THE EFFECT OF INTRODUCTION OF AFRICAN WORMWOOD (ARTEMISIA AFRA) ON THE RHIZOSPHERE OF AGRICULTURAL LANDS

BY

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DECLARATION

I declare that this dissertation describes my original work, except where specific acknowledgement is made to the work of others.

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Place: Alice.

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ABSTRACT

The herbaceous aromatic plant locally known as "mhlonyane" (*Artemisia afra*) is indigenous to the Eastern Cape region of South Africa. Owing to the importance that this plant has always had in local traditional medicine and, more recently, the discovery that it is the source of a valuable oil, there has been great interest in its introduction as a commercial "crop". It has thus been produced successfully over the last decade on an experimental basis. Since the plant is indigenous to the area, it can be produced on normal arable land with little management input. Financial returns from even a poor crop of the plant can be more than 50-fold the return from the more conventional crops such as maize or sorghum. However, the sustainability of this high profit production system is in question as there is evidence to suggest that there may be significant deleterious effects on the soil, resulting from the introduction of this plant.

Growth inhibition of a number of crop plants and grasses, in particular, are thought to be linked with this plant genus. Experiments were carried out to establish whether the development of bare patches around the growth zones of the plant were the result of the accumulation of allelochemicals from the exudates or decomposition products of the tissue of this plant. Extracts from the leaves, stems and roots of the plant, as well as aqueous extracts of the soil on which the plants were growing were assayed for their potential inhibitory effects on the germination and seedling vigour of the seeds of selected food crops, represented by a grass, maize (*Zea mays* cv. SNK 247), a broad-leaved dicotyledon, speckled sugar bean (*Phaseolus vulgaris*) and a forage grass species, Italian rye grass (*Lolium perenne*). Distilled water and aqueous extracts of soil from adjoining land with similar soils on which, however, *Artemisia* had never been grown, were used as controls. The extracts from the soils on the test sites and the plant extracts showed statistically significant ($P < 0.05$) inhibitory effects. However, increased dilutions (1:1, 1:2, 1:2.5 and 1:5) of the "original" extracts resulted in proportional increases in germination and dry matter yield, indicating a marked decrease in inhibition. In the control extracts, germination and seedling vigour occurred without signs of impairment. The leaf extracts were the most inhibitory, followed by extracts from the other parts of the plant, the stems and roots, between which there was no significant ($P < 0.05$) difference. The soil extracts showed the

least efficacy. Inhibitory effect was markedly reduced after the 1:2 dilution of all original extracts tested on rye grass. This was less pronounced with maize and bean. Generally, effect on germination was more pronounced than on seedling vigour, indicating that germination is the main limiting factor and once seeds are able to germinate, affected seedlings manage to adjust gradually and approach "normal" growth. Analyses were also carried out to establish the nature of the compounds present in the soils on which *Artemisia* had been grown in comparison to similar soil left fallow. Soil analyses included physical tests, particularly structural stability of soil aggregates to examine the effect of released compounds from roots of *Artemisia afra* on the rhizosphere of "normal" agricultural lands. Root exudates of *A. afra* significantly ($P < 0.05$) increased the structural stability of aggregates of soil planted to this shrub. Organic compounds in soil planted to *A. afra* were, in general, a mixture of unsaturated hydrocarbons, carbonyl compounds and benzenes. The compounds were either absent or present only in small amounts in the control soils. These compounds are therefore, with a high degree of certainty, considered to be responsible for the allelopathic effects associated with *Artemisia afra*.

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1. INTRODUCTION

Interaction between higher plants is very complex with several phases including the concept of competition and that of biochemical inhibition or allelopathy (Muller, 1966). Interactions among and between plant species can be attributed to such specific factors as competition for nutrients, water and light; susceptibility or immunity to insects and diseases including viruses; and the effect of other environmental stresses. However, it has become increasingly apparent that there are other interactions which involve released substances from one plant, that have an influence on another plant, particularly an inhibitory effect known as allelopathy (Tukey, 1969; Putnam and Duke, 1974; Whittaker and Feeny, 1971). Specifically, Rice (1983) defined allelopathy as any direct or indirect harmful effect by one plant on another through the production of chemical compounds that escape into the environment.

These allelopathic interactions between plants also include effects mediated through associated micro-organisms (Putnam, 1984). Allelopathy has been recognized as a powerful ecological factor capable of altering the structure, function and composition of many plant communities. Plants shown to exhibit allelopathic effects include aromatic shrubs of the genera; *Artemisia, Eucalyptus* and *Salvia* species (Putnam, 1984). One striking characteristic about such aromatic shrubs is the release of chemical compounds into the rhizosphere (root zone) in phytotoxic concentrations which results in the growth inhibition of other plants.

The biochemical substances involved in allelopathic interactions have been termed allelochemicals and they belong to a group of materials collectively known as secondary metabolites, which are widely distributed in plants (Putnam,1985). Such chemicals enter the soil by rain leachings from foliage, by exudation from living plant parts, during decomposition of plant residues or as microbial by-products of residue decomposition (Tukey, 1969; Rice, 1974; Harborne, 1982).

It has been suggested that allelopathy may be involved in shifts in species distribution and the increase in dominance by certain annual and perennial weeds in a variety of agro-ecosystems (Muller, cited in Mersie and Singh, 1987). It is obvious to anyone concerned with agriculture that weeds often exert harmful interference on crop growth. Although weed scientists have performed numerous weed:crop 'competition studies', seldom has allelopathy been considered or mentioned and certainly never eliminated as a possible mechanism (Putnam, 1984), especially as it relates to weed control (Leather, 1987).

Special interest has been devoted to chemical characterization of the compounds excreted from higher plants into soil and to the determination of their phytotoxic activity. These investigations were aided by the introduction of improved methods of chemical identification, such as paper and gas chromatographic techniques, so that it became possible more and more frequently to identify even small amounts of liberated compounds (Börner, 1960; Saleh, 1985).

In an attempt to elucidate the allelopathic influences of both higher and lower plants, Akehurst, cited in Rice (1979) suggested that toxins might be produced by blooms of algae and that these might cause algal succession by inhibiting some species and not others. He also stated that long chain fatty acids have long been reported to be important allelochemicals produced by algae. These compounds were recently reported to be potent toxins in decaying residues of a higher plant, *Polygonum aviculare* (Alsaadawi *et al.*, 1983).

Muller, cited in Rice (1985) established that several species of *Salvia* and *Artemisia* have been found to produce volatile compounds which inhibited growth of test plants in a *Salvia* and *Artemisia* stand.

Since plant chemicals are involved in allelopathy, it is of interest to identify potential allelochemicals, monitor their release into the environment, as well as the quantity and extent of release. It is also important to assess the mechanisms of inhibition of growth of other associated plants.

2. LITERATURE REVIEW

2.1. Chemical Nature of Allelopathic Agents

Allelopathic compounds consist of a wide variety of chemical types. Rice (1985) established that these allelochemicals arise either through the acetate or shikimic acid pathway. He also stated that these compounds range from very simple volatiles and aliphatic compounds to complex, multi-ringed aromatic compounds.

This is illustrated in fig. 1.

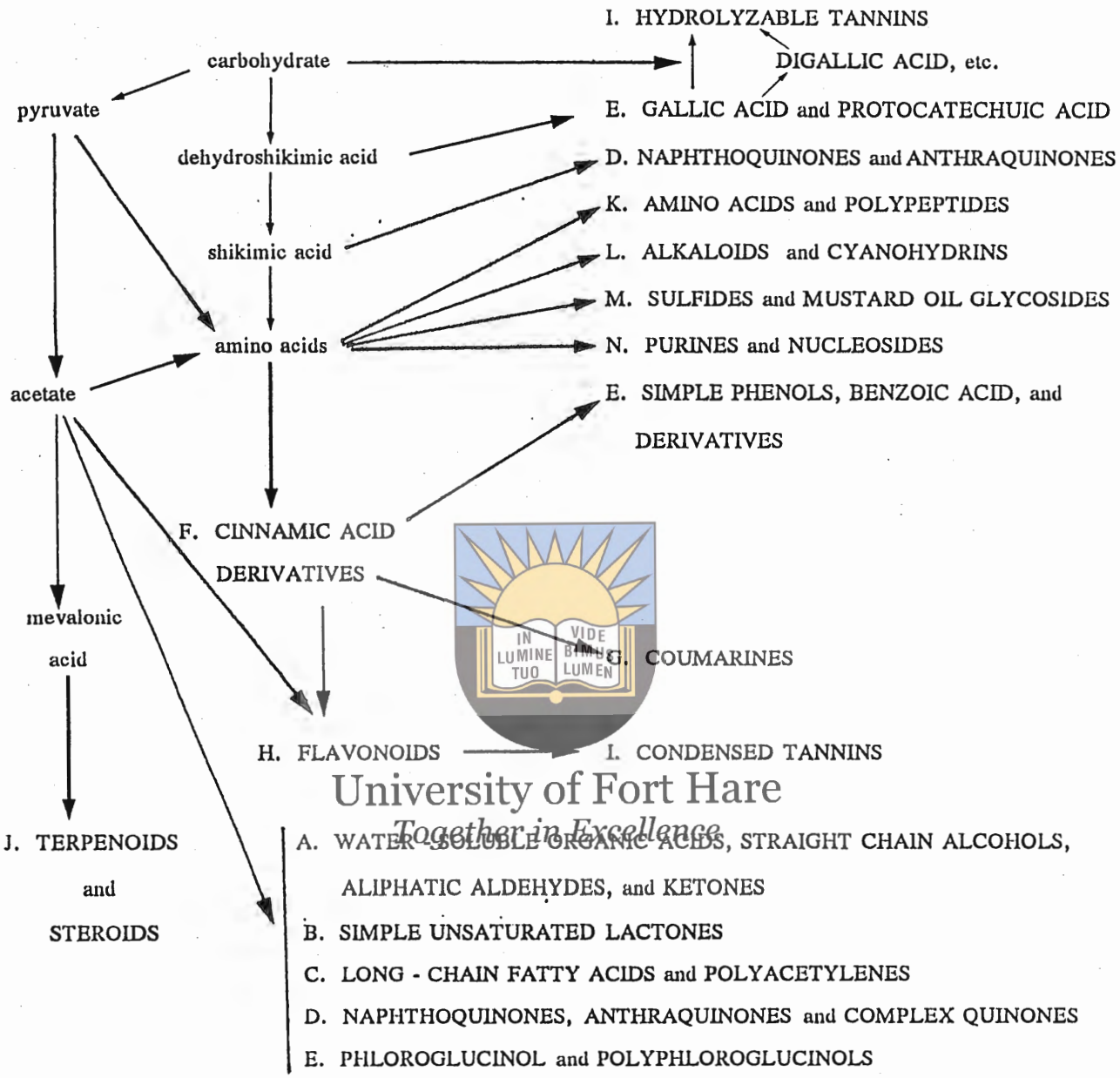

Fig 1. Chemical categories of allelopathic compounds with probable major biosynthetic pathways leading to their production. Source : Rice (1985)

Several organic acids such as malic, citric, acetic and tartaric acids in fruits, are often concentrated enough to inhibit germination of seeds inside the fruits and the presence of acetaldehyde in unripe grains of corn and unripe seeds of peas prevents them from germination (Evenari, 1949). The type of allelochemical present has been found to vary with different plant species. Patrick (1971) discovered that acetic and butyric acids were among the toxins produced during decomposition of rye residues and salts of acetic, propionic and butyric acids were the major phytotoxins produced in decaying wheat straw (Börner, 1960; Tang and Waiss JR., 1978).

Rice (1985) stated that Juglone (5-hydroxy-1, 4-naphthoquinone) is the only quinone identified as an allelopathic compound from higher plants. In an experiment to investigate the toxic influence of Walnut trees on other plants in their immediate vicinity, Gries (1943) showed that *Juglans nigra* and *Juglans cinerea* contain large quantities of hydrojuglone in the root bark as well as in the leaves and in the fruit flesh. He stated that this compound, which is not phytotoxic, is oxidized immediately on exposure to the air into toxic juglone.

Muller (1966) reported that the two most common groups of inhibitors in plants center about the terpenes and the phenols and that each of these groups is biosynthetically related, the terpenes through the isoprene pattern and the phenols through benzene. In agreement with this, Rice (1985) stated that simple phenols, phenolic acids derived from benzoic acid and those derived from cinnamic acid have been the most commonly identified allelopathic compounds produced by higher plants and the most common allelopathic compounds identified in soil under allelopathic plants are p-hydroxybenzoic, vanillic, p-coumaric and ferulic acids. This is confirmatory to the findings of Börner (1960) who stated that, experiments on the chemical structure of inhibiting compounds from cold water extracts of straw of barley, rye and wheat and also alcoholic extracts of roots of these plants, contain ferulic acid (4-hydroxy-3-methoxycinnamic acid), p-coumaric acid (4-hydroxycinnamic acid), vanillic acid (4-hydroxy-3-methoxybenzoic acid) and p-hydroxybenzoic acid.

Coumarins are lactones of o-hydroxycinnamic acid in which side chains often are isoprenoids. Coumarin, esculin and psoralen (a furanocoumarin) all strongly inhibit seed germination and

these are produced by a variety of legumes and cereal grains (Rice, 1985).

Flavonoids are widespread in higher plants and a few have been implicated in allelopathy. It has been observed that numerous flavonoids and their glycosides are inhibitory to nitrifying bacteria (Rice and Pancholy, 1974).

Robinson (1983) stated that higher plants produce a great variety of terpenoids but only a few have been implicated in allelopathy. However, monoterpenoids are the major components of essential oils of higher plants and they are the predominant terpenoid inhibitors that have been identified. Many fungi (Owens, 1969) and algae (Fenical, 1975) also produced terpenoid allelochemicals.

Muller *et al.* (1964) discovered that the volatile compounds in the leaves of aromatic shrubs, including *Artemisia californica,* inhibited the growth of oat (*Avena fatua* L.) and cucumber (*Cucumis sativus* L.). Further experimentation indicated that the volatile terpenes could escape into the atmosphere, become adsorbed onto soil particles and, possibly, enter the seedling through the waxy cuticle of the root epidermis (Muller, 1965, 1966). Phytotoxicity by terpenes is highly dependent on weather conditions, particularly temperature and moisture (Kelsey *et al.*, 1978). This confirms the observation of Halligan (1975) who stated that soil toxicity from adsorbed terpenes diminishes rapidly after wetting, therefore, any terpenes that might have been adsorbed during a short dry period would have been lost during an ensuing rainy period. This explanation was given, following an experiment on soils of *Artemisia tridentata* subspecies vaseyana, in which relatively no monoterpenes were found, due to a high moisture content of the soil. Another reason given for the low terpene concentration was that the soil used for the analysis (sandy loam) had an abundance of sand and large particles that lack fixation sites. Although monoterpenes were minimal in the soils, three sequiterpene lactones (arbusculin -A, -B and -C), two coumarins (unbelliferone and isoscopoletin) and an unknown flavonoid were identified in the soils beneath sagebrush plants (Kelsey *et al.*, 1978).

Graven *et al.* (1990) reported that the major chemical components of *Artemisia afra* volatile oil are: camphene, 1,8 cineole, α-Thujone, ß-Thujone, camphor and α-pinene. In a capillary gas chromatographic/mass spectrometric analysis of *Artemisia monosperma* and *Artemisia judaica* fresh plants, Saleh (1985) reported that volatile oil of *Artemisia monosperma* was found to be made up primarily of highly unsaturated hydrocarbons and aromatic acetylenic compounds with 3-methyl-3-phenyl-1,4 pentadiyne being the major component. Volatile oil of *Artemisia judaica*, on the other hand, was found to be a mixture of esters, ketones and aldehydes in which pipertone is the major component.

There are only a few instances in which amino acids have been implicated in allelopathy and, in most cases, the specific amino acids have not been identified (Rice, 1985). Owens *et al.* (1972) reported that Rhizobitoxine, a non-protein amino acid is produced by certain strains of *Rhizobium japonicum* and several of the phytotoxins produced by pathogenic micro-organisms are polypeptides and related glycopeptides.

Niemann, cited in Börner (1960) showed that the seeds and fruits of numerous plant species excrete ninhydrin-positive compounds during the swelling period. Later, other authors successfully identified numerous liberated amino acids and sugars from swelling seeds of *secale cereale*, *Triticum aestivum* and *Hordeum vulgare*, as well as *Trifolium repens*, *Lolium perenne* and *Artemisia absinthium* (Linskens and Knapp, cited in Börner, 1960). In a study by Putnam and Schmidt (1959) on the free amino acid fraction of soils, they reported small but detectable amounts of free amino acid. However, McCalla and Haskins (1964) stated that, under usual soil conditions, it is unlikely that free amino acid concentrations will become sufficiently high to be toxic to plant growth.

Cyanohydrins have been implicated in allelopathy in several instances. Akazawa *et al.* (1960) found that dhurrin, a cyanohydrin, occurs in grain sorghum seedlings and the seedlings contain enzymes that hydrolyze dhurrin to glucose, HCN (hydrogen cyanide) and p-hydroxybenzaldehyde. Both HCN and p-hydroxybenzaldehyde are potent allelochemicals (Putnam, 1984). HCN and benzaldehyde are produced by the hydrolysis of amygdalin which

has been identified as being responsible for the peach re-plant problem in old peach orchards (Patrick, 1955; Börner, 1960).

Many antibiotics produced by various micro-organisms are nucleosides. Among these are nebularine, cordycepin and nucleocidin (Rice, 1985). In higher plants, purines have also been found to be involved in allelopathy. One of the known purines shown to be involved is caffeine (Chou and Waller, 1980). Evenari, (1949) included caffeine as an alkaloid and one of the most potent in the inhibition of seeds.

2.2. Factors Affecting Amounts of Allelopathic Compounds Produced by Plants

Certain factors are known to affect the amounts of allelopathic compounds released by plants. These include soil moisture, levels of ethylene and amount of phosphorus.

Gilmore (1977) examined the effects of soil moisture stress on loblolly pine (*Pinus taeda*) and reported that α-pinene concentration was found to increase in water-stressed loblolly pine whereas ß-pinene, myrcene and limonene decreased. In a similar test, Rice (1979) stated that camphene concentration increased with soil moisture stress in one test year and decreased during the second test year.

Sarkar and Phan (1974) reported that the amount of total phenols in carrot roots increased five-fold after a 3-day exposure to ethylene, and seven-fold after a 7-day exposure. In their test, they also observed that more than four new phenols were produced which do not occur normally in carrot tissues. This mechanism could certainly enhance the effect of ethylene as an allelopathic agent. A large increase in the concentration of the isomers of chlorogenic acid was observed in extracts of sunflowers grown under phosphate-deficient conditions (Koeppe *et al.*, 1976). Moreover, more phenolic compounds were leached from the living intact roots, dried roots and tops of phosphate-deficient plants than from phosphate-sufficient ones (Rice, 1979).

2.3. Some Mechanisms of Action of Allelopathic Agents

Evidence has been accumulating over many years that plants can influence each others' growth by means of exudates (Rice, 1974). However, there is still only fragmentary evidence on the particular processes in the receiving plant, that are primarily influenced by the exudate (Newman and Miller, 1977). One relatively common mechanism of action of allelopathic compounds is the reduction in uptake of minerals by plants (Rice, 1979).

Muller (1966) postulated that inhibition of seedling growth by terpenes is due to the accumulation of this compound in the cutin of root epidermis as the roots come into contact with soil particle surfaces. Dissolved terpenes move through lipid layers of the cell wall into the protoplast where they disrupt vital functioning of cellular components. Though the nature of this inhibition is not fully known, Muller (1965) observed what appears to be a failure of cell division in severely inhibited seedlings. Thus the terpenes, like several other kinds of plant growth inhibitors, seem to suppress mitosis. Mersie and Singh, (1987) reported that lantana weed residue inhibit growth and reduce dry weight of roots of corn, wheat, soybean, velvetleaf and virginia pepperweed. Two possible explanations for the observed effect were the inhibition of cell division and cell elongation in roots.

Danks *et al.* (1975) also investigated the mechanism of action of phenolic acids in rose cell suspension cultures. They observed that, the phenolic acids did alter the flow of carbon from protein and carbohydrates to lipids in the cultures, explaining the growth and germination inhibition caused by phenolic compounds.

In an attempt to elucidate the effect of allelopathic compounds on mineral absorption, Yoder and Scheffer (1973) exposed susceptible corn roots to the host-specific toxin of *Helminthosporium carbonum* Ullst. race 1. They observed that corn roots removed nitrate from solution and accumulated it in tissues twice as fast as did control roots. After further tests, they concluded that the enhanced nitrate accumulation was caused by increased uptake rather than by decreased nitrate metabolism or decreased nitrate leakage. In a similar test, Frick *et al.* (1976, 1977)

reported that the *Helminthosporium maydis* race T toxin inhibited K^+ uptake by corn roots. Inhibition was more pronounced in highly susceptible lines.

Another and more direct way, that allelochemicals may inhibit ion absorption by plants is by inhibiting plasma membrane-bound ATPases that are involved in ion transport (Balke and Hodges, 1977). Balke, cited in Rice (1979) surveyed various phenolic compounds for their effects on K^+ absorption by excised oat (*Avena sativa*) roots and on ATPase activity of plasma membrane vesicles isolated from oat roots. He found that the flavonoids were generally more inhibitory than phenolic acids at a 10^{-4}M concentration. The most inhibitory phenolic tested was juglone with a 79% inhibition of K^+ absorption and a 42% inhibition of ATPase activity. Glass (1973) showed that various phenolic compounds, applied at low concentrations, could reduce phosphorus uptake by detached roots of barley and, since some of these substances occur in plants and soils, they could be involved in allelopathy.

In an experiment to examine the effect of root exudates of *Anthoxanthum odoratum*, rye grass (*Lolium perenne*), narrow-leaved ribwort (*Plantago lanceolata*) and white clover (*Trifolium repens*) on the rate of phosphorus uptake by these plants, Newman and Miller (1977) observed a clear reduction in phosphorus uptake by all species from *Plantago* leachate and increased uptake by *Anthoxanthum* from *Trifolium* leachate. This led to the conclusion that, among species which commonly grow together, root exudates from one plant can influence phosphorus uptake by another. In some cases, phosphorus uptake is promoted, relative to the control and, in other cases, it is inhibited.

It has been shown that benzoic acids are capable of inhibiting active potassium and phosphate absorption as well as depolarizing cell membrane electrical potentials (Glass, 1973, 1974; Glass and Dunlop, 1974). It was later established that the major factor in the inhibition of phosphate uptake by the benzoic acids is their lipid solubility (Glass, 1974). Gajic *et al.* (cited in Rice 1979) reported that addition of 0.8 to 3g/ha. of agrostemmin, an extract from *Agrostemma githago*, significantly increased amounts of easily available phosphorus and potassium in manured eroded Chernozems and hydromorphic black soils in Yugoslavia. Results in

unmanured soils were variable, but, at least some concentrations of agrostemmin increased amounts of available phosphorus and potassium.

Rhizobitoxine, a substance secreted by certain strains of the bacterium *Rhizobium japonicum*, firstly detected in the root nodules of soybeans has greatly revolutionized the weedicide industry by the indispensable mechanism with which it acts (Anon, 1969). The substance, very poisonous to young plants, has a high affinity for an enzyme that normally promotes the synthesis of protein in plant growth processes. It inactivates the enzyme on contact, halting further plant development. It is effective in applications as dilute as 85.2 ml/acre, but causes little harm to mature plants.

2.4. Decomposition of Allelopathic Compounds in Soil

It has been established that many factors, both genetic and environmental, affect the amounts of potential phytotoxins produced by all plants and micro-organisms which, if present in appropriate concentrations, can either inhibit or stimulate the growth of other plants. Once the phytotoxins are produced and escape into the environment, they begin to be decomposed either by micro-organisms or by chemical action not involving micro-organisms (Rice, 1979). Thus, potential allelopathic effects depend basically on the relative rates of addition of the allelochemicals to the environment and decomposition or inactivation. It then becomes abundantly clear that if allelopathic compounds which are released into the environment were not decomposed, probably no plants would survive.

In a comprehensive work done by Henderson and Farmer (1955) several fungi isolated from soils under a variety of vegetational types were found to attack p-hydroxybenzaldehyde, ferulic acid, syringaldehyde and vanillin. These compounds were used as the sole source of carbon by the organisms tested. Vanillin and ferulic acid were converted to vanillic acid before the breaking of the benzene ring, syringaldehyde was converted to syringic acid and p-hydroxybenzaldehyde was converted to p-hydroxybenzoic acid (Henderson, 1956). These intermediate products were found to be attacked by adaptive enzymes and the formation of these

enzymes was greatly decreased by the antibiotic citrinin. All of the intermediate products are toxic to many organisms. So allelopathic effects could continue to be exerted for a prolonged period even during decomposition (Rice, 1979).

Kunc (1971) investigated decomposition of vanillin in a Chernozem soil and reported that it decomposes via vanillic and protocatechuic acid before the aromatic ring opens. During incubation of the soil with vanillin, the number of bacteria capable of using vanillin as the sole carbon source increased. Of the twenty-one strains isolated, fifteen were identified as *Pseudomonas* spp., five as *Cellulomonas* spp. and one as *Achromobacter* sp. Ferulic acid is one of the major compounds resulting from decomposition of lignin and is thus one of the more important phenolic compounds in most soils (Rice, 1979).

It has been established that tannins are common and generally very inhibitory allelopathic compounds against most test plant species. Nevertheless, there are micro-organisms which can decompose at least some of these highly diverse compounds (Lewis and Starkey, 1968, 1969; Grant, 1976). Condensed tannins are especially difficult to decompose and, as a result, they accumulate in soil over a long period of time (Rice and Pancholy,1973). Grant (1976) isolated a strain of *Penicillium adametzi* Zal. from enrichment cultures with condensed tannins as the carbon source. This *Penicillium* grew well on the low-molecular weight tannins. However, it grew poorly on higher molecular weight condensed tannins which indicates it would decompose such compounds very slowly.

Schreiner and Skinner; Fraps; and Funchess, (cited in McCalla and Haskins 1964) showed that the harmful effect of dihydroxystearic acid could be largely overcome by adequate mineral fertilization of the plant. However, toxic effects of coumarin and vanillin were not prevented by the application of inorganic nutrients. Skinner and Noll (1916) reported that vanillin and salicyclic aldehyde persisted for several months on a silty clay loam soil. However, in the presence of $NaNO_3$, these compounds were found to quickly decompose. They also observed that neither vanillin nor salicyclic aldehyde persisted in limed soil. Truog and Sykora (cited in McCalla and Haskins 1964) explained that finely divided soil materials such as lime, soil,

kaolin, or quartz largely neutralized the phytotoxicity of vanillin or guanidine carbonate.

2.5. The Role of Allelopathy in Agriculture

The concept that one plant can influence the growth of another is well known in agricultural plant science (Tukey, 1969). Plant to plant chemical interactions have been well recognized in commercial agriculture and, in fact, form the basis of many common agricultural practices. It is currently being utilized in modern plant science in the development of bioassay systems for detecting growth regulators, detection and eradication of disease and in fruit storage and ripening (Tukey,1969).

The return of plant residues to the soil has long been recognized as being of agronomic importance, especially in relation to soil tilth and maintenance of organic matter. Such practices, however, sometimes result in deleterious effects on succeeding crops. Toxicities arising from such residues provide challenging problems and opportunities for agronomists and weed scientists. Where stubble-mulch farming has also been practiced for soil and water conservation, toxins from the stubble have proven toxic to certain rotational crops (McCalla and Haskins, 1964). In recent years, there has been a movement toward conservation tillage (including no-tillage) practices which preserve surface plant residues. These residues can influence crop emergence, growth and productivity and have similar influence on weed emergence and growth. Recent work by Putnam and De Frank (1983) and Putnam (1985) indicates that management of selected crop residues eg. rye *Secale*, wheat *Triticum* and sorghum species can greatly reduce weed germination and growth.

2.5.1. Allelopathic effects of crop plants on other crop plants

Certain crop plants have been found to produce compounds inhibitory to the growth of the same and other crop plants (Rice, 1985). Willis (1985) substantiated the findings of McCalla and Haskins (1964) by establishing that phytotoxic allelopathic effects on plants have been associated with certain agronomic practices where specific plant species grown in rotations were found to

be compatible, while others were incompatible with those that followed.

The auto-inhibitory effect displayed by some crop plants has been demonstrated in a number of studies. For example, the unharvested parts of rice plants are customarily mixed with the soil through ploughing or other mechanical manipulation, because this has been thought to be beneficial. It has been commonly observed, however, that productivity of the second rice crop in a paddy is less than that of the first crop. After a comprehensive study on the effects of decomposing rice residues in soil on the growth of rice plants, Chou and Lin (1976) observed that aqueous extracts of decomposing rice residues in soil inhibited radicle growth of rice seedlings and growth of rice plants. The authors then concluded that the decline in productivity of rice relative to the first crop was due primarily to the allelopathic effects of decaying rice residues in the paddy soil. This confirms the findings of McCalla and Dulley (1949), who suggested that the detrimental effects of crop residues on subsequent crops might be due to a combination of toxins from the residues and from micro-organisms that were caused to grow more profusely by substances in the residues.

Wheat (*Triticum aestivum* L.) is a crop which has been found to undergo auto-inhibitory effect. A study by Kimber (1973) to examine the inhibitory effect of wheat straw residues on subsequent wheat crops revealed that both nitrogen immobilization and toxins affect the yield of wheat when it is grown in the presence of excess straw residues. He also pointed out that addition of nitrogen did not overcome the effects of the straw in several types of experiments and finally, inferred that this added effect was due to toxins derived from the rotting straw (Rice, 1979).

Walker and Jenkins (1986) reported that sweet potato (*Ipomoea batatas* (L.) Lam.) interferes with crop growth. They observed that sweet potato plant residues inhibited the growth of sweet potato vine cuttings and cowpeas (*Vigna unquiculata* (L.) Walp.). Subsequent research by Walker *et al.* (1989) showed the mechanism of growth inhibition to be reduced uptake of Ca, Mg and S by sweet potato plant residues.

Leon, cited in Rice (1979) observed that the growth of Sorghum (*Sorghum vulgare* Pers.) in

Senegal, West Africa, decreased markedly when it follows sorghum grown in sandy soil but not in soils high in montmorillonite. For an explanation of this observation, he concluded that the native microflora in the sandy soils of Senegal were not able to detoxify the soil fast enough to prevent inhibition of subsequent crops of sorghum in the same soil.

Guenzi and McCalla (1962) also reported that corn (*Zea mays* L.) residues were inhibitory to seed germination and seedling growth of wheat. Later in 1966, these authors, in a search for chemical inhibitors, identified several phenolic acids in mature corn plant residues and all were found to be inhibitory to wheat seedling growth. Guenzi *et al.* (1967) also reported that the toxicity of decaying corn residues remained high during the first 22 weeks of decomposition in field tests, but declined thereafter.

2.5.2. Allelopathic effects of weeds on crop plants

In the past few years, with the increase in some of the more important weed grasses, it has been observed under field conditions that specific weed grasses tend to predominate (Schreiber and Williams, (1967). Approximately 75 species of weeds have been reported to have allelopathic potential but many of those have not been tested against crop plants (Putnam 1984; Rice 1985). One might, however, speculate that aggressive perennial weed species that quickly gain dominance may do so by allelopathic mechanisms (Putnam,1985).

Naqvi (1972) found in greenhouse and field tests, that Italian rye grass (*Lolium multiflorum* Lam.) suppresses germination and growth of many species in its vicinity. Later, Naqvi and Muller (1975) reported that leachates from the above ground parts caused by artificial rain and of soil previously occupied by Italian rye grass and decomposing residues were toxic to seedling growth of oats, Bromus species lettuce and Trifolium species.

Holm, cited in Rice (1985) listed purple nutsedge (*Cyperus rotundus*) as one of the ten worst seeds in the world. Rice (1985) established that interference by this weed caused reductions in yields of various crops ranging from 23 to 89% even on the basis of average effects of various

weed densities. It is noteworthy, that numerous workers have found purple nutsedge to be strongly allelopathic. Friedman and Horowitz (1971) observed that soil previously infested with this weed for 9 to 12 weeks significantly reduced germination of mustard, barley and cotton seeds.

Growth and yield reductions of corn by infestations of giant foxtail (*Setaria faberii* Herrm.) have been well documented and these reductions were always ascribed to competition until Schreiber and Williams (1967) demonstrated that decaying roots of giant foxtail inhibited growth of maize roots markedly. This result occurred in spite of addition of sufficient nitrogen to reduce the short term effect of a high C/N ratio of the added organic matter, indicating an allelopathic effect.

There is considerable evidence now to suggest that some of the more aggressive perennial weed species, including quackgrass (*Agropyron repens*) and yellow nutsedge (*Cyperus esculentus*) may impose allelopathic influences, particularly through toxins released from their residues (Putnam, 1985). Recent studies also indicate that quackgrass residues release compounds that are inhibitory to root growth, nodulation and nitrogen fixation on a number of legumes. This confirms the findings of Schreiber and Williams (1967) referred to above. Rasmussen and Einhellig (1975) demonstrated that aqueous extracts of milkweed (*Asclepias syriaca* L.) leaves significantly inhibited growth of grain sorghum seedlings and also observed that reduced concentrations of the extracts resulted in proportional increases in yield. They isolated two phenolic toxins, both of which inhibited seed germination of sorghum and radish.

2.5.3. Possible use of allelopathy in biological weed control

Research in allelopathy has focused mainly in the areas of agriculture, forestry, phytopathology, patterning of vegetation, algal succession and old-field succession. Successful biological control of weeds is limited to a few instances where introduced predators have reduced populations of a target species (Putnam and Duke, 1974). These authors stated that no biological methods have gained wide acceptance for selective use in agronomic crops. They further stated that, although

plant breeders have successfully incorporated both insect and disease resistance into cultivars of many crops, there has been no concerted effort to develop crops with superior competitive ability with weeds. However, an observation made by Muller, cited in Putnam (1985) indicated that, black mustard (*Brassica nigra*) can form almost pure stands after invading annual grasslands of coastal Southern California. This was attributed to inhibitors released from the dead stalks and leaves which do not allow germination and growth of other plants. Similar results could therefore be exploited with crops, specifically to achieve almost pure stands of crops (over weeds) by use of an allelopathic mechanism.

Direct utilization of principles of allelopathy as a weed management strategy has been recently attempted by some weed scientists (Putnam, 1985). Two documented approaches cited in (Putnam, 1985) are as follows: firstly, to screen for allelopathic types in germplasm collections of crops, the idea being to ultimately transfer this character into cultivars by either conventional breeding or other genetic transfer techniques. Superior weed suppressing types have been reported from searches of cucumber (*Cucumis sativus*), (Putnam and Duke, 1974); oat (*Avena sativa*), (Fay and Duke, 1977); sunflower (Leather, 1983) and soybean collections (Massantini *et al.*, cited in Putnam, 1985). This approach may be particularly suited to crop plants that are maintained in high density monocultures (growing crops in pure stands) i.e turfgrasses, forage grasses, or legumes. Another approach has been to utilize allelopathic rotational crops or companion plants in annual or perennial cropping systems. Living rye (*Secale cereale* L.) and its residues have been shown to provide nearly complete suppression of a variety of agro-ecosystem weeds (Barnes and Putnam, 1983). Similarly, residues of sorghum, barley (*Hordeum*), wheat (*Triticum*) and oats can provide exceptional suppression of certain weed species (Putnam and DeFrank, 1983).

Another input on screening was done by Fay and Duke (1977). The authors screened three thousand accessions of the U.S.D.A. World Collection of *Avena* subspecies germplasm for their ability to exude scopoletin, a naturally occurring compound shown to inhibit root growth. They observed that plants grown in close association with the toxic accession exhibited severe chlorosis, stunting and twisting, indicative of chemical (allelopathic) effect rather than simple

competition.

Swain (1977) reported that over 10, 000 low-molecular weight secondary products have already been isolated from higher plants and fungi. Some of these chemicals or their analogs could provide important new sources of agricultural chemicals for the future.

There is considerable interest within industry on at least two approaches involving allelochemicals for weed control. One involves the production of crops (perhaps through genetic engineering) which can either themselves suppress associated weeds or provide a source of chemicals to suppress the weed (Putnam, 1985). Another approach mentioned by the author is to produce natural herbicides through batch culture with micro-organisms. Two phosphonated amino acid herbicides (bialophos and glufosinate) have already been discovered using this approach.

Classic seed burial studies indicate that several weed species maintain viability after 80 years, with one species (*Verbascum blattaria* L.) still germinating after 100 years, due to long viability caused by chemical inhibitors contained in their seed coats (Putnam, 1984; 1985). These specialized allelopathic chemicals induce dormancy and also prevent decay of the seeds by micro-organisms (Rice, 1977). Rice (1985) explained that this effect is due to unsaturated lactones and phenolic compounds present in many seeds which are potent antimicrobial compounds. Fruits and seeds are also known to contain diverse germination inhibitors including phenolic compounds, flavonoids and/or their glycosides and tannins. Unique methods to destroy inhibitors could then provide an excellent weed management strategy (Putnam, 1985).

It should perhaps be noted that, as Putnam (1985) observed, the major challenges to weed scientists are to minimize the negative impacts of weed allelopathy on crop growth and yield and to exploit allelochemicals as additional crop protection strategies.

2.6. Roles of Allelopathy in Natural Ecosystems

2.6.1. Patterning of vegetation

Patterning refers to the spatial arrangements of individuals which are visually apparent in the field, such as bare areas under certain tree species (Rice, 1979). Many ecologists have attempted to explain the patterning of vegetation and the general distribution of plants largely on the basis of competition. There is little doubt that competition always plays a role in spatial distribution, but there is growing evidence that allelopathy probably plays a role also in most, if not all, spatial distributions of plants (Bell and Muller, 1973). This is observed where distinct zones of inhibition are present under and adjacent to a variety of woody species and, often, toxins from their litter are implicated (del Moral and Muller, 1970).

In various ecosystems, plants tend to pattern themselves as nearly pure stands, or as individuals spaced in rather specific densities or configurations. This is characteristic of several weeds in the mustard family which tend to form almost pure stands after invading grasslands or agricultural lands (Putnam, 1984). Many desert species show obvious zones of inhibition around which few, if any, aliens are allowed to invade. These patterns often cannot be adequately explained by competition alone and are probably caused by a combination of factors including allelopathy. The phenomenon happens with herbaceous plants as well as woody shrubs and trees (Putnam, 1985). Ecologically significant biochemical interaction among plants may take a variety of forms, ranging from stimulation to inhibition (Muller, 1966).

2.6.1.1. Patterning due to allelopathic effects of grasses

Rasmussen and Rice, cited in Rice (1985) observed that a small grass, *Sporobolus pyramidatus*, often extended the size of its stands in the University of Oklahoma Golf course from a few plants to large areas in a short time in spite of a heavy stand of bermuda grass, (*Cynodon dactylon*), a more robust plant. They subsequently obtained overwhelming evidence that *S. pyramidatus* is able to spread rapidly into heavy sods of bermuda grass or buffalo grass

(*Buchloe dactyloides*) because it produces toxins that are exuded from living roots or diffuse from decaying roots or shoots and inhibit seed germination and growth of these species. Chou and Young (1975) surveyed twelve species of subtropical grasses for the presence of phytotoxins. Six phenolic acids were identified in ether extracts of the twelve species and they were differentially distributed in the grasses. Most of the compounds were also found in soil collected from under the various species in the field and control soil with no grasses or other herbs had significantly lower concentration of the phytotoxins than did the grass soils. The authors suggested, therefore, that the phytotoxins in soils under the test grasses probably originated from the grasses due to leaching from the plants during rain, exudation from the roots, decomposition of grass residues, or combinations thereof.

It has been observed that the grass, Prostrate knotweed (*Polygonum aviculare*), rapidly encroaches into bermuda grass lawns and the bermuda grass dies in patches of prostrate knotweed while bermuda grass at the edges of the knotweed patches turns yellow. Alsaadawi and Rice (1982) collected soil samples from under these two grasses and performed germination experiments on bermuda grass seeds using soil from under bermuda grass as controls. They further performed seed germination and seedling growth tests of bermuda grass, using root exudates, decaying roots and shoots of prostrate knotweed. In all these tests, seed germination and seedling growth of bermuda grass were inhibited. Alsaadawi *et al.* (1983) isolated eleven allelochemicals inhibitory to growth of bermuda grass from soil under prostrate knotweed and none of these occurred in soil under bermuda grass. Four of the compounds were found to be phenolics and seven were long-chain fatty acids.

In a phytotoxic compound study on grasslands in California, Panova, cited in Rice (1979) found that the 0-10 cm horizon which was most saturated with roots had the greatest inhibitory activity regardless of the floristic composition of the particular steppe community under study.

Newman and Rovira (1975) conducted a study on possible allelopathic interactions, using four grass and four forb species. They observed that growth inhibition was significant when compared with controls. However, analysis of plants for N, P & K showed that the growth

reductions were not due to nutrient deficiencies. Rice (1979) therefore, stated that only organic compounds have been considered to be allelopathic agents. He also established that most, if not all, organic compounds which are inhibitory at a certain concentration are stimulatory to the same allelopathic process at lower concentrations.

2.6.1.2. Patterning due to allelopathic effects of shrubs

Numerous investigators have presented evidence that many species of *Artemisia* have allelopathic effects against neighbouring species and a considerable volume of recent research on allelopathy in shrubs as related to grasslands, has concerned this genus. Much of this research has focused on the identification of phytotoxins produced by this shrub and also the mechanisms of action (Rice, 1979). Hoffman and Hazlett (1977) tested aqueous extracts of *Artemisia tridentata* litter and foliage extracts of this and several other species of *Artemisia* against seed germination of numerous species associated with *Artemisia* under field conditions. Volatile compounds from the foliage were also tested against seed germination. The authors reported numerous instances where germination was inhibited in some species, while in others, germination was stimulated. They then concluded that their results suggest possible allelopathic influences of *Artemisia* on species distribution patterns in *Artemisia* dominated vegetation.

The phenomenon of patterning of vegetation was clearly demonstrated in a natural, uncultivated grassland in Southern California by Muller (1966). The author reported bare areas of inhibition adjacent to a soft chaparral vegetation formed by *Salvia leucophylla* and *Artemisia californica* shrubs. He observed that under and between *Salvia* and *Artemisia* shrubs and in the first two meters beyond the shrub crowns, the soil may be completely barren of herbs or it may exhibit sparsely scattered stunted seedlings of a few annual species. The third to sixth meters beyond the shrub bear dense but stunted few species. However, more than six to ten meters beyond the shrubs, this inhibited vegetation gradually merges with normal grassland, including robust plants of species not found closer to the shrubs. The inhibition phenomenon extends far beyond the reach of the shrub roots whose lateral extension barely reaches the closest inhibited herbs, falling several meters short of uninhibited grassland. This, Muller concluded, suggests that the toxic

principle of the shrub is a volatile material which moves in a vapour state. The volatiles involved were identified as terpenes, in the forms α-pinene, ß-pinene, camphene, cineole and camphor.

Auto-toxicity is probably of wide significance in plant succession in many kinds of vegetation. Muller (1966) observed a localization of young *Salvia* and *Artemisia* shrubs outside the area of mature shrubs and stated that seedlings of either *Salvia* or *Artemisia* are rarely found among the mature shrubs even if there occurs abundant open space within the stand. He observed deterioration in the interior of the older and larger stand and what seem to be areas of bare soil. This led him to suggest that the same allelopathic influence that restricts the growth of annual herbs also causes the deterioration of the shrubs and inhibits the establishment of their seedlings.

2.6.2. Allelopathic activities of micro-organisms

Soil micro-organisms produce a tremendous variety of organic substances during the decomposition of plant and animal residues and numerous studies have shown that some of these substances are phytotoxic (McCalla and Haskins, 1964). Phytotoxins can have very important effects on higher plants, indirectly, through their effects on micro-organisms in the soil, which are important to the growth of the higher plants in both beneficial and detrimental ways (Rice, 1979).

Kushnir, cited in Rice (1979) found large numbers of bacteria, actinomycetes and fungi in the rhizosphere of (*Crambe cordifolia* Stev.) and (*Heracleum sosnowski* Mand.), even though these two plants are strongly allelopathic. He also indicated that several of the bacteria and fungi were found to synthesize allelopathic compounds. This confirms the experimental results of Patrick (1955) who indicated that, excreted non-toxic compounds of higher plants may be decomposed or transferred by micro-organisms to phytotoxic ones. He also stated that, amygdalin, a natural constituent of peach root bark, is non-toxic on peach seedlings, while the break-down product, benzaldehyde, shows a considerable toxic effect.

Microbes in the rhizosphere can also produce toxic compounds by enzymatic degradation of conjugates or polymers present in the plant tissue. Examples of this phenomenon are the action of microbes on the cyanogenic glycosides of johnsongrass (*Sorghum halepense*) and *Prunus* species to produce toxic H C N and the corresponding benzaldehydes (Conn, 1980).
Some of the toxic compounds produced by micro-organisms include organic and amino acids, numerous antibiotics and substances such as gibberellin. Toxic inorganic compounds also produced by soil micro-organisms include nitrites, carbon dioxide and hydrogen sulfide (McCalla and Haskins, 1964).

Turner, cited in Rice (1979) stated that many of the compounds produced by fungi are important phytotoxins which can inhibit the growth of higher plants and of other micro-organisms. He also mentioned that many of these compounds are important antibiotics which are used in the treatment of human ailments. Examples are quinones, including the tetracycline antibiotics such as aureomycin (Whittaker and Feeny, 1971). In plants, antibiotics may inhibit germination, root growth and production by plants of foliage, pigment and plastids (McCalla and Haskins, 1964). Antibiotics produced *in vitro* by soil micro-organisms may not be produced by them in soil due to either inappropriate nutritional or other ambient conditions, or in cases where they are produced, they may be quickly inactivated by adsorption to soil colloids, by chemical or by microbial degradation (Elad and Misaghi, 1985). On the other hand, polar lipids and, to some extent, volatile ammonia, produced in soil by actinomycetes and other micro-organisms during chitin microbial decomposition, inhibited the saprophytic and pathogenic activities of *Rhizoctania solani* Kuhn in soil (Sneh and Henis cited in Katz *et al.*, 1987).

In germination tests performed on *Latuca sativa* and *Anastatica hierochuntica*, it was observed that various isolates of actinomycetes inhibited germination of the test seeds at various rates from 0 to 96% (Katz *et al.*, 1987). Krasil'nikov, cited in McCalla and Haskins (1964) observed that some strains of actinomycetes caused chlorosis of plants such as corn and that, fungi of the genus *Fusarium* caused chlorosis of grapevines. Hodgson *et al.* (1947) found that filtrates from crown gall bacteria produced wilting in tomatoes.

Penicillium urticae (Bainer), a fungus, isolated from sub-surface tilled soil at Alliance, Nebraska was found to produce a toxic material which caused severe stunting of germinating corn. Kent and Heatley, cited in Norstadt and McCalla (1963) identified the phytotoxic substances to be patulin, an unsaturated lactone. They observed that adding patulin to the soil in amounts necessary to suppress plant growth to about 50 percent of normal, caused rapid development of a new microbial population. Many colonies of mould appeared on the surface of the soil. Isolates of these colonies grown in potato dextrose broth developed substances toxic to corn seedlings. Microscopic examination showed the isolates did not belong to the penicillia. Thus, although soils with the larger amounts of organic matter have a greater ability to neutralize the phytotoxic effect of patulin, they may have the potential to develop altered microbial populations that may produce substances strongly toxic to germinating seeds.

Vaughan *et al.* (1983) stated that many phytotoxic allelochemicals produced by the decomposition of plant residues, no matter how toxic they may be, are soon inactivated, destroyed or transformed by the soil microflora. Another fact, which confirms the assumption of the participation of micro-organisms in 'soil sickness' is that, toxicity can be removed by steaming soil or treating with chemicals, procedures primarily affecting most micro-organisms (Börner, 1960).

2.6.3. Allelopathic effects on micro-organisms

Organic materials which diffuse from roots are probably restricted to the immediate rhizosphere and do not exercise a direct influence on other plants. However, it has been shown that these materials do affect the micro-organisms of the rhizosphere and may indirectly affect the health and nutrition of the plants (Börner, 1960). In addition, Swain (1977) stated that some of the secondary compounds (eg. juglone) that occur naturally in specific plants (in addition to being effective allelopathic agents), can also selectively inhibit the growth of micro-organisms and thus, protect the plant against attack or invasion by disease organisms. One such plant protection mechanism is the production of butyric acid and other phenolic acids during decomposition of rye or timothy residues in soil, which have been shown to have nematicidal

properties that affect plant parasitic nematodes but not saprophytic species (Sayre *et al.*, cited in Patrick, 1986).

In shoot and root growth tests performed on 21 plant species exposed to the vapours of *Artemisia tridentata*, Weaver and Klarich (1977) observed that respiration rates of soil microbes also declined logarithmically with exposure to increasing amounts of volatile *Artemisia tridentata* exudates.

Moore-Landecker and Stotzky (1974) reported that, during the process of bean and cucumber seed germination, certain unidentified volatile compounds evolve which reduce spore formation in some fungi. Schenck and Stotzky (1975) also observed that germinating seeds (widely separated taxonomically), evolved volatile metabolites which increase the growth of a variety of bacteria and fungi when bubbled through an inoculated culture medium. They therefore concluded that, since the influence on various micro-organisms was similar, the volatile effect may probably not be species specific. They also observed an increase in the number of micro-organisms in the rhizosphere and near seeds (the spermosphere) and deduced that it may be due, in part, to volatiles and not exclusively to soluble compounds released by the plants.

2.6.4. Algal allelopathy and its ecological role

The incidence of allelopathy in the aquatic environment has also been demonstrated by a number of scientists. Keaton (1978) observed that as lakes age, there is a replacement of diatom blooms of mesotrophic lakes by the blue-green algal blooms, more typical of eutrophic lakes. Further examination of the relationship between blue-green algal populations in Linsley Pond, Connecticut and diatom populations revealed that, cell-free filtrates of axenic or bacterized cultures of the dominant blue-green algae from the pond inhibited the growth of diatoms isolated from the same pond. Filtrates of cultures of planktonic bacteria growing on ^{14}C-glycolate, were found to contain high molecular weight organic compounds. Subsequently, it was demonstrated that in both nonaxenic cultures of algae and in lake water, bacteria utilize low molecular weight extracellular metabolites of algal origin and form higher molecular weight compounds. Such

chemical interactions can result in compounds, either inhibitory or stimulatory to both algae and bacteria.

Murphy *et al.* (1976) reported that excretion of hydroxamate chelators by certain blue-green algae ties up iron and makes it unavailable to other algae, thus inhibiting their growth. This, they stated, is definitely an allelopathic action because the resulting iron deficiency resulted from the addition of an organic compound to the environment.

Boiko, cited in Rice (1979) observed that water extracts and volatile excretions of certain species of lichens and moss were inhibitory to radish seed germination, whereas extracts of some moss species were found to be slightly stimulatory.

Booth, cited in Lund (1967), reported that in man-made 'deserts' or 'dust bowls', algal crusts may bind soil particles together and so prevent erosion and permit re-colonization by Angiosperms. This was confirmed later by Cameron, cited in Lund (1967), who observed a reduction in soil erosion by algal crust formation in Arizona. A soil aggregate stability test conducted by McCalla (1946) revealed that growth and decline of fungal hyphae parallels the rise and fall respectively of aggregate stability, showing the role of fungi as effective soil binding agents, though their influence is of short duration (Griffiths and Jones, cited in Molope *et al.*, 1987).

The allelopathic interaction of *Artemisia tridentata*, *Artemisia californica* and other *Artemisia* species have been thoroughly investigated (Halligan, 1973; Hoffman and Hazlett, 1977). The predominant aromatic species found in the Eastern Cape region of South Africa is African wormwood (*Artemisia afra*). It occurs mainly in rangelands but occasionally found in croplands. However, there is no evidence of research on the phytotoxic compounds associated with this species and its impact on agronomic crops as well as the soil, hence the need for this study.

3. MATERIALS AND METHODS

3.1. Introduction

Routine analyses were done on the different soils to check for trends with respect to nutrient status, pH, organic matter content and physical properties, including structural stability of soil aggregates.

With regard to the effect of differences in soil chemicals arising from the presence or absence of *Artemisia*, one laboratory and one greenhouse experiment were conducted in 1994. The first experiment involved seed germination and seedling vigour tests, using maize (*Zea mays* cv. SNK 247), speckled sugar bean (*Phaseolus vulgaris*) and Italian rye grass (*Lolium perenne*) as indicator plants. Both hot and cold water extracts of the test plant (*Artemisia afra*) materials were used in these tests. The second set of experiments involved the extraction and identification of organic compounds from the soil on which the test plants grew. The identification was done by chromatographic techniques.

3.2. Soils

3.2.1. The soil environment

The study was conducted on two plots established for the CENTOIL (Ciskei Essential Oils) project, initiated during 1973. Soils from the plots have been completely described by Van Averbeke (1991) and the descriptions have been included in the appendices (Appendix A). The classification of the soils is given below:

SOIL NAME

1. South Africa[1] FORM: Oakleaf SERIES: Jozini* (now known as Ritchie)
   FAO[2] : Orthic Luvisol
   Soil Taxonomy[3] : Typic Haplustalf

2. South Africa FORM: Estcourt SERIES: Rosemead* (now known as Haarlem)
   FAO : Eutric Planosol
   Soil Taxonomy : Aquic Arenic Haplustalf

3.2.2. Soil physical properties

Samples were taken from sites close to *Artemisia* plants on patches where the presence of *Artemisia* had resulted in virtual exclusion of any other plants (Plate 1).

3.2.2.1. Soil sampling procedures

An auger of 8cm internal diameter was used to sample soils from 8 randomly chosen 'spots' around the plants at the two *Artemisia* sites and at 0-15, 15-30 and 30-45cm depths. For 'controls', 8 randomly selected spots with similar soils on adjoining land which had no previous record of *Artemisia* production were sampled at similar depths. Individual soil samples, per site, from the various depths were air dried and ground to pass a 2mm sieve. Structural stability tests were performed on soil aggregates (by the wet sieving method) from 0-15cm depth, employing the method of Sumner (1958). Dry bulk density tests were performed on samples taken (by the core method) from 0-15 and 15-30cm depths, employing the method of

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1 Van Averbeke (1991)

2 Van Averbeke (1991)

3 Van Averbeke (1991)

* These soil Series are no longer in use. They have been replaced by soil Families and the new Family names shown in brackets.

Blake and Hartge (1986). In order to estimate the quantity of combustible organic material in the soil, percentage loss on ignition tests were performed on the various soil samples taken from 0-15cm depth, employing the method of Blakemore, Searle and Daly (1987).

Plate 1: *Artemisia afra* shrub. Bare area between adjacent shrubs depicts zone of inhibition.

3.2.3. Soil moisture

Soil samples were also taken from different parts of the sampling site at different depths and were sealed in aluminium cylinders and weighed. Weighed samples were dried in an oven for 48 hours at 105°C and re-weighed thereafter. Soil moisture content at the time of sampling was then calculated on a wet basis.

3.2.4. Assessment of biological changes

Samples were taken from the soil surface by scraping with a spade, for determination of

presence or absence of macroscopic growths such as fungi, algae etc that may affect soil surface properties. The samples were also observed under a microscope to identify the growth found.

3.2.5. Soil chemical properties

3.2.5.1. Routine chemical analyses

Soil chemical analyses procedures employed on each sample were as follows: pH by the glass electrode method of Blakemore *et al.* (1987); extractable phosphorus by the Bray 1 method of Jackson (1958); organic carbon content by the Walkley-Black method (Allison, 1965).

3.2.5.2. Analyses for organic compounds

Organic compounds were extracted from both *Artemisia* and control soils by equilibrating 200g soil samples with 250mls of anhydrous ethyl ether for 48hrs. The supernatant was decanted, dried with a spatula full of anhydrous sodium sulphate and the ether evaporated with a rotary evaporator (BUCHI Rotavapor R-114) to obtain approximately 2mls of concentrated soil extract (Muller and Muller, 1964) (Plate 2). The following tests were performed on the soil extracts obtained.

3.2.5.2.1. Infrared (IR) Spectroscopy: A Perkin Elmer-1600 Infrared spectrophotometer was used for infrared analysis of the soil extracts. Spectra obtained were compared with published infrared spectra of commercial compounds (Saleh, 1985).

3.2.5.2.2. Nuclear Magnetic Resonance (NMR): NMR analysis was performed on the soil extracts using a Bruker AMX 400 NMR instrument. In this analysis, ether was completely evaporated from the sample. Each dried sample was dissolved in 0.5ml chloroform-D and about 0.05ml of tetramethylsilane (TMS) was added (Silverstein *et al.*, 1981). The resulting solution was filtered through fine cotton wool and analyzed.

3.2.5.2.3. Gas Chromatography and Mass Spectrometry (GC-MS):

A Jeol AX505W double focusing mass spectrometer, integrated with a Hewlett Packard HP5890 gas chromatograph was used. 0.3μl of each extract was injected into the GC. The flow rate of the carrier gas (helium) was 1ml/min. Chromatographic conditions were as follows: injection port and interface temperature, 250°C; column temperature, 100°C for 1 min and programmed to 250°C at the rate of 5°C/min. All spectra were recorded in the electron impact ionization (EI) mode at 70 eV. Identification of the organic components in each sample was carried out by the interpretation of their mass spectra (MS), by an MS search against the NIST/NIST library and by comparison of their retention times (R_ts) with authentic compounds (Saleh, 1985).

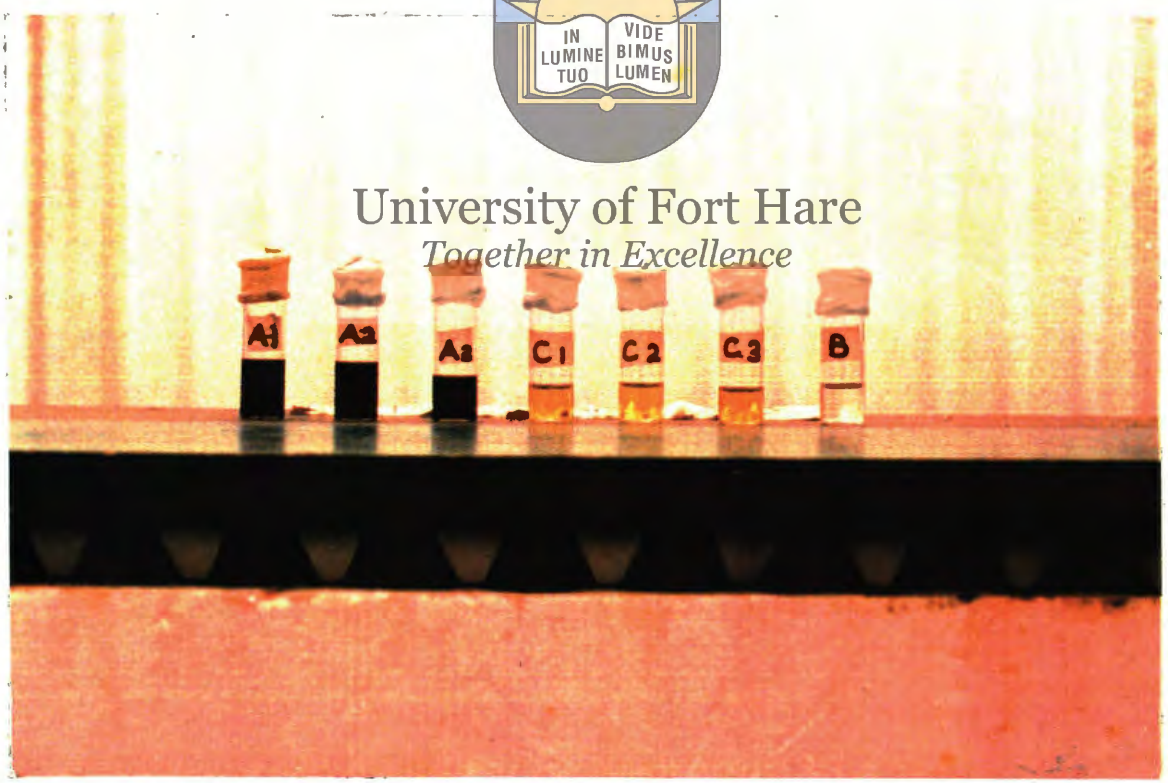


Plate 2: Concentrated diethyl ether extracts of soil planted to *A. afra* (A1, A2 & A3), control soil (C1, C2 & C3) and blank ethyl ether (B).

3.2.6. Experimental design and statistical analyses

The experimental design was a completely randomized one with 4 treatments and 6 replications for dry bulk density and 4 treatments with 8 replications for each of the following tests: organic carbon content, extractable phosphorus, pH, loss on ignition and structural stability of soil aggregates. The different treatments consisted of soils from under *Artemisia* (from both sites) and control soils.

All data were analyzed using the Student Edition of Statistix 4.1. Analysis of variance (ANOVA) was used to test for significance ($p \leq 0.05$) of treatments and differences due to changes in the rhizosphere were compared using Fisher's protected LSD (Gomez and Gomez, 1984).

3.3. Greenhouse experiments

Thirty-two *Artemisia* plants, approximately 30cm high were randomly chosen from the test site for this experiment on 30 March 1994. A hole, 30cm in diameter and 20cm deep was dug around each selected test plant (to minimize soil disturbance) and plants, with their roots and soil intact, were transferred into pots, 25cm in diameter and 20cm deep with narrow slits at the base and weighed. To obtain a total weight of 6kg, excess soil was removed from the sides. Pots were placed in trays and were kept in a glass fibre greenhouse under natural lighting conditions. All pots received 150ml of full nutrient solution (Aquafert 1 and 2), twice a week, for a period of 8 months. In order to prevent chemical compounds from being leached out of the soil, potted plants were supplied with moisture by a wick mechanism from the base of the pots with the water moving up the root zone by capillary action.

3.4. Germination tests

Seeds of agronomic crops (maize and bean) and a forage crop (rye grass), economically important in the Eastern Cape region of South Africa, were chosen as indicator seeds in this study. The plants from the greenhouse pot experiment were harvested and each plant was cut to separate the leaves from the stem and the roots. The different parts from all the plants were then further cut into 0.5cm segments and pooled for extraction of the sap. These were blended for 30 seconds in a Phillips HR 2810 blender (8g in 100ml water) according to the method of Katz *et al.* (1987). Half of the plant materials were blended in hot water and the other half in cold. These were separately transferred into evaporating basins and after a minute, the supernatant liquid was decanted. Pastes, obtained from the blended material were separately transferred into a 250μm metal sieve and filtered, using the decanted liquid and no additional water. The filtrates obtained were considered "original extracts" and various dilutions (1:1, 1:2, 1:2.5 and 1:5) (or 100, 200, 250 and 500%, respectively) of the original extracts were made, stored in closed glass bottles and kept at room temperature. Soil on which the test plants grew in the greenhouse (151.2g of soil) was soaked overnight. One set was soaked in cold water and another in hot water (180ml) according to the method of Kelsey *et al.* (1978), then filtered through a Whatman number 30 filter paper and diluted as described above. For 'control', similar soil from an adjoining land which had not been previously planted to *Artemisia* was used. The extracts were used for germination tests by soaking the indicator seeds in the various extracts for 6hrs prior to placing on discs of filter paper for germination. The discs were moistened with plant and/or soil extracts. For maize and bean, 40 seeds (10 in each of 4 petri dishes) were used per treatment. For rye grass, 200 seeds (50 in each of 4 petri dishes) were used per treatment. Six mls of appropriate extract was added daily to the seeds in each petri dish, to keep them moist. A secondary control was treated in the same manner, except that distilled water was used instead of plant or soil extract. Petri dishes and contents were maintained at 25°C. Germination percentages were recorded at 9, 15, 19 and 25 days after sowing (DAS). The experimental design was a completely randomized one with 50 treatments and 4 replications per treatment, for each indicator. The treatments consisted of the various dilutions from both cold and hot water extracts of *A. afra* plant parts, soil from under *A. afra*,

as well as extracts from the control soil.

3.4.1. Seedling vigour tests

3.4.1.1. General

Growth performance of indicator seeds was tested using two separate experiments. The first involved watering the indicator seedlings with cold and hot water extracts of test plant leaves, water extracts from soil under test plants, as well as control soil (as in germination experiment). The second involved the growth of indicator seeds in pots containing soils on which *Artemisia afra* had been grown for at least 8 months. (The plants were transplanted from the field from plots where *Artemisia* had been growing for the past 22 years. The 8-month period was to off-set any major leaching losses due to the very heavy rains of the two previous growing seasons-1992/93 and 1993/94).

3.4.1.2. Seedling vigour test (extracts)

In this experiment, seedlings of the indicators were grown as 'plugs' in cavity trays (Plate 3) on raised rails (Brutsch, 1994). "Hygromix", a commercial growth medium composed of peat moss, vermiculite and polystyrene beads was used. Original extracts obtained from the blended test plant leaves, as well as extracts from *Artemisia* and control soils, were diluted (as in germination tests). Seeds were also soaked in extracts for 6hrs before planting. For maize and bean, 40 seeds (10 in each of 4 rows) were planted per treatment. Each row had 10 cavities and one seed was sown per cavity. For rye grass, 2000 seeds (500 in each of 4 rows and 50 seeds per cavity) were sown for each treatment. After emergence, seedlings were thinned to 10 per cavity (most vigorous ones). Six mls. of appropriate extract was added to each cavity on a daily basis. All plants received 6mls of full nutrient solution once every week. Trays were kept in a glass fibre greenhouse with daily temperature in the 15-30°C range. After 4 weeks of growth, the seedlings were harvested, washed under gentle flow of water and dried in an oven at 60°C. Total dry matter yield was recorded for each replicated treatment. The experimental design was

a completely randomized one with 30 treatments and 4 replications per treatment, for each indicator. The treatments consisted of the various dilutions from cold water extracts of *A. afra* leaves, soil from under *A. afra*, as well as extracts from the control soil.

Plate 3: Bean (left) and maize (right) seedlings grown as "plugs" in cavity trays on raised rails.

3.4.1.3.Seedling vigour test (soils)

In this experiment, potted *Artemisia afra* plants grown in the greenhouse were harvested after a period of 8 months and the indicator seeds (maize and bean) were planted in these soils. Ten maize and 10 bean seeds were separately planted in each pot. Pots were irrigated with 250 mls of distilled water thrice per week. For controls, similar soils collected from adjoining land with no previous record of *Artemisia* production were used. Control soils were of the same masses as *Artemisia* soils.

3.4.1.4. Evaluation of seedling growth : Since seedlings had to be harvested before they matured, it was necessary to use growth parameters that reflect biomass production. For this, plant height and stem diameter were selected and recorded at 14 and 28 days after emergence (DAE).

Stem diameter : a pair of vernier callipers was used to measure the diameter of stems (just below cotyledon) of three plants from each pot and the average was recorded.

Plant height : a meter rule was used to measure the height (tip of tallest leaf) of three plants from each pot and the average was recorded.

Germination percentages were recorded 14 DAE. Whole plants in each pot were harvested 28 DAE and roots were gently washed on a 250μm sieve under running water. Shoots were separated from roots and both parts were dried in an oven at 60°C. Dry matter yield per pot was recorded. The experimental design was a completely randomized one with 2 treatments (*Artemisia* or control soil) and 4 replications per treatment, for each indicator.

All data were analyzed using the Student Edition of Statistix 4.1. Analysis of variance (ANOVA) was used to test for significance ($p \leq 0.05$) of treatments and differences due to inhibition were compared using Fisher's protected LSD (Gomez and Gomez, 1984).

4. RESULTS

4.1. Soil analytical data

These analyses were done in 1989 by Van Averbeke (1991). The data have been included in the present study to give a general view of some of the physical and chemical properties of both soils.

4.1.1. Particle size analysis

It is clear from the data (Table 1) that there were large differences in the particle size distributions of the soils at the various depths. The Estcourt soil Form generally had higher clay content than the Jozini, at all depths. The clay content in the B21 horizon of the Estcourt was more than 300% the clay content in the B21 horizon for the Jozini.

Table 1. Particle size analysis for Fort Hare Jozini and Alice Rosemead (Estcourt)

Fort Hare Jozini	Lab. No.	67/89	68/89	69/89	70/89	71/89
	Horizon	Ap	A3	B1	B21	B3
	Coarse earth > 2mm (%)	0	0	0	0	0
	Fine earth < 2mm (%)	100	100	100	100	100
	Particle size distribution (%)					
	co Sa	0.1	0.0	0.0	0.0	0.0
	me Sa	0.3	0.1	0.4	0.0	0.0
	fi Sa	11.3	9.3	5.7	5.8	5.3
	vf Sa	36.5	32.1	33.2	28.4	29.7
	Total Sand	**48.2**	**41.5**	**39.5**	**34.2**	**35.0**
	co Si	26.2	29.0	33.5	32.1	32.5
	fi Si	12.0	15.5	12.9	16.1	15.1
	Total Silt	**38.2**	**44.5**	**46.4**	**48.1**	**50.3**
	Clay	13.6	13.9	14.3	17.8	17.4
	TOTAL	**100.0**	**99.9**	**100.1**	**100.1**	**100.0**
Alice Rosemead (Estcourt)	Lab. No.	15/89	16/89	17/89	18/89	19/89
	Horizon	A1P	A12	E	B21	C
	Coarse earth > 2mm (%)	25	33	60	10	15
	Fine earth < 2mm (%)	75	67	40	90	85
	Particle size distribution (%)					
	co Sa	15.6	22.9	32.0	0.6	0.1
	me Sa	1.0	1.5	1.6	1.3	0.1
	fi Sa	10.2	9.5	7.3	3.5	1.0
	vf Sa	22.3	21.4	15.8	8.4	8.8
	Total Sand	**49.1**	**55.3**	**56.7**	**13.8**	**10.0**
	co Si	18.9	18.2	16.4	6.1	10.7
	fi Si	13.0	10.8	9.1	3.7	33.1
	Total Silt	**31.9**	**29.0**	**25.5**	**9.8**	**43.8**
	Clay	18.7	15.7	17.9	76.5	46.2
	TOTAL	**100.0**	**100.0**	**100.1**	**100.1**	**100.0**

Source: Van Averbeke (1991)

4.1.2. Other soil physical properties

4.1.2.1. Structural stability of soil aggregates

The different treatments (*Artemisia* and control soils) showed varying stability effects when tested on the various sieve sizes (Table 2). Differences between the treatments were very significant ($P < 0.05$). In the Estcourt soil Form, the mass of aggregates retained on the 5.0-2.0mm sieve size of the control soil (30.7g) was much higher than was the case for the Jozini (1.9g).

Table 2. Structural stability of soil aggregates for two soil Forms as affected by *A. afra*.

SOIL FORM	TREATMENT	PERCENTAGE WATER STABLE AGGREGATES		
		Sieve size (mm)		
		5.0-2.0	2.0-1.0	1.0-0.5
Jozini	*Artemisia*	1.2	7.4	3.3
	control	1.9	2.6	4.1
	LSD (0.05)	1.0	0.6	0.6
	CV (%)	1.2	2.4	3.5
Estcourt	*Artemisia*	46.2	10.7	3.5
	control	30.7	6.6	2.9
	LSD (0.05)	1.8	0.6	0.2
	CV (%)	1.0	1.5	1.4

4.1.2.2. Dry bulk density

There were significant ($P < 0.05$) differences in bulk density between the *Artemisia* and control soils in the Estcourt soil Form, at both depths (Table 3). However, there were no significant ($P < 0.05$) differences between the treatments in the Jozini soil Form.

Table 3. Dry bulk density of two soil Forms as affected by *Artemisia afra.*

SOIL FORM	TREATMENT	DRY BULK DENSITY (g/cm³)	
		Depths (cm)	
		0-15	15-30
Jozini	*Artemisia*	1.47	1.45
	control	1.46	1.45
	LSD (0.05)	NS	NS
	CV (%)	0.22	0.30
Estcourt	*Artemisia*	1.68	1.68
	control	1.63	1.61
	LSD (0.05)	0.04	0.05
	CV (%)	0.46	0.64

NS : Not significant

4.1.2.3. Soil moisture

Soil moisture content at the time of sampling, calculated on a wet basis was estimated to range from 5.3 at 15cm depth to 6.9% at 45cm depth.

4.1.2.4. Assessment of biological changes

Growth of some lower plants, including moss was observed on the surface of the soil at the *Artemisia* sites. Microscopic examination showed the moss samples to be *Trichostomum brachydontium* (Plate 4).

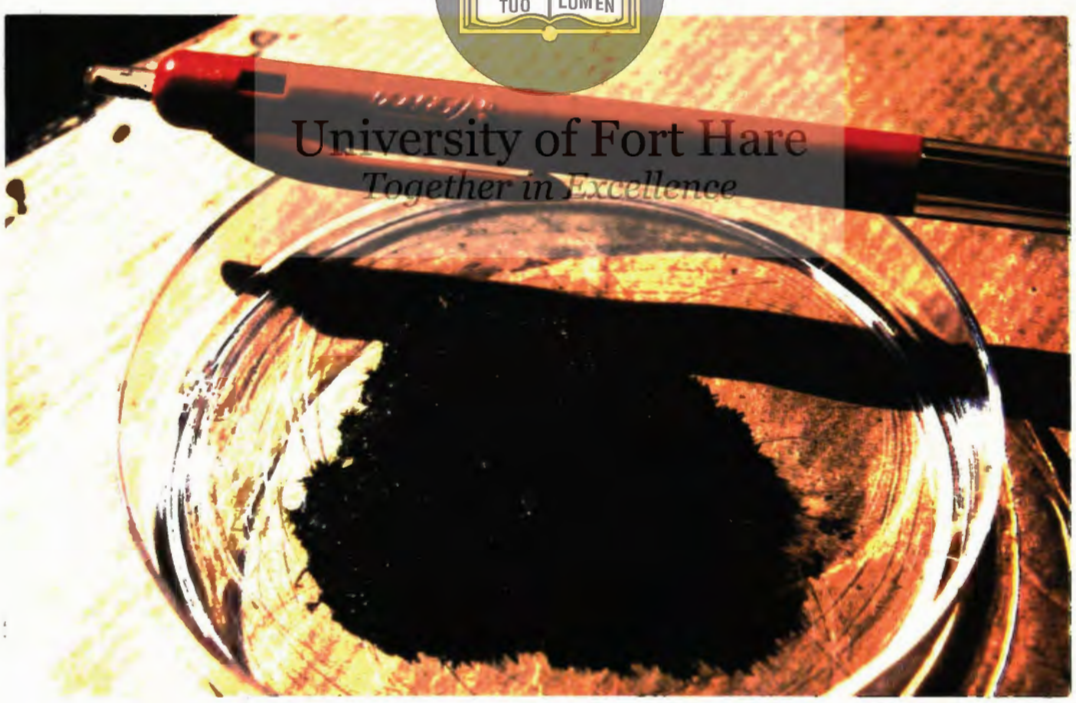


Plate 4: Macrophotograph of moss growing on the soil surface at *Artemisia* sites.

4.1.3. Chemical data

Apart from the B21 horizon of the Estcourt which had CEC value more than 2 x the value for the Jozini, CEC, sum of cations, organic carbon percentage and electrical conductivity values were generally greater in the Jozini soil Form than was the case for the Estcourt. pH values for both soil Forms were rather similar at the various depths (Table 4).

Table 4. Chemical analysis for Fort Hare Jozini and Alice Rosemead (Estcourt)

Fort Hare Jozini	Lab. No.		67/89	68/89	69/89	70/89	71/89
	Horizon		Ap	A3	B1	B21	B3
	Exchangeable cations (me/100g soil)						
	Ca		4.80	5.20	6.00	6.40	5.60
	Mg		3.20	3.60	4.50	4.80	4.40
	K		1.36	0.88	0.74	0.10	0.16
	Na		0.06	0.14	0.24	0.52	0.54
	S - value (me/100g soil)		9.42	9.82	11.48	11.82	10.70
	Exchangeable acidity (me/100g soil)		0.09	0.06	0.05	0.05	0.00
	Sum of cations (me/100g clay)		69.3	70.6	80.3	66.4	61.5
	CEC (me/100g soil)		9.5	9.9	11.5	11.9	10.7
	Organic carbon (%)		0.84	0.39	0.39	0.49	0.49
	pH	water 1:2.5	6.2	6.9	6.9	6.7	7.2
		KCL 1:2.5	5.3	5.9	6.1	6.1	6.1
	EC 25°C mS/m		30	63	83	174	56
Alice Rosemead (Estcourt)	Lab. No.		15/89	16/89	17/89	18/89	19/89
	Horizon		A1p	A12	E	B21	C
	Exchangeable cations (me/100g soil)						
	Ca		3.4	3.2	2.8	8.8	-
	Mg		2.7	2.5	2.9	12.9	14.8
	K		0.8	0.7	0.8	3.2	3.6
	Na		0.3	0.3	0.4	1.5	3.0
	S - value (me/100g soil)		7.2	6.7	6.9	26.4	-
	$CaCO_3$ (%)		0.0	0.0	0.0	0.0	12.6
	Exchangeable acidity (me/100g soil)		0.06	0.0	0.0	0.0	0.0
	Sum of cations (me/100g clay)		38.6	42.6	38.5	30.1	-
	CEC (me/100g soil)		7.3	6.7	6.9	26.4	39.0
	Organic carbon (%)		0.53	0.43	0.28	0.43	0.10
	pH	water 1:2.5	6.9	7.1	7.0	7.8	8.5
		KCL 1:2.5	6.0	6.0	6.0	6.1	6.8
	EC 25°C mS/m		23	22	24	78	118

Source: Van Averbeke (1991)

4.1.4. Other soil chemical properties

4.1.4.1. Soil pH

There were no significant ($P < 0.05$) differences in pH between the *Artemisia* and control soils in the Jozini soil Form (Table 5). Similarly, no significant differences were obtained between the treatments in the Estcourt soil Form at the 15-30 and 30-45cm depths.

Table 5. pH of two soil Forms at three depths as affected by *Artemisia afra.*

SOIL FORM	TREATMENT	pH		
		Depths (cm)		
		0-15	15-30	30-45
Jozini	*Artemisia*	5.70	5.80	5.96
	control	5.75	5.85	5.99
	LSD (0.05)	NS	NS	NS
	CV (%)	0.37	0.40	0.26
Estcourt	*Artemisia*	5.65	5.73	5.81
	control	5.76	5.84	5.93
	LSD (0.05)	0.05	NS	NS
	CV (%)	0.20	0.42	0.41

NS : Not significant

4.1.4.2. Organic matter content

For both soil Forms, organic matter content was higher in the test soil than in the control and these differences were statistically significant ($P < 0.01$) (Table 6).

Table 6. Organic matter content of two soil Forms as affected by *Artemisia afra*. (0-15 cm. only)

SOIL FORM	TREATMENT	* ORGANIC MATTER CONTENT (%)
Jozini	*Artemisia*	1.06
	control	0.71
	LSD (0.05)	0.07
	CV (%)	1.68
Estcourt	*Artemisia*	1.30
	control	0.76
	LSD (0.05)	0.17
	CV (%)	3.49

* Organic matter (%) = Organic carbon (%) x 1.724

4.1.4.3. Extractable phosphorus

The extractable phosphorus content in the test soil was higher than that in the control for both soil Forms. However, the difference was not significant ($P < 0.05$) in the Jozini soil Form (Table 7).

Table 7. Extractable phosphorus content of two soil Forms as affected by *Artemisia afra*. (0-15 cm. only)

SOIL FORM	TREATMENT	EXTRACTABLE PHOSPHORUS (ppm)
Jozini	*Artemisia*	27.10
	control	23.10
	LSD (0.05)	NS
	CV (%)	3.81
Estcourt	*Artemisia*	38.00
	control	24.25
	LSD (0.05)	6.88
	CV (%)	4.67

NS : Not significant

4.1.4.4. Analyses for organic compounds

The various parts of *A. afra* as well as soil under this shrub contained water-soluble substances which varied in their inhibitory effects against the indicator seeds tested. Organic compounds in soil planted to *A. afra* were, in general, a mixture of unsaturated hydrocarbons, carbonyl compounds and benzenes. Figs. A, B (blank) and C show the contrast between the two soils and the blank ether. The hydrocarbon nature of the organic compounds was confirmed from the IR and NMR spectra of the ether extracts. The major benzene compounds identified are as follows:

1: Benzene, 1,1-(dichloroethenylidene) bis 4-chloro $[C_{14}H_8Cl_4]$

2: Benzene, 1-chloro-3-2,2-dichloro-1-(4 chlorophenyl)ethyl- $[C_{14}H_{10}Cl_4]$

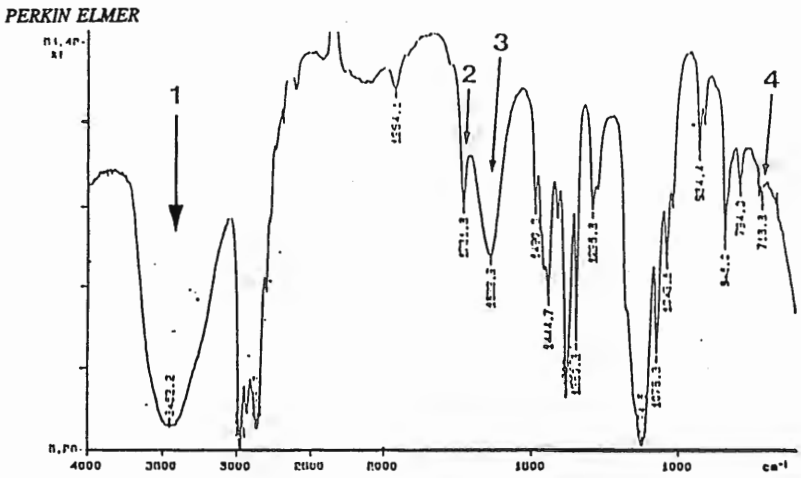
3: Benzene, 1,1-(2,2,2-trichloroethylidene) bis 4-chloro $[C_{14}H_9Cl_5]$

The arrows in Figs.A (*Artemisia*) and C (control) show the peaks for the major compounds found in the ether extracts. The peaks in the control spectra are weak in comparison to those in the test (*Artemisia*) soil. These weak spectra signify lower concentrations of the compounds present in the control extract.

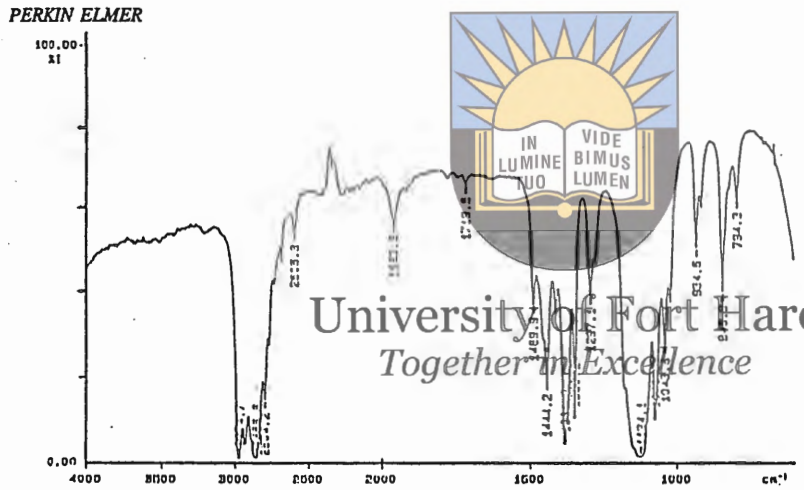
The peak numbers indicate the absorption bands for the compound groupings.

Peak number	compound groupings [1]
1.	region for alcohols and phenols
2.	region for carbonyl groups
3.	region for unsaturation
4.	region for out of plane band in aromatics.

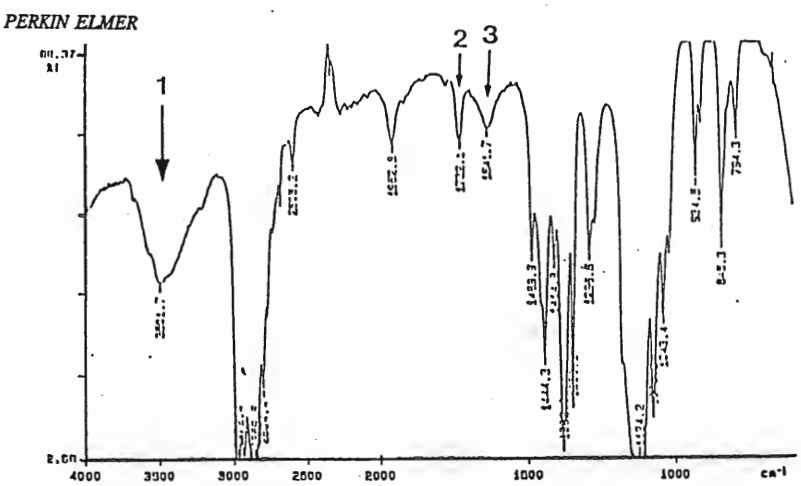
[1] Silverstein *et al.*, (1981)



Y: 1 SCAN, 4.0cm^{-1} Fig. A. Infrared spectrum of *Artemisia* soil extract.



X: 1 SCAN, 4.0cm^{-1} Fig. B. Infrared spectrum of ethyl ether (blank).



Y: 1 SCAN, 4.0cm^{-1} Fig. C. Infrared spectrum of control soil extract.

4.2. Loss on ignition

Percentage loss on ignition was higher in the test soil than in the control for both soil Forms. These differences were statistically significant ($P < 0.01$) (Table 8).

Table 8. Percentage loss on ignition of two soil Forms as affected by *Artemisia afra*. (0-15 cm. only)

SOIL FORM	TREATMENT	LOSS ON IGNITION (%)
Jozini	*Artemisia*	2.35
	control	2.09
	LSD (0.05)	0.13
	CV (%)	1.20
Estcourt	*Artemisia*	2.85
	control	2.08
	LSD (0.05)	0.32
	CV (%)	2.73

4.3. Germination Tests

No significant differences ($P < 0.05$) were found between the cold and hot water extracts averaged over all dilutions. However, for the purposes of illustration, both have been included in the text in tabular forms.

4.3.1. Agronomic crops

Tables 9 and 10 show the results of the various cold and hot water extracts respectively, on seed germination. Averaged over the different days of recording, there were statistically significant ($P < 0.05$) differences between effects of the distilled water control and the dilutions of the various plant parts (Table 9).

Table 9. Germination percentages of maize, bean and rye grass seeds moistened with different cold water extracts at various dilutions.

Plant part/ soil	Treatment/ dilutions	Indicator Crop											
		Maize DAS				Bean DAS				Rye Grass DAS			
		9	15	19	25	9	15	19	25	9	15	19	25
Leaves	original	5.0	22.5	30.0	40.0	2.5	20.0	22.5	27.5	3.5	13.0	18.5	25.0
	1:1	10.0	30.0	47.5	50.0	22.5	47.5	55.0	60.0	5.0	19.0	28.5	41.5
	1:2	30.0	57.5	65.0	65.0	20.0	45.0	60.0	62.5	13.5	38.0	57.5	82.0
	1:2.5	17.5	47.5	70.0	72.5	40.0	67.5	75.0	75.0	15.0	47.5	65.0	79.5
	1:5	20.0	55.0	75.0	82.5	40.0	67.5	80.0	80.0	27.0	64.0	78.0	82.5
	control	90.0	97.5	97.5	97.5	97.5	100.0	100.0	100.0	37.0	84.5	90.0	90.0
	LSD(0.05)	11.2	11.0	12.3	13.4	9.9	11.5	7.1	9.3	4.5	6.2	6.3	7.2
	CV (%)	5.3	2.9	2.6	2.7	3.6	2.7	1.5	1.9	3.6	1.9	1.5	1.5
Stems	original	7.5	25.0	40.0	47.5	7.5	20.0	25.0	35.0	6.5	18.5	28.0	37.5
	1:1	22.5	55.0	60.0	60.0	27.5	60.0	60.0	60.0	10.0	25.0	33.5	47.5
	1:2	27.5	57.5	72.5	72.5	27.5	52.5	67.5	70.0	16.5	44.5	66.5	79.0
	1:2.5	20.0	57.5	75.0	82.5	40.0	67.5	77.5	77.5	20.0	51.5	67.0	80.0
	1:5	32.5	80.0	90.0	90.0	47.5	77.5	77.5	77.5	26.5	58.5	74.0	83.5
	control	90.0	97.5	97.5	97.5	97.5	100.0	100.0	100.0	37.0	84.5	90.0	90.0
	LSD(0.05)	13.1	10.9	11.0	11.5	6.8	13.0	13.0	13.6	4.2	6.4	6.6	8.1
	CV (%)	5.3	2.4	2.1	2.1	1.5	3.1	2.6	2.6	2.9	1.8	1.5	1.6
Roots	original	7.5	35.0	42.5	45.0	7.5	22.5	30.0	40.0	3.0	14.0	22.5	32.0
	1:1	17.5	45.0	55.0	60.0	30.0	42.5	50.0	52.5	5.5	22.0	30.5	43.5
	1:2	35.0	60.0	70.0	72.5	30.0	55.0	67.5	67.5	14.5	43.5	63.0	80.5
	1:2.5	25.0	62.5	75.0	77.5	32.5	70.0	80.0	80.0	17.0	49.0	68.5	83.0
	1:5	25.0	62.5	80.0	85.0	47.5	70.0	75.0	75.0	24.5	59.5	74.0	79.0
	control	90.0	97.5	97.5	97.5	97.5	100.0	100.0	100.0	37.0	84.5	90.0	90.0
	LSD(0.05)	12.4	13.9	11.4	12.6	8.4	14.3	15.4	14.6	3.7	6.9	6.7	8.9
	CV (%)	5.0	3.1	2.2	2.3	3.0	3.2	3.1	2.9	2.9	2.0	1.6	1.8
Soils	original	30.0	62.5	70.0	70.0	17.5	35.0	42.5	42.5	12.5	28.5	38.0	44.0
	*original	75.0	92.5	92.5	92.5	75.0	97.5	97.5	97.5	32.0	68.0	80.5	82.5
	1:1	35.0	67.5	82.5	82.5	32.5	55.0	62.5	67.5	11.5	31.0	43.5	61.5
	1:2	32.5	67.5	87.5	87.5	35.0	62.5	72.5	77.5	17.5	46.5	64.5	81.0
	1:2.5	30.0	67.5	77.5	82.5	37.5	67.5	72.5	77.5	20.5	55.5	74.0	84.0
	1:5	45.0	90.0	90.0	90.0	45.0	75.0	80.0	85.0	27.0	57.5	73.5	80.0
	**control	90.0	97.5	97.5	97.5	97.5	100.0	100.0	100.0	37.0	84.5	90.0	90.0
	LSD(0.05)	15.2	14.2	10.6	10.2	11.7	15.1	10.7	14.3	4.8	6.2	7.1	7.0
	CV (%)	4.0	2.3	1.6	1.5	3.1	2.7	1.8	2.3	2.7	1.5	1.4	1.2

* : undiluted extract of control soil.

** : distilled water used to moisten seeds (secondary control).

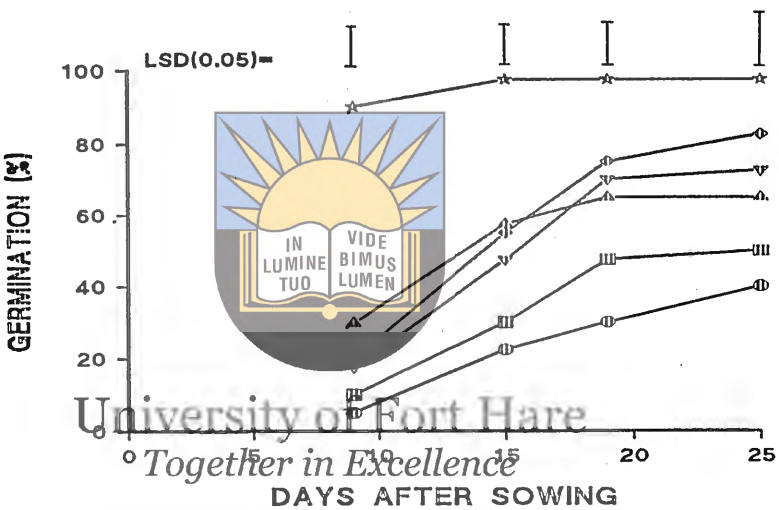
Table 10. Germination percentages of maize, bean and rye grass seeds moistened with different hot water extracts at various dilutions.

Plant part/ soil	Treatment/ dilutions	Indicator Crop											
		Maize DAS				Bean DAS				Rye Grass DAS			
		9	15	19	25	9	15	19	25	9	15	19	25
Leaves	original	0.0	15.0	27.5	35.0	0.0	2.5	10.0	15.0	1.5	12.0	20.0	29.5
	1:1	12.5	35.0	45.0	52.5	15.0	42.5	52.5	57.5	2.5	16.5	29.0	41.5
	1:2	27.5	62.5	72.5	72.5	15.0	52.5	62.5	65.0	15.5	40.5	61.5	81.0
	1:2.5	20.0	50.0	70.0	72.5	30.0	55.0	67.5	67.5	18.5	46.0	61.5	82.0
	1:5	22.5	52.5	70.0	82.5	42.5	62.5	75.0	75.0	25.0	62.5	79.0	83.5
	control	90.0	97.5	97.5	97.5	97.5	100.0	100.0	100.0	37.0	84.5	90.0	90.0
	LSD(0.05)	10.6	9.6	12.6	13.3	9.9	12.2	11.8	12.4	4.0	4.9	5.8	7.4
	CV (%)	5.0	2.5	2.7	2.6	4.0	3.2	2.6	2.7	3.3	1.5	1.4	1.5
Stems	original	10.0	45.0	52.5	55.0	17.5	27.5	37.5	47.5	5.5	17.5	26.0	38.5
	1:1	20.0	50.0	55.0	60.0	22.5	50.0	60.0	62.5	9.5	26.0	34.0	47.0
	1:2	35.0	60.0	72.5	75.0	17.5	52.0	67.5	72.5	17.0	44.0	66.5	79.0
	1:2.5	27.5	70.0	85.0	85.0	45.0	72.5	85.0	85.0	19.5	49.5	69.5	82.5	
	1:5	50.0	90.0	92.5	92.5	47.5	75.0	82.5	82.5	24.0	58.0	74.0	82.5
	control	90.0	97.5	97.5	97.5	97.5	100.0	100.0	100.0	37.0	84.5	90.0	90.0
	LSD(0.05)	11.2	10.9	11.8	13.3	8.9	12.3	13.0	10.8	4.8	7.8	8.2	7.1
	CV (%)	3.9	2.2	2.1	2.5	2.2	2.2	2.4	2.0	3.5	2.3	1.9	1.4
Roots	original	10.0	42.5	52.5	55.0	0.0	32.5	37.5	52.5	2.0	17.5	24.0	33.5
	1:1	20.0	47.5	60.0	60.0	22.5	50.0	60.0	60.0	7.0	22.0	31.5	45.0
	1:2	32.5	67.5	72.5	72.5	22.5	55.0	65.0	65.0	15.0	37.0	54.0	74.0
	1:2.5	25.0	57.5	77.5	80.0	40.0	72.0	75.0	75.0	16.5	45.0	65.5	81.0
	1:5	30.0	65.0	80.0	87.5	55.0	72.5	82.5	82.5	21.5	62.0	80.5	86.5
	control	90.0	97.5	97.5	97.5	97.5	100.0	100.0	100.0	37.0	84.5	90.0	90.0
	LSD(0.05)	13.1	17.4	12.3	12.4	16.9	14.5	11.8	11.2	3.7	5.8	6.2	7.1
	CV (%)	5.1	3.7	2.3	2.2	5.5	3.1	2.3	2.1	3.1	1.8	1.5	1.4
Soils	original	27.5	60.0	75.0	75.0	10.0	37.5	45.0	45.0	12.5	26.0	35.5	47.0
	*original	70.0	90.0	90.0	90.0	70.0	95.0	95.0	95.0	30.5	68.0	78.0	80.0
	1:1	35.0	67.5	77.5	77.5	35.0	57.5	67.5	70.0	13.0	30.5	43.0	60.0
	1:2	37.5	72.5	85.0	85.0	32.5	60.0	70.0	70.0	21.0	49.5	68.5	80.0
	1:2.5	35.0	75.0	92.5	92.5	37.5	65.0	77.5	77.5	19.0	57.0	75.0	84.0
	1:5	45.0	90.0	92.5	92.5	45.0	70.0	72.5	72.5	25.5	63.5	79.5	84.5
	**control	90.0	97.5	97.5	97.5	97.5	100.0	100.0	100.0	37.0	84.5	90.0	90.0
	LSD(0.05)	12.4	17.9	11.2	11.2	15.3	12.7	13.9	13.9	4.7	6.2	6.3	8.4
	CV (%)	3.2	2.9	1.6	1.6	4.2	2.3	2.4	2.4	2.6	1.5	1.2	1.4

* : undiluted extract of control soil.

** : distilled water used to moisten seeds (secondary control).

Fig. 2 shows the effect of *Artemisia* leaf extracts on the germination of maize seeds, as shown in Plate 5. At 9 DAS, germination percentage for distilled water (control) was 90, whereas the next lower value (1:2 dilution) was 30. This shows a strong inhibitory effect associated with *A. afra* leaf extracts during the early stages of germination. Thereafter, the inhibitory effect diminishes.



Fig(2). Effect of different concentrations of A. afra leaf extracts on germination of maize seeds at four different dates after sowing.

EXPLANATION OF SYMBOLS

(★)distilled water
(Φ)1:5 dilution
(▽)1:2.5 dilution
(△)1:2 dilution
(III)1:1 dilution
(Φ)original undiluted extract

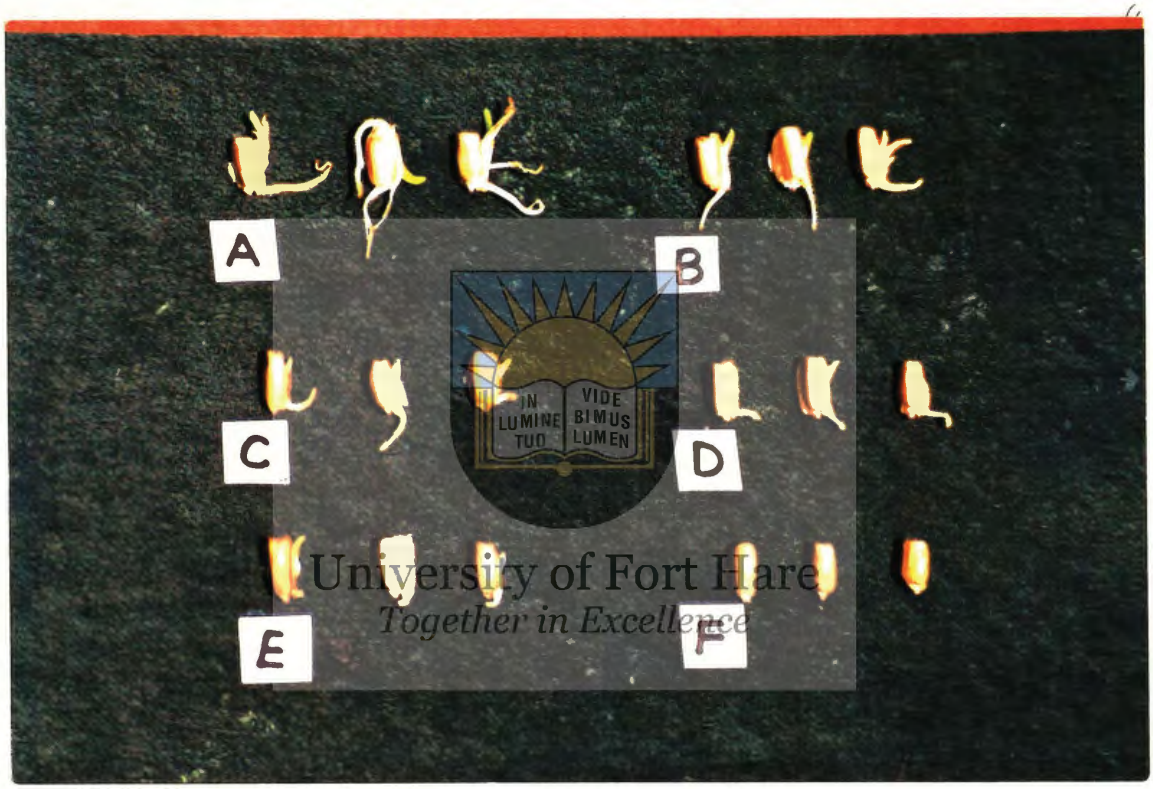
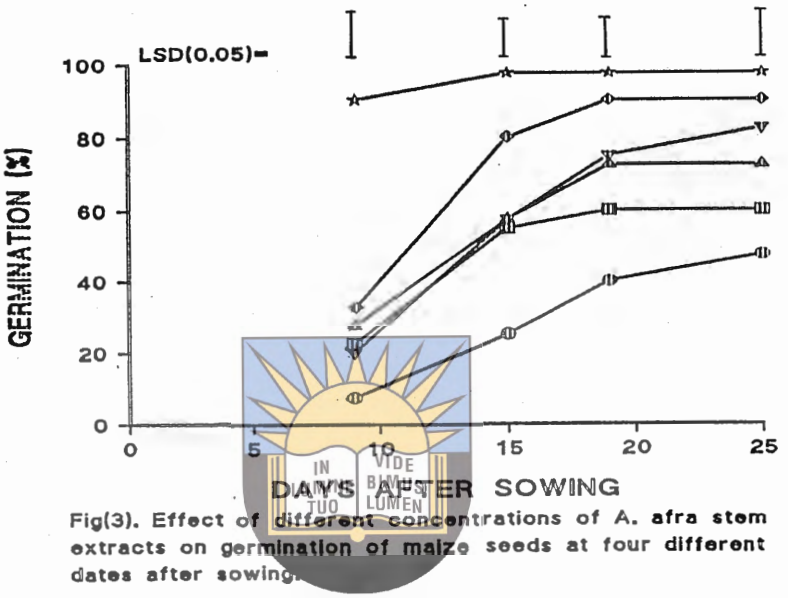


Plate 5: Radicle and plumule lengths of maize seeds as affected by cold water extracts of *A. afra* leaves at various dilutions (7 DAS).

[A] control (distilled water)
[B] 1:5 dilution
[C] 1:2.5 dilution
[D] 1:2 dilution
[E] 1:1 dilution
[F] original undiluted extract.

Fig.3 shows the effect of *Artemisia* stem extracts on the germination of maize seeds. Similar to the effect in fig.2, germination percentage at 9 DAS was 90 for the distilled water (control) and 32.5 for the next lower value (1:5 dilution).

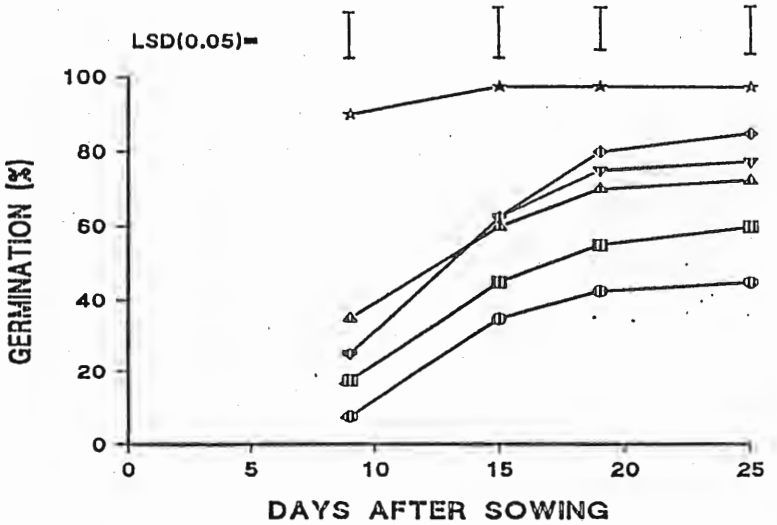


Fig(3). Effect of different concentrations of A. afra stem extracts on germination of maize seeds at four different dates after sowing.

EXPLANATION OF SYMBOLS

(*)distilled water
(Φ)1:5 dilution
(v)1:2.5 dilution
(m)1:1 dilution
(o)original undiluted extract

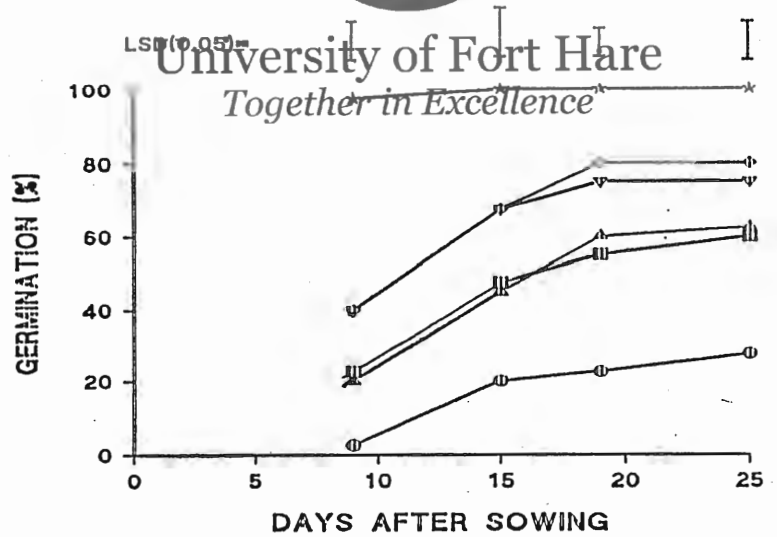
The effect of extracts from the roots of *Artemisia* on the germination of maize seeds is shown in fig.4. At the various dates after sowing, the statistical results show no differences between the 1:2, 1:2.5 and 1:5 dilutions. At 9 DAS, germination percentages for the distilled water and the next lower value (1:2 dilution) are 90 and 35 respectively. Fig.5 shows the effect of *Artemisia* leaf extracts on the germination of bean seeds, as shown in Plate 6. At 9 DAS, a difference of 95% was observed between germination means for distilled water (control) and the undiluted extract. At the various dates after sowing, no significant ($P < 0.05$) differences existed between the 1:1 and the 1:2 dilutions. Similarly, the statistical results show no significant ($P < 0.05$) differences between the 1:2.5 and 1:5 dilutions for all four dates after sowing.



Fig(4). Effect of different concentrations of A. afra root extracts on germination of maize seeds at four different dates after sowing.

EXPLANATION OF SYMBOLS

(★)distilled water
(Φ)1:5 dilution
(▽)1:2.5 dilution
(△)1:2 dilution
(▥)1:1 dilution
(⊕)original undiluted extract



Fig(5). Effect of different concentrations of A. afra leaf extracts on germination of bean seeds at four different dates after sowing.

EXPLANATION OF SYMBOLS

(★)distilled water
(Φ)1:5 dilution
(▽)1:2.5 dilution
(△)1:2 dilution
(▥)1:1 dilution
(⊕)original undiluted extract

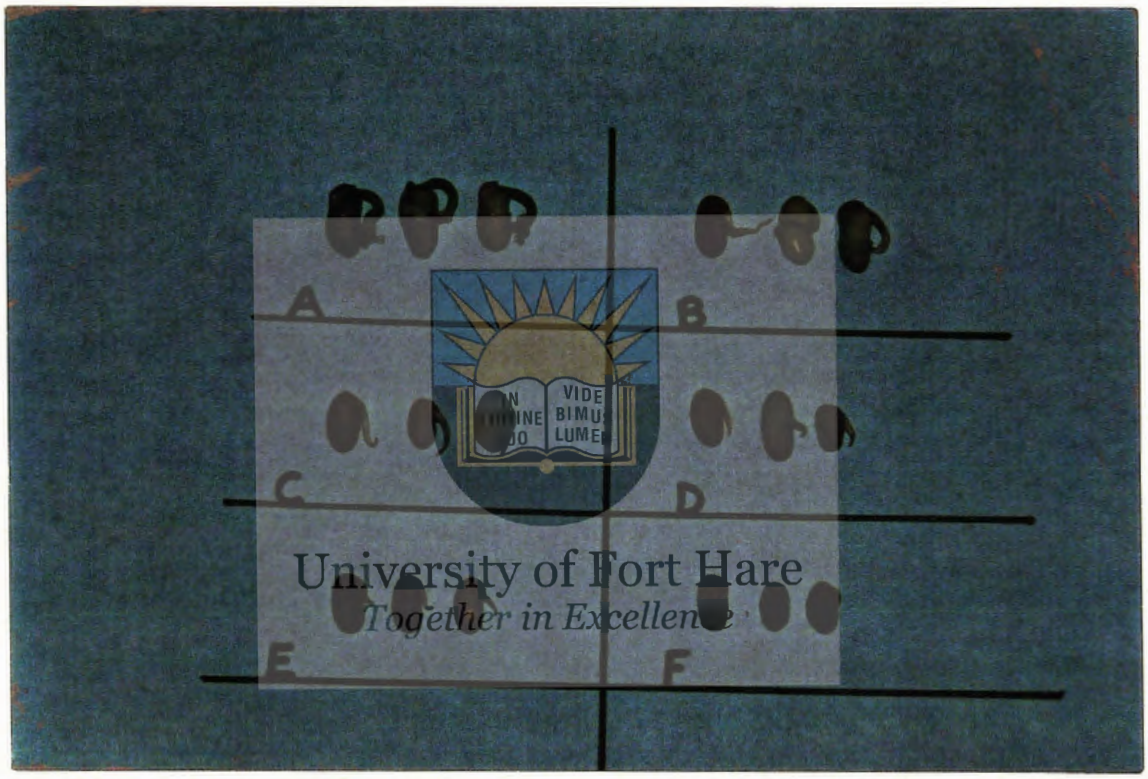
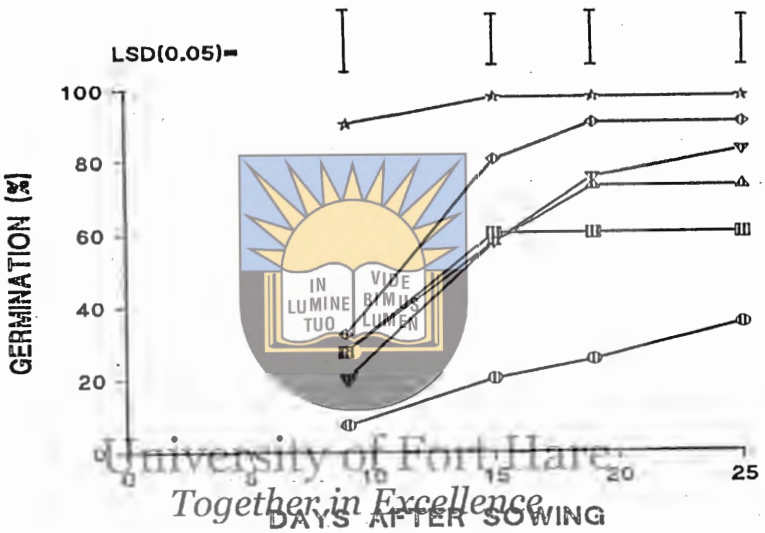


Plate 6: Radicle and plumule lengths of bean seeds as affected by cold water extracts of *A. afra* leaves at various dilutions (5 DAS).

[A] control (distilled water)
[B] 1:5 dilution
[C] 1:2.5 dilution
[D] 1:2 dilution
[E] 1:1 dilution
[F] original undiluted extract.

Fig.6 shows the effect of *Artemisia* stem extracts on the germination of bean seeds. A difference of 90% was observed between germination means for the distilled water effect and the undiluted extract. At 15 DAS, germination percentages for the 1:1 dilution and the undiluted extract are 60 and 20 respectively, which is a relatively large difference between the two concentrations.

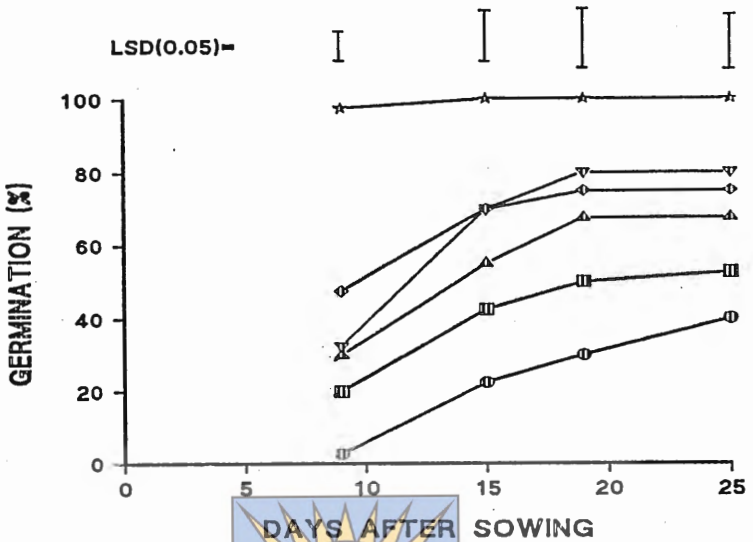


Fig(6). Effect of different concentrations of A. afra stem extracts on germination of bean seeds at four different dates after sowing.

EXPLANATION OF SYMBOLS

(☆)distilled water
(◇)1:5 dilution
(▽)1:2.5 dilution
(△)1:2 dilution
(□)1:1 dilution
(○)original undiluted extract

Fig.7 shows the effect of *Artemisia* root extracts on the germination of bean seeds. A difference of 95% was observed between germination means for distilled water (control) and the undiluted extract, as in fig.5. Apart from the 25 DAS where no significant ($P < 0.05$) difference existed between the 1:1 dilution and the original extract, the statistical results show differences between effects of these two solutions at 9, 15 and 19 DAS.

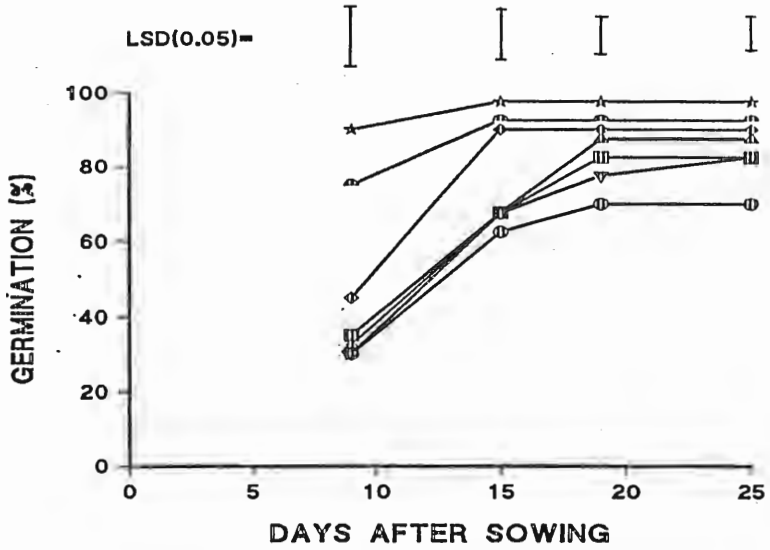


Fig(7). Effect of different concentrations of A. afra root extracts on germination of bean seeds at four different dates after sowing.

EXPLANATION OF SYMBOLS

(★)distilled water (△)1:2 dilution
(◇)1:5 dilution (▽)1:1 dilution
(▽)1:2.5 dilution (○)original undiluted extract

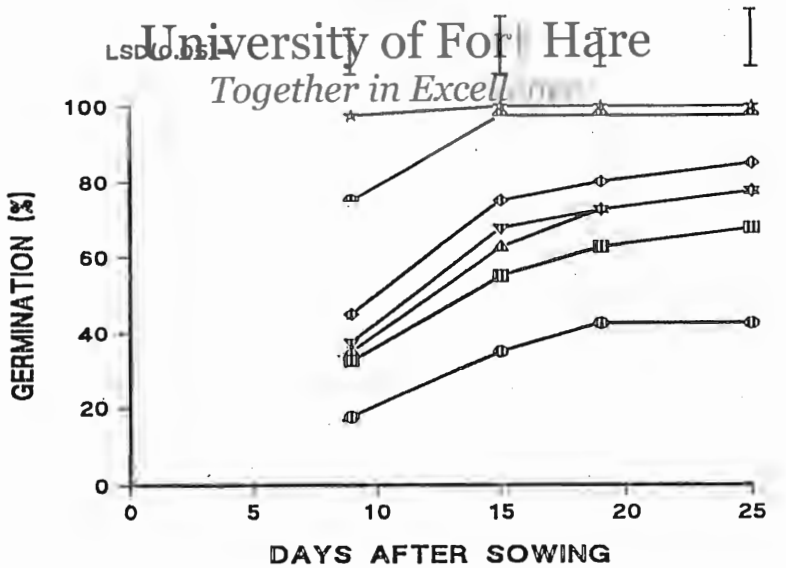
The effect of extracts from soil planted to *Artemisia* on the germination of maize seeds is shown in fig.8. At 9 DAS, no significant ($P < 0.05$) difference was observed between effects from the distilled water and, that from the control soil extract. Similarly, no significant ($P < 0.05$) differences existed between means of the various dilutions at 9 DAS. Fig.9 shows the effect of extracts from soil planted to *Artemisia* on the germination of bean seeds. Unlike the maize, significant ($P < 0.05$) differences were observed between effects from the distilled water and that from the control soil extract, at 9 DAS. Whereas no significant differences were observed between the 1:2, 1:2.5 and 1:5 dilutions, clear significant difference existed between effect of the original extract and that from the various dilutions.



Fig(8). Effect of different concentrations of extracts from soil planted to A. afra on germination of maize seeds at four different dates after sowing.

EXPLANATION OF SYMBOLS

(*)distilled water
(Φ)1:5 dilution
(▽)1:2.5 dilution
(△)1:2 dilution
(▥)1:1 dilution
(●)original undiluted control soil extract
(Φ)original undiluted Artemisia soil extract

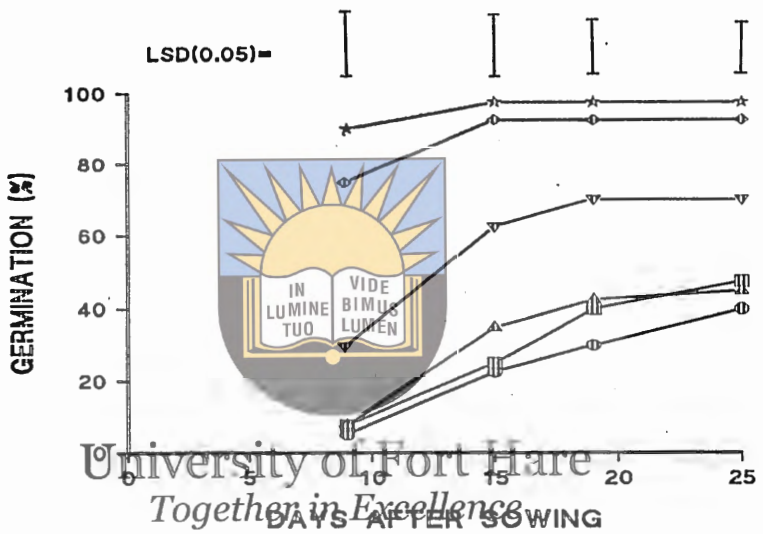


Fig(9). Effect of different concentrations of extracts from soil planted to A. afra on germination of bean seeds at four different dates after sowing.

EXPLANATION OF SYMBOLS

(*)distilled water
(Φ)1:5 dilution
(▽)1:2.5 dilution
(△)1:2 dilution
(▥)1:1 dilution
(●)original undiluted control soil extract
(Φ)original undiluted Artemisia soil extract

Fig.10 shows the effect of extracts from the various parts of *Artemisia*, as well as extracts from soil planted to this shrub, on maize seeds. For all four dates of recording, no significant ($P < 0.05$) differences were observed between effects of distilled water and the extract from the control soil. Similarly, no clear significant ($P < 0.05$) differences existed between effects of extracts from the various parts of the shrub. However, there was a significant ($P < 0.05$) difference between effect of extract from soil planted to *Artemisia* and, the other extracts.



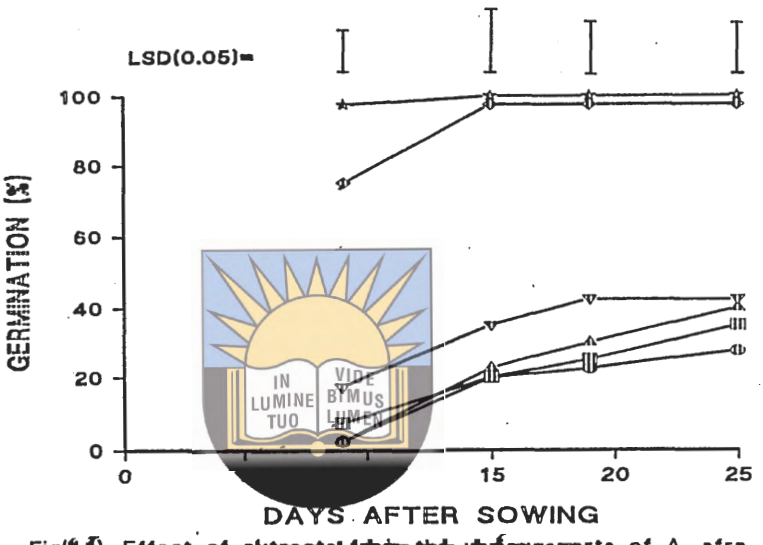
Fig(10). Effect of extracts from the various parts of A. afra and soil planted to this shrub on germination of maize seeds at four different dates after sowing.

EXPLANATION OF SYMBOLS

(*)distilled water
(Φ)control soil extract
(▽)Artemisia soil extract
(Φ)leaf extract
(▥)stem extract
(△)root extract

The effect of extracts from the various parts of *Artemisia*, as well as extracts from soil planted to this shrub, on bean seeds is shown in fig.11. At 15 DAS, there was no significant difference between the effect of distilled water and extract from the control soil. However, the statistical results show significant ($P < 0.05$) difference between the effect of extract from the control soil and extracts from the various parts of the shrub, including soil planted to the shrub. No significant difference was observed between the effect of extract from soil planted to *Artemisia*

and, extracts from the various parts of the shrub at 15 DAS. A percentage difference of 80 was observed between germination means for distilled water (100%) and *Artemisia* leaf extract (20%) at 15 DAS.



Fig(II 1). Effect of extracts from the various parts of A. afra and soil planted to this shrub on germination of bean seeds at four different days after sowing.

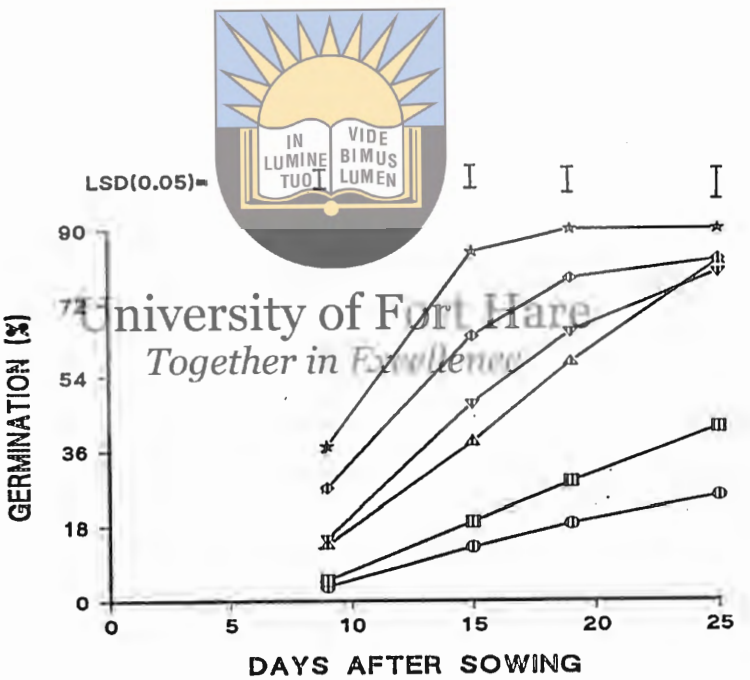
EXPLANATION OF SYMBOLS

(*)distilled water
(◇)control soil extract
(▽)Artemisia soil extract
(⊕)leaf extract
(⊞)stem extract
(△)root extract

For both agronomic crops tested, *A. afra* leaf extracts were found to be the most inhibitory. There was no significant ($P < 0.05$) difference between the potency of stem and root extracts. Extract from soil planted to *A. afra* was less inhibitory than was the case for the plant parts. However, extract from soil planted to *A. afra* was found to be more inhibitory than extracts from the control soil. Generally, inhibition effect decreased gradually with increase in dilution.

4.3.2. Forage crop

Effect of extracts from the various parts of *A. afra* followed a similar trend to the agronomic crops, in which significant ($P < 0.05$) differences were obtained between distilled water and the various dilutions of extracts. For all extracts assayed, differences due to dilution effect were significant ($P < 0.05$) among the means during the first 19 DAS. However, such differences were markedly reduced thereafter. Fig.12 shows the effect of *Artemisia* leaf extracts on the germination of seeds of rye grass. At 19 DAS, significant ($P < 0.05$) differences were observed among the effects of the various dilutions. All means were significantly ($P < 0.05$) different from each other. The highest mean (90%) was obtained from the effect of distilled water, whilst the lowest (18.5%) was from the original undiluted extract.

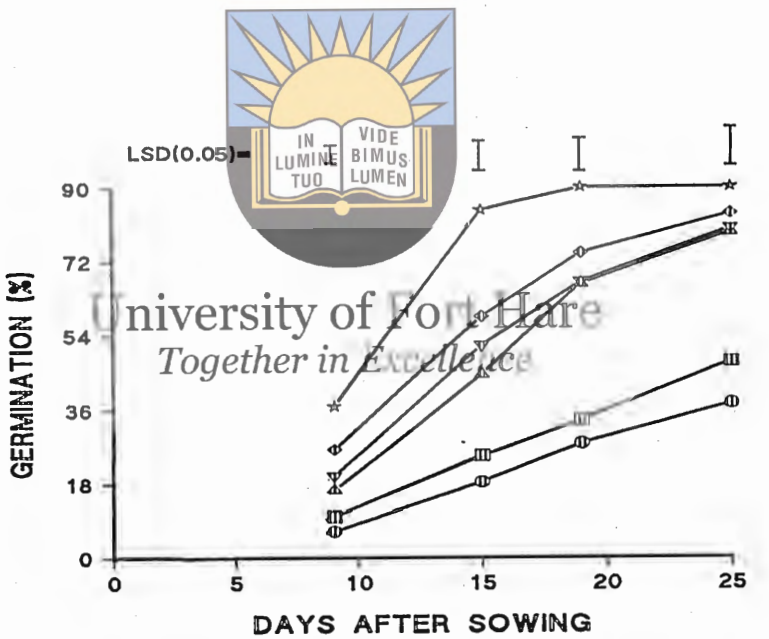


Fig(12). Effect of different concentrations of A. afra leaf extracts on germination of seeds of rye grass at four different dates after sowing.

EXPLANATION OF SYMBOLS

(★)distilled water
(Φ)1:5 dilution
(▽)1:2.5 dilution
(△)1:2 dilution
(▥)1:1 dilution
(⊕)original undiluted extract

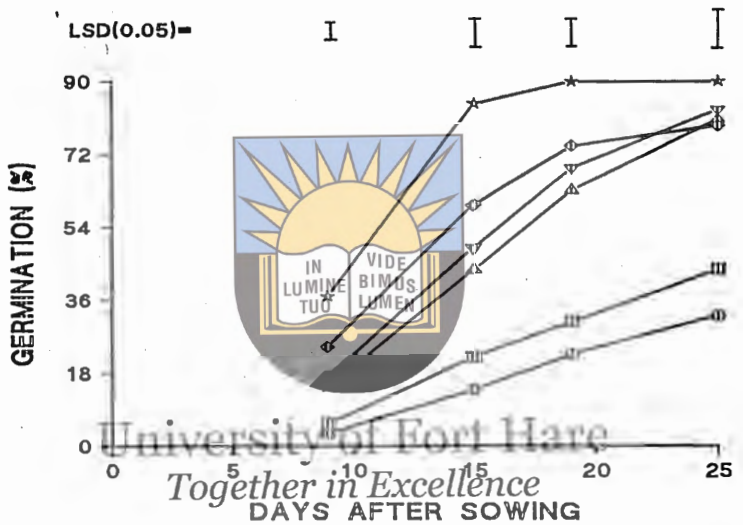
Fig.13 shows the effect of *Artemisia* stem extracts on the germination of seeds of rye grass. Significant ($P < 0.05$) differences were apparent among the effects of the various dilutions at 15 DAS. All means were significantly ($P < 0.05$) different from each other. The highest mean (84.5%) was obtained from the effect of distilled water and, the lowest (18.5%), from the original undiluted extract. The effect of *Artemisia* root extracts on the germination of seeds of rye grass is shown in fig.14. Apart from the 1:2 and 1:2.5 dilutions which showed no significant ($P < 0.05$) difference between their mean values, differences existed among the effects of the other dilutions at 15 DAS. The effect for distilled water gave the highest mean (84.5%) and that for the original undiluted extract, the lowest (14%).



Fig(13). Effect of different concentrations of A. afra stem extracts on germination of seeds of rye grass at four different dates after sowing.

EXPLANATION OF SYMBOLS

(*)distilled water
(◇)1:5 dilution
(▽)1:2.5 dilution
(△)1:2 dilution
(▯)1:1 dilution
(○)original undiluted extract

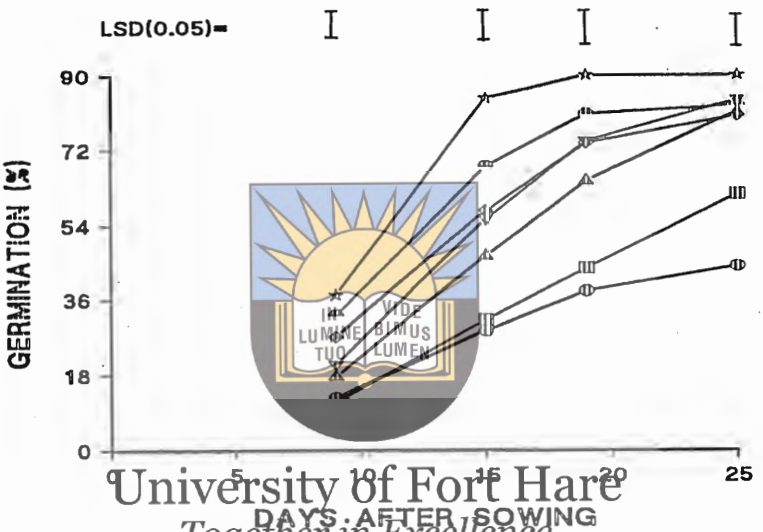


Fig(14). Effect of different concentrations of A. afra root extracts on germination of seeds of rye grass at four different dates after sowing.

EXPLANATION OF SYMBOLS

(⋆)distilled water
(Φ)1:5 dilution
(▽)1:2.5 dilution
(△)1:2 dilution
(Ⅲ)1:1 dilution
(Θ)original undiluted extract

The effect of extracts from soil planted to *Artemisia* on the germination of seeds of rye grass is shown in fig.15. Among the 1:2, 1:2.5, 1:5 dilutions and the control soil extract, no significant ($P < 0.05$) differences were observed at 25 DAS. There were significant differences between the above dilutions and the distilled water control (90%) as well as the original undiluted extract (44%) from soil planted to *Artemisia*.

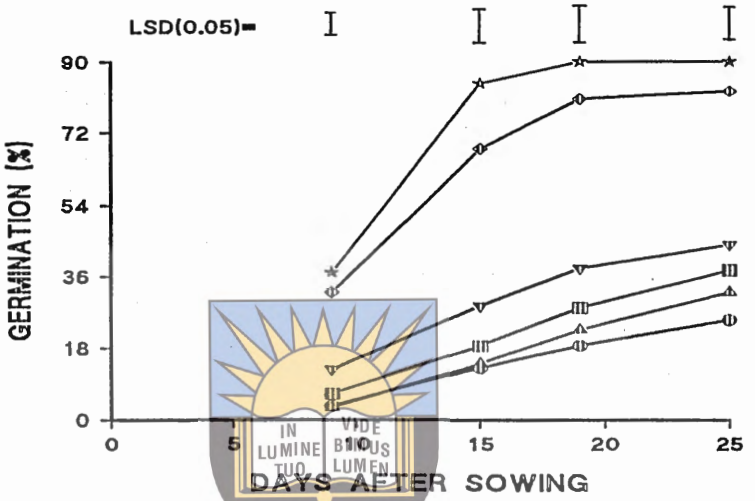


Fig(15). Effect of different concentrations of extracts from soil planted to A. afra on germination of seeds of rye grass at four different dates after sowing.

EXPLANATION OF SYMBOLS

(⋆)distilled water
(⋄)1:5 dilution
(▽)1:2.5 dilution
(△)1:2 dilution
(▫)1:1 dilution
(▬)original undiluted control soil extract
(⊕)original undiluted Artemisia soil extract

Fig.16 shows the effect of extracts from the various parts of *Artemisia*, as well as extracts from soil planted to this shrub, on the seeds of rye grass. The statistical results show that significant ($P < 0.05$) differences existed among the two soil extracts and the distilled water control, the latter showing the highest germination percentage at 9 DAS. The extracts from the various parts of the shrub showed significantly ($P < 0.05$) different effects. The roots, stems and leaves gave 3%, 6.5% and 3.5% germination respectively, at 9 DAS.



Fig(16). Effect of extracts from the various parts of A. afra and soil planted to th... on germination of seeds of rye grass at four different dates after sowing.

EXPLANATION OF SYMBOLS

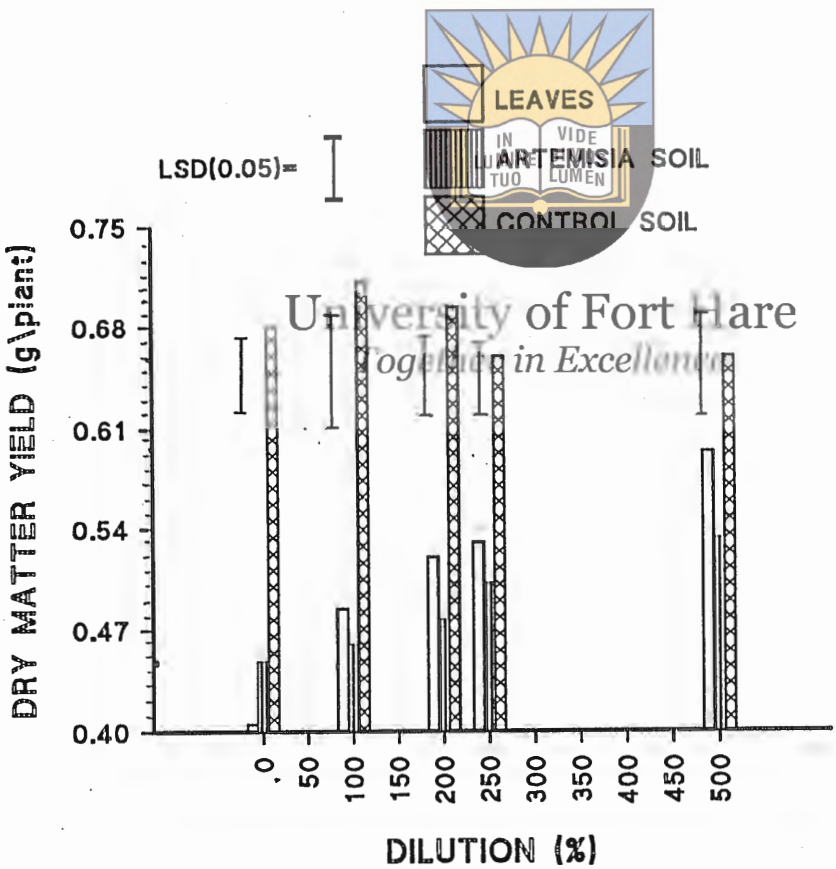
(*)distilled water
(o)control soil extract
(▽)Artemisia soil extract
(◐)leaf extract
(III)stem extract
(△)root extract

In general, the 1:2 dilution was the least inhibitory for the various extracts assayed. This is demonstrated by the germination percentages for leaf extract at 25 DAS, which were 82.0 as against 82.5 and 90.0 for control soil extract and distilled water respectively (Table 9).

4.4. Seedling Vigour Tests

4.4.1. Extracts

The effect of cold water extracts from leaves of *Artemisia* as well as from soil planted to this shrub, on the seedling vigour of maize is shown in fig.17a. Averaged over all dilutions, the statistical results show ($P < 0.01$) that the leaf extracts had the same effect as the extracts from soil under *Artemisia*. Significant differences were observed between extracts of the test (*Artemisia*) and control soils. Plates 7 and 8 show the harvested seedlings of maize and bean, grown as "plugs" in cavity trays on raised rails, as affected by extracts from the various parts of *A. afra*, as well as extract from soil planted to *A. afra*.



Fig(17a). Seedling vigour as shown by dry matter yield of maize treated with three different cold water extracts.

Plate 7: Effect of *A. afra* plant and/or soil extracts on the growth of maize seedlings grown as "plugs" in cavity trays.

1 SC, 1 LC, 1 RC- original undiluted extracts from *A. afra* stems, leaves and roots respectively.

1 SoC, 1 S;C - original undiluted extracts from soil planted to *A. afra* and control soil respectively.

2 LC - 1:1 dilution of original leaf extract.

CT - distilled water (secondary control).

Plate 8: Effect of *A. afra* plant and/or soil extracts on the growth of bean seedlings grown as "plugs" in cavity trays.

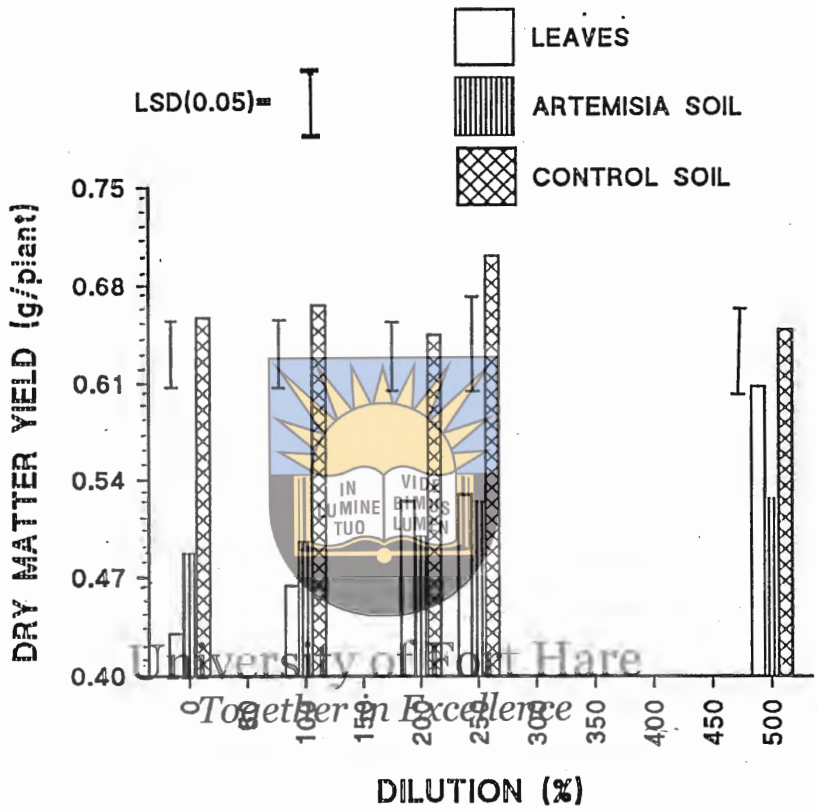
1 SC, 1 LC, 1 RC- original undiluted extracts from *A. afra* stems, leaves and roots respectively.

1 SoC, 1 S;C - original undiluted extracts from soil planted to *A. afra* and control soil respectively.

2 LC - 1:1 dilution of original leaf extract

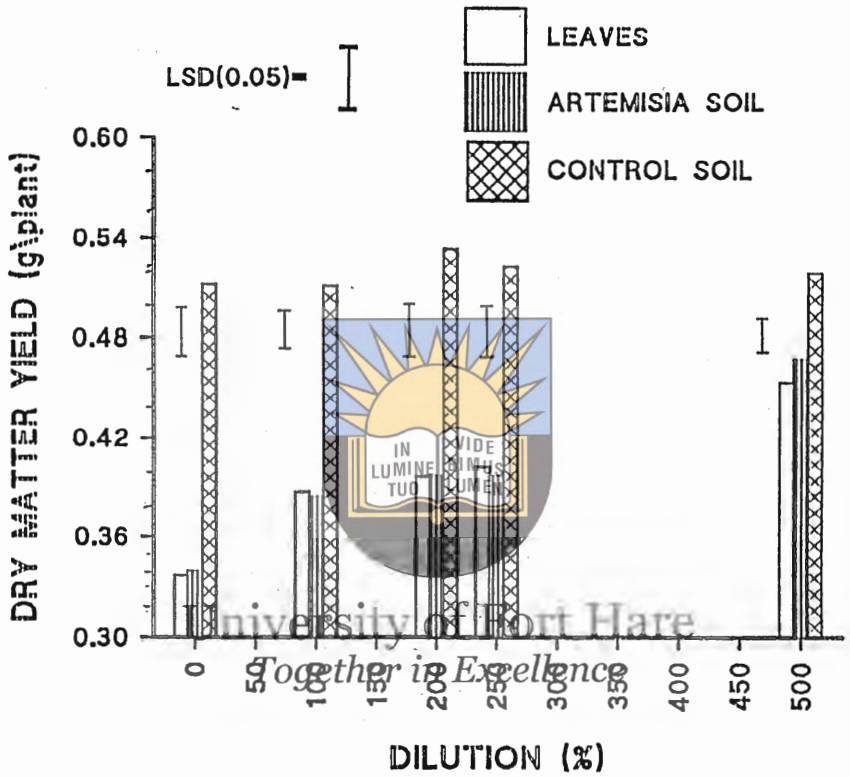
CT - distilled water (secondary control).

Fig.17b shows the effect of hot water extracts, (unlike cold extracts as in 17a), on the seedling vigour of maize. Averaged over all dilutions, the statistical results show ($P < 0.01$) that the hot water extracts had the same effect as the cold water extracts.

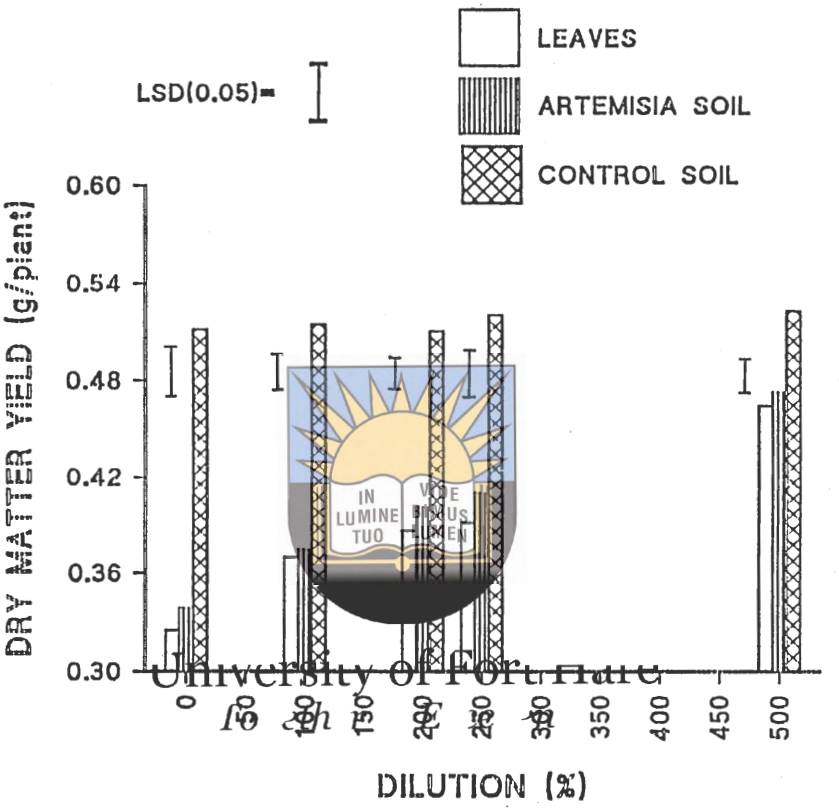


Fig(17b). Seedling vigour as shown by dry matter yield of maize treated with three different hot water extracts.

Figs. 18a and 18b show the effect of extracts from cold and hot water respectively, from the leaves of *Artemisia*, as well as from soil under this shrub, on the seedling vigour of beans. As in figs. 17a and 17b, the statistical results show ($P < 0.01$) that the hot water extracts had the same effect as the cold water extracts. Also, leaf extracts had the same effect as the extracts from soil under *Artemisia*. Significantly greater means were obtained from the effect of control soil extract than was the case for soil under *Artemisia*.

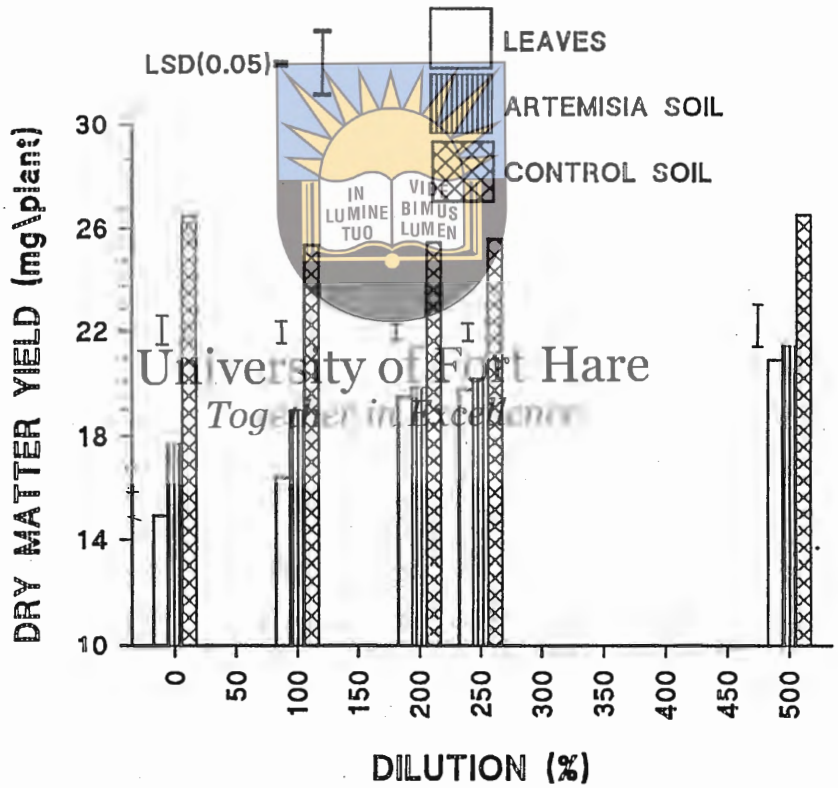


Fig(18a). Seedling vigour as shown by dry matter yield of bean treated with three different cold water extracts.

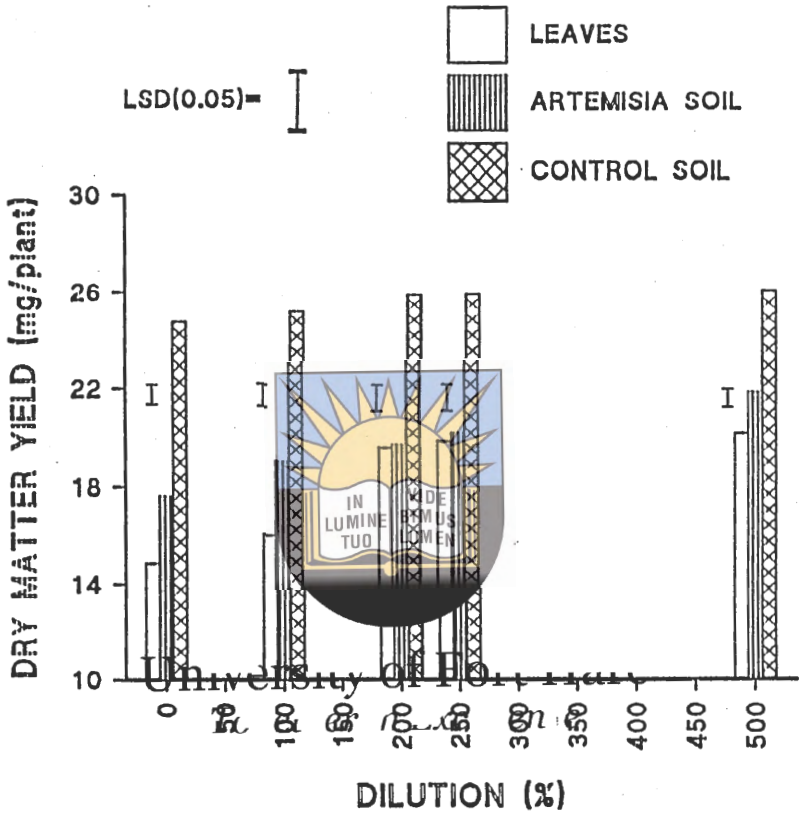


Fig(18b). Seedling vigour as shown by dry matter yield of bean treated with three different hot water extracts.

The effect of cold and hot water extracts from the leaves as well as from soil under *Artemisia* on the seedling vigour of rye grass is shown in figs. 19a and 19b respectively. The statistical results show that the hot water extracts had the same effect as the cold water extracts. The effect of extracts from the test soil was significantly different from that of the control soil at all dilutions. The 100% dilution and the original extract were the only concentrations which showed significant differences between the effect of leaf and test soil extracts. Other dilutions did not show any significant ($P < 0.01$) differences between the two extracts.



Fig(19a). Seedling vigour as shown by dry matter yield of rye grass treated with three different cold water extracts.



Fig(19b). Seedling vigour as shown by dry matter yield of rye grass treated with three different hot water extracts.

4.4.2. Soils

Table 11 shows the effect that soils planted to *A. afra* have on some growth parameters of maize and bean seedlings. For both agronomic crops, germination percentages were much lower in the *Artemisia* soils than was the case for the controls and the differences were statistically significant ($P < 0.05$). There was a 50% difference between the germination means of the two treatments and this was observed for both crops. In contrast, stem diameters for both crops were significantly ($P < 0.05$) greater in the *Artemisia* soils than in the controls. For maize, plant height was greater in the test soils than was the case for the control soils, for both 14 and 28 DAE, while for the bean, plant height was less in the test soils than in the controls. There was no particularly clear trend observed for shoot and root dry matter yields for both crops. In maize for instance, shoot dry matter yield was greater in the test soil than was the case for the control, while the reverse was true for the root dry matter yield. In bean, both shoot and root dry matter yields were greater in the controls than was the case for the *Artemisia* soils. However, these differences were not statistically significant ($P < 0.05$).

Table 11. Germination and growth parameters for maize and bean as affected by soil previously occupied by *Artemisia afra*.

CROP	DAYS AFTER EMERGENCE	SOIL	GERMINATION (%)	STEM DIAMETER (mm)	PLANT HEIGHT (mm)	DRY MATTER (g)	
						SHOOT	ROOT
Maize	14	*Artemisia*	37.50	7.76	60.50	-	-
		control	87.50	6.05	50.53	-	-
		LSD(0.05)	45.01	0.89	6.21	-	-
		CV (%)	11.31	2.02	1.76	-	-
	28	*Artemisia*	-	12.68	88.00	21.85	9.66
		control	-	8.39	75.50	19.66	10.16
		LSD(0.05)	-	2.77	8.14	NS	NS
		CV (%)	-	4.14	1.56	9.42	10.74
Bean	14	*Artemisia*	40.00	3.90	19.44	-	-
		control	90.00	3.40	25.25	-	-
		LSD(0.05)	12.99	0.47	3.29	-	-
		CV (%)	3.14	1.99	2.31	-	-
	28	*Artemisia*	-	4.39	39.50	8.08	1.98
		control	-	4.00	43.75	10.88	2.45
		LSD(0.05)	-	0.16	NS	NS	NS
		CV (%)	-	0.61	1.64	7.15	5.44

NS : Not significant.

5. DISCUSSION

5.1. Soil analytical data

5.1.1. Particle size distribution

The differences in particle size between the two sites is attributed to differences in parent material (Table 1). The recent alluvial deposit of the Jozini soil Form resulted in a high fine sand and silt fraction, whereas the mudstone of the Estcourt soil Form had abundance of clay sized particles.

5.1.2. Other soil physical properties

Table 2 reveals relatively high differences between the aggregate stability values for *Artemisia* soils and those of the control soils for both soil Forms. The relatively low difference between the effect of treatments in the Estcourt soil Form (5.0-2.0 mm. sieve size) is most probably attributable to the presence of small Fe/Mn concretions of the order of 1-2 mm. diameter which are homogeneously mixed with the soil (*in situ*) and are a characteristic feature associated with that soil Form (Van Averbeke, 1991). These concretions may have contributed to the high mass of aggregates retained on the 5.0-2.0 mm. sieve size.

As reported by Van Averbeke (1991) that the aggregates of the surface horizon of the Jozini soil samples from the control sites were found not to be water-stable and they degrade rapidly following wetting, it was, however, observed in the present study that aggregates of the same soil, planted to *A. afra*, were very stable in water and did not degrade rapidly on wetting. This suggests that *A. afra* shrubs have a stabilizing effect on the aggregates of these soils. One most probable mechanism by which this is achieved may be the release of exudates from the roots of the shrubs into the rhizosphere, where they influence the binding of soil particles. Pojasok and Kay (1990) reported that root exudates promote the release of polyvalent cations into the soil solution and their increased concentrations result in increased aggregate stability.

The moss which were found growing on the soil surface at the *Artemisia* sites may also have contributed to the stability of the aggregates by binding soil particles, as reported by Lund (1967), for fungi.

The significant differences in bulk densities, observed between the treatments in the Estcourt soil is probably the result of the effects of root exudates and other organic compounds found in the rhizosphere of the soils under *Artemisia*. This notion is further strengthened by the results for the loss on ignition, discussed below.

5.1.3. Chemical

Soil pH values for the tests done by Van Averbeke (1991) and those for the current study followed the same trend, in which pH increased with depth. In the current study, the minimal differences in pH values and lack of statistical significance between the test and control soils for both soil Forms, suggests that growth inhibition of plants in the vicinity of *A. afra* shrubs may not be attributed to changes in soil pH.

The significant differences in organic matter content suggest that, under *Artemisia*, there is considerably more accumulation of organic matter in the soil. The conditions under which the experiments were conducted were such that there was no meaningful contribution of organic material from leaf fall. It would therefore be reasonable to conclude that the higher levels of organic matter in the *Artemisia* soils were the result of accumulations not from above but from within the root zone, in which case, the only possible sources would be root exudates or increased microbial biomass or both. The duration of the experiment was also such that any possible major contribution from the decomposition of root tissue can be discounted.

Although in the Jozini soil Form, there was no significant difference between the phosphorus levels of the two treatments, the actual amount of phosphorus was higher in the test soil. In the Estcourt soil Form, the differences were even greater and statistically significant. With regard to plant nutrition, it should be noted that the presence of certain allelochemicals in the

rhizosphere can impair the plant's ability to take up phosphorus. Glass (1975) found that P uptake in barley roots was impeded by hydroxy-benzoic acids, which are known to be allelochemicals. The presence of *Artemisia* therefore has an effect on soil P levels. The actual differences between the two soil Forms are likely to be due to intrinsic differences in other soil properties.

Apart from the sharp increases in the CEC values for the B21 and C horizons of the Estcourt soil Form, CEC and sum of cations were quite uniform in the various horizons for both soil Forms (Table 4). The sharp increases in CEC are considered to be related to the high clay contents in the B21 and C horizons.

Generally, electrical conductivity values increased with depth. Sharp increases were observed in the B21 horizon for both soil Forms. This is possibly also a result of the increase in clay content.

From the chemical data presented in Table 4, it is abundantly clear that differential soil fertility can be ruled out as being contributory to the observed growth inhibition in the vicinity of *Artemisia afra* shrubs. This increases the likelihood of the mechanism responsible being the release of allelochemicals from the various parts of *A. afra* shrubs into the rhizosphere, toxifying the soil and rendering it unsuitable for the growth of other plants in the neighbourhood.

5.2. Loss on Ignition

Loss on ignition gives a measure of the combustible organic material in the soil which, in turn, can also influence such properties as soil binding and therefore aggregate stability and, to a degree, bulk density. The results from this experiment clearly show that the introduction of *Artemisia* in soils has an important influence on this soil parameter. An important corollary of these results is that non-humified organic materials may also have an important role in the binding processes in the soils under *Artemisia*.

5.3. Analysis for organic compounds

It was of interest to note that none of the various terpene compounds identified by Graven *et al.* (1990) in the volatile oil of *A. afra* fresh plants were found in soil under the shrub in this study. A possible reason is that, organic compounds which may enter the soil from the above ground parts of the plant may undergo various transformations, aided by micro-organisms and, be changed into other complexes quite different from the parent compounds. These may or may not be toxic to plants. Cheng (1992) found that soil compounds can be transformed by micro-organisms, resulting in new products which may be either more toxic or more complex in structure, as well as simple in structure and less toxic.

Although phenols, which have been shown in the literature to be one of the two main groups of plant inhibitors, were not specifically identified in this study, it should be borne in mind that they are biosynthetically related to benzene (Muller, 1966), which happens to be the major group of compounds identified in this study.

5.4. Germination tests

The statistical significance between the effect of extracts from the various parts of the test plant and the distilled water control clearly indicates the presence of inhibitory components associated with this aromatic shrub. Further, the significance of effects between the test soil and control soil indicates that the inhibitory component was present in the test soil, but may be either absent or at concentrations too low in the control soil to cause inhibition of germination. The fact that aqueous extracts of soil under the test plants were not as effective as those of the plant materials, strongly supports the hypothesis that there are toxic components concentrated in the plant material, particularly the leaves which may get into the rhizosphere upon the death and decomposition of the plant materials. It is thought that phytotoxins found in the soil may have entered the soil due to leaching from the plants during rain, exudation from the roots, decomposition of residues, or combinations thereof. It is worth mentioning that, since test plants were kept in a greenhouse and under restricted atmospheric conditions, the contribution from

leaf fall and decay would, in this case, have been minimal, while leaching losses from the plants due to rainfall were practically absent. This, therefore, implies that root exudates would have in all probability, contributed immensely to the toxicity of the soil under the test plants.

The fact that maize responded more noticeably than bean to increased dilutions of extracts from *A. afra*, suggests that the crops have different thresholds of sensitivity to allelochemicals.

Maize is also known to recover from water stress more quickly than bean. This is mainly due to the physiology of the maize plant. It is, therefore, also probable that the quick recovery of maize from the phytotoxicity of *A. afra* extracts may be partly a function of the physiology of the maize plant.

Extracts from the control soil seem to have had some negative effects on the germination of seeds of rye grass. The apparent mild toxicity of the control soil could be related to the fact that the seeds of rye grass are much smaller than those of maize and bean, thus providing far less mass to counter the effects of allelochemicals found in the soil, even in small amounts, at least at germination.

5.5. Seedling vigour test (extracts)

In the germination bioassay, it was established that aqueous extracts from leaves of *Artemisia afra* were more potent than those from soil under this shrub. However, in the seedling vigour tests, there was no significant difference ($P < 0.05$) between the potencies of these two extracts. This may be attributed mainly to the differences in the biochemical processes involved in germination and early seedling development. The high potency of the leaf extracts observed during the germination tests may possibly be due to severe suppression of enzymes responsible for normal seed germination. The disruption of the enzymatic process can result in nearly complete suppression of seed germination (Putnam, 1985). Thereafter, the effect of the inhibitors diminishes with time. In addition, the physiology of the plant, once it has germinated, determines how quickly it can overcome the effect of the inhibitors (Smith and Martin, 1994).

Similar to the germination test, increased dilutions of the extracts resulted in proportional increases in dry matter yield. The statistical significance between the effects of test and control soils indicates that the active inhibitory component was present in the test soil, but may be either absent or at concentrations too low in the control soil to retard seedling vigour. This further suggests that the toxic principle may be mainly inhibitory to seed germination and has most impact on the biochemical pathways active in the germination stage but not necessarily thereafter but perhaps, only inhibits the establishment of seedlings at much greater concentrations than achieved in this study. This evidence also strongly suggests that the bare areas in the vicinity of *Artemisia* shrubs are related to this allelopathic effect.

5.6. Seedling vigour test (soils)

The very poor germination of both maize and bean (37.5 and 40% respectively) when planted in soils previously occupied by *Artemisia afra* demonstrates the toxicity of soils under *A. afra* shrubs. The 50% difference in germination for both maize and bean (relative to the control) is indicative of this effect.

The observed trend regarding stem diameter, plant height and biomass yield shows the inherent differences in the growth patterns of the crops, which may also be a reflection of their differences in sensitivity to allelochemicals. Bean seem to have a higher threshold of inhibition than maize. The results show that 28 DAE, the bean seedlings have not been able to overcome the toxic effect of soils planted to *Artemisia afra*, or are close to the threshold for overcoming the toxic effect. The results of this test suggest that the germination process is the main determining factor and, once seeds have been able to germinate, they manage to approach "normal" growth, all other environmental and nutritive factors remaining unchanged.

6. CONCLUSIONS

The introduction of *Artemisia afra* on agricultural land results in changes in the rhizosphere. The study showed that the changes affect both the physical and chemical properties of the soil. Since microbial ecology is greatly affected by the physical and chemical composition of the soil, it is highly probable that the microbial ecology of soils planted to *A. afra* would also change. However, in the present study, no investigations were carried out to assess the effect of *A. afra* on the microbial spectrum of the soil but the degree of physical and chemical changes observed makes it more than likely that the microbiology of the soil would also change. The inclusion of microbial studies at this stage was outside the scope defined for this study, which was preliminary in nature, to identify the less complex chemical and physical interactions before attempting the more complex microbiological ones. The latter, however, is considered to constitute a very fertile area for further research. The organic carbon content of any soil is known to have a direct bearing on the number and activity of microbes present in that soil. In the study, soils planted to *A. afra* showed greater levels of organic carbon than was the case for control soils. This would suggest a greater number and increased activity of microbes in the *Artemisia* soils. Since organic matter is known to play a significant role in soil particle binding, aggregates of soils with greater levels of organic carbon are prone to be more stable when wetted than those with little amounts of organic carbon.

In the study, aggregate stability of two soil Forms, Jozini and Estcourt, was enhanced by *A. afra* shrubs. Evidence from the study strongly suggests that root exudates were most probably responsible for binding of soil particles, in a similar way to that reported in the literature by Pojasok and Kay (1990). The stability of the aggregates under wet sieving conditions may also be the result of the exudates containing significant levels of hydrophobic substances which would protect the aggregates against slaking. A detailed investigation of the nature of the exudate materials was well beyond the scope of the present study but is, clearly, an area in need of research.

One of the major differences between soils on which *Artemisia* grew and control soils is that, on the surface of the former, a thin but stable crust, covered with the moss (*Trichostomum brachydontium*) developed. The stability of the crust has also been attributed to soil particle binding by the moss, in a way similar to that of fungi reported in the literature. Microbial changes in any soil could have far reaching effects on the utility and potential of the soil. Monitoring of soil management, based on an appreciation of microbial ecology is therefore extremely important. The role of such biota as moss in stabilizing soil aggregates is therefore an area which also merits more direct investigation. Results from the study thus generally indicate that the structural stability of soils is enhanced when agricultural lands are planted to *A. afra*. An important practical implication of this would be its potential applications in protecting soils against erosion and physical degradation.

It has also been shown from the study that extracts from the various parts of *A. afra* are inhibitory to germination and early seedling development. In view of this deleterious effect of the sap, great caution should be exercised when consideration is being given to the cultivation of the shrub close to cropping lands. For this reason, perhaps, even small-scale farmers and households which cultivate the plants for medicinal purposes need to be advised of the risk associated with cultivating the shrubs concurrently with other crops meant for subsistence, on the same piece of land.

An important possible practical application of the allelopathic principle established in relation to this plant is that of weed control. Management of weeds by utilizing natural plant products rather than chemicals is ecologically and socially desirable. Considering the challenges faced by small-scale farmers and the weedicide industry regarding the successful control/eradication of certain weeds, it becomes of prime importance to develop mechanisms of employing natural products from allelopathic plants at certain threshold concentrations, necessary to suppress the growth of some of the more prevalent weeds in commercial agriculture, under various weather conditions. This is an area which needs more detailed investigation, with a view to identifying natural weedicides which, in addition to their ecological benefits, could also significantly reduce production costs.

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8. APPENDICES

University of Fort Hare
Together in Excellence

TABLE C1. Description of the Fort Hare Jozini ecotope.

PROFILE DESCRIPTION OF THE FORT HARE JOZINI

SOIL NAME
South Africa [1] : Oakleaf Jozini (now known as Oakleaf Ritchie)
FAO [2] : Orthic Luvisol
Soil Taxonomy[3] Typic Haplustalf

DATE: 05/09/89
AUTHOR: W. Van Averbeke
LOCATION: Ciskei (South Africa), Alice, Fort Hare Research Farm, land No D2, border strip No 13, 10m north west of the farm ring road.

LATITUDE: 32^0 47′ 51" S LONGITUDE: 26^0 50′ 55" E

ALTITUDE: 508m

LANDFORM
General: Dissected coastal plateau
Regional: Alluvial valley of the Tyume River
Local: Most recent alluvial terrace

SLOPE: Flat or nearly flat land, sloping gently down in a South Eastern direction with a gradient of 0,5%.

VEGETATION AND LAND USE: Annually cropped land which has been irrigated at least for the last 10 years, presently under oats.

CLIMATE:
Mean annual temperature: 18,1^{0}C
Mean annual rainfall: 575 mm with a distinct winter minimum and more or less pronounced spring and autumn maxima.

PARENT MATERIAL: Recent alluvial deposit consisting mainly of fine sand and silt, but containing significant amounts of clay. Mineralogically the sand fraction consists mainly of quartz and lesser amounts of plagioclase, rock fragments and iron and manganese oxides. In the clay fraction

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1 Macvicar, De Villiers, Loxton, Verster, Lambrechts, Merryweather, Le Roux, Van Rooyen and Von Harmse (1977)

2 FAO (1974)

3 USDA (1975)

both 1:1 and 2:1 type clay minerals are present.

DRAINAGE

Superficial: No run-off occurs except during continued heavy rains

Permeability: rather slow; behaves more like a clayey soil than a sandy soil (Russell, 1982)

Internal drainage: water drains readily but not rapidly
Drainage class: Class 4, well drained (FAO, 1977)

MOISTURE CONDITIONS OF THE PROFILE: dry up to a depth of 0,5m ; slightly moist up to a depth of 3m and moist lower down.

GROUND WATER TABLE: By augering to a depth of 4m it was established that there is no permanent water table present in the rooting zone of this soil. A few faint grey and some high chroma mottles found at a depth of 3,5m and lower suggest that temporary saturation of the soil at depths greater than 3,5m might occur following flooding, excessive rain or irrigation.

EVIDENCE OF EROSION: There are no indications of any wind or water erosion occurring at this site and generally soil material tends to accumulate. During periods of flooding new material is deposited by the river. The frequency of floods used to be about once every ten years but the recent damming of the Tyume river should reduce the flooding hazard considerably.

HUMAN INFLUENCE: Locally, the Oakleaf type soils situated in bottomland positions are regarded as having a high cropping potential. Most of these soils have been cultivated for a considerably long time. Where cultivation involved the application of organic matter and chemical fertilizer, the fertility of the plough layer has been improved. Phosphorus levels (127ppm, Bray 1) and (280 ppm, ISFEI) and potassium levels (532 ppm) in the top soil of the experimental land are particularly high.

SPECIAL FEATURES: The aggregates of the surface horizon are not water stable and degrade rapidly following wetting. Rain or irrigation generally results in the formation of surface crust, which reduces the infiltrability of the soil. Traffic is responsible for the presence of a plough pan, which adversely affects the permeability of the soil. As a result temporary aeration problems can occur in the surface layer following irrigation. The susceptibility of this soil to crusting and soil compaction could be related to the texture of the soil, which is characterised by a high fine sand faction.

Bennie and Krynauw (1985) list a high fine sand content as one of the factors affecting the susceptibility of soils to compaction and soil crusting, the latter being the compaction of surface layer.

BRIEF DESCRIPTION OF THE PROFILE: The texture of the profile is a more or less uniform, fine sandy clay loam, characterised by a small and gradual increase in clay content with depth. The upper 0,5 m of the profile is brown. Lower down in the profile the colour changes to dark brown due to the illuviation of clay and organic matter. The soil is more than 4 m deep and generally allows for a normal distribution of the roots. However, the presence of a plough pan at a depth of 300 to 500 mm has a limiting effect on root development and water movement. In this layer roots preferentially grow in fissures that separate the weak to moderately developed coarse angular blocks. There is abundant evidence of faunal activity especially in the subsoil layers.

PROFILE DESCRIPTION

Ap	0-300 mm	Dry: dark brown (10 YR 3/3) moist and brown (10 YR 5/3); fine sandy loam; generally massive but in places weak medium subangular blocky; hard (dry), friable (moist), slightly sticky and slightly plastic (wet); many fine roots; clear smooth transition, broken in places.
A3	300-500 mm	Dry; dark brown (10 YR 3/3/) moist and brown (10 YR 5/3) dry; fine sandy loam; weak to moderate coarse angular blocky; hard to very hard (dry), friable to firm (moist), slightly sticky and slightly plastic (wet); many fine roots preferentially growing interpedal but also intrapedal; gradual smooth transition.
B1	500-700	Dry; dark brown (10 YR 3/3) moist and brown (10 YR 5/3) dry; fine sandy clay loam; massive; slightly hard to hard (dry), friable to firm (moist); lightly sticky and slightly plastic (wet), patchy thin very dark brown (10 YR 3/2) moist/dry cutans of clay and organic matter; many fine roots; increased number of macro pores; undant evidence of faunal activity; gradual smooth transaction.
B21	700-1200	Slightly moist; dark brown to very dark greyish brown (10 YR 3/2,5) moist and brown (10 YR 5/3) dry; slightly hard to hard (dry), friable to firm (moist), slightly sticky and slightly plastic (wet), broken thin very dark brown (10 YR 3/2) moist/dry cutans of clay and organic matter; few to many fine roots; abundant evidence of faunal activity, mostly worm casts, often filled or partially filled with darker (10 YR 3/2) moist/dry soil material.
B3	1200-1800+	Slightly moist; dark brown to very dark greyish brown (10 YR 3/2,5) moist and brown (10 YR 5/3) dry; fine sandy clay loam; weak coarse subangular blocky; slightly hard to hard (dry); friable to firm (moist), slightly sticky and slightly plastic (wet); patchy thin cutans of clay and organic matter; few fine roots; abundant evidence of faunal activity; few charcoal fragments at 1500 mm.

Table C7. Description of the Alice Rosemead ecotope.

PROFILE DESCRIPTION OF THE ALICE ROSEMEAD ECOTOPE

South Africa FORM: Estcourt SERIES: Rosemead (now known as Haarlem)
FAO : Eutric Planosol
Soil Taxonomy : Aquic Arenic Haplustalf

LOCALITY: Alice, Fort Hare Farm, 32° 47' 0"S, 26° 51' 00"E

CLIMATE: Semi-arid

PARENT MATERIAL: No. of kinds: single
Lithology: mudstone
Underlying material: mudstone
Mode of acculation: combination of colluvium and weathering in situ

FACTORS OF SOIL FORMATION: Weathering:
(a) physical: strong
(b) chemical: strong

TOPOGRAPHY: Terrain morphological unit: 4, old terrace
Slope:
Kind: straight
Aspect: 120° ESE

VEGETATION: Annually cropped land

HORIZON	DEPTH (mm)	DESCRIPTION
Alp	0-230	Dry; dark brown (10 YR 3/3) moist and greyish brown (10 YR 5/2) dry; loam; massive to weak, fine to medium subangular blocky; hard (dry), very small Fe/mn concretions, many fine roots, gradual smooth transition.
A12	230-390	Dry; non-homogeneous, dark brown (10 YR 3/3) moist matrix colour and greyish and brown (10 YR 5/2) dry matrix colour with few rusty mottles; silt loam; massive; hard (dry); few to many Fe/Mn concretion, many fine roots; gradual smooth transition.

E	390-420	Dry; dark brown (10 YR 4/3) moist and pale brown (10 YR 6/3) dry; silt loam; loose (dry); very many small Fe/Mn concretion, many fine roots; abrupt smooth transition.
B21	420-660	Dry; non-homogeneous, very dark brown (10 YR 2/3) moist and dark brown (10 YR 3/3) dry; clay; strong medium prismatic; hard; continuous very dark brown cutans on ped faces; few to many, small Fe/Mn concretions, many fine roots in upper part of horizon decreasing gradually with depth; gradual smooth transition.
C	660-1350+	Dry; non-homogeneous, patchy colours, yellowish brown (10 YR 5/4) moist matrix colour and yellowish brown (10 YR 5/4) dry matrix colour; coarse angular blocky; hard (dry); many Fe/Mn concretions, few large lime nodules consisting of hard core and a soft mantle, roots extend to a depth of 900 mm.

Coefficient of variation and standard error results for aggregate stability by the wet sieving technique.

SOIL FORM	TREATMENT	SOIL REPLICATES	Percentage water stable aggregates			
			5.0-2.0 mm.	2.0-1.0 mm.	1.0-0.5 mm.	5.0-0.5 mm.
Jozini	*Artemisia*	1	35.8	7.8	3.1	46.7
		2	35.2	6.9	4.0	46.1
		3	33.9	8.0	2.4	44.3
		4	36.2	7.8	3.5	47.5
		5	36.0	7.6	3.8	47.4
		6	33.6	8.2	3.3	45.1
		7	36.1	5.9	3.6	45.6
		8	34.6	7.2	2.9	44.7
		Mean	35.2	7.4	3.3	45.9
		S.E	1.03	0.75	0.52	1.21
		C.V (%)	2.9	10.1	15.8	2.6
	control	1	1.9	2.4	4.0	8.3
		2	1.4	2.8	4.7	8.9
		3	2.1	2.5	4.4	9.0
		4	1.9	2.8	3.2	7.9
		5	1.6	2.6	4.2	8.4
		6	2.5	3.0	3.7	9.2
		7	2.0	2.4	4.3	8.7
		8	1.6	2.4	4.6	8.6
		Mean	1.9	2.6	4.1	8.6
		S.E	0.35	0.23	0.50	0.42
		C.V (%)	18.4	8.8	12.2	4.9
Estcourt	*Artemisia*	1	46.2	10.8	3.8	60.8
		2	45.5	10.7	3.7	59.9
		3	44.5	11.2	3.7	59.4
		4	46.0	11.8	3.6	61.4
		5	47.2	11.3	3.3	61.8
		6	45.8	9.5	3.5	58.8
		7	49.0	11.0	3.3	63.3
		8	45.7	9.3	3.3	58.3
		Mean	46.2	10.7	3.5	60.5
		S.E	1.34	0.87	0.21	1.68
		C.V (%)	2.9	8.1	6.0	2.78
	control	1	29.5	6.9	3.1	39.5
		2	29.8	7.4	3.4	40.6
		3	28.5	7.7	3.2	39.4
		4	29.8	6.7	2.6	39.1
		5	31.6	6.1	2.5	40.2
		6	33.0	5.2	2.7	40.9
		7	30.0	7.0	3.0	40.0
		8	33.2	5.7	2.6	41.5
		Mean	30.7	6.6	2.9	40.2
		S.E	1.72	0.86	0.33	0.82
		C.V (%)	5.6	13.0	11.4	2.0

Electrical conductivity of water used= 12.7mSm^{-1} (at 25°C)
pH of water used= 7.0 (at 16°C)

APPENDIX B2

Coefficient of variation and standard error results for dry bulk density by the core method.

SOIL FORM	TREATMENT	SOIL REPLICATES	DRY BULK DENSITY (g/cm³)	
			Depths (cm)	
			0-15	15-30
Jozini	*Artemisia*	1	1.47	1.46
		2	1.44	1.42
		3	1.47	1.44
		4	1.49	1.47
		5	1.50	1.48
		6	1.46	1.45
		Mean	1.47	1.45
		S.E	0.02	0.02
		C.V (%)	1.5	1.5
	control	1	1.45	1.44
		2	1.44	1.42
		3	1.48	1.47
		4	1.47	1.45
		5	1.47	1.45
		6	1.46	1.44
		Mean	1.46	1.45
		S.E	0.01	0.02
		C.V (%)	1.0	1.1
Estcourt	*Artemisia*	1	1.71	1.69
		2	1.67	1.64
		3	1.70	1.70
		4	1.66	1.65
		5	1.67	1.67
		6	1.69	1.70
		Mean	1.68	1.68
		S.E	0.02	0.03
		C.V (%)	1.2	1.6
	control	1	1.64	1.62
		2	1.66	1.65
		3	1.59	1.58
		4	1.62	1.62
		5	1.65	1.63
		6	1.62	1.58
		Mean	1.63	1.61
		S.E	0.03	0.03
		C.V (%)	1.6	1.7

Coefficient of variation and standard error results for loss on ignition of two soil Forms.

SOIL FORM	TREATMENT	SOIL REPLICATES	LOSS ON IGNITION (%)
Jozini	*Artemisia*	1	2.2
		2	2.2
		3	2.4
		4	2.5
		5	2.2
		6	2.4
		7	2.6
		8	2.3
		Mean	2.4
		S.E	0.15
		C.V (%)	6.4
	control	1	1.8
		2	2.0
		3	2.3
		4	2.0
		5	2.1
		6	2.2
		7	2.4
		8	1.9
		Mean	2.1
		S.E	0.20
		C.V (%)	9.7
Estcourt	*Artemisia*	1	3.0
		2	2.4
		3	3.0
		4	2.5
		5	2.8
		6	3.0
		7	3.2
		8	2.9
		Mean	2.9
		S.E	0.27
		C.V (%)	9.6
	control	1	2.4
		2	2.2
		3	1.9
		4	1.9
		5	2.3
		6	2.2
		7	1.8
		8	1.9
		Mean	2.1
		S.E	0.23
		C.V (%)	10.9

Coefficient of variation and standard error results for organic carbon content of two soil Forms.

SOIL FORM	TREATMENT	SOIL REPLICATES	ORGANIC CARBON (%)
Jozini	*Artemisia*	1	0.62
		2	0.68
		3	0.65
		4	0.62
		5	0.60
		6	0.60
		7	0.58
		8	0.59
		Mean	0.60
		S.E	0.03
		C.V (%)	5.4
	control	1	0.42
		2	0.39
		3	0.40
		4	0.39
		5	0.42
		6	0.41
		7	0.41
		8	0.45
		Mean	0.40
		S.E	0.02
		C.V (%)	4.8
Estcourt	*Artemisia*	1	0.73
		2	0.71
		3	0.68
		4	0.69
		5	0.61
		6	0.77
		7	0.91
		8	0.91
		Mean	0.80
		S.E	0.11
		C.V (%)	14.4
	control	1	0.45
		2	0.45
		3	0.45
		4	0.44
		5	0.46
		6	0.42
		7	0.42
		8	0.45
		Mean	0.40
		S.E	0.01
		C.V (%)	3.4

APPENDIX B5

Coefficient of variation and standard error results for extractable phosphorus content of two soil Forms.

SOIL FORM	TREATMENT	SOIL REPLICATES	EXTRACTABLE PHOSPHORUS (ppm)
Jozini	*Artemisia*	1	16.8
		2	23.6
		3	28.8
		4	22.8
		5	30.4
		6	21.2
		7	23.6
		8	17.6
		Mean	23.1
		S.E	4.78
		C.V (%)	20.7
	control	1	32.0
		2	25.2
		3	28.0
		4	27.2
		5	28.0
		6	28.0
		7	28.0
		8	20.4
		Mean	27.1
		S.E	3.29
		C.V (%)	12.1
Estcourt	*Artemisia*	1	30.8
		2	34.0
		3	44.4
		4	29.2
		5	42.4
		6	36.8
		7	50.8
		8	35.6
		Mean	38.0
		S.E	7.33
		C.V (%)	19.3
	control	1	17.6
		2	21.6
		3	30.4
		4	27.2
		5	30.4
		6	22.4
		7	19.2
		8	25.2
		Mean	24.3
		S.E	4.86
		C.V (%)	20.0

Coefficient of variation and standard error results for the pH of two soil Forms.

SOIL FORM	TREATMENT	SOIL REPLICATES	pH		
			Depths (cm)		
			0-15	15-30	30-45
Jozini	*Artemisia*	1	5.7	5.9	6.0
		2	5.7	5.7	6.0
		3	5.7	5.9	5.9
		4	5.6	5.7	6.0
		5	5.7	5.8	6.0
		6	5.7	5.8	6.0
		7	5.8	5.8	5.9
		8	5.7	5.8	5.9
		Mean	5.7	5.8	6.0
		S.E	0.05	0.08	0.05
		C.V (%)	0.9	1.3	0.9
	control	1	5.6	5.8	6.1
		2	5.8	5.9	5.9
		3	5.7	5.8	5.9
		4	5.7	5.8	5.9
		5	5.9	5.9	6.0
		6	5.6	5.7	6.1
		7	5.8	6.0	6.0
		8	5.9	5.9	6.0
		Mean	5.8	5.9	6.0
		S.E	0.12	0.09	0.08
		C.V (%)	2.1	1.6	1.4
Estcourt	*Artemisia*	1	5.8	6.0	6.0
		2	5.7	5.7	5.8
		3	5.6	5.6	5.7
		4	5.6	5.7	5.9
		5	5.6	5.8	5.8
		6	5.7	5.7	5.9
		7	5.7	5.7	5.8
		8	5.5	5.6	5.6
		Mean	5.7	5.7	5.8
		S.E	0.09	0.13	0.12
		C.V (%)	1.6	2.2	2.1
	control	1	5.9	5.9	6.0
		2	5.8	5.9	6.0
		3	5.7	5.8	5.9
		4	5.7	5.7	5.8
		5	5.8	5.8	5.9
		6	5.7	5.8	5.9
		7	5.8	6.0	6.0
		8	5.7	5.8	5.9
		Mean	5.8	5.8	5.9
		S.E	0.07	0.09	0.07
		C.V (%)	1.3	1.6	1.2

APPENDIX B7

Structural stability of soil aggregates for two soil Forms as affected by *Artemisia afra* (wet sieving technique. 5.0-2.0mm sieve size only).

ANALYSIS OF VARIANCE TABLE FOR JOZINI

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	7	2.93000	0.41857	0.55	0.7752
Treat	1	4435.56	4435.56	5836.26	0.0000
Error	7	5.32000	0.76000		
Total	15	4443.81			

Grand Mean = 18.525 Standard Error = 0.2179

Coefficient of Variation = 1.4% LSD(0.05) = 1.0

ANALYSIS OF VARIANCE TABLE FOR ESTCOURT

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	7	18.0244	2.57491	1.17	0.4194
Treat	1	968.766	968.766	441.23	0.0000
Error	7	15.3694	2.19563		
Total	15	1002.16			

Grand Mean = 38.456 Standard Error = 0.3704

Coefficient of Variation = 1.0% LSD(0.05) = 1.8

APPENDIX B8

Dry bulk density of two soil Forms as affected by *Artemisia afra* (0-15cm only).

ANALYSIS OF VARIANCE TABLE FOR JOZINI

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	5	0.00277	0.00055	4.61	0.0594
Treat	1	0.00030	0.00030	2.50	0.1747
Error	5	0.00060	0.00012		
Total	11	0.00367			

Grand Mean = 1.4667 Standard Error = 0.0032

Coefficient of Variation = 0.22% LSD(0.05) = 0.0163

ANALYSIS OF VARIANCE TABLE FOR ESTCOURT

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	5	0.00167	0.00033	0.48	0.7797
Treat	1	0.00853	0.00853	12.31	0.0171
Error	5	0.00347	0.00069		
Total	11	0.01367			

Grand Mean = 1.6567 Standard Error = 0.0076

Coefficient of Variation = 0.46% LSD(0.05) = 0.0391

APPENDIX B9

pH of two soil Forms as affected by *Artemisia afra* (0-15cm only).

ANALYSIS OF VARIANCE TABLE FOR JOZINI

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	7	0.07000	0.01000	1.40	0.3341
Treat	1	0.01000	0.01000	1.40	0.2753
Error	7	0.05000	0.00714		
Total	15	0.13000			

Grand Mean = 5.7250 Standard Error = 0.0211

Coefficient of Variation = 0.37% LSD(0.05) = 0.0999

ANALYSIS OF VARIANCE TABLE FOR ESTCOURT

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	7	0.08438	0.01205	5.87	0.0163
Treat	1	0.05063	0.05063	24.65	0.0016
Error	7	0.01437	0.00205		
Total	15	0.14938			

Grand Mean = 5.7063 Standard Error = 0.0113

Coefficient of Variation = 0.20% LSD(0.05) = 0.0536

APPENDIX B10

Organic matter content of two soil Forms as affected by *Artemisia afra* (0-15cm only).

ANALYSIS OF VARIANCE TABLE FOR JOZINI

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	7	0.00677	0.00097	0.27	0.9453
Treat	1	0.50410	0.50410	142.86	0.0000
Error	7	0.02470	0.00353		
Total	15	0.53558			

Grand Mean = 0.8862 Standard Error = 0.0149

Coefficient of Variation = 1.68% LSD(0.05) = 0.0702

ANALYSIS OF VARIANCE TABLE FOR ESTCOURT

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	7	0.10684	0.01526	0.74	0.6506
Treat	1	1.12891	1.12891	54.60	0.0002
Error	7	0.14474	0.02068		
Total	15	1.38049			

Grand Mean = 1.0294 Standard Error = 0.0359

Coefficient of Variation = 3.49% LSD(0.05) = 0.1700

APPENDIX B11

Extractable phosphorus content of two soil Forms as affected by *A. afra* (0-15cm only).

ANALYSIS OF VARIANCE TABLE FOR JOZINI

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	7	133.280	19.0400	1.30	0.3684
Treat	1	64.0000	64.0000	4.38	0.0748
Error	7	102.400	14.6286		
Total	15	299.680			

Grand Mean = 25.100 Standard Error = 0.9562

Coefficient of Variation = 3.81% LSD(0.05) = 4.5220

ANALYSIS OF VARIANCE TABLE FOR ESTCOURT

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	7	305.270	43.6100	1.29	0.3730
Treat	1	756.250	756.250	22.36	0.0021
Error	7	236.790	33.8271		
Total	15	1298.31			

Grand Mean = 31.125 Standard Error = 1.4540

Coefficient of Variation = 4.67% LSD(0.05) = 6.8765

APPENDIX B12

Loss on ignition of two soil Forms as affected by *Artemisia afra* (0-15cm only).

ANALYSIS OF VARIANCE TABLE FOR JOZINI

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	7	0.36938	0.05277	4.65	0.0300
Treat	1	0.27562	0.27562	24.31	0.0017
Error	7	0.07937	0.01134		
Total	15	0.72437			

Grand Mean = 2.2187 Standard Error = 0.0266

Coefficient of Variation = 1.20% LSD(0.05) = 0.1259

ANALYSIS OF VARIANCE TABLE FOR ESTCOURT

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	7	0.36750	0.05250	0.72	0.6595
Treat	1	2.40250	2.40250	33.14	0.0007
Error	7	0.50750	0.07250		
Total	15	3.27750			

Grand Mean = 2.4625 Standard Error = 0.0673

Coefficient of Variation = 2.73% LSD(0.05) = 0.3183

APPENDIX C1

Effect of *A. afra* leaf extract at various dilutions on the germination of maize seeds at 25 DAS

ANALYSIS OF VARIANCE TABLE

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	3	145.833	48.611	0.62	0.6138
Treat	5	8870.83	1774.17	22.57	0.0000
Error	15	1179.17	78.611		
Total	23	10195.8			

Grand Mean = 67.917 Standard Error = 1.810

Coefficient of Variation = 2.67% LSD(0.05) = 13.36

APPENDIX C2

Effect of extracts from soil planted to *A. afra* at various dilutions and soil from an adjoining crop land (control) on the germination of maize seeds at 25 DAS

ANALYSIS OF VARIANCE TABLE

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	3	525.000	175.000	3.71	0.0309
Treat	6	1892.86	315.476	6.68	0.0008
Error	18	850.000	47.222		
Total	27	3267.86			

Grand Mean = 86.071 Standard Error = 1.299

Coefficient of Variation = 1.51% LSD(0.05) = 10.21

APPENDIX C3

Effect of *A. afra* leaf extract at various dilutions on the germination of bean seeds at 25 DAS

ANALYSIS OF VARIANCE TABLE

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	3	83.333	27.778	0.74	0.5471
Treat	5	11800.0	2360.00	62.47	0.0000
Error	15	566.667	37.778		
Total	23	12450.0			

Grand Mean = 67.500 Standard Error = 1.255

Coefficient of Variation = 1.86% LSD(0.05) = 9.264

APPENDIX C4

Effect of extracts from soil planted to *A. afra* at various dilutions and soil from an adjoining crop land (control) on the germination of bean seeds at 25 DAS

ANALYSIS OF VARIANCE TABLE

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	3	210.714	70.238	0.76	0.5313
Treat	6	9135.71	1522.62	16.47	0.0000
Error	18	1664.29	92.460		
Total	27	11010.7			

Grand Mean = 78.214 Standard Error = 1.817

Coefficient of Variation = 2.32% LSD(0.05) = 14.29

APPENDIX C5

Effect of *A. afra* leaf extract at various dilutions on the germination of seeds of rye grass at 25 DAS

ANALYSIS OF VARIANCE TABLE

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	3	13.8333	4.61111	0.20	0.8937
Treat	5	14257.5	2851.50	124.64	0.0000
Error	15	343.167	22.8778		
Total	23	14614.5			

Grand Mean = 66.750 Standard Error = 0.9763

Coefficient of Variation = 1.46% LSD(0.05) = 7.21

APPENDIX C6

Effect of extracts from soil planted to *A. afra* at various dilutions and soil from an adjoining crop land (control) on the germination of seeds of rye grass at 25 DAS

ANALYSIS OF VARIANCE TABLE

Source	Degrees of Freedom	Sum of Squares	Mean Square	F-value	Prob
Rep	3	111.429	37.1429	1.66	0.2109
Treat	6	6263.71	1043.95	46.68	0.0000
Error	18	402.571	22.3651		
Total	27	6777.71			

Grand Mean = 74.714 Standard Error = 0.8937

Coefficient of Variation = 1.20% LSD(0.05) = 7.03