	1.67	35.00	1.07
Farmers' dressed + 2 sprays (Ether Fr.)	1.30	38.33	1.06
Farmers', 1 spray (Ether Fr.)	1.48	32.67	1.05
Farmers', 2 spray (Ether Fr.)	1.34	35.67	1.07
Farmers' (Control)	1.34	29.33	1.06
Copper oxychloride (0.1% a.i.)	3.26	49.33	1.04
Streptomycin sulphate (0.1% a.i.)	3.33	92.67	1.08

eans of 3 replicates

. ext) = Crude extract (volatile oil) of *E. citriodora*

ther Fr.)= Ether fraction of *E. citriodora* volatile oil

	0.05	0.01
pod infection	0.07	0.09
disease severity	1.27	1.70
disease incidence	13.73	18.36

were treated (seed dressed or foliar sprayed) with volatile oil or ether fraction of *E. citriodora* and that in the untreated control plots. The severity of common bacterial blight on beans was significantly (P = 0.05) higher in plots sown with artificially inoculated seeds which received various treatments than in plots sown with farmers' seeds (Appendix 11a). There was no significant (P = 0.05) difference in common bacterial blight infection in plots planted with farmers' seeds which were dressed and those plots planted with seeds which were not dressed but received foliar sprays of the volatile oil or ether fraction of *E. citriodora*. No significant (P = 0.05) difference was observed in severity of common bacterial blight in plots sown with farmers' seeds which were treated (seed dressed and foliar sprayed) with the volatile oil and those treated with the ether fraction of *E. citriodora* extract. Streptomycin sulphate (0.1% a.i) and copper oxychloride (0.1% a.i) gave a lower common bacterial blight severity than plant extracts but it was not significantly (P = 0.05) lower than in plots planted with inoculated bean seeds which were later sprayed once with the volatile oil or sprayed twice with the ether fraction of *E. citriodora* extract.

Plots planted with inoculated dressed seeds which received two foliar sprays of the ether fraction of *E. citriodora* had the highest common bacterial blight incidences. The lowest disease incidences were recorded in plots planted with farmers dressed seeds which were sprayed once with *E. citriodora* volatile oil.

Common bacterial blight incidence in plots planted with inoculated seeds which were dressed with the volatile oil from *E. citriodora* was not significantly (P = 0.05) different from that in plots where bean plants received foliar spray only. There was no significant (P = 0.05) difference in common bacterial blight incidence in plots planted with inoculated seeds which were later sprayed with *E. citriodora* volatile oil and that in plots sprayed with the ether fraction of *E. citriodora* extract. There was a significantly (P = 0.05) higher common bacterial blight incidence in plots planted with inoculated seeds which were dressed or foliar sprayed with ether fraction or volatile oil from *E. citriodora* when compared to that in plots planted with farmers' seeds receiving similar treatment (Appendix 11b). Common bacterial blight incidence was not significantly (P = 0.05) different in plots planted with farmers' seeds which were dressed with the *E. citriodora* volatile oil or ether fraction of the same plant extract when compared to that in plots planted with farmers' seeds which were only sprayed using the plant extracts. Foliar spray with copper oxychloride reduced common blight incidence significantly (P = 0.05) when compared to that in plots planted w th inoculated seeds receiving other treatments. No significant (P = 0.05) difference in disease incic ence was observed in plots sown with inoculated seeds treated wth streptomycin sulphate when compared to plot sown with inoculated seeds, dressed or sprayed with e her fraction or volati e oil from *E. citriodora* .

The highest pod infection was recorded in plots planted with inoculated dressed seeds which were sprayed twice with the volatile oil from *E. citriodora*. The lowest pod infection was observed in plots planted with farmers seeds which received two foliar sprays of *E. citriodora* volatile oil. There was no significant (P = 0.05) difference in pod infection of bean plants in plots planted with inoculated seeds which were dressed or sprayed with ether fraction or volatile oil from *E. citriodora* when compared to the untreated control plots (Appendix 11c). No significant (P = 0.05) difference was observed in pod infection in plots planted with inoculated seeds which were dressed or sprayed with the two plant extracts when compared to that in plots planted with farmers' seeds which received similar treatments. Pod infection of bean plants treated with copper oxychloride or streptomycin sulphate was not significantly (P=0.05) lower than that of bean plants in plots treated with the plant extracts (seed dressed or foliar sprayed).

4.7 Effect of plant extracts on total yield and yield components from common bacterial blight infected bean plants in 1992 long rains season:

The bean plants treated with crude extracts (volatile oil) and ether fraction from *E. citriodora* during the March-June 1992 growing season were harvested and the following yield parameters recorded viz. yield per hectare, number of seeds per 20 plants,number of pods per 20 plants,number of seeds per 10 pods and 100-seed weight (Table 14).

The highest yield/hectare was observed in plots planted with inoculated seeds which were later sprayed twice with the volatile oil from *E. citriodora*. The lowest yield/hectare was observed in plots planted with dressed inoculated seeds which later received two foliar sprays of *E. citriodora* volatile oil. Yield per hectare from plots sown with inoculated seeds which were dressed with *E. citriodora* volatile oil was significantly ($P=0.05$) higher than that from plots which received similar treatments followed by foliar sprays.

(Appendix 12a). There were significiantly (P=0.05) higher yields per hectare from plots planted with inoculated seeds which were dressed with the volatile oil from *E. citriodora* only when compared to those from plots planted with seeds dressed with the ether fraction of the same plant extract. There was significantly (P=0.05) higher yield/hectare from plots planted with farmers' seeds which were dressed with *E. citriodora* volatile oil, followed later by two sprays when compared to those from plots sown with farmers' seeds which later received one foliar spray. Plots sown with farmers seeds which received no treatment gave 2774.34 kg/ha which was not significantly (P=0.05) lower than that from plots treated with the ether fraction or the volatile oil from *E. citriodora.*

The F-test for the number of seeds per 20 plants indicated that there was no significant (P=0.05) difference among plots receiving various treatments (Appendix 12b). Comparison of means which was done using `the least significant difference' (LSD) indicated that there were variations in the treatment means. The highest number of seeds/20 plants was observed in plots planted with dressed inoculated seeds which were later sprayed twice with the ether fraction of the volatile oil from *E. citriodora*. The lowest number of seeds/20 plants was observed in plots sown with inoculated seeds which were later sprayed once with the same plant extract. The number of seeds/20 plants from plots planted with inoculated seeds which were

dressed with *E. citriodora* volatile oil was not significantly (P=0.05) different from those obtained from plots which received similar treatments but followed by a foliar spray. No significant (P=0.05) difference was noted in the number of seeds/20 plants from plots planted with inoculated seeds which were dressed with *E. citriodora* volatile oil when compared to those from plots planted with inoculated seeds which were dressed with the ether fraction of the same plant extract. There was no significant (P=0.05) difference in the number of seeds/20 plants harvested from plots sown with inoculated seeds which were treated with either the volatile oil or ether fraction from *E. citriodora* when compared to those from the untreated control plots. Plots which were foliar sprayed with copper oxychloride produced a significantly (P=0.05) lower number of seeds/20 plants when compared to those from plots planted with seeds which were dressed with the volatile oil from *E. citriodora*. No significant (P=0.05) difference in the number of seeds/20 plants was observed in plots planted with farmers' seeds which were dressed with the ether fraction or volatile oil from *E. citriodora* and those which were from plots foliar sprayed with either of the plant extracts. There was no significant (P=0.05) difference in the number of seeds/20 plants harvested from plots sown with farmers' seeds which were treated (seed dressed or foliar sprayed) with the ether fraction or the volatile oil from *E. citriodora* and those from the untreated control plots.

There was an indication that the number of pods from plants receiving different treatments was not significantly (P = 0.05) different (Appendix 12c). Plots planted with dressed inoculated seeds which were later sprayed twice with the ether fraction of *E. citriodora* volatile oil produced the highest number of pods/20 plants. Bean plants in plots planted with inoculated seeds which later received two foliar sprays of the ether fraction of *E. citriodora* volatile oil produced the lowest number of pods/20 plants. The number of pods/20 plants from plots planted with inoculated seeds which were dressed only or later sprayed with the volatile oil or ether fraction from of *E. citriodora* was not significantly (P = 0.05) higher than that of the untreated control plots. There was no significant (P=0.05) difference in the number of pods/20 plants from plots planted with artificially inoculated seeds when compared to those from plots planted with farmers' seeds which were dressed or sprayed with ether fraction or volatile oil. The volatile oil from *E. citriodora* applied on farmers' seeds did not significantly (P = 0.05) increase the number of pods/20 plants when compared to those from plots treated with ether fraction of the same plant extract. The untreated control did not show a significantly (P = 0.05) lower number of pods/20 plants when compared to bean plants from plots treated with copper oxychloride, streptomycin sulphate, ether fraction and crude extracts (volatile oil) from *E. citriodora*.

The highest number of seeds/10 pods was observed in plots planted with dressed inoculated seeds which received one foliar

spray of *E. citriodora* volatile oil. The lowest number of seeds/10 pods was observed in plots planted with dressed inoculated seed which received two foliar sprays of *E. citriodora* volatile oil and in plots sown with inoculated seeds which were foliar sprayed with copper oxychloride or streptomycin sulphate. There was no significant (P = 0.05) difference in seed number/10 pods harvested from bean plants from plots receiving different treatments (Appendix 12d). The number of seeds per 10 pods from plots planted with dressed inoculated seeds were not significantly (P = 0.05) different from those harvested from plots receiving foliar sprays,irrespective of the plant extract used. There was no significant (P = 0.05) difference in seed number/10 pods in plots planted with farmers seeds which were dressed when compared to those from plots which received foliar spray of the two plant extracts. No significant (P = 0.05) difference was noted in seed number/10 pods obtained from the untreated control plots and those from plots treated differently.

Plots planted with dressed inoculated seeds which were later sprayed once with *E. citriodora* volatile oil produced the highest 100-seed weight. The lowest 100- seed weight was observed in plots planted with farmers' seeds which were later sprayed once with the ether fraction of *E. citriodora* volatile oil. The weight of 100-seeds from plots treated with the volatile oil or ether fraction of *E. citriodora* was not significantly (P = 0.05) different from the untreated control

plots (Appendix 12e). Plots sown with inoculated seeds which received seed treatment or later foliar treatment with either volatile oil or ether fraction of *E. citriodora* gave 100-seed weight which was comparable to that obtained from plots sown with farmers' seeds and received similar treatments. The 100-seed weight of beans from plots treated with copper oxychloride or streptomycin sulphate was not significantly ($P = 0.05$) different from those harvested from plots treated with the plant extracts.

5 DISCUSSION

The detection of antibiotic substances in higher plants, that are active against a wide range of microorganisms would play an important role in reducing the economic losses caused by the microorganisms. In this study, locally available plants from various families were tested against *P. syringae pv. phaseolicola and X. campestris pv. Phaseoli* so as to have some information on their antibiotic activity. These plant materials were found to have a marked antibacterial activity against *P.syringae* pv. *phaseolicola* and *X.campestris* pv. *phaseoli.* Most of the work done on plant extracts involve *in vitro* tests but very few workers have done any conclusive work on *in vivo* tests. Most of the plant extracts used in this study have been used medicinally, in perfumery, and as insect repellents (Uphof, 1968; Howes, 1974; Usher, 1984).

5.1 Antibacterial activity *in vitro*

Tests done on the various plant extracts using zonal inhibition technique indicated that extracts from *E. citriodora, N. inodorum, C. lucistanica, T. minuta* and *S. chamaecyparissus* were active against the test pathogens. Although the 5 plant extracts showed marked antibacterial activity, some differences in their potency was noted. *E. citriodora* volatile oil showed the highest activity. The extract produced a zone of growth inhibition on *X. campestris* pv. *phaseoli* that measured

32.83 mm excluding the disc diameter, as compared to streptomycin sulphate that produced a growth inhibition zone measuring 35.33 mm on the same test pathogen. The results of this investigation show that the antibacterial property is not family, genera or species specific. The bioactivity of the volatile oils of these plants does not come as a surprise since volatile oils in general have been associated several times with antimicrobial and antiseptic property. (Heywood and Chant, 1982; Hethelyi; *et al.*, 1987; Oloke and Kolawole, 1987; Dellacassa *et al*, 1989).

The volatile oils of *Eucalyptus* sp. have been used as antiseptic and to relieve colds (Heywood and Chant, 1982). In 1988, Jacob *et al.* also demonstrated that Eucalyptus leaf extract could be used to control the pre-emergency damping off of *Solanum melongena*. *Eucalyptus citriodora* extract was also noted to inhibit the growth of *Escherichia coli and Staphylococcus aureus*, although no antimicrobial activity against *Pseudomonas aeruginosa* was observed (Dellacassa *et al*, 1989). *Eucalyptus* sp. have been reported to have a wide spectrum of bioactivity. (Tokin, 1951 as quoted by Korzybski *et al.* (1967) investigated the effects of volatile phytonicides from 400 species of plants for protocidal properties. The highest degree of activity was shown by *E. citriodora, E. cinerea, E. gunnii, Santolina chamaecyparissus, Allium cepa, A. Sativum, A. fistulosum, A. rotundum, Eugenia apiculata, Cedrus*

atlantica, Platanus orientalis, Paeonia arborea and *Zelkowa carpinifolia.*

Tagetes minuta extacts extracted using chloroform and methanol showed no bioactivity while the volatile oil showed a marked bioactivity. The use of different solvents for extraction cannot be overemphasized because the antibiotic substance may be soluble in one solvent and not in another. This leads to varying results given on the same plant by different authors. The volatile oils of *T. minuta* have been found to be active against bacteria and fungi by other investigators. Hethelyi *et al.* (1987) demonstrated that the multiplication of 39 microorganisms could be inhibited by the volatile oil of *T. minuta, Pseudomonas syringae* pv. *pisi, P. syringae* pv. *tabaci, Xanthomonas campestris* pv. *vesicolor*, some fungi and gram positive bacteria showed total inhibition. The results agree with the current findings which indicate that *T. minuta* extract could inhibit the growth of *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli* by forming inhibition zones. The essential oil of *T. minuta* is not only bioactive to bacteria but also acts as an insect repellent (Heywood and Chant, 1982).

S. chamaecyparissus oil was found to have a lower activity compared with *E. citriodora* and *T. minuta* oils. However, such plant extracts should not be ignored because they may be containing a very active compound with low diffusability through the medium (Skinner, 1955). The antimicrobial property

of this plant has been used in traditional herbal medicine. The flowers are used to treat ringworm which is a fungal disease. The plant is also used as an insect repellent (Usher, 1984).

The acqueous extracts of *N. inodorum* showed marked antibacterial activity against halo blight and common blight pathogens. Garlic which is from the same family has been found to give similar results. Ark and Thompson (1959) demonstrated that extracts from this plant could inhibit the growth of *P. syringae* pv. *phaseolicola* and *Colletctrichum lindemuthianum* in *in vitro* and *in vivo* tests.

Antibiotic principles have also been isolated from vegetables used as food. In an investigation carried out by Pederson and Fisher (1944), the juices of cabbage, onion, celery and chinese cabbage were found to contain substances which had an inhibitory and bactericidal action toward bacteria normally present upon the surfaces of vegetables for instance *Pseudomonas spp.*

In our study, common bacterial blight pathogen (*X. campestris* pv. *phaseoli*) was more sensitive than *P. syringae* pv. *phaseolicola*. Many *Pseudomonas* species are resistant to a number of antibacterial agents (Palleroni, 1984). Similar observations were made by Mishenkova *et al.* (1983) who found that *Xathomonas campestris* pv. *phaseoli* was more sensitive than *Pseudomonas syringae* pv. *lachrymans* and *P. syringae* pv.

atrofascians to volatile oils from *Calendula officinalis* and *Thymus serphyllum*. Although the plant extracts were less active than streptomycin sulphate *in vitro*, they gave good results worthy pursuing further.

5.2 Antibiotic activity of separated fractions

When the crude extract (volatile oil), ether and hexane fraction of *E. citriodora, T. minuta, C. lucistanica* and *S. chamaecyparissus* were tested for antibacterial activity, the crude forms of *T. minuta* and *E. citriodora* were found to be more active on *P. syringae* pv. *phaseolicola* than the separated fractions. *T. minuta* crude extract produced a growth inhibition zone on *X. campestris* pv. *phaseoli* which was not significantly different from that produced by the ether fraction. Such observations were made by Oloke and Kolawole (1987) who investigated the activity of the crude extracts, three purified groups of compounds from the volatile oil of *Aframomum melegueta* against several bacterial and fungal strains. The crude oil showed comparable activity to that of paradols against the test pathogens.

The ether fractions had a higher antibacterial activity than hexane fractions. Some activity was also noted in all hexane fractions except that from *S. chamaecyparissus*. This indicates that there are more then one active compounds, some of which are eluted by ether and others by hexane. The reduction in activity of the extracts after fractionation may be explained in a similar manner. The active compounds in ether and hexane

fractions may be acting synergistically in crude extracts. The marked bioactivity of the ether fraction of the crude extracts for instance that from *E. citriodora* may be as a result of some oxygenated compounds showing a peak at 2700-2800 wavelengths which is absent in hexane fraction. (Appendix 13 a,b,c). The peak is not pronounced in the crude form . Penfold and Grant (1923) as quoted by Skinner (1955) suggested that the antimicrobial activity of *Eucalyptus* oil is partly attributed to the presence of an oxygenated compound known as cineol. According to Satwalekar *et al.*(1957) as quoted by Watt and Breyer-brandwijk (1962). *E. citriodora* oil contains an antibiotic principle citriodol in addition to 7-monomethyl-ethers of aromadendrin, kaempferol and ellagic acid. These have marked pharmacodynamic effects. Ekundayo *et al.* (1990) suggested that the antimicrobial property of the essential oil of *Vitex agnus - castus* could be attributed mainly to the presence of 1, 8, cineole, a compound reported to be in *Eucalyptus* species. Hethelyi *et al.* (1987) also suggested that *T. minuta* oil with high levels of ketone functional group such as dehydrotagetone and tagetone showed a high antimicrobial activity.

In general, terpenoids and essential oils containing them are known to have a wide range of biological and clincial properties and are considered to be of some medicinal and pharmaceutical importance (Thomas 1989).

5.3 Minimum inhibitory concentration of *E. citriodora* and *T. minuta* extracts

Further investigations on the crude extracts (volatile oil) from *E. citriodora* and *T. minuta* indicated that the latter was more active using tube dilution technique. Skinner (1955) emphasized on the need to investigate further not only on plant extracts that produce large growth inhibition zones but also those producing small ones. The tube dilution technique revealed that *T. minuta* extract could completely inhibit the growth of *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli* at 0.20% concentration whereas *E. citriotora* volatile oil caused inhibition of halo blight pathogen and common blight pathogen at 0.78% and 0.39% concentrations respectively. This indicates that if the active compounds could be isolated, they may cause inhibition at very low concentrations. Similar work by Hethelyi *et al* (1987) indicated that undiluted *T. minuta* oil could cause total inhibition of the test pathogens as described earlier but 10% concentration of the oil could not inhibit microbial growth.

The test of minimum inhibitory concentrations of the ether frations of *E. citriodora* and *T. minuta* indicated that it lies between 1.56 and 3.13%; and 6.25 and 12.5% concentrations respectively. Concentration of 50% of ether fraction produced an inhibition zone that measured 34.75 mm in diameter which was surprisingly wider than that of the undiluted form *E. citriodora*. This may be due to the incorporation of water into the ether fraction thus increasing the diffusability through the medium.

5.4 Effect of temperature on the antibiotic activity of plant extracts.

The plant extracts showed no significant difference in activity after subjecting them to various temperatures. This suggests that the active compound(s) is heat stable. Similar findings were reported by Atkinson (1946) who found that leaf and stem extracts of *Drosera* and extracts from the berries of Persoonias could not be destroyed by heating at 100^{c} for at least 45 minutes. Crude extracts kept at 4^{c} retained their activity for at least 8 months, but the bacterial substance present in cabbage, onion, celery and chinese cabbage could be destroyed by heating. (Pederson and Fisher,; 1944).

5.5. *In vivo* test for the control of bean bacterial blights.

In the greenhouse trials, the volatile oils from *E. citriodora* and *T. minuta* could reduce halo blight and common blight severity significantly (P=0.05) when used a seeddress for 8 hrs. The effectiveness of Copper oxychloride and that of Streptomycin sulphate to control halo blight and common bacterial blight of beans was not significantly (P=0.05) higher than that of *E. citriodora* and *T. minuta* volatile oil when used for seed dressing.Among the extracts tested, the volatile oil of *E. citriodora* gave the best results. Similar observations were made by Jacob *et al.* (1988) who found that *Eucalytus* leaf

extract as a 30 minute seedsoak prior to sowing was effective against *Pythium aphanidermatum* on brinjals. Foliar sprays did not reduce halo blight and common blight severity significantly when compared to the untreated control plants under greenhouse conditions. There is an indication that the volatile oils from *E. citriodora* and *T. minuta* can be more effective when used for seed dressing than when used as foliar sprays. This may be due to reduced ability of plant extracts to penetrate into plant tissues. On exposure to the atmosphere, the active compounds may be converted into inactive forms, thus rendering the plant extracts ineffective against the two bacteria. It is also speculated that when plant extracts are exposed to sunlight some of the compounds are inactivated by being converted to other compounds (Maffei and Chialva, 1990). However, very few workers have investigated the antimicrobial activity of plant extracts *in vivo* therefore more work in this area is highly recommended since it is of importance to the farmers and could be more environmentally friendly.

The phytotoxicity observed on bean plants sprayed with ether fractions of *T. minuta* and *E. citriodora* may be attributed to traces of di-ethylether which may have remained during evaporation invacuo. Di-ethyether at 100%, 50%, 25% was found to inhibit germination of seeds. It therefore needs to be stressed that before testing plant extracts for antibacterial properties, any solvent used in their extraction should be evaporated completely.

Field trails on the control of halo blight in season II indicated that dressing bean seeds and later spraying bean plants with *E. citriodora* volatile oil at seedling stages and at pod filling stage reduced disease severity significantly when compared to bean plants which were sprayed with the ether fraction from the same plant extract. *In vitro* tests by Oloke and Kolawole (1987) showed similar results. The volatile oil from *Aframomum melegueta* had a higher antibiotic activity against several bacterial and fungal strains than the purified compounds. Dellacassa *et al.* (1989) suggested that the volatile oils from *Eucalyptus* spp. contain several compounds with antibiotic activities which work together. In the current study high disease severity in plots treated with the ether fraction of *E. citriodora* volatile oil may be as a result of separation of some compounds which work synergistically. Foliar spray with copper oxychloride reduced halo blight incidence significantly when compared to bean plants in plots which were treated with the ether fraction or volatile oil from *E. citriodora*.

Although *X. campestris* pv. *phaseoli* was more sensitive in *in vitro* tests than *P. syringae* pv. *phaseolicola*, the control of the former under field conditions using the volatile oils from *E. citriodora* was not noticeable. There was no significant difference in severity of common bacterial blight in plots planted with inoculated seeds which were treated (seed dressed or foliar sprayed) with the volatile oil or ether fraction from *E. citriodora* and that in the untreated control plots. It is

speculated that the volatile oil may have reduced ability to penetrate through the plant tissue thus reducing its effectiveness.

It was observed that some bean seeds which were dressed with the volatile oil from *E. citriodora* produced a high yield. For instance there was a significantly higher yield/hectare from plots planted with dressed inoculated seeds when compared with those plots with similar seeds which were foliar sprayed only (season 1). Plots planted with those dressed seeds had a significantly higher number of pods and number of seeds/20 plants when compared to the untreated control plots but there was no corresponding reduction in disease severity. This is an area that can be taken as the next challenge but probably

should put more emphasis on disease progress under various treatments as opposed to one single record of disease severity.

6. Conclusions and Recommendations

This study has revealed that some plant extracts were very promising in controlling *P. syringae* pv. *phaceolicola* and *X. campestris* pv *phaseoli* and should be considered for further investigations. There is thus need to popularize organic/degradable pesticides which are hoped to be of no harm to mankind and animals. The safety of these chemicals, if available is suspected to be due to their biodegradability.

Moreover, these plants which would be the major sources of the proposed chemicals have been used as herbal medicine, food, food flavours and perfumes. Their availability is safeguarded by farmers benefiting from the sale of plants to an intrepreneur for extraction.

Current market pesticides have been known to cause environmental concern since some are non-biodegradable and cause health impairment to man and animals. Since these plants are locally available, their use for the control of plant diseases would go a long way in reducing the cost of imported pesticides.

Future work should possibly include gram positive bacteria e.g *Clavibacter michiganense* subsp. *michiganense* which causes bacterial canker of tomatoes. Some workers have found a better response when using gram positive bacteria than in gram negative bacteria (Tsuchiya *et al.*, 1944; Carlson and Douglas, 1948; Dellacassa *et al.* 1989).

Further investigations should be done to establish whether there is any difference in amount of active compounds among tissues, plants of the same genus and plant species from different ecological areas. Pure compounds should be isolated from the active plant extracts to establish the active compound.

Since plants are attacked by viruses, fungi, bacteria etc, some work should be done to investigate the effect of plant extracts on all these phytopathogens and also to establish the mode of action.

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7 Appendices

Appendix 1: Zones of growth inhibition produced by plant extracts on *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli*

ANOVAR TABLE

Source	df	ss	mss	F value
Treatment	13	6298.936	484.53354	295.73709**
Extracts	6	615.8839	1025.9807	626.21162**
Pathogens	1	12.87054	12.87054	7.8555884 **
Extracts x Pathogens	6	130.18158	21.69603	13.242813 **
Error	28	45.875	1.6383929	
Total	41	6344.811		

** Significant at 1 % level.

Appendix 2: The activity of crude extract , ether and hexane fractions on halo blight and common blight bacteria

ANOVAR TABLE.

Source	df	ss	mss	F value
Pathogen (A)	1	50.344141	50.344141	139.53189 **
Plant extract (B)	4	3943.7969	985.94923	2732.619 **
Plant species (C)	3	1254.823	418.27433	1159.2731 **
(A X B)	4	37.797759	9.4494398	26.189704 **
(A X C)	3	49.157459	16.38532	45.424308 **
(B X C)	12	2203.4406	183.62005	508.91428 **
(A X B X C)	12	156.36474	13.030395	36.114542 **
Error	120	43.296891	0.3608074	
Total	159	7739.0215		

** Significant at 1 % level.

Appendix 3: Zones of growth inhibition produced by diluted ether fractions of the essential oils of *E. citriodora* and *T. minuta*.

ANOVAR TABLE

Source	df	ss	mss	F value
Pathogen (A)	1	0.035714	0.035714	0.2376218 ns
Extract conc.(B)	6	9351.4063	1558.5677	10369.877 **
Extract type (C)	1	1914.0089	1914.0089	1234.794 **
A X B	6	0.620486	0.1034143	0.6880636 ns
A X C	1	2.580386	2.580386	17.168511 **
B X C	6	1268.7723	211.46205	1406.9556 **
A X B X C	6	6.20094	1.03349	6.8762908 **
Error	84	12.625	0.1502976	
Total	111	12556.25		

** Significant at 1% level.

ns not significant at 5% and 1% level.

Appendix 4 a: Effect of temperature on the activity of *T. minuta* and *E. citriodora* extracts* on *P. syringae* pv. *phaseolicola* and *X. Campestris* pv. *phaseoli*.

Test pathogen (A)	Extract type (C)	Temperature °C (B) 0	10	20	30	40	50	60	70	80	90	100	121	Means AxC	Mean(A)
P. syringae pv. *phaseolicola*	*E. citriodora*	31.00	31.50	31.25	32.50	32.50	33.75	30.25	32.25	32.50	33.75	32.50	33.75	32.29	
	T. minuta	16.75	16.50	16.00	16.00	16.75	17.25	16.00	16.50	16.25	17.75	16.25	17.50	16.63	24.46
	Means (AxB)	23.88	24.00	23.63	24.25	24.63	25.50	23.13	24.38	24.38	25.75	24.38	25.63		
X. campestris pv. *phaseoli*	*E. citriodora*	34.25	34.50	34.50	33.75	31.50	34.00	32.00	32.00	33.50	32.50	33.25	33.00	33.23	
	T. minuta	22.00	20.50	22.50	22.50	23.25	22.00	22.75	21.50	22.50	23.25	23.00	23.00	22.40	27.82
	Means(AxB)	28.13	27.50	28.50	28.13	27.38	28.00	27.38	26.75	28.00	27.88	28.13	28.00		
															Mean(C)
Means (AxC)	*E. citriodora*	32.63	33.00	32.88	33.13	32.00	33.88	31.13	32.13	33.00	33.13	32.88	33.38		32.81
	T. minuta	19.38	18.50	19.25	19.25	20.00	19.63	19.38	19.00	19.38	20.15	19.63	20.25		19.51
	Mean (B)	26.01	25.75	26.07	26.19	26.00	26.76	25.26	25.57	26.19	26.82	26.26	26.82		

*Means of 4 replicates

	0.05	0.01		0.05	0.01
LSD(A)	0.58	0.76	LSD(AC)	0.82	1.07
LSD(B)	1.42	1.86	LSD(BC)	2.00	2.63
LSD(C)	0.58	0.76	LSD(ABC)	2.83	3.72
LSD(AB)	2.00	2.63			

Appendix 4 b: Effect of temperature on the activity of *T. minuta* and *E. citriodora* extracts on *P. syringae* pv. *phaseolicola* and *X. campestris* pv. *phaseoli*.

ANOVAR TABLE.

Source	df	ss	mss	F value
Pathogen (A)	1	533.33333	533.33333	127.78702 **
Temperature (B)	11	42.00	3.8181918	0.9148389 **
Extract (C)	1	8453.5208	8453.5208	2025.4692 **
(A X B)	11	33.91667	3.083336	0.7387687 ns
(A X C)	1	275.52087	275.52087	66.014984 **
(B X C)	11	30.9792	2.8162909	0.6747851 ns
(A X B X C)	11	40.72913	3.7026482	0.8871569 ns
Error	144	601	4.1736111	
Total	191	10011		

** Significant at 1% level.

ns not significant at 5% and 1% level.

Appendix 5a: Disease severity of halo blight infected bean plants treated with plant extracts in the green house.

ANOVAR TABLE

Source	df	ss	mss	F value
Treatments	14	959.93435	68.566739	39.4006247 **
Error	30	52.20735	1.740245	
Total	44	1012.147		

** Significant at 1% level.

Appendix 5b: Disease severity of common blight infected bean plants treated with plant extracts in the green house.

ANOVAR TABLE.

Source	df	ss	mss	F value
Treatments	14	751.41761	53.672686	12.509243 **
Error	30	128.71927	4.2906422	
Total	44	880.13688		

** Significant at 1% level.

Appendex 6: Meteorological data.

Month	1991 Temp. (oC) Max.	Min.	Total rainfall (mm)	1992 Temp.(oC) Max.	Min	Total rainfall (mm)
JAN.	24.4	12.7	33.9	24.0	12.0	4.7
FEB.	25.2	13.1	0.4	26.6	13.2	70.2
MAR.	26.1	13.7	84.8	22.6	14.2	5.6
APR.	23.9	14.4	158.3	24.3	14.8	401.7
MAY.	22.4	14.8	281.4	22.5	13.4	216.5
JUN.	21.9	12.6	12.5	21.3	12.4	20.6
JUL.	20.3	9.9	12.9	19.9	11.3	29.4
AUG.	22.0	10.1	40.3	19.7	10.5	3.8
SEP.	23.9	10.0	2.8	22.9	11.5	16.3
OCT.	25.0	13.0	21.6	23.6	12.8	70.5
NOV.	22.7	13.5	199.4	21.8	13.6	112.6
DEC.	22.8	13.3	50.7	*	*	*

* Data for December was not collected since the month was not over.

Data provided through the courtesy of Mr. kinyua, Kabete agrometeorological station.

Appendix 7a: Disease severity of halo blight infected beans treated with extracts from *E.citriodora.*

ANOVAR TABLE.

Source	df	ss	mss	F value
Treatment	19	146.93787	7.7335721	15.101073 **
Block	2	0.02272133	0.0136066	0.0265692 ns
Error	38	19.460587	0.5121207	
Total	59	166.42567		

** Significant at p=0.01

ns Not significant

Appendix 7b: Disease incidence of halo blight infected bean treated with extracts from *E. citriodora.*

ANOVAR TABLE

Source	df	ss	mss	F value
Treatment	19	67035	3528.1579	15.463668 **
Block	2	163.33334	81.66667	0.3579392 ns
Error	38	8669.9997	228.15789	
Total	59	75868.333		

** Significant at p = 0.01

ns Not significant.

Appendix 7c: Pod infection of halo blight infected beans treated with extracts from *E. citriodora*. ANOVAR TABLE

Source	df	ss	mss	F value
Treatment	19	120.26582	6.32978	37.692396
Block	2	0.0245633	0.0122816	0.0731344 **
Error	38	6.3814367	0.1679325	
Total	59	126.6718		

** Significant at p = 0.01

ns Not significant.

Appendix 8a: Effect of plant extracts on the yield per hectare

ANOVAR TABLE.

Source	df	ss	mss	F value
Treatment	**19**	**86512496**	**455328.9**	**24.699****
Block	**2**	**23562.84**	**11781.42**	**0.639 ns**
Error	**38**	**700521.06**	**18434.77**	
Total	**59**	**9375333.5**		

Appendix 8b: Effect of plant extracts on number of seeds harvested from 20 plants in 1991.

ANOVAR TABLE.

Source	df	ss	mss	F value
Treatment	19	693516.33	36500.859	3.0903568 **
Block	2	12574.60	6287.3	0.5323162 ns
Error	38	448826.07	11811.212	
Total	59	1154917.00		

Appendix 8c: Effect of plant extracts on the number of pods per 20 plants.

ANOVAF TABLE.

Source	df	ss	mss	F value
Treatment	19	57304.7	3016.0368	3.9250335 **
Block	2	1194.4	597.2	0.7771887 ns
Error	38	29199.6	768.41053	
Total	59	87698.7		

Appendix 8d: Effect of plant extracts on the number of seeds per 10 pods.

ANOVAF TABLE.

Source	df	ss	mss	F value
Treatment	19	165.25	8.6973684	1.1754873 ns
Block	2	30.40	15.20	2.0543463 ns
Error	38	281.16	7.3989474	
Total	59	477.25		

Appendix 8e: Effect of plant extracts on 100-seed weight.

ANOVAR TABLE.

Source	df	ss	mss	F value	
Treatment	19	398.18	20.956842	1.8185181	ns
Block	2	39.737	19.8685	1.7240778	ns
Error	38	437.917	11.524132		
Total	59	759.52			

** Significant at p = 0.01

ns Not significant

Appendix 9a: Disease severity of beans treated with *E. citriodora* extract in 1992 growing season.

ANOVAR TABLE.

Source	df	ss	mss	F value	
Treatment	23	305.76684	13.29421	19.022241	**
Block	2	1.4405882	0.7202941	1.0306447	ns
Error	46	32.148352	0.6988772		
Total	71	339.35578			

Appendix 9b: Disease incidence of beans treated with *E.citriodora* extract in 1992 growing season.

ANOVAR TABLE.

Source	df	ss	mss	F value
Treatment	23	46205.208	2008.9221	9.2506774 **
Block	2	827.08333	413.54167	1.9042752 ns
Error	46	9989.5837	217.16486	
Total	71	57021.875		

Appendix 9c: Pod infection of beans treated with *E. citriodora* extract in 1992 growing season.

ANOVAR TABLE.

Source	df	ss	mss	F value
Treatment	23	0.0479111	0.0020830913	1.1310166 ns
Block	2	0.0172111	0.00860555	4.6723923 **
Error	46	0.0847221	0.0018417867	
Total	71	0.1498444		

Appendix 10a: Yield per hectare of halo blight infected bean plants treated with *E. citriodora* extract in 1992 growing season.

ANOVAR TABLE.

Source	df	ss	mss	F value
Treatment	23	3212720.3	139683.49	2.527**
Block	2	20803.66	10401.83	0.188 ns
Error	46	2542876	55279.91	
Total	71	5776400		

Appendix 10b: number of seeds from halo blight infected bean plants treated with *E citriodora* extract in 1992 growing season.

ANOVAR TABLE.

Source	df	ss	mss	F value
Treatment	23	127061.65	5524.4196	0.3926264 ns
Block	2	470808.03	235404.02	16.730411 **
Error	46	647239.32	14070.42	
Total	71	1245109		

Appendix 10c: Number of pods from halo blight infected bean plants treated with *E. citriodora* extract in 1992 growing season.

ANOVAR TABLE.

Source	df	ss	mss	F value
Treatment	23	9781.6528	425.28925	0.4886899 ns
Block	2	25929.861	12964.931	14.8977 **
Error	46	40032.139	870.26389	
Total	71	75743.653		

Appendix 10d: Number of seeds/10 pods from halo blight infected bean plants treated with *E. citriodora* extract in 1992 growing season.

ANOVAR TABLE.

Source	df	ss	mss	F value
Treatment	23	484.31945	21.057367	0.720639 ns
Block	2	46.52778	23.26389	0.796152 ns
Error	46	1344.1389	29.22041	
Total	71	1874.9861		

Appendix 10e: Weight of 100 seeds from halo blight infected bean plants treated with *E.citriodora* extract in 1992 growing season.

ANOVAR TABLE.

Source	df	ss	mss	F value
Treatment	23	351.79649	15.2955	0.8640945 ns
Block	2	187.57731	93.788655	5.2984388 **
Error	46	814.2546	17.701187	
Total	71	1353.6284		

** Significant at p = 0.01

ns Not significant

Appendix 11a: Disease severity of common blight infected bean plants treated with *E.citriodora* extract in 1992 growing season.

ANOVAR TABLE.

Source	df	ss	mss	F value
Treatment	23	200.47504	8.7163061	14.692079 **
Block	2	0.88229194	0.4114597	0.6935505 ns
Error	46	27.290221	0.5932656	
Total	71	228.58818		

Appendix 11b: Disease incidence of common blight infected bean plants treated with *E.citriodora* extract in 1992 growing season.

ANOVAR TABLE.

Source	df	ss	mss	F value
Treatment	23	60363.111	2624.4831	37.845539 **
Block	2	10.02778	5.01389	0.0723012 ns
Error	46	3189.9723	69.347225	
Total	71	63563.111		

Appendix 11c: Pod infection of common blight infected bean plants treated with *E.citriodora* extract in 1992 growing season.

ANOVAR TABLE.

Source	df	ss	mss	F value	
Treatment	23	0.0245986	0.0010695043	0.6583277	ns
Block	2	0.0118694	0.0059347	3.6530734	*
Error	46	0.07477305	0.0016245773		
Total	71	0.1111986			

Appendix 12: Yield components of common blight infected bean plants treated with *E.citriodora* extract in 1992 growing season.

a). Yield per hectare

ANOVAR TABLE.

Source	df	ss	mss	F value	
Treatment	23	591:063.5	21350.587	1.398593	ns
Block	2	15797.04	7898.52	0.517536	ns
Error	46	702041.16	15261.76		
Total	71	5631901.7			

b). Number of seeds from 20 plants.

ANOVAR TABLE.

Source	df	ss	mss	F value	
Treatment	23	253710.32	11030.883	1.1829201	ns
Block	2	48835.361	24417.681	2.6184816	ns
Error	46	428955.97	9325.129		
Total	71	731501.65			

c). Number of pods in 20 plants.

ANOVAR TABLE.

Source	df	ss	mss	F value	
Treatment	23	12691.653	551.811	0.6266765	ns
Block	2	2960.0083	1480.0042	1.6807999	ns
Error	46	40504.639	880.53562		
Total	71	56156.3			

d). Number of seeds per 10 pods.

ANOVAR TABLE.

Source	df	ss	mss	F value	
Treatment	23	452.4444	19.671496	0.8127946	ns
Block	2	80.027778	40.013889	1.6533096	ns
Error	46	1113.3056	24.202296		
Total	71	1645.7778			

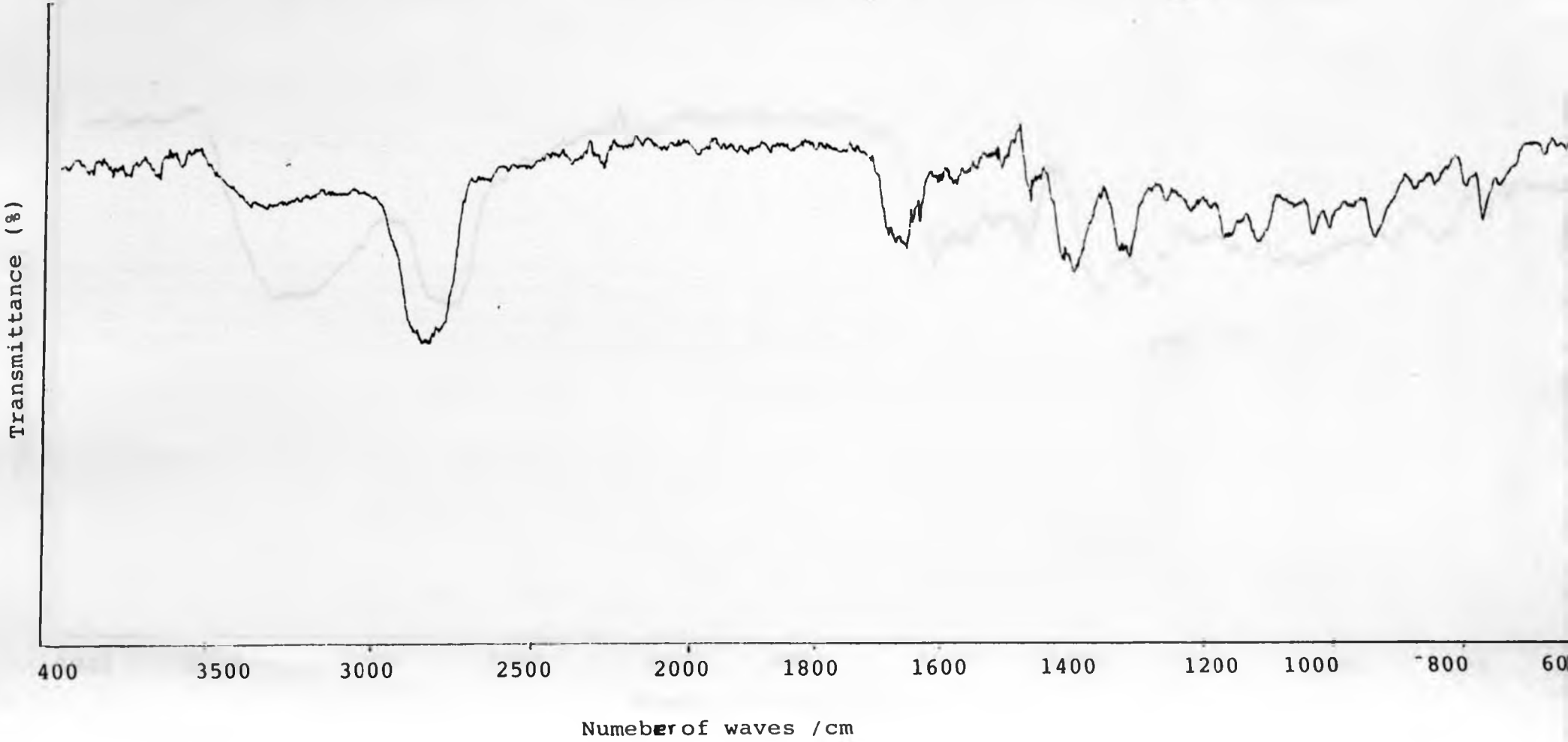
e). **Hundred seed weight.**

ANOVAR TABLE.

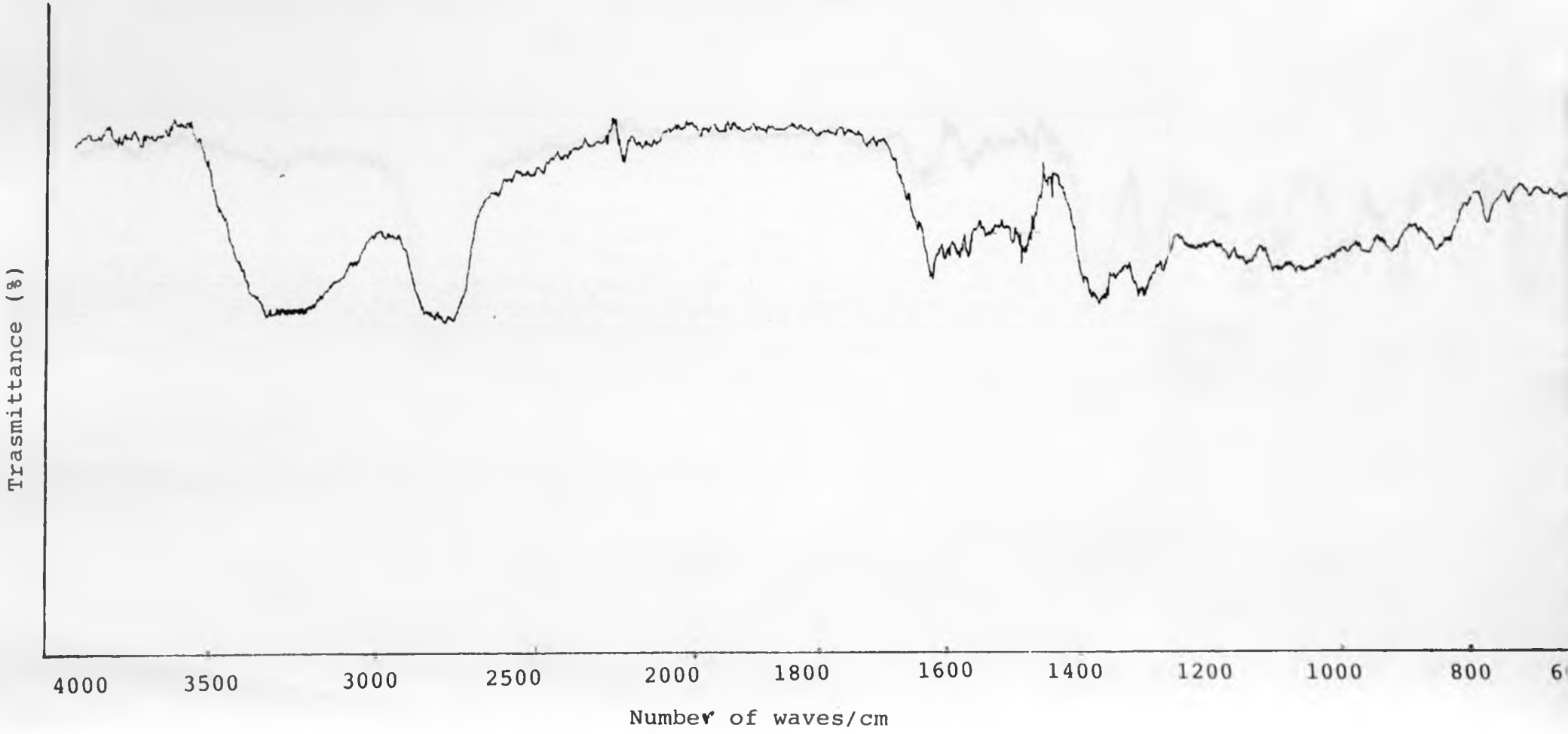
Source	df	ss	mss	F value	
Treatment	23	475.06392	20.654953	0.631514	ns
Block	2	127.39918	63.69959	1.9475805	ns
Error	46	1504.5238	32.707039		
Total	71	2106.9869			

ns Not significant at p = 0.01.

Appendix 13a: Infra red analysis of the essential oil of the essential oil of E. citriodora



Appendix 13b. Infra red analysis of ether fraction of E. citriodora oil.



Appendix 13c: Infra red analysis of Hexane fraction of E. citriodora oil