A BIOLOGICAL CONTROL APPROACH OF *ARMILLARIA* ROOT ROT OF TEA.

BY

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SUMMARY

Field, greenhouse and laboratory experiments were conducted between June 1987 and September 1991 to investigate: (i) effects of soils amended with locally available organic agricultural wastes (ii) effects of sub-lethal doses of basamid (iii) effects of commonly used inorganic fertilizers in tea (iv) effects of age and surface area of coffee pulp (v) effects of soil moisture in coffee pulp amended soil on the proliferation of *Trichoderma* spp. in Kenya tea soils (vi) antibiotic activities of *Trichoderma* spp. in Kenya tea soils on *Armillaria mellea* and (vii) the potential of coffee pulp in the management of *Armillaria* root rot of tea.

The organic amendments, saw dust, coffee pulp and husks in a 10:1 ratio, filter press cake, cow manure, sheep manure and goat manure in this study influenced the population dynamics of soil microorganisms differently both in the greenhouse and field. Coffee pulp and husks significantly (P=0.05) enhanced *Trichoderma* proliferation more than the other organic amendments. The enhanced proliferation of *Trichoderma* spp. (*T. koningii, T. harzianum and T. longibrachiatum*) by coffee pulp was rate dependant with 16 and 22 grams carbon giving the highest populations without significant (P=0.01) differences between them. The peak *Trichoderma* proliferation was on the fifth month after treatment application, implying that for sustenance of high *Trichoderma* populations in the soil for biological control of *Armillaria* root rot of tea, a second or even third application would be necessary. The coffee pulp had the added advantage of significantly (P=0.05) enhancing high

proliferation of non-*Trichoderma* fungi and bacteria, which in turn could make synergistic antagonism against *A. mellea* possible.

The coffee pulp and husks in a 10:1 ratio was as effective as basamid in the management of *Armillaria* root rot of tea in the field. Unlike basamid, coffee pulp significantly (P=0.05) enhanced tea yields.

The usage of basamid at different rates, some of them sub-lethal to *Trichoderma* and inorganic fertilizers NPKS 25:5:5:5, NPK 20:10:10, S/A and DAP at 150 kg N/ha was significantly inferior to coffee pulp in the enhancement of microbial proliferation including that of *Trichoderma* spp. Possible contributory factors to the superior enhancement effect of the coffee pulp to microbial proliferation include adequate food reservoir and optimal soil pH for microbial activities.

Fresh and one-month-old coffee pulp and husks enhanced significantly (P=0.05) more microbial proliferation including that of *Trichoderma* spp. more than the older pulps. The population proliferation with age was quadratic and dependant on carbon content.

Increase in coffee pulp and husks surface area, improved the material's ability to enhance *Trichoderma* proliferation and that of other microorganisms only for a short duration. This implies that in order to sustain high *Trichoderma* populations, more frequent applications of the ground coffee pulp and husks would be required than when the normal coffee pulp is used.

Dry soils at 15 to 25% moisture content significantly hampered *Trichoderma* proliferation. Wet soils at 35 to 50% moisture content significantly (P=0.05) enhanced *Trichoderma* spp. proliferation. The better survival of *Trichoderma* spp. in very wet coffee pulp amended soils, indicated their tolerance to anaerobiosis. The results suggested therefore that for maximum biocontrol benefit of coffee pulp due to proliferation of *Trichoderma*, application should be restricted to very wet months of the year.

Metabolites of *Trichoderma* isolates significantly (P=0.05) inhibited mycelial and rhizomorphal growth of *A. mellea*. *T. koningii* (isolate 9), *T. longibrachiatum* (isolate 3) and *T. harzianum* (isolate 4) had the highest antibiotic activities against *A. mellea*. A mixture of metabolites of the 9 isolates did not exhibit synergistic effects.

CHAPTER ONE

1.0 Introduction

The tea plant, *Camellia sinensis* (L.) O. Kuntze is an important crop in the agriculturally based economy of Kenya. It ranks second to coffee, *Coffeae arabica* L. as an earner of foreign exchange and provides a livelihood for more than a million people (Anon, 1987). Genotype (Hadfield, 1966; Magambo and Omolo, 1982; Nyirenda and Ridpath, 1984; Stuart, 1930), environmental factors (Barua and Das, 1979; Carr, 1977; Fordham and Palmer-Jones, 1977; Herd and Squire, 1976; Laycock, 1969; Squire, 1978; and Tanton, 1979, 1981, 1982[a], 1982[b]), husbandry practices (Mwakha and Anyuka, 1982; Barua, 1961; Othieno, 1980 and Templer, 1978), hormonal physiology (Barua, 1969; Kulasegaram, 1969 and 1971; Kulasegaram and Kathiravetpillai, 1972 and 1974), diseases and pests (Carr, 1972; Mouli *et al*, 1986; Venkata Ram, 1970; Sudoi, 1984; Arulpragasam *et al*, 1987; Ando *et al*, 1986; Ando *et al*, 1987), influence the performance of tea. Among diseases of tea, *Armillaria* root rot caused by *A. mellea* Vahl ex. Fr. is the most prevalent in Kenya (Onsando, 1986[a]). The disease is anticipated to increase in some of the Nyayo Tea Zones which have fields along the forest belt where inoculum of *A. mellea* is high.

Management strategies against *Armillaria* root rot include, avoidance of sites with a high disease incidence when establishing new plantations (Gibson, 1975), use of soil fumigants like Carbon disulphide, Methyl bromide, Shell D.D. and Metham sodium (Venkata Ram and Devasahayam Joseph, 1974), removal of all substrate sources

from the soil by uprooting and destroying all root remnants (Horne, 1974; Sokolov, 1964), ring barking of forest trees for a period of 1 to 2 years to reduce starch reserves in the roots (Gibson and Goodchild, 1960[b]; Goodchild, 1959; Leach, 1939), use of host resistance is another possible strategy (Morquer and Touvet, 1972; Gibson, 1960; Armitage and Barnes, 1968; Risbeth, 1972[b]) and cultural practices such as adequate land preparation and sanitation (Anon, 1986).

Avoidance of sites with high incidence of *Armillaria* root rot is not practical in Kenya due to high pressure on land. Because of this, forest areas where the inoculum is high are being utilized. Use of soil fumigants is hazardous to the environment and is expensive. Use of conventional fungicides in Kenya has also been unsuccessful (Templer and Machaga, 1974). Removal and distribution of substrate sources is machinery and labour intensive and hence very expensive. Ring barking requires experienced personnel, who can avoid making either too shallow or too deep cuts. Shallow cuts permit bark regeneration while deep cuts result into rapid death of the trees (Leach, 1939; Redfern, 1968). Identification of good resistant genotypes has not been successful (Armitage and Barnes, 1968; Morquer and Touvet, 1972; Gibson, 1960[a]). Adequate land preparation which has the element of uprooting alternate hosts and removal of all roots that could act as substrate is expensive.

In view of the constraints of the existing management methods, there is need to develop alternative control strategies which are environmentally sound and economically sustainable. Considering the non-polluting nature of biological control, management of

Armillaria root rot using biological control agents would be an ideal alternative worthy exploring. Organisms with antagonistic activities against *A. mellea* include *Trichoderma viride* (Bliss, 1951; Anon, 1974; Ohr, 1973; Munnecke, *et al* 1973). However, antagonistic activities of these organisms have not been utilized in the management of Armillaria root rot. This is because saprophytic soil microorganisms with putative biocontrol activity, if introduced into the soil, would not establish or maintain populations any higher than those occurring naturally (Garrett, 1965; Risbeth, 1976; Cook, 1990). In order for *Trichoderma* to be commercially useful, ways of encouraging its population build-up need to be sought. This, therefore, calls for manipulation of soil conditions to proliferate existing antagonists to *Armillaria*. In light of this, this study was undertaken to investigate:

(i) Effects of soils amended with organic agricultural wastes and sub-lethal doses of basamid on the proliferation of *Trichoderma* spp.

(ii) Effects of commonly used inorganic fertilizers in tea on the proliferation of *Trichoderma* spp.

(iii) Effects of age and surface area of coffee pulp on proliferation of *Trichoderma* spp.

(iv) Effects of soil moisture on the proliferation of *Trichoderma* spp. in coffee pulp amended soils.

(v) Antibiotic activities of *Trichoderma* spp. in Kenya tea soils on *Armillaria mellea*.

(vi) The potential of the organic amendment with the highest potency to proliferate *Trichoderma* spp. in field management of Armillaria root rot of tea.

CHAPTER TWO

LITERATURE REVIEW

1.1. *Armillaria* root rot

Armillaria root rot is caused by the fungus, *Armillaria mellea* Vahl ex. Fr. It is an important disease on many tree species and fruit trees throughout the world (Patton and Riker, 1959; Day, 1929; Swift, 1968; Wargo and Shaw III, 1985; Vaartaja, 1968; Bliss, 1951; Marsh, 1952; Risbeth, 1968; Wilbur *et al*, 1971). The disease is a major tea production constraint in many tea growing areas including Kenya (Leach, 1939; Gibson and Goodchild, 1960[b]; Hulburt, 1957; Venkata Ram and Devasahayam Joseph, 1974; Onsando, 1986[a]). In plantations the distribution of *Armillaria* root rot is patchy (Aggregated) and the pattern of spread is predominantly radial, emanating from the source of infection (Kable, 1974; Ono, 1970; Swift, 1972). Stress and loss of vigour of the host plant is an important predisposing factor to infection (Wargo and Houston, 1974; Wargo *et al*, 1972; Batra, 1974). Cases of infection of non-stressed trees are, however, known (Patton and Riker, 1959).

A tea bush infected with this fungus is usually yellow and shorter than adjacent healthy bushes (Plate 1). Because similar symptoms are associated with other tea root diseases (Onsando, unpublished), the diagnostic symptoms and signs of the disease are cracking of the bark at the collar region of a tea plant (Plate 2),

a sheet of creamy, white mycelia (plate 3) often with fan-like striation beneath the cracked and dry bark at the collar region or on roots (Fox, 1990). Because of the cracking of the bark at the collar region, *Armillaria* root rot is often referred to as "Root splitting disease" (Anon, 1974; Venkata Ram and Devasahayam Joseph, 1974). At high elevations (over 2,000m above sea level, a.s.l), dark brown to black rhizomorphs develop (Plate 4). The typical honey coloured mushrooms of *A. mellea* rarely grow from the base of infected trees in Kenya (Gibson, 1960[a]).

1.2. *Armillaria mellea*

Armillaria mellea Vahl.ex.Fr. belongs to the class of Basidiomycetes and in the sub-class Homobasidiomycetes due to lack of cross walls on the basadium (Webster, 1980). Because the basidia of *A. mellea* are borne on a well defined hymenium, it is placed in the series Hymenomycetes and the order Agaricales (Webster, 1980). This order includes the fungi whose fruiting bodies are commonly called mushrooms or toadstools (Pegler, 1977). The fungus is a complex of morphologically distinct species but closely related (Anderson and Ellrich, 1979). The taxon can be divided into genetically isolated distinct biological species. At least ten species have been determined and each one possesses bifactorial heterothallism (Anderson and Ullrich, 1979). Different clones of *A. mellea* have been reported in a ponderosa pine forest in South Central Washington (Adams, 1974; Shaw III and Roth, 1976). Clones of *A. mellea* collected from areas remote from each other have shown



Plate 1: *Armillaria* infected tea bush showing yellowing and stunted growth relative to the surrounding bushes



Plate 2: *Armillaria* infected tea bush at advanced stages showing diagnostic cracking of the bark at the collar region.



Plate 3: *Armillaria* infected tea bush showing diagnostic sheet of creamy white mycelia beneath the cracked dry bark



Plate 4: *Armillaria* infected tea bush showing dark brown to black rhizomorphs.

remarkable differences in virulence and disease epidemiology (Wilbur *et al*, 1971).

Armillaria mellea, though a destructive pathogen is known to have a low rate of spread (Shaw III, 1976). The fungus spreads by means of rhizomorphs and/or mycelia by way of root contact (Pearce and Malasczuk, 1990; Worall *et el*, 1986; Kile, 1981; Pearce *et al*, 1986). The rhizomorphs are responsible for underground spread of the fungus (Leach, 1937; Redfern, 1973; Worrall, 1986; Morrison, 1982), but when absent or rare mycelial transfer at root contact is the main means of spread (Swift, 1968; Risbeth, 1986; Kile 1981; Pearce *et al* 1986; Podger *et al* 1978; Shearer and Tippett, 1988). The role of spores in the dissemination of *A. mellea* is not certain (Swift, 1968; Swift, 1972; Risbeth, 1964 and 1971).

Rhizomorphs which are commonly known as boot laces (Anon, 1986), are highly differentiated aggregations of hyphae with a well developed apical meristem (Motta, 1969). They grow under the bark, along the surface of infected roots and into the soil (Anon, 1986; Garrett, 1956[a]; Worrall *et el*, 1986; Pearce and Malasczuk, 1990). Both the subterranean and sub-cortical types of rhizomorphs occur at high elevations (over 2,000m a.s.l) in Kenya (Gibson, 1960[a]). This is because temperatures above 26°C do not favour rhizomorph development (Risbeth, 1986; Risbeth, 1978). Besides temperature, factors such as moisture, aeration, pH, soil depth, soil organic content and soil chemical composition influence activities of rhizomorphs of *Armillaria* spp. (Swift, 1968; Redfern 1973; Morrison, 1974, 1976 and 1982).

In forest plantations, stumps and roots of felled trees provide a good food-base for the fungus and hence a good source of inoculum (Hubert, 1950; Risbeth, 1972[a]; Risbeth, 1979; Morrison, 1972; Shaw III, 1975). The fungus can survive in woody debris for decades (Redfern, 1971) and in dormant state deep in the soils (Munnecke, *et al*, 1970). This saprophytic nature of *A. mellea* make reduction of inoculum by fallowing difficult, particularly if fallowing cannot be practiced for many years. Infection by *A. mellea* is related to the quantity and quality of inoculum (Garrett, 1956[b]; Risbeth, 1972[b]). Infected young trees die much faster, but older ones tend to remain chronically infected (Hintikka, 1974; Shaw III and Roth, 1976).

1.3. Control and management

Control and management of *Armillaria* root rot are made difficult by the complexity of the soil environment and economic constraints (Shaw III and Roth, 1978; Wargo and Shaw III, 1985). However, avoidance of sites with a high disease incidence, when establishing new plantations has been suggested (Gibson, 1975). Cultural practices such as digging a trench around disease foci to block further underground spread of *Armillaria* have been recommended in forestry and horticultural industries (Horne, 1974; Roth *et al* 1977; Sokolov, 1964; Shaw III and Roth, 1974; Bliss, 1951). Removal of substrates from the soil by removing diseased trees, uprooting and destroying stumps and root remnants has also been recommended (Horne, 1974; Roth *et al*, 1977; Sokolov, 1964). Starch exhaustion in the roots of *Armillaria* alternate host plants

before planting tea, either by leaving the stumps *in situ* for a long time (40 years) or ring barking the plants for a period of 1 to 2 years prior to uprooting is a preventive means of managing *Armillaria* (Leach, 1939; Goodchild, 1959; Gibson and Goodchild, 1960[b]; Risbeth, 1972[a]). But ring barking of alternate tree hosts is very often done wrongly (Leach, 1939) and does not work all the time (Redfern 1968). Identification of good resistant genotypes has been unsuccessfully tried (Morquer and Touvet, 1972; Gibson, 1960; Armitage and Barnes, 1968; Risbeth, 1972[b]). Armillatox, a phenolic emulsion containing 48% active ingredient is marketed for specific use against *Armillaria* (Bray, 1970; Pawsey and Rahman, 1974 and 1976). Trials in Kenya and Tanzania with Armillatox were, however, unsuccessful (Templer and Machaga, 1974). Maneb, Sodium Pentachlorophenate (NaPcP), Methyl bromide, Vortex, Chloropicrin, Carbon disulphide and Vapam have been used for the control of *Armillaria* root rot (Pawsey and Rahman, 1976; Rykowski, 1974; Filip and Roth, 1977). The efficacy of Carbon disulphide on *Armillaria* of citrus has been attributed to the enhancement of colonization of the root rhizosphere and rhizoplane by *Trichoderma*, a fungus whose possible antagonistic activity against *A. mellea* has been indirectly implied (Bliss, 1951). A toxin from savanna soils in Rhodesia that inhibits growth of *Armillaria* rhizomorphs has been isolated (Boughey *et al*, 1964 and swift, 1970). Olembo (1972) reported a heat-liable soil factor within *Armillaria*-free sites in East Africa that inhibited growth of *Armillaria*.

Chemical management of *Armillaria* root rot has not been very successful due to the fact that the disease is usually confined to

small numbers of trees in each plantation (Johnson and Thomas, 1975). The small patches with the fungal inoculum within a plantation are usually too small for elaborate evaluation of chemicals against the disease. This is further compounded by the adverse effects of chemicals to the environment and the applicator (Crossland and Stephenson, 1988; Chet, 1990).

Biological control is an attractive management strategy of *Armillaria* root rot (Risbeth, 1976). It is the action of parasites, predators or pathogens in maintaining another organism's population density at a lower level than would occur in their absence (De Bach, 1964). In forest trees, two mycophagous nematodes greatly reduced mortality of *Pinus ponderosa* Laws. (Rifle, 1973). Peno *et al* (1975) noted differences in the populations of antagonistic organisms to *Armillaria* in the rhizosphere of health and *Armillaria* infected *P. strobus* L. Studies *in vitro* have shown that Mycorrhizal fungi can inhibit development of *Armillaria* (Eghbaltalab *et al*, 1975). Leach, (1937) noted that *Rhizoctonia lamellifera* Small prevented *Armillaria* from colonizing tea roots. *Trichoderma viride* Pers. ex. S.C. Gray has been implicated although without direct evidence to be a possible contributor to reduction of *Armillaria* on tea (Anon, 1974), and citrus (Bliss, 1951). Although antibiosis of *Trichoderma viride* against *A. mellea* of citrus has been documented (Ohr, *et al*, 1973; Munnecke *et al*, 1973; Bliss, 1951), there is no direct work utilizing its antibiotic activity as a management strategy of *Armillaria* root rot of tea. However higher populations of *Trichoderma* spp. have been observed in fumigated soils against *Armillaria* root rot (Anon, 1974). In

order for this antibiosis of *Trichoderma* to be commercially useful, ways of encouraging its population in *Armillaria* infested soils are necessary.

In spite of the difficulty in establishing or maintaining populations of introduced saprophytic soil microorganisms with putative biocontrol activities to levels higher than those occurring naturally (Garret, 1965; Cook, 1990), successful introduction of the microparasite *Sporidesmium sclerotivorum* uecker against *sclerotina sclerotiorum* Libert de Bary has been achieved (Ayers and Adams, 1979; Adams an Ayers, 1982). Perhaps the greatest progress towards biological control of soil-borne plant pathogens in the past 25 years is with antagonists introduced with the planting material, that is biological control with plant associated microorganisms (Risbeth, 1963; Kerr, 1972; Cook, 1990).

1.4. Soil amendments and microbial activities

Organic matter added to the soil in the form of animal manures, crop residues and composts has been known to suppress soil-borne plant diseases (Chung, 1989; Baker, 1962; Latham and Watson, 1967; Huber and Watson, 1970; Burke, 1954; Williams and Hacks, 1957; Mankau, 1963; Lewis and Papavizas, 1975). For example, alfalfa meal and cotton waste controlled avocado root rot *Phytophthora cinnamomi* Rands (Zentimeyer, 1963; Gilpatrick, 1969). Recent greenhouse studies on root rots due to *Phytophthora cinnamomi* Rands have attributed biocontrol activity to hyphomycetes such as *Aspergillus flavus* Link and *Penicillium funiculosum* Thom (Tsao, 1988). Container media amended with hard wood bark compost suppressed *Rhizoctonia* damping off of radish (Chung Y.R., 1989;

Nelson and Hoitink, 1983; Nelson *et al*, 1983). Composted larch bark is used by vegetable growers in the Nagano valley of Japan for the control of Fusarium brown rot of Chinese yam (Sekiguchi, 1977). Composts prepared from tree leaves or rice hulls have been used for the management of club root of chinese cabbage (Tamura and Taketani, 1977). Ammoniated Douglas fir bark stored in piles until decomposed, provided a high degree of control of *Phytophthora fragariae* Hickman of strawberry (Hoitink and Fahy, 1986). Incorporation into soil of composted municipal sludge (CMS) controlled *Aphanomyces* root rot of pea, *Rhizoctonia* root rot of bean, cotton and radish, *Fusarium* wilt of cucumber, and *Phytophthora* crown rot of pepper (Lumsden *et al*, 1983). Compost amended media, particularly those amended with composted bark, suppress major soil-borne plant pathogens (Chet *et al*, 1983; Gugino *et al*, 1973; Hoitink *et al*, 1976; Spencer and Benson, 1981 and 1982; Stephens and Stebbins, 1985).

Agricultural organic amendments have been used to reduce the populations of plant parasitic nematodes. Examples include; Root knot nematodes (Khan *et al*, 1979; Mishra and Prassad, 1978; Sitaramaiah and Singh, 1978), citrus nematodes, *Tylenchulus semi-pentrans* cobb and *Rotylenchus reniformis* Linford and Oliveira (Badra and Mohamed 1979; Mankau, 1963), Cyst nematodes of potatoes, *Globodera rosto chiensis* (Wollen) Behrens (Van der Laan, 1956), *Helicotylenchus* spp. and *Pratylenchus* spp. (Hunt *et al*, 1973; D'Errico and Maio, 1980).

Although physical, chemical and biological interactions in the soil are so complex and varied for easy determination of specific

effects of organic amendments responsible for disease control (Huber and Watson, 1970), there is evidence implicating microbial participation (Burke, 1954; Williams and Hacks, 1957; Chung, 1989; Baker, 1962, Latham and Watson, 1967). The mechanism of biological control as enhanced by organic amendments include pathogen exposure to high temperature, toxic decomposition products, antagonism and enhanced host resistance (Alexander 1977; Baker and Cook, 1974; Bollen, 1969; Burge *et al*, 1981; Hointik and Fahy, 1986; Linderman and Gilbert, 1975).

Sub-lethal or lethal temperatures as a result of composting of organic amendments have reduced diseases caused by *Rhizoctonia solani* Kühn (Hoitink *et al*, 1976; Yuen and Raabe, 1984), *Verticillium albo-atrum* Reincke and Berthold (Staffeldt, 1959), *V. dahliae* Kleb. (Sterne *et al*, 1979; Winkler, 1972); *Phytophthora cinnamomi* Rands (Hoitink, *et al*, 1976), *P. cryptogea* Pethybridge and Lafferty (Wijnen, *et al*, 1983), *Botrytis cinera* Fr. (Hoitink, 1976; Ylimaki *et al*, 1983) *Sclerotium rolfisi* Sacc. (Yuen and Raabe, 1984), *Armillaria mellea* (Vahl. ex. Fr.) Kummer (Yuen and Raabe, 1984).

Toxic products of organic material decomposition such as ammonia, ethylene, carbon dioxide, organic acids, dimethyl sulphide and dimethyl disulphide have fungitoxic effects on soil-borne plant pathogens (Schippers and Bouman, 1973; Gilpatrick, 1969; Sonoda, 1977; Papavizas and Lewis, 1971; Smith, 1976; Walker, 1971).

An increased supply of potassium, phosphorus, nitrogen or calcium, during decomposition of organic materials improves the

nutritional status of host plants (Alexander, 1977; Nakasaki *et al*, 1985; Tisdale *et al*, 1985). Changes in the nutritional status of a plant, in turn, influence its response to pathogen invasion (Baker and Cook, 1974; Nayudu and Walker, 1960; Perrenound, 1977). For example, host resistance is boosted through formation of thick layers of potassium silicate as in cereals which act as barriers to pathogen invasion (Kirkby and Mengel, 1978; Perrenound, 1977). Adequate supplies of calcium have been shown to suppress effects of *Rhizoctonia solani* Kühn on beans due to formation of calcium pectate (Bateman, 1964; Henis and Katana, 1974).

Besides improving nutritional status of the host, the supply of nutrients enhances microbial activities and hence antagonism (Alexander, 1977; Baker and Cook, 1974; Shipton, 1977). Antagonism encompasses competition (Clark, 1968), hyperparasitism (Alexander, 1977; Mankau, 1980; Lockwood, 1977; Hunter *et al*, 1977), predation (Baker and Cook, 1974); lysis (lockwood, 1977; Lamana and Malette, 1965), antibiosis (Gottlieb and Shaw, 1970) and cross protection (Asher, 1978; Baker *et al*, 1978; Deacon, 1973; Deacon, 1976; Gutteridge and Slope, 1978; Wong and Siviour, 1979).

Use of organic amendments for biological control of soil-borne plant pathogens is mainly at greenhouse and/or experimental stage. For these materials to be of commercial significance, specific amendments for specific diseases have to be determined, and the optimal soil conditions for enhancement of specific antagonists worked out.

Inorganic soil amendments are widely used in agriculture as fertilizers and to change the physiochemical properties of the soil

(Henis and Katan, 1974). Inorganic amendments affect the interaction between soil-borne pathogens and saprophytic soil residents in plant debris and in the rhizosphere (Henis and Katan, 1974; Chapman, 1965; Davey and Danielson, 1968; Garret, 1956[a]; Garret, 1970; Sadasivan, 1965; Christensen and Brett, 1985; Huber *et al*, 1980; Taylor *et al*, 1983; Trollidenier, 1981; Engel, 1988). This subsequently affects the survival ability of some pathogens (Garrett, 1970). Addition of nitrogenous fertilizers to the soil for example, decreased the population of antibiotic producing organisms in maize field but not in wheat fields (Huber *et al*, 1968). The form of nitrogen influences disease development. For example, NH_4-N enhanced antagonism against take-all of wheat pathogen, *Gaeumannomyces graminis* var. *tritici* (Sacc) Arx and Olive (Smiley, 1977). The suppression of diseases in certain cases due to the source of N has been attributed to pH changes in the rhizosphere (Smiley and Cook, 1973). The pH changes have in turn been thought to stimulate activities of antagonists (Smiley, 1977). Unless environmental conditions are conducive (Weindling and Fawcett, 1936), inoculation of the soil with antagonists is generally ineffective as a biological control measure. Therefore the need to address the antagonists of *Armillaria* directly cannot be overemphasized.

The requirement of nitrogen by the tea plant far exceeds that of phosphorus and potassium due to the regular plucking (Beevers, 1976; Willson, 1975). In Kenya nitrogen is applied to tea in form of compound fertilizer, Nitrogen:Phosphorus:Potassium

and Sulphur (NPKS 25:5:5:5), Sulphate of Ammonia and urea (Wanyoko, 1983). The compound fertilizer avails nitrogen in both $NO3^-$ and $NH4^+$ form. Whereas the yield responses to these fertilizers are documented severally (Willson, 1967; Othieno, 1983; Wanyoko, 1983), no effort has been made to address the effect of these on the microbial ecology of tea soils with emphasis to microbial antagonists that could emerge and help in management of *Armillaria*. Since no yield advantage has been attributed to any of the types of fertilizers (Wanyoko, 1988), the probable microbial benefits should be addressed.

1.5. *Trichoderma* spp. and biological control

The genus *Trichoderma* was first described by Persoon in 1794 (Rifai, 1969). It is recognized as one of the commonest genera of fungi in the majority of soils (Aytoun, 1952; Danielson and Davey, 1973). A number of *Trichoderma* species have been associated with mycoparasitism of a wide range of soil-borne plant pathogens. Examples include, *Rhizoctonia solani* Kühn (Elad, *et al*, 1980; Elad, *et al*, 1981; Hadar, *et al*, 1979; Lewis and Papavizas, 1987), *Sclerotinia sclerotirum* (Libert) de Bary (Trufmann and Keane, 1990), *Fusarium oxysporum* Jarvis and Shoemaker (Sivan *et al*, 1987). Suppressiveness in soils to soil-borne plant pathogens has been in many cases, attributed to some *Trichoderma* species (Henis, *et al*, 1979; Chet and Baker, 1981, Liu and Baker, 1980). *Trichoderma harzianum* Rifai retard sporulation of *Cochliobolus sativus* (Ito Kurib.) Dreschsl ex. Dastur as well as parasitize it (Biles and Hill, 1988). *Trichoderma* harzanium 85 and *T. viride* I, reduced

fruit rots of strawberries to the level achieved by a fungicide dichlofluanid (Transomo and Dennis, 1977).

Introduction of *Trichoderma* with sublethal doses of fumigants such as Pentachloronitrobenzane (PCNB) and Methyl bromide, has shown better results than use of *Trichoderma* alone for management of soil-borne plant pathogens (Curt, *et al*, 1977; Hader, et al, 1979; Strashnov, *et al*, 1985; Elad, *et al*, 1982[a]). This has been attributed to the higher tolerance of *Trichoderma* to sub-lethal doses of a fumigant, which kills most of the other soil fungi and bacteria leaving *Trichoderma* to flourish with minimal competition (Munnecke, *et al*, 1973; Ohr, 1973).

Many isolates of *Trichoderma* have been shown to produce non-volatile and volatile antibiotics active against a wide range of fungi (Dennis and Webster, 1971[a], 1971[b]). Specific *Trichoderma* spp. like *T. viride* Pers. ex. S.F. Gray produces mycolytic enzymes consisting mainly of ß-1,3-glucanase and chitinase at optimum pH and temperature (Nobuo and Ogawa, 1968; Rodriguez-kabana, *et al*, 1978). *Trichoderma harzanium* Rifai, also excretes ß-1,3-glucanase and chitinase when grown on cell walls of *Sclerotium rolfisii* Sacc. as a sole carbon source (Elad, *et al*, 1982[b]). Direct parasitism by way of coiling of *Trichoderma hamatum* (Bon.) Bain on *Rhizictonia solani* Kühn and *Pythium* spp. resulting into lysis of the pathogen has been observed (Chet, *et al*, 1981).

While the potential of *Trichoderma* spp. as biological control agents of soil-borne plant pathogens is phenomenal, control of *Armillaria* by introducing biocontrol organisms is difficult (Risbeth, 1976). This is attributed to the positional advantage of

Armillaria over the introduced biocontrol agents and the difficulty in maintaining effective populations of antagonistic organisms (Risbeth, 1976). Attempts to maintain effective populations of antagonistic organisms is therefore important.

CHAPTER THREE

MATERIALS AND METHODS

2.0. Introduction

Field, greenhouse and laboratory experiments were conducted between June 1987 and September 1991 to investigate: (i) effects of soils amended with organic agricultural wastes and sub-lethal doses of basamid on proliferation of *Trichoderma* spp. (ii) effects of commonly used inorganic fertilizers in tea on the proliferation of *Trichoderma* spp. (iii) effects of age and surface area of coffee pulp on proliferation of *Trichoderma* spp. (iv) effects of soil moisture on the proliferation of *Trichoderma* spp. in organic material-amended soils (v) antibiotic properties of *Trichoderma* spp. in soils in Kenya tea fields on *Armillaria mellea* and (vi) the potential of the organic amendment with the highest potency to proliferate *Trichoderma* spp. in the field management of *Armillaria* root rot of tea.

Unless mentioned, all the greenhouse and laboratory experiments were repeated twice to ensure reproducibility of the results. All graphic regression lines in this thesis denote "best fit" curves.

2.1. Experiment 1

Experiment 1 was conducted in the greenhouse at the Tea Research Foundation of Kenya (TRFK) to investigate effects of soils amended with organic materials (Table 1) and Basamid, a soil sterilant on the proliferation of *Trichoderma* spp. The TRFK lies at an altitude of 2178m above sea level, latitude 0° 22'S and longitude 35° 21'E.

Table 1: Organic materials used in Experiment 1 and their chemical composition

	Chemical composition		
Organic amendment	% C	% N	C:N
Saw dust	37.00	0.18	205.00
Coffee pulp and husks (10:1 ratio).	33.0	1.92	17.00
Filter press cake (Sugar-cane processing by-product).	17.00	0.88	19.30
Cow manure	27.00	2.57	10.50
Sheep manure	27.00	1.92	14.10
Goat manure	17.00	1.94	8.80

2.1.1 Determination of organic carbon

A modified Black's method (Black, 1965) for determination of soil organic carbon was adopted. One gram of each organic material was ground in a porcelain mortar with a pestle. A fine sample of 0.1g of each material was transferred into a 500 ml conical flask. Ten millilitres of 1N solution of potassium dichromate ($K_2Cr_2O_7$) were added to each flask and the organic materials were dispersed by a gentle swirl of the flasks. Twenty milliliters of concentrated

sulphuric acid were added rapidly and the flask swirled gently at first and then vigorously for one minute. The flasks were allowed to stand on a bench for 30 minutes, then 200ml of deionized water were added to each flask. Four drops of O-phenathroline-ferrous complex indicator (Ferroin) were added to each flask. The resultant solution in each case was titrated with 0.5 N solution of Ferrous sulphate ($FeSO_4$) to attain an end point of red (maroon) colour against a white background.

The amount of organic carbon in each organic amendment was determined according to the following formula using a correction factor (F=1.33)

$$\frac{\text{Milliequivalents } K_2Cr_2O_7 - \text{Milliequivalents } FeSO_4 / X\ 0.003\ X\ 100\ X\ f}{\text{Grams air dried amendment}}$$

For saw dust twice the volume of $K_2Cr_2O_7$ and conc. H_2SO_4 were used. The organic materials in all cases including the blank were processed in triplicate, so the mean titre volume in each case was used in the calculation.

2.1.2. Determination of total nitrogen

Kjeldahl's method but slightly modified (Vogel, 1961) for determination of leaf total nitrogen was adopted. All the organic materials (Table 1) were air dried and 0.1g of powdered material transferred to a clean dry Kjeldahl flask. Approximately 0.006 of powdered catalyst (Selenium powder) and 1 ml of analytical concentrated sulphuric acid were added. This was followed by gentle heating on digesters until frothing stopped, then the heating temperatures were raised and heating continued until the acid solution was clear and for another 30 minutes thereafter. The

contents were left to cool and the flask neck was rinsed with 2 ml of water to wash deposits into the bulb.

Distillation was carried out by Markam's distillator where 40% sodium hydroxide was added and a stopper replaced quickly to avoid danger of ammonia gas escaping. The ammonia was distilled into conical flasks containing 5 mls of 2% boric acid, 2 drops of indicator (Bromocresol green) and 12 mls of water. Twenty millilitres of the distillate were collected for titration.

For titration, N/28 Hcl was used. As a standard, for a basis of comparison, 0.3g borax was dissolved in water and diluted to 50 mls of which 10 mls were pipetted into a conical flask and 1 drop of indicator (Bromocresol green) added and the resultant solution titrated with Hcl. The end point was judged by comparing the colour change with the colour of 20 mls reference solution plus one drop of the indicator. The Hcl was run until the two solutions showed the same colour.

The percentage nitrogen was determined by a formula below:

$$\% N = \frac{V \times N \times 14}{10 \times W}$$

where N = normality of Hcl
W = weight of organic amendment sample.
V = volume of Hcl used in titrating the distillate.

The chemical composition of the organic amendments used was as in Table 1.

2.1.3. Soil collection and treatment application

Soils naturally infested with *A. mellea* were collected from a field at the TRFK, Kericho. Both the topsoil and subsoil were taken using a soil sampler and were mixed thoroughly.

One thousand four hundred grams wet weight of soil (equivalent to 870g dry weight) with about 37% moisture content were thoroughly mixed with each of the mist sprayed organic materials (Table 1), at different carbon rates of 36, 22, 16 and 8 grams. Basamid, a soil fumigant was used at 3.0, 2.0 1.5 and 1.0 grams per pot, respectively. Each mixture was put in a pot of 15-cm-diameter and 14-cm-depth and watered to saturation except for the basamid soil mixture. The water saturated mixtures were left to drain for 40 hours. Pots containing the Basamid soil mixture were covered with polythene for 7 days to prevent escape of fumes. They were then treated like the others after the 7th day.

The organic materials and their rates constituted the treatments replicated three times in a completely randomized block design.

To keep the moisture level at about 37% the pots with the contents were weighed every day and water was added appropriately to restore the initial weights (Onsando and Alvarez, 1987).

Temperature and relative humidity were taken daily at 9.00 a.m., 12.00 noon and 3.00 p.m. during the experimental period. The temperature means for 9.00 a.m., 12.00 noon and 3.00 p.m. were 18, 22 and 20°C, respectively, while relative humidity values were 54, 45 and 55%, respectively, and mean minimum and mean maximum temperatures were 10.4°C and 26.1°C respectively.

2.1.4. Soil sampling for microbial bioassay

Soil for microbial analysis was sampled using a sterilized 10 mm cork borer and a glass rod. Each sampling was done at three random sites per pot and at three depths of 3, 6 and 8 cm. The samples were mixed and a 1g sub-sample taken for serial dilution. Each serial dilution was plated in triplicate. The plates were incubated at room temperature (16-22°C) and the microbial counts taken on the 4th day for *Trichoderma*, while *Penicillium* and other fungi were taken on the 8th day.

The population dynamics of *Trichoderma* spp. and other fungi in the amended soils was monitored using modified Martin's rose bengal-streptomycin agar medium (Martin, 1950), consisting of KH_2PO_4, 0.5g; K_2HPO_4 0.5g; Mg $SO_4.7H_2O$, 0.5g; Peptone, 0.5g; Yeast extract, 0.5g; Dextrose, 10g; Rose bengal, 0.5g; Agar, 17g and one litre distilled water. Streptomycin sulphate was added to the medium before pouring at a concentration of 40mg/litre. The bacterial isolation and population determination on the other hand was monitored by a weak peptone-dextrose agar consisting of $MgSO_4.7H_2O$, 0.2g; KH_2PO_4, 0.5g; $Fe_2(SO_4)_3.9H_2O$, Trace; Dextrose, 1.5g; Peptone , 0.05g; Agar, 17g; and 1 litre distilled water. In both media, a tenfold serial dilution technique (Taylor, 1962), was used to quantify the respective microorganisms.

2.2. Experiment 2

Experiment 2 was carried out to investigate effects of inorganic fertilizers on proliferation of *Trichoderma* spp. in previously non-treated soils and coffee pulp-amended soils, at the

rate of 16g carbon. Unless specified, all other procedures in this experiment were similar as those of experiment 1.

The fertilizers (Table 2) were used at the rate of 150 kg N/ha, which is the commercial recommendation for tea in Kenya. The quantity of each fertilizer per pot was mathematically determined. The calculation was based on soil weight and not volume due to the thorough mixing of the fertilizers with the respective soils.

The treatments which were as indicated in Table 2 and their rates together with the control were replicated four times in a completely randomized block design.

Table 2: Fertilizers and their rates of application. Experiment 2

Fertilizers	Rates per pot (Grams)
Nitrogen:Phosphorus:potassium:Sulphur (NPKS) 25:5:5:5	1.50
Nitrogen:Phosphorus:potassium (NPK) 20:10:10	1.89
Sulphate of Ammonia (as)	1.79
Diammonium phosphate (DAP)	2.09
Control	0.00

2.2.1. Soil sampling for microbial activity determination

Soil sampling was done by a sterile cork borer at three randomly-selected sites per pot. The soil from three sites was mixed in a sterile glass petri dish, then a sub-sample of 1.7g wet weight which was equivalent to 1g dry weight was taken for analysis from each pot.

Soil microbial activity was determined by hydrolysis of Fluorescein Diacetate (FDA) through microbial enzymes such as proteases, lipases and esterases. The product of hydrolysis Fluorescein was quantified spectrometrically. The method was used as described by Schnure and Rosswell (1982).

The soil sample and 0.5 mls of Fluorescein Diacetate (FDA) were simultaneously added to 100 mls of phosphate buffer solution in 250 mls sterile conical flasks.The flasks were incubated on an orbital shaker (SGM-300) at 24°C for 120 minutes after which further hydrolysis of FDA was arrested by adding acetone to give a final concentration OF 50% vol/vol.

The soil samples/buffer suspension were centrifuged at 3000 rpm (REMI centrifuge T & L) and the resulting supernatant filtered through Whatman's Filter Paper No.3. The filtrate in each case was

put through a spectrophotometer (double beam uv spectrophotometer, 5000 series) to determine absorbance at 490 nm. For any off-range readings the samples were diluted with an equal amount of buffer solution throughout. The optical density (O.D.) readings were tabulated per treatment.

The FDA reagent was prepared by dissolving 0.04g of FDA in 20 mls of acetone (2 mg/ml) and the stock stored at -20°C. The phosphatic buffer (pH 7.6) on the other hand was prepared by mixing 0.2M of K_2HPO_4 and KH_2PO_4 at a ratio of 87 mls and 13 mls respectively, and then diluting the resultant solution with sterile deionized water to a total volume of 200 mls (Gueffroy, 1975).

2.3. Experiment 3

Experiment 3 was initiated to investigate the effect of age of coffee pulp on the proliferation of *Trichoderma* spp.

The coffee pulp and husks in a 10:1 ratio for this experiment were collected from the Coffee Research Foundation of Kenya, Sub-station, Koru. The sub-station is in Kisumu district at an altitude of 1559M. Fresh pulp was collected in November 1990. A portion of this pulp was sun-dried immediately and stored in a dry place. The rest was kept outside the TRFK greenhouse and allowed to undergo natural decomposition. After one month, the pulp was mixed thoroughly then another portion was taken for sun drying. The second patch was taken after two months and it underwent the same treatment. The subsequent patches were removed after three then four months, respectively. The five sun-dried patches constituted 5 treatments.

The organic carbon and nitrogen content for the five patches of pulp (Table 3) were determined as described in experiment 1 sections 2.1.1. and 2.1.2., respectively.

Table 3: The carbon and nitrogen contents and carbon nitrogen ratio of coffee pulp at different ages, Experiment 3

Age of pulp	C	N	C:N
Fresh	40.87	2.33	17.5
1-month old	43.54	3.42	12.7
2-month old	45.40	3.42	13.2
3-month old	46.99	3.63	12.9
4-month old	47.75	3.47	13.8
Control	3.02	0.06	50.3

2.3.1. Amendment of the pulp with soil

The coffee pulp was used at 16 grams carbon to 1400g soil at a moisture content of 38%. The pulp was moistened by a fine misty spray of water, and then mixed with soil appropriately. The soil-pulp mixture was put in 15-cm-diameter and 14-cm-depth plastic pots. There were four treatments (Table 3) and a control (plain soil). Each treatment was replicated three times in a completely randomized block design and kept in the greenhouse.

All other procedures regarding isolation and quantification of *Trichoderma*, *Penicillium*, *Mucor*, other fungi and bacteria were carried out as described in Experiment 1, section 2.1.4. Microbial activity determination was carried out as described in Experiment 2, section 2.2.1.

Temperature (Max. and Min.) and relative humidity were taken daily at 9.00 a.m., 12.00 noon and 3.00 p.m. during the experimental period. The temperature means for 9.00 a.m., 12.00 noon and 3.00 p.m. were 21, 23 and 22°C, respectively. The relative

humidity values were 62, 49 and 62%. The mean minimum and mean maximum temperatures were 11°C and 30°C. At the end of the experiment, chemical analysis of soils (Table 4) was carried out as described in Experiment 1, section 2.1.1., respectively.

Table 4: Chemical analysis of soils amended with different ages of coffee pulp and a control at the end of the experiment

Age of coffee pulp	N (ppm)	C(ppm)	C:N	pH
Fresh	771.3	46100	59.8	4.3
1 month old	646.0	46600	72.1	4.5
2 months old	773.0	52300	67.7	4.2
3 months old	605.0	54100	89.4	4.4
4 months old	683.0	53200	77.9	4.3
Control	589.0	30200	51.3	4.4

2.4. Experiment 4

Experiment 4 was conducted to investigate effects of coffee pulp surface area on the proliferation of *Trichoderma* spp. The experiment was conducted in the TRFK greenhouse. The coffee pulp surface area was increased by reducing the pulp particle size with the use of electric coffee blender. The treatments of ground coffee pulp and filter press cake (Plate 1) were replicated four times in a completely randomized block design.

Soil collection and treatment application, maintenance of soil moisture and microbial bioassays were carried out as described in Experiment 1, sections, 2.1.3. and 2.1.4., respectively.

Temperature and relative humidity were taken daily at 9.00 a.m., 12.00 noon and 3.00 p.m., during the experimental duration.

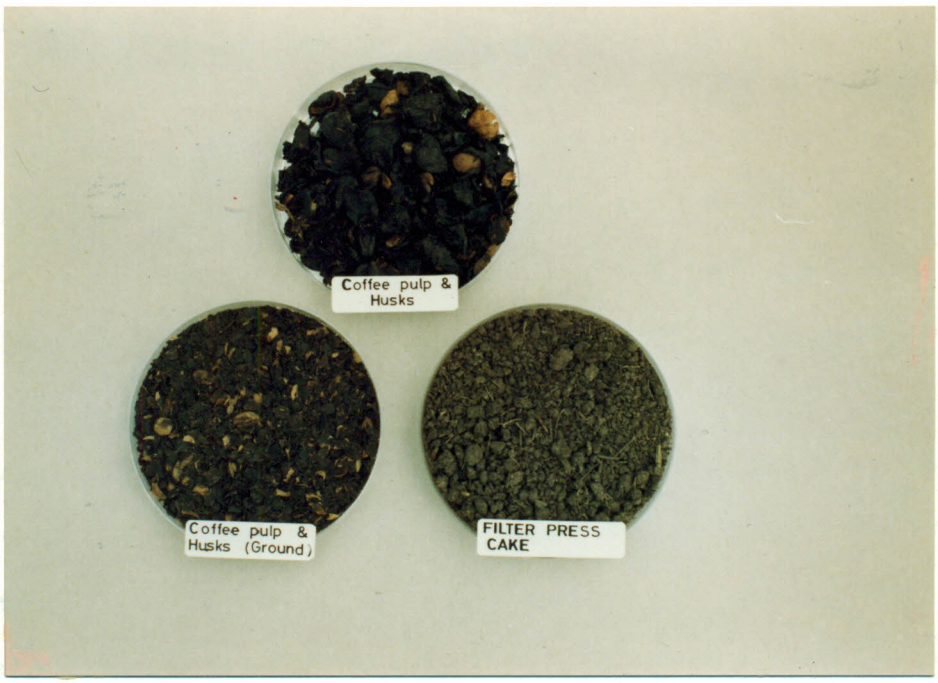


Plate 5: Particle size, reduction of ground coffee pulp as it compares to normal coffee pulp and filter pres cake

The temperature means for 9.00 a.m., 12.00 noon and 3.00 p.m. were 20, 24 and 20°C, respectively. The relative humidity values on the other hand were 66, 52 and 67%.

2.5. Experiment 5

Experiment 5 was conducted to determine effects of sub-lethal doses of the soil fumigants (Basamid) on the proliferation of *Trichoderma* spp. The lethal dose was pre-determined in a separate experiment and it was 0.3g per 1.5 kg of soil. The experiment was conducted in the TRFK greenhouse. Basamid was used at two rates of 0.2 and 0.1 grams per 15-cm diameter and 14-cm depth plastic pots containing 1400 grams of soil at moisture level of 38%. Coffee pulp and filter press cake used at the rate of 16 grams carbon were also included in this experiment for comparison purposes. The four treatments and a control were replicated three times in a completely randomized block design.

Soil collection, treatment application, maintenance of soil moisture and microbial bioassays were carried out as described in Experiment 1, sections 2.1.3. and 2.1.4., respectively.

Temperature and relative humidity were taken daily at 9.00 a.m., 12.00 noon and 3.00 p.m. during the experimental period. The temperatures for 9.00 a.m., 12.00 noon and 3.00 p.m. were 19, 24 and 21°C, respectively. The relative humidity values were 64, 51 and 62%.

2.6. Experiment 6

Experiment 6 was carried out to investigate effects of soil moisture on the proliferation of *Trichoderma* spp. in soils amended

with coffee pulp. The coffee pulp (ground) was used at the rate of 16 grams of carbon/870 grams of soil (dry weight) per 15-cm-diameter and 14-cm-depth pot.

Soil collection, treatment application, maintenance of soil moisture and microbial bioassays were carried out as described in Experiment 1, sections 2.1.3. and 2.1.4., respectively. Soil microbial activity was determined as described in Experiment 2, section 2.2.1.

Temperature and relative humidity in the greenhouse were taken daily at 9.00 a.m., 12.00 noon and 3.00 p.m. during the experimental period. The temperature means for 9.00 a.m., 12.00 noon and 3.00 p.m. were 19, 25 and 18°C, respectively. The mean relative humidity values were 64, 44 and 49%. The mean minimum and mean maximum temperatures were 11 and 28°C.

2.6.1. Varying soil moisture levels

Water amounts of 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mls were added to samples of 10g air dried soil (pH 4.3 and 9.8% water content) amended with coffee pulp at the rate of 16 grams of carbon. The soil/organic amendment samples were weighed then oven-dried at 110°C for 12 hours to determine their dry weights and hence moisture levels.

On the basis of the determined water content levels, a standard curve of the amount of water added was plotted against the resulting moisture levels (Figure 1). Using the curve, the amount of water needed to give the experimental moisture levels of 15, 25, 35, 40 and 50% was determined. The information was extrapolated for

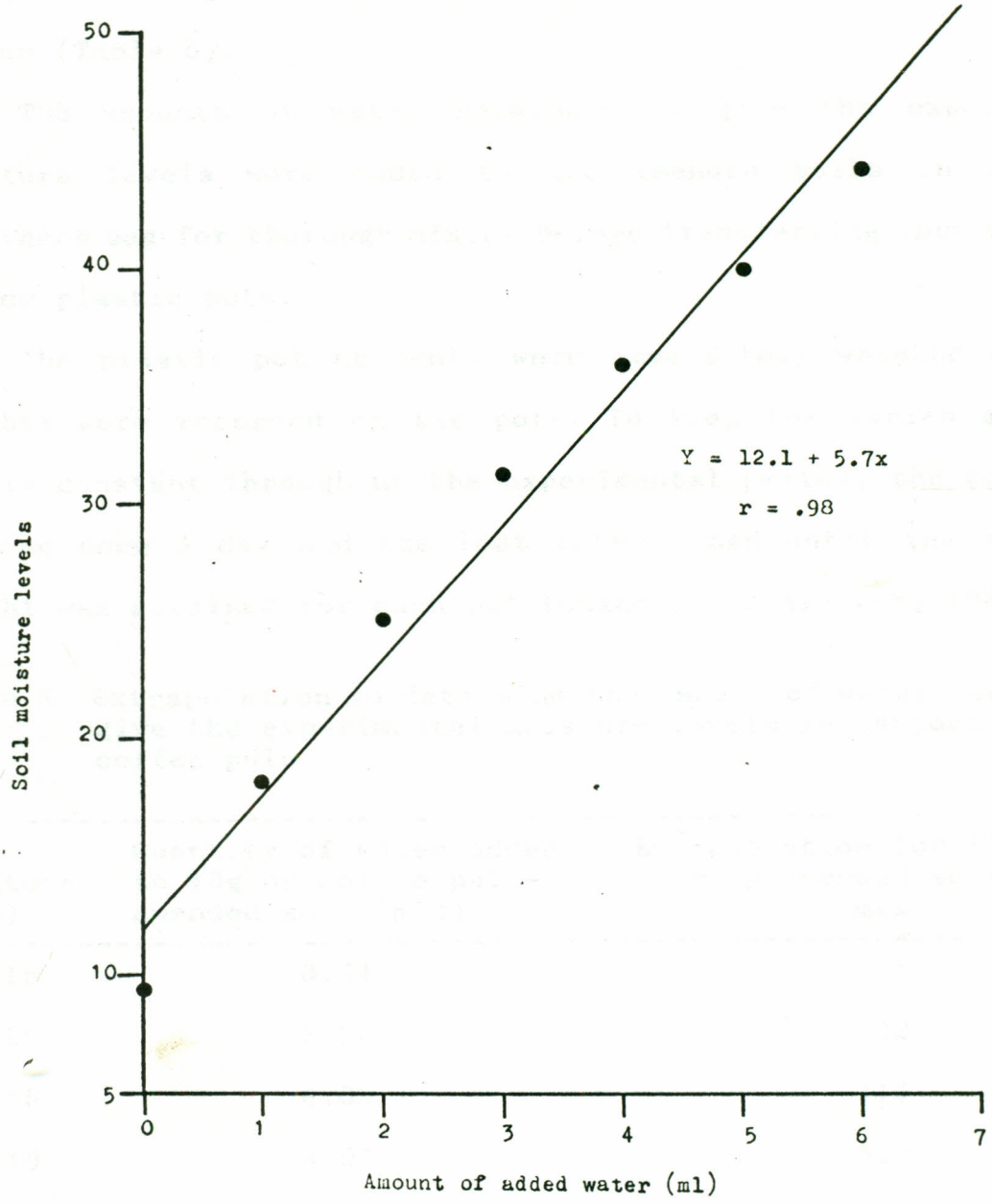


Fig 1 : A standard curve of amount of water added to 10g air dried soil/coffee pulp amendment mixture and resulting moisture levels.

870 grams of dry soil plus the coffee pulp amendment at 16 grams carbon (Table 5).

The amounts of water necessary to give the experimental moisture levels were added to the amended soils in a clean polythene bag for thorough mixing before transferring into the 15cm x 14cm plastic pots.

The plastic pot contents were immediately weighed and the weights were recorded on the pots. To keep the varied moisture levels constant throughout the experimental period, the pots were weighed once a day and the lost water added until the original weight was obtained for each pot (Onsando and Alvarez, 1987).

Table 5: Extrapolation to determine the amount of water needed to give the experimental moisture levels in respect to coffee pulp

Moisture (%)	Quantity of water added to 10g of coffee pulp-amended soil (mls)	Extrapolation for 1025.5g of pulp amended soil mls
15	0.54	55
25	2.26	232
35	4.00	410
40	4.90	503
50	6.65	682

2.6.2. Soil sampling for microbial population and activity determination

Because of the moisture variation among the treatments, soil sampling was done at 0.63g and 1.0g dry weights (Table 6) for microbial population and activity determination, respectively.

Table 6: The actual soil weights for microbial population and activity determination from different soil moisture treatments.

Soil moisture (%)	Actual soil weight (wet) for pop. determination (=0.63 dry wt)	Actual soil weight (wet) for activity determination (=1.0 dry weight)
15	0.74	1.18
25	0.84	1.33
35	0.96	1.54
40	1.05	1.67
50	1.26	2.00

2.7. Experiment 7

Experiment 7 was initiated to assess the antibiotic properties of *Trichoderma* spp. in soils in Kenya tea fields to *Armillaria mellea*. This was done by both vegetative and metabolite interactions of the fungi.

2.7.1. Soil sampling for *Trichoderma* isolation

The soils for this experiment were taken from representative tea fields both East and West of the Kenya Rift valley (Figure 2). The samples taken East of Rift Valley were from Meru, Embu, Kirinyaga, Nyeri and Kiambu. Those taken from West of the Rift Valley were from Kitale, Kakamega, Nandi, Kericho and Kisii, (Fig. 2). Both the top-soil and sub-soil were taken using a soil sampler from three different locations per district and three sites for each field. The samples were thoroughly mixed, then a sub-sample was taken for *Trichoderma* isolation. The isolation was done using modified Martin's rose bengal-streptomycin agar medium (Martin, 1950). Streptomycin sulphate was added to the medium after

autoclaving at a concentration of 40 mg/litre. Pure cultures of the isolates were sorted on the basis of macroscopic appearance such as colour and colour intensity, mode of sporulation and intensity of sporulation on Potato Dextrose Agar (PDA) of the colonies. Species identification was done at C.A.B. International Mycological Institute, Kew.

2.7.2 Isolation of *Armillaria mellea*

Ten isolates of *Armillaria* were obtained from dying or dead tea bushes *Camellia sinensis* from all the tea growing areas in Kenya (Fig. 2). Primary isolation of *Armillaria* was by Malt Extract Agar, consisting of Malt Extract, 3%; Glucose, 2%; Peptone, 0.5% Agar, 2%; and Orthophenyl phenol (OPP), 0.006%.

Two distinct morphological groups of isolates were recognized among the ten *Armillaria* isolates. These groups differed consistently in regard to their mycellia and rhizomorph characters (Mwangi *et al*, 1989). A representative of each of the two morphological groups were re-designated as *Armillaria* isolates M and N. The M and N isolates were from East and West of Kenya Rift Valley, respectively.

2.7.3 Extraction of the tea root bark extract

Two hundred grams of fresh tea root bark of 4-year-old seedling tea plants were macerated in 300 mls of 95% ethanol containing 50 mg potassium metabisulphate, a reducing agent, to stop conversion of catechins to theaflavins (TF) and thearubigins (TR). The maceration was done by using a Kenwood blender for 10 minutes at ambient temperature. The blended mixture was filtered

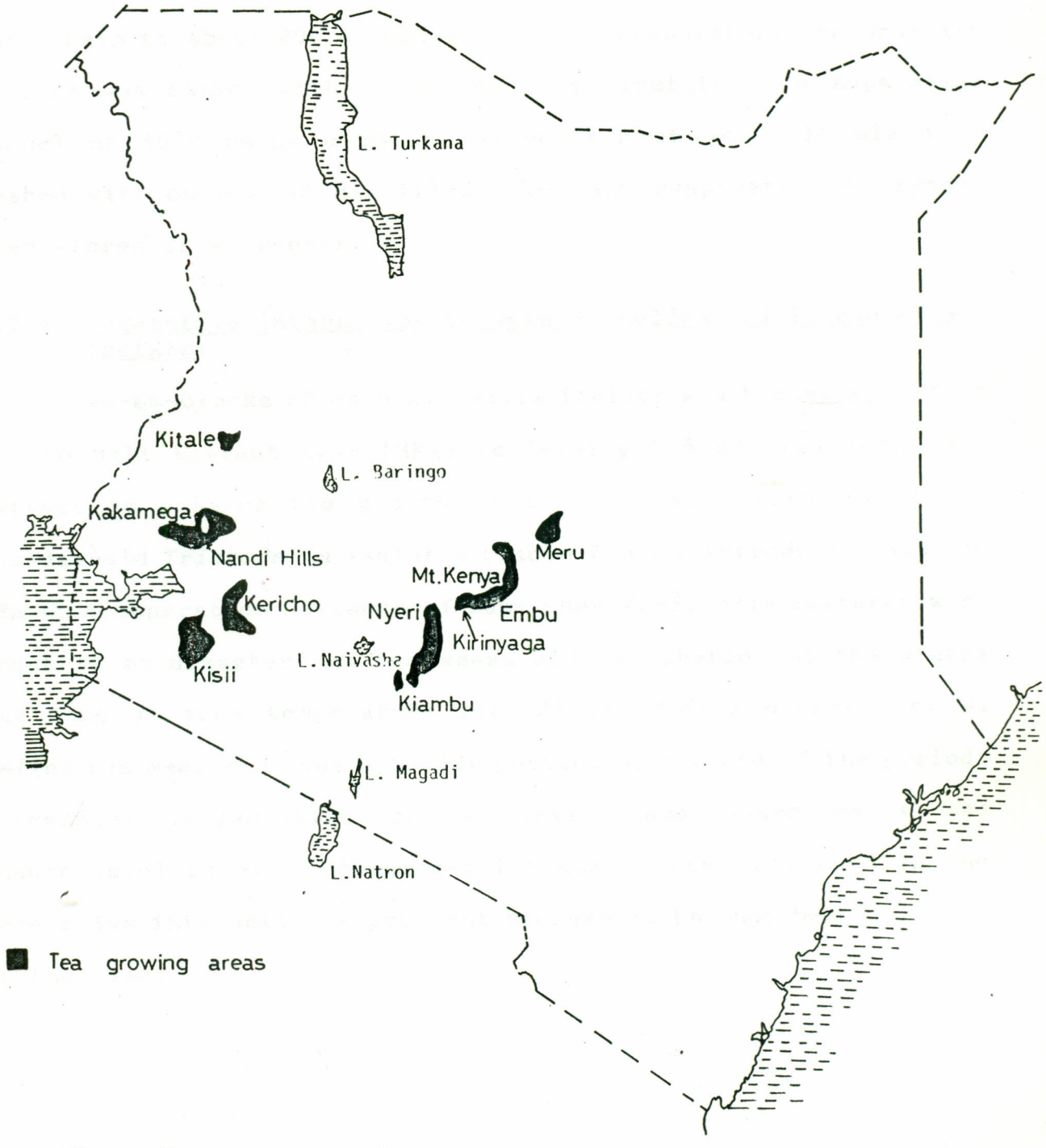


Fig. 2 : Kenya tea growing areas.

under reduced pressure. The filtrate was evaporated at 40°C in a water bath to about 200 ml using a rotary evaporator. The brownish extract was shaken with 150 ml of ethyl acetate in a separating funnel at 40°C using a rotary evaporator at about 10 mls then washed with 50 mls of distilled water and evaporated to dryness then stored in a freezer.

2.7.4. Vegetative interaction between *A. mellea* and *Trichoderma* isolates

Five-mm-blocks of each *A. mellea* isolate was transferred from PDA to Malt Extract Agar (MEA) containing 0.5 g/l tea root bark extract to enhance the growth of the fungus. Five-mm-blocks of one-day-old *Trichoderma* isolates (Table 7) were introduced into the MEA at a separation distance of 25mm when *Armillaria* cultures were about 10 mm diameter and two weeks old. Incubation of the plates was done at room temperature (18°-21°C). Radial diameter of *A. mellea* was measured over a 20-day period. At the end of the period, successful re-isolation of *A. mellea* was noted as + and unsuccessful as -. *T. harzianum* isolate 10 was not used in the vegetative interaction experiment because it had not been isolated at the time.

Table 7: *Trichoderma* isolates used for vegetative and metabolite interaction studies with *A. mellea*.

Species name	Isolate number	IMI* number
T. koningii	1	339493
T. koningii	2	339494
T. longibrachiatum	3	339495
T. harzianum	4	339496
T. harzianum	5	339497
T. koningii	6	342180
T. koningii	7	342181
T. koningii	8	342182
T. koningii	9	342183
T. harzianum	10	342184
T. harzianum	11	342185

IMI* International Mycological Institute, Kew.

2.7.5. Extraction of *Trichoderma* metabolites

The *Trichoderma* isolates (Table 7) used in this experiment were grown on Potato Dextrose Agar (PDA) for three days and four 5-mm-blocks were taken from the colony periphery for inoculation into Richard's nutrient solution.

Two and a half litres of Richard's nutrient solution consisting of KNO_3, 10g; KH_2PO_4, 5.0g; $MgSO_4.7H_2O$, 1g; Sucrose 50g per litre of deionized water were made. The nutrient solution was sub-divided into 100 ml aliquots and then put into 250ml

Erlenmeyer flasks, making up 25 flasks in all which were auto-claved at 121°C, 1 bar for 20 minutes.

The inoculation of *Trichoderma* isolates into Richard's nutrient solution was carried out in a laminar flow cabinet in duplicate. This gave rise to 11 pairs of inoculants plus the twelfth pair whose inoculant was a mixture of *Trichoderma* isolates. The control was not inoculated. The broth cultures were kept at 23°C on an orbital shaker for three weeks.

The *Trichoderma* metabolites were harvested in two successions and cold sterilized using sterile millipore filter membranes (0.2µm, 47mm, Ref. No. 401 312, D-3354 Dassel, W. Germany). To remove larger particles before cold sterilization, Whatman's filter paper No.42 was used. To achieve complete sterilization, isolates with heavy sporulation were filtered twice. The millipore filtration apparatus was initially autoclaved then washed with sterile distilled water after each sample and the external surfaces sterilized by absolute ethanol.

The *Trichoderma* isolate filtrates containing metabolites were emptied into sterile 100 ml bottles with screw caps.

2.7.6. Assessment of *Trichoderma* metabolites for antibiosis against *Armillaria mellea*

Two litres of Malt Extract Agar consisting of Malt extract 30g; Peptone, 10g; Agar, 21g per litre of deionized water were melted then sub-divided into 150ml aliquotes in 500ml flasks with magnetic bars inside then autoclaved at 121°C, 1 bar and for 20 minutes. The respective filtrates of *Trichoderma* isolates were incorporated into the 150 ml Malt Extract Agar at 50-60°C then

mixed thoroughly with the help of a magnetic stirrer and poured into sterile plastic plates in 4 replications. The pouring was done in a laminar flow cabinet.

Armillaria mellea isolates M and N that had been subcultured on Malt Extract Agar, were inoculated to the plates in replicates of 4 for isolate M and replicates of three for isolate N.

Radial mycelial growth and rhizomorphal growth were recorded at weekly intervals. *Armillaria mellea* biomass (dry weight) was also recorded. This was determined by melting the agar in boiling water, leaving rhizomorphal and mycelial growth. The two were rinsed in distilled water, blotted dry and kept in the oven at 105°C for 12 hours. Also nutrient broth colours (pigments), nutrients broth pH and *Trichoderma* biomass (dry weight) were recorded at harvesting time.

2.8. Experiment 8

Experiment 8 was initiated to evaluate the efficiency of coffee pulp and husks on management of the *Armillaria* root rot under field conditions. Coffee pulp was used in the field because of its ability to enhance proliferation of the population of *Trichoderma* spp. as was revealed in Experiment 1.

This experiment was situated on the Gakoe Tea Estate in Kiambu district, Central Kenya. The site for the experiment was chosen because of its high *Armillaria* root rot incidence.

2.8.1. Field preparation for the experiment

A small patch in the field with uniform disease distribution was chosen for this experiment. The tea bushes in the experimental

area had died of *Armillaria mellea*. The tea bushes were uprooted and the roots chopped into small pieces which were left *in situ*. The experimental area was dug to a depth of 40cm and all soil removed to allow for application of treatments. Basamid and the coffee pulp were each applied in two layers of 20 and 40cm deep at the rate of 666g product for Basamid and 102kg wet coffee pulp and husks at a ratio of 10:1 per plot (3 x 4 bushes). The rest of the soil was returned to fill up the place. The Basamid plots were covered with a polythene sheet for three weeks to minimize the escape of the fumigant fumes.

2.8.2. Tea planting

The plants (Clone 6/8) were planted four months after application of treatments. There were 12 plants per plot in a spacing of 1.22 x 1.22m. The plots were separated from one another by a guard row of 1.22m width. There were three treatments all replicated three times in a completely randomized block design.

For planting Diammoniun phosphate (DAP) was used at the rate of 15g per planting hole. Compound fertilizer NPKS 25:5:5:5 was used for yearly top dressing at the rate of 150 kg N/ha.

The tea was brought to bearing by pegging method (anon, 1987). All the other cultural practices were as outlined in the Tea Growers Handbook, 4th Edition (Anon, 1987).

Subsequent applications of the coffee pulp at 102 kg wet weight were incorporated into the soil but this time between the rows (across and up and down) starting the second year and yearly thereafter.

2.8.3. Soil Sampling and microbial population determination

Soil sampling for microbial population monitoring was done once in two months. Three randomly selected sites within a plot were sampled using a soil sampler at two soil depths of 5 to 15cm and 15 to 30cm. All these samples were mixed together and a sub-sample was taken for microbial analysis.

The microbial analysis was carried out as described in Experiment 1, section 2.1.4.

2.8.4. Evaluation of tea plant performance

The tea plant performance was assessed by measuring the stem girths 1 cm from the ground level, recording number of branches per plant, plant foliage circumference in the fourth year and harvestable tea yield. The stem girths were determined by use of a vernier calliper while the plant foliage circumference was determined by use of a rope tied to the stem at ground level then stretched full length to the periphery of the foliage and turned round to mark a circle on the ground corresponding to the extent of foliage spread. The diameter of the circle was then measured using a tape measure in centimetres. The diameter was then used to calculate the circumference of foliage spread. For harvestable tea yield, the experimental plots were plucked when the rest of the estate tea was being plucked. The conventional two leaves and a bud were plucked every time and weighed. The green leaf weight was converted into kilograms made tea per hectare using a conversion factor of 0.225 (Anon, 1987).

2.8.5. Disease assessment

Armillaria root rot incidence was assessed in the third and fourth years of the experiment. This was done by counting the number of tea bushes that died due to *Armillaria* expressed as a percentage of the total number of bushes per plot.

2.8.6. Soil and plant nutrient analysis

Soil and leaf nitrogen content was determined by Kjeldahl's method (Vogel, 1961).

2.8.7. Other soil properties determinations

Other soil properties examined during the fourth year of the experiment were: bulk density, soil reaction (pH) and organic matter content.

The soil bulk density was determined by the core method (Blake in Black, 1965).

pH values of the soils were determined in 1:1 soil water suspension using an ELL pH meter (Anon, 1987).

Organic matter contents of the soil were determined by the Walkey-black method (Black, 1965).

CHAPTER 4

RESULTS

3.0. Experiment 1

Effects of soil amended with saw dust, coffee pulp and husks in a 10:1 ratio, filter press cake, cow manure, goat manure and basamid, a soil sterilant, on the proliferation of *Trichoderma* spp. and other soil microorganisms were investigated in this experiment.

3.1. *Trichoderma* spp.

Populations of *Trichoderma* spp. (Table 7) per gram of soil were significantly (P=0.01) different throughout the experimental duration (Table 8).

Coffee pulp and husks significantly (P=0.01) enhanced highest proliferation of *Trichoderma* spp. at all rates of carbon used except 8 gc throughout the experimental duration (Table 8). The filter press cake which was second best at the rates of 16-36 g of carbon (gc) was as good as coffee pulp but only at its lowest rate of 8 gc (Table 8). The other organic amendments were significantly (P=0.01) inferior to the two irrespective of the rates used and were no better than the control throughout the experimental duration (Table 8). Among the other organic amendments, saw dust was the poorest in enhancing *Trichoderma* proliferation.

The peak *Trichoderma* population proliferation in coffee pulp amended soil was on the 5th month (Fig.3).

The rates of organic amendments influenced significantly (P=0.01) the proliferation of *Trichoderma* only in the case of coffee pulp and filter press cake. The 22 and 16 gc rate of coffee

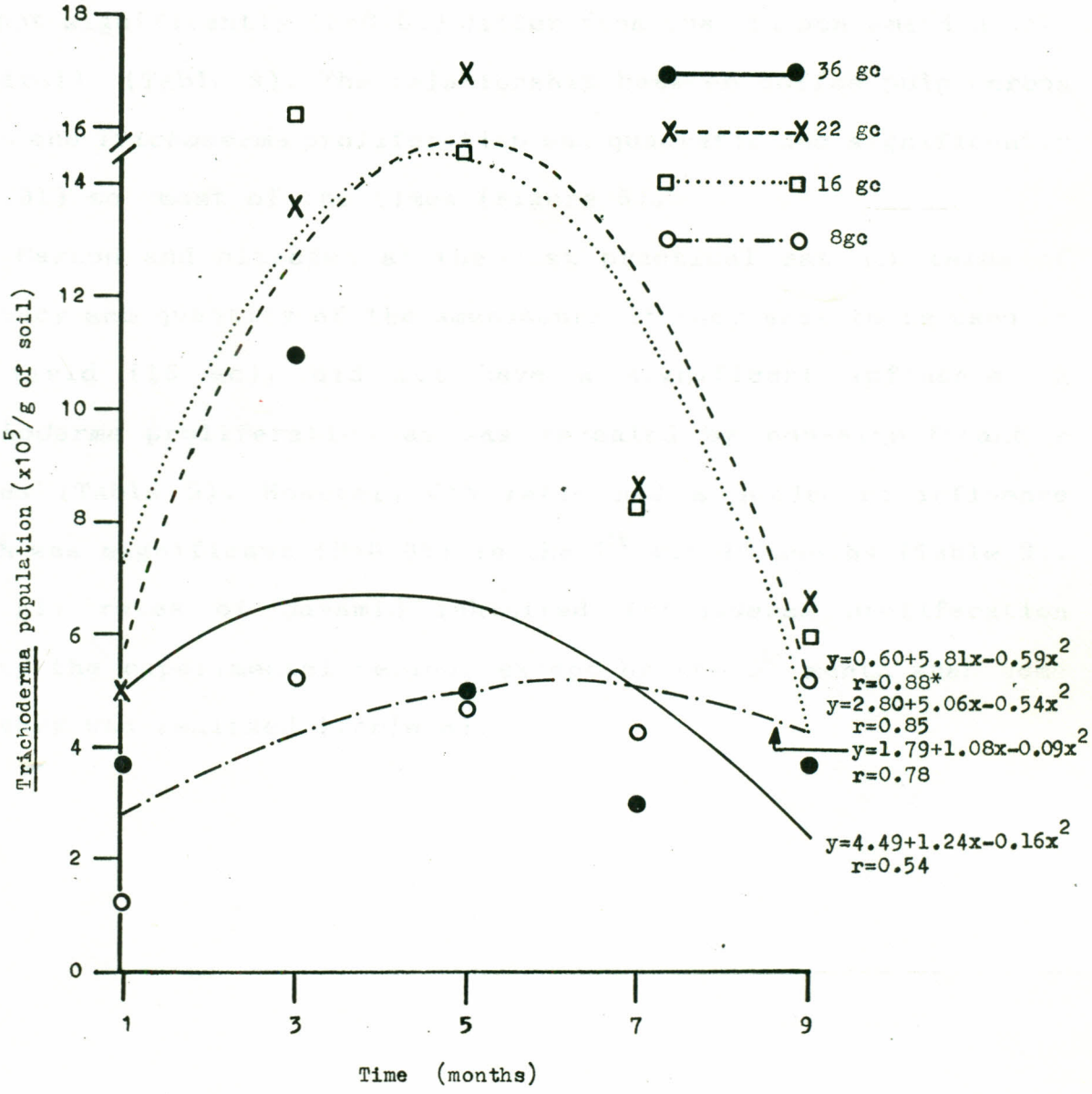


Fig. 3 : Regression analysis between Trichoderma population in soil amended with coffee pulp at different rates of carbon in time (months).

pulp and filter press cake enhanced more *Trichoderma* proliferation than the 36 and 8 gc rates in most cases (Table 8, Figure 4). However, between 22 and 16 gc rates for coffee pulp there was no significant (P=0.01) difference (Table 8). Proliferation of *Trichoderma* in soil with cow manure, sheep manure or goat manure did not significantly (P=0.01) differ from that in non-amended soil (control) (Table 8). The relationship between coffee pulp carbon rates and *Trichoderma* proliferation was quadratic and significantly (P=0.01) so most of the times (Figure 5).

Carbon and nitrogen at the most practical rate in terms of efficacy and quantity of the amendments if they were to be used in the field (16 gc), did not have a significant influence on *Trichoderma* proliferation as was revealed by non-significant r values (Table 9). However, C:N ratio had a quadratic influence which was significant (P=0.05) in the 7^{th} and 9^{th} months (Table 9).

All rates of basamid inhibited *Trichoderma* proliferation during the experimental period, except by the 9^{th} month when some recovery was realized (Table 8).

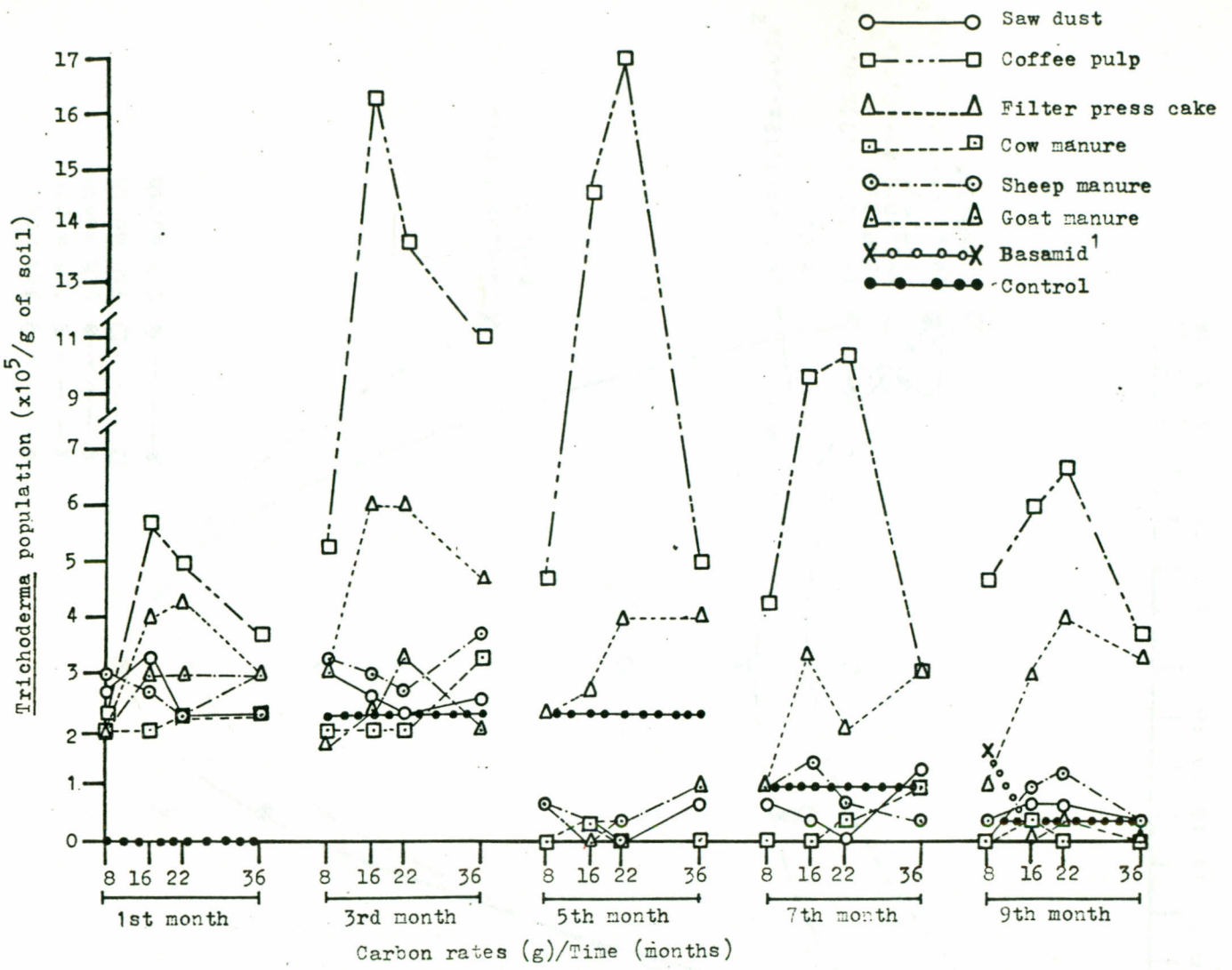


Fig. 4 : Rates of organic amendments and the proliferation of Trichoderma population in time (months).

[1]Basamid was used at 1.0, 1.5, 2.0 and 3.0 g

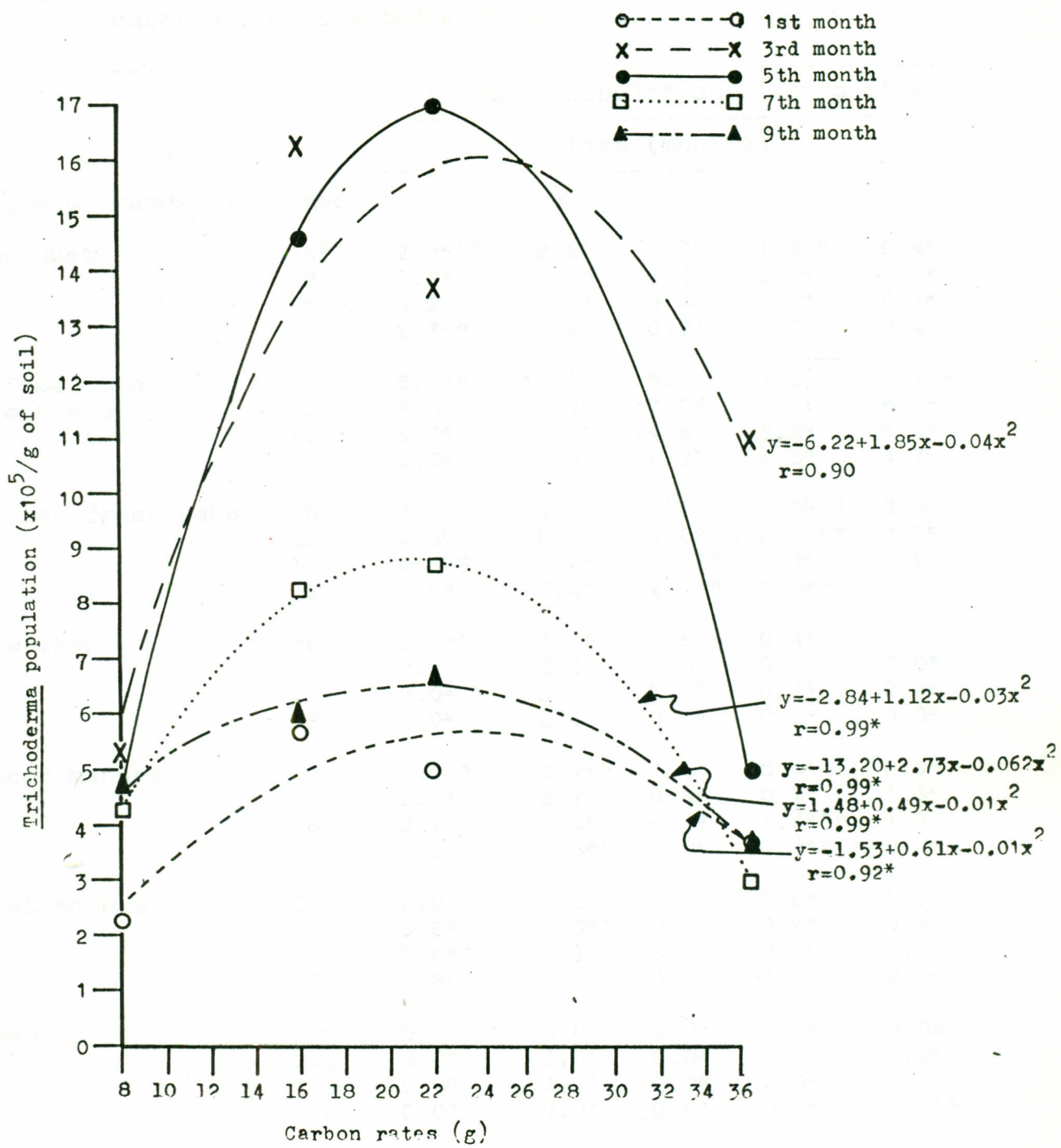


Fig. 5 : Regression analysis between carbon rates of coffee pulp and Trichoderma population in time (months).

* Significant at P=0.01.

Table 8: *Trichoderma* populations in soil amended with organic materials or treated with basamid.

Organic amendments/Rates		*Trichoderma* population x 10^5/g of soil				
		Time (months)				
		1	3	5	7	9
Saw dust	36[2]	2.3^{ab}[1]	2.6^{a}	0.7^{a}	1.3^{ab}	0.4^{a}
	22	2.3^{ab}	2.3^{a}	0.0^{a}	0.0^{a}	0.7^{a}
	16	3.3^{ab}	2.6^{a}	0.4^{a}	0.4^{a}	0.7^{a}
	8	2.7^{ab}	3.0^{a}	0.7^{a}	0.7^{a}	0.4^{a}
Coffee pulp and husks	36	3.7^{ab}	11.0^{d}	5.0^{b}	3.0^{bcd}	3.7^{bc}
	22	5.0^{c}	13.7^{d}	17.0^{d}	8.7^{e}	6.7^{d}
	16	5.7^{c}	16.3^{e}	14.6^{c}	8.3^{c}	6.0^{d}
	8	2.3^{ab}	5.3^{c}	4.7^{b}	4.3^{d}	4.7^{c}
Filter press cake	36	3.0^{ab}	4.7^{abc}	4.0^{b}	3.0^{bcd}	3.3^{bc}
	22	4.3^{c}	6.0^{c}	4.0^{b}	2.0^{abc}	4.0^{bc}
	16	4.0^{bc}	6.0^{c}	2.7^{ab}	3.3^{cd}	3.0^{b}
	8	2.0^{a}	3.0^{a}	2.3^{ab}	1.0^{ab}	1.0^{a}
Cow manure	36	2.3^{ab}	3.3^{ab}	0.0^{a}	0.4^{a}	0.0^{a}
	22	2.7^{ab}	2.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
	16	2.0^{a}	2.0^{a}	0.3^{a}	0.0^{a}	0.4^{a}
	8	2.0^{a}	2.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Sheep manure	36	3.0^{ab}	3.7^{ab}	1.0^{a}	0.4^{a}	0.4^{a}
	22	2.3^{a}	2.7^{a}	0.4^{a}	0.7^{a}	1.3^{a}
	16	2.7^{ab}	3.0^{a}	0.4^{a}	1.4^{abc}	1.0^{a}
	8	3.0^{ab}	3.3^{ab}	0.0^{a}	1.0^{ab}	0.0^{a}
Goat manure	36	2.0^{a}	2.0^{a}	1.0^{a}	0.0^{a}	0.0^{a}
	22	3.0^{ab}	3.3^{ab}	0.4^{a}	0.4^{a}	0.4^{a}
	16	3.0^{ab}	2.3^{a}	0.0^{a}	0.4^{a}	0.0^{a}
	8	2.3^{ab}	1.7^{a}	0.7^{a}	0.4^{a}	0.0^{a}
Basamid	3.0[3]	0.0^{d}	0.0^{f}	0.0^{a}	0.0^{a}	0.0a
	2.0	0.0^{d}	0.0^{f}	0.0^{a}	0.0^{a}	0.0^{a}
	1.5	0.0^{d}	0.0^{f}	0.0^{a}	0.0^{a}	0.0^{a}
	1.0	0.0^{d}	0.0^{f}	0.0^{a}	0.0^{a}	1.6^{ab}
Control	0.0	2.3^{ab}	2.3^{a}	1.0^{a}	0.4^{a}	0.0^{a}

[1] Means followed by the same letter are not significantly different from each other (P=0.01) according to Duncan's Multiple range test.

[2] Organic amendment rates in grams of carbon

[3] Basamid rates in grams

Table 9: Correlation coefficients (r) in a quadratic regression analysis between *Trichoderma* populations and carbon (c), nitrogen (n) and C:N ratio of organic amendments at 16 gc

Chemical component	Time (months) 1	3	5	7	9
Carbon (c)	0.37	0.28	0.33	0.21	0.21
Nitrogen (n)	0.58	0.43	0.38	0.51	0.35
C:N ratio	0.68	0.64	0.57	0.73*	0.78*

* Significant (P=0.05).

3.2. Total fungal population

Besides *Trichoderma* spp. other fungi were recovered from the amended and non-amended soils. *Penicillium* spp. were the most predominant fungi recovered. Others were *Mucor* spp. and occasionally *Aspergillus* spp.

Populations of fungi per gram of soils were significantly (P=0.01) different throughout the experimental duration (Table 10).

All carbon rates of coffee pulp and husks significantly (P=0.01) enhanced the highest proliferation of fungi most times (Table 10). The second highest enhancement was that of filter press cake followed by goat manure (Table 10). Between coffee pulp and filter press cake significant (P=0.01) differences were realized mainly on the 1st and 3rd months (Table 10). Goat manure, however, was not significantly different from filter press cake most times (Table 10). The other organic amendments had significantly (P=0.01) inferior enhancing effect on the proliferation of fungi at all rates of carbon throughout the experimental period (Table 10).

Fungal proliferation in the coffee pulp amended soil was at the peak in the 5th month as was the case with *Trichoderma* (Fig. 6).

The rates of organic amendments influenced significantly (P=0.01) the proliferation of fungi only in the cases of coffee pulp, filter press cake and goat manure (Table 10). For these three, the proliferation of fungi increased with increase in rates of organic carbon most times (Table 10).

Basamid completely inhibited fungal proliferation at all rates, except by the 5th month (Table 10).

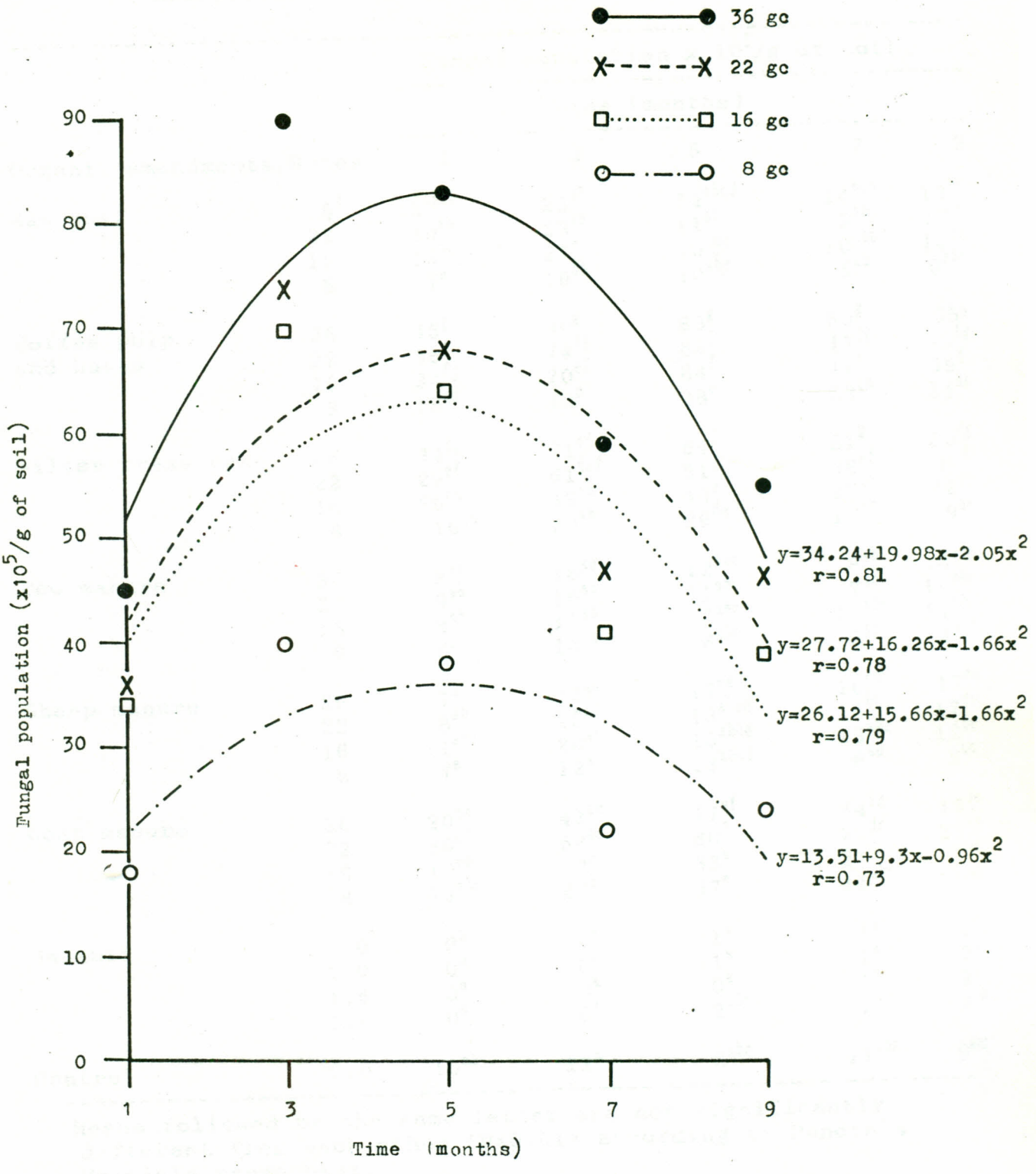


Fig. 6 : Regression analysis between fungal population in soil amended with coffee pulp at different rates of carbon in time (months).

Table 10: Total fungal populations in soil amended with organic materials or treated with basamid

Organic amendments/Rates		*Fungal* population x 10^5/g of soil				
		Time (months)				
		1	3	5	7	9
Saw dust	36[2]	12^{abcl}	23^{ab}	14^{abcd}	14^{bcd}	13^{bc}
	22	10^{ab}	23^{ab}	11^{abc}	9^{ab}	13^{bc}
	16	15^{bc}	27^{b}	10^{abc}	10^{abc}	14^{cd}
	8	7^{a}	16^{ab}	10^{abc}	5^{ab}	6^{abc}
Coffee pulp and husks	36	45^{g}	90^{g}	83^{g}	59^{g}	55^{g}
	22	36^{g}	74^{fg}	68^{f}	47^{fg}	46^{fg}
	16	34^{fg}	70^{ef}	64^{f}	41^{f}	39^{f}
	8	18^{cd}	40^{c}	38^{e}	22^{cd}	24^{de}
Filter press cake	36	34^{fg}	71^{ef}	64^{f}	51^{g}	50^{fg}
	22	29^{ef}	61^{def}	51^{f}	38^{ef}	41^{f}
	16	25^{de}	48^{cd}	40^{e}	27^{de}	22^{de}
	8	10^{ab}	20^{ab}	26^{de}	17^{cd}	19^{de}
Cow manure	36	8^{ab}	18^{ab}	12^{abc}	8^{ab}	8^{abc}
	22	9^{ab}	16^{ab}	11^{abc}	7^{ab}	10^{abc}
	16	8^{ab}	17^{ab}	6^{abc}	12^{abc}	11^{abc}
	8	6^{a}	14^{ab}	6^{abc}	3^{ab}	4^{abc}
Sheep manure	36	7^{a}	13^{ab}	17^{cd}	16^{cd}	17^{de}
	22	8^{ab}	15^{ab}	13^{abcd}	14^{bcd}	17^{de}
	16	11^{abc}	20^{ab}	15^{abcd}	13^{abc}	12^{bc}
	8	7^{a}	12^{a}	13^{abcd}	8^{ab}	9^{abc}
Goat manure	36	20^{de}	43^{cd}	47^{ef}	44^{fg}	19^{de}
	22	30^{ef}	58^{de}	60^{f}	27^{de}	25^{de}
	16	19^{cd}	42^{c}	33^{e}	31^{e}	27^{e}
	8	13^{abc}	27^{b}	37^{e}	11^{abc}	15^{cd}
Basamid	3.0[3]	0^{h}	0^{a}	1^{a}	1^{a}	2^{a}
	2.0	0^{h}	0^{a}	1^{a}	1^{a}	2^{a}
	1.5	0^{h}	0^{a}	0^{a}	1^{a}	2^{a}
	1.0	0^{h}	0^{a}	2^{abc}	1^{a}	3^{ab}
Control	0.0	10^{ab}	12^{a}	8^{abc}	11^{abc}	9^{abc}

[1] Means followed by the same letter are not significantly different from each other (P=0.01) according to Duncan's Multiple range test.

[2] Organic amendment rates in grams of carbon

[3] Basamid rates in grams

3.3. Total bacterial population

Populations of bacteria per gram of soil were significantly (P=0.01) different throughout the experimental duration (Table 11).

Filter press cake carbon rates enhanced the highest proliferation of bacterial populations followed by those of coffee pulp in most cases. The enhancement effects of the filter press cake and the coffee pulp were not significantly different in most cases. The generally higher rates of goat manure enhancement effect did not significantly (P=0.01) differ from that of filter press cake and coffee pulp (Table 11). The other organic amendments were significantly inferior and not better than the control in the enhancement of the proliferation of bacteria (Table 11).

The peak bacteria proliferation by coffee pulp as determined by regression analysis was on the 4^{th} month unlike the case of *Trichoderma* and other fungi (Figure 7) at all carbon rates.

The rates of organic amendments influenced significantly (P=0.01) the proliferation of bacterial population most of the times (Table 11). This was more pronounced for filter press cake, coffee pulp and goat manure, in that order. Except for the 7^{th} and 9^{th} months, bacterial proliferation increased with increase in rates of carbon of the organic amendment most of the times (Table 11).

Basamid completely inhibited the bacterial proliferation up to the 3^{rd} month. By the 5^{th} month, exceptionally high bacterial populations were recovered (Table 11).

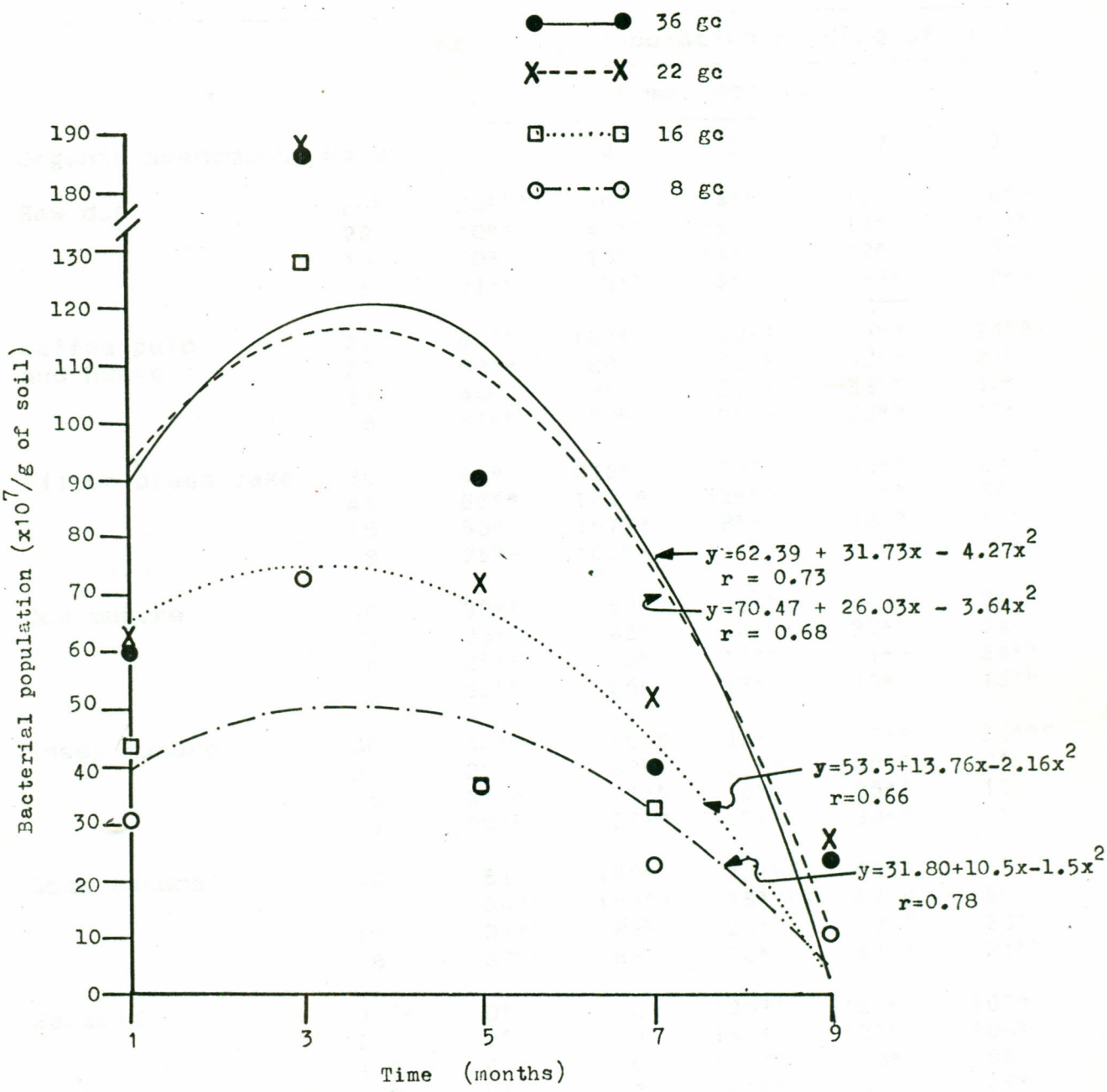


Fig. 7 : Regression analysis between bacterial population in soil amended with coffee pulp at different rates of carbon in time (Months).

Table 11: Total bacterial populations in soil amended with organic materials and treated with basamid

Organic amendments/Rates		Bacterial population x 10^7/g of soil				
		Time (months)				
		1	3	5	7	9
Saw dust	36[2]	28^{ab}[1]	30^{ab}	14^{ab}	15^{ab}	6^{ab}
	22	10^{bc}	44^{ab}	16^{ab}	14^{ab}	10^{ab}
	16	20^{a}	18^{a}	14^{ab}	12^{a}	9^{ab}
	8	21^{ab}	19^{ab}	16^{ab}	13^{ab}	6^{ab}
Coffee pulp and husks	36	60de	187^{e}	90^{cd}	40^{ab}	24^{abc}
	22	63de	188^{e}	72^{cd}	52^{ab}	27^{bc}
	16	44^{c}	128^{cd}	37^{ab}	33^{ab}	13^{ab}
	8	31^{ab}	73^{b}	36^{ab}	23^{ab}	11^{ab}
Filter press cake	36	65^{e}	212^{e}	73^{cd}	43^{ab}	28^{bc}
	44	56de	169de	52^{bcd}	39^{ab}	34^{c}
	16	50^{d}	157de	38^{ab}	43^{ab}	17^{abc}
	8	35^{bc}	103^{bc}	46^{abcd}	52^{ab}	12^{ab}
Cow manure	36	32^{ab}	66^{ab}	38^{ab}	48^{ab}	24^{abc}
	22	23^{ab}	46^{ab}	34^{ab}	32^{ab}	34^{c}
	16	25^{ab}	25^{ab}	15^{ab}	41^{ab}	24^{abc}
	8	22^{ab}	26^{ab}	19^{ab}	13^{ab}	13^{ab}
Sheep manure	36	33^{ab}	96^{ab}	43^{cd}	33^{ab}	20^{abc}
	22	29^{ab}	82^{bc}	22^{ab}	26^{ab}	31^{c}
	16	35^{bc}	71^{ab}	16^{ab}	35^{ab}	15^{abc}
	8	26^{ab}	26^{ab}	12^{a}	33^{ab}	11^{ab}
Goat manure	36	51^{d}	152^{d}	52^{bcd}	68^{cd}	30^{c}
	22	54de	169de	45^{abcd}	57^{bc}	59^{d}
	16	31^{ab}	88^{bc}	24^{ab}	72^{cd}	30^{c}
	8	30^{ab}	88^{bc}	32^{ab}	47^{ab}	21^{abc}
Basamid	3.0[3]	0^{f}	0^{f}	135de	120^{e}	107^{e}
	2.0	0^{f}	0^{f}	143^{e}	122^{e}	104^{e}
	1.5	0^{f}	0^{f}	102de	138^{e}	96^{c}
	1.0	0^{f}	0^{f}	65^{cd}	97^{cde}	86^{e}
Control	0.0	32^{ab}	36^{ab}	17^{ab}	15^{ab}	14^{abc}

[1] Means followed by the same letter are not significantly different from each other (P=0.01) according to Duncan's Multiple range test.

[2] Organic amendment rates in grams of carbon

[3] Basamid rates in grams

4.0. Experiment 2

Effects of commonly used inorganic fertilizers in tea on the proliferation of *Trichoderma* spp. were investigated in this experiment. Because of known efficacy in the proliferation of *Trichoderma* spp. of coffee pulp and husks (Exp. 1), and for comparison purposes, it was also used.

4.1. *Trichoderma* spp.

Populations of *Trichoderma* per gram of soil were significantly (P=0.01) different at most of the bioassay times (Table 12).

Coffee pulp and husks significantly (P=0.01) enhanced the highest proliferation of *Trichoderma* population throughout the experimental period, except in the first week (Table 12). Although inorganic fertilizers enhanced higher proliferation of *Trichoderma* more than the control, the differences were not significant.

4.2. Total fungal population

Populations of total fungi per gram of soil were significantly (P=0.01) different at most of the bioassay times (Table 13).

Coffee pulp and husks significantly (P=0.01) enhanced the proliferation of total fungal population throughout the experimental period except in the first week (Table 13). Although inorganic fertilizers enhanced higher proliferation of the total fungal population than the control, the differences were not significant (Table 13).

4.3. Total bacterial population

Bacterial populations were significantly (P=0.01) different, with coffee pulp and husks supporting the highest bacterial populations (Table 14).

Table 12: *Trichoderma* population in soil amended with inorganic fertilizers and coffee pulp and husks in a 10:1 ratio.

	Trichoderma population x 10^5/g of soil					
	Time of bioassay (months)					
Treatments	1st wk	1st month	2nd mth	3rd mth	4th mth	5th mth
Coffee pulp and husks	1.3	11.3[a1]	10.3[a]	10.3[a]	8.8[a]	8.8[a]
NPKS[3]	1.5	1.5[b]	1.8[b]	1.5[b]	1.5[b]	1.3[b]
NPK[4]	1.3	1.5[b]	1.8[b]	1.5[b]	1.5[b]	1.3[b]
S/A[5]	1.3	1.5[b]	1.8[b]	1.8[b]	1.8[b]	1.3[b]
DAP[6]	1.3	1.5[b]	2.0[b]	1.5[b]	1.5[b]	1.5[b]
Control	1.0	1.0[b]	1.3[b]	0.8[b]	0.8[b]	0.5[b]
	NS[2]					

[1] Means followed by the same letter are not significantly different from each other (P=0.01) according to Duncan's Multiple Range test.

[2] Non significant

[3] NPKS: Nitrogen : Phosphorus : Potassium : Sulphur

[4] NPK: Nitrogen: Phosphorus : Potassium

[5] S/A: Sulphate of Ammonia

[6] DAP: Diammonium Phosphate

Table 13: Total fungal population in soil amended with inorganic fertilizers and coffee pulp and husks in a 10:1 ratio.

	Total fungal population x 10^5/g of soil					
	Time of bioassay					
Treatments	1st wk	1st mth	2nd mth	3rd mth	4th mth	5th mth
Coffee pulp and husks	6.0	128.0a[1]	103.3[a]	144.0[a]	112.5[a]	113.3[a]
NPKS[3]	8.0	7.5[b]	6.3[b]	8.8[b]	8.5[b]	12.5[b]
NPK[4]	7.8	8.5[b]	5.8[b]	10.3[b]	9.0[b]	10.3[b]
S/A[5]	6.3	6.8[b]	6.0[b]	10.8[b]	9.0[b]	11.3[b]
DAP[6]	7.0	5.8[b]	7.0[b]	10.3[b]	8.3[b]	12.3[b]
Control	7.0	5.3[b]	4.5[b]	6.8[b]	4.8[b]	7.8[b]
	NS[2]					

[1] Means followed by the same letter are not significantly different from each other (P=0.01) according to Duncan's Multiple Range test.

[2] Non-significant

[3] NPKS: Nitrogen : Phosphorus : Potassium : Sulphur

[4] NPK: Nitrogen: Phosphorus : Potassium

[5] S/A: Sulphate of Ammonia

[6] DAP: Diammonium Phosphate

Table 14: Total bacterial population in soil amended with inorganic fertilizers and coffee pulp and husks in a 10:1 ratio

	Total bacterial population x 10^7/g of soil					
	Time of bioassay					
Treatments	1st wk	1st month	2nd mth	3rd mth	4th mth	5th mth
Coffee pulp and husks	8.8^{c} [1]	62.3^{a}	86.0^{a}	60.0^{a}	30.0^{a}	53.8^{a}
NPKS[2]	6.8^{ab}	7.5^{b}	10.8^{a}	5.8^{b}	7.0^{b}	6.3^{b}
NPK[3]	4.5^{a}	5.0^{b}	11.8^{b}	4.3^{b}	8.0^{b}	4.3^{bc}
S/A[4]	5.8^{ab}	6.0^{b}	13.5^{b}	5.8^{b}	9.0^{b}	4.8^{bc}
DAP[5]	11.0^{c}	5.3^{b}	12.0^{b}	5.3^{b}	8.5^{b}	4.3^{bc}
Control	8.5^{bc}	5.8^{b}	13.5^{b}	5.5^{b}	8.5^{b}	2.5^{c}

[1] Means followed by the same letter are not significantly different from each other (P=0.01) according to Duncan's Multiple Range test.

[2] NPKS: Nitrogen : Phosphorus : Potassium : Sulphur

[3] NPK: Nitrogen: Phosphorus : Potassium

[4] S/A: Sulphate of Ammonia

[5] DAP: Diammonium Phosphate

4.4. Microbial activity

Microbial activities as determined by fluorescein diacetate hydrolysis through enzymatic action were significantly (P=0.01) different throughout the experimental duration (Table 15).

Coffee pulp and husks enhanced significantly (P=0.01) the highest microbial activity in most cases (Table 15). Although the inorganic fertilizers supported slightly higher microbial activity than the control, the differences were not significant (Table 15).

4.5. Effect of coffee pulp and inorganic fertilizers on soil pH at the termination of the experiment

Coffee pulp amended soils had significantly (P=0.01) higher soil pH at the termination of the experiment than inorganic fertilizer amended soils and control (Table 16). The inorganic amendments had an acidifying effect which was significant (P=0.01) compared to the control (non-amended soil) and coffee pulp. A regression analysis between mean pH values and mean *Trichoderma* populations on monthly basis was significant (P=0.01) and quadratic throughout the experimental duration with r-values ranging from 0.97 to 0.99 (Table 16).

Table 15: Effects of coffee pulp and husks in a 10:1 ratio and inorganic fertilizers on microbial activity.

	Microbial activity (A 490 nm)/g of soil					
	Time of bioassay					
Treatments	1st wk	1st mth	2nd mth	3rd mth	4th mth	5th mth
Coffee pulp and husks	0.292	0.675[a1]	0.672[a]	0.614[a]	0.615[a]	0.569[a]
NPKS[3]	0.244	0.445[b]	0.359[b]	0.374[b]	0.424[b]	0.472[ab]
NPK[4]	0.236	0.413[b]	0.379[b]	0.338[b]	0.408[b]	0.320[bc]
S/A[5]	0.248	0.391[b]	0.343[b]	0.355[b]	0.403[b]	0.377[bc]
DAP[6]	0.317	0.395[b]	0.322[b]	0.371[b]	0.391[b]	0.294[c]
Control	0.259	0.337[b]	0.324[b]	0.348[b]	0.434[b]	0.354[c]
	NS[2]					

[1] Means followed by the same letter are not significantly different from each other (P=0.01) according to Duncan's Multiple Range test.

[2] Non-significant

[3] NPKS: Nitrogen : Phosphorus : Potassium : Sulphur

[4] NPK: Nitrogen: Phosphorus : Potassium

[5] S/A: Sulphate of Ammonia

[6] DAP: Diammonium Phosphate

Table 16: Effect of coffee pulp and husks in a 10:1 ratio and inorganic fertilizers on soil pH at the termination of the experiment and regression of the pH with *Trichoderma* populations.

		Trichoderma population x 10^5/g of soil				
		Time of bioassay (Months)				
Treatments	pH values	1	2	3	4	5
Coffee pulp and husks	4.5[a1]	11.3[a1]	10.3[a1]	10.3[a1]	8.8[a1]	8.8[a1]
NPKS[2]	3.9[b]	1.5[b]	1.8[b]	1.5[b]	1.5[b]	1.3[b]
NPK[3]	4.0[b]	1.5[b]	1.8[b]	1.5[b]	1.5[b]	1.3[b]
S/A[4]	3.9[b]	1.5[b]	1.8[b]	1.8[b]	1.8[b]	1.3[b]
DAP[5]	3.9[b]	1.5[b]	2.0[b]	1.5[b]	1.5[b]	1.5[b]
Control	4.2[c]	1.0[b]	1.3[b]	0.8[b]	0.8[b]	0.5[b]
Regression statistic (r-values)		0.99	0.99	0.99	0.99	0.98

[1] Means followed by the same letter are not significantly different from each other (P=0.01) according to Duncan's Multiple Range test.

[2] NPKS: Nitrogen : Phosphorus : Potassium : Sulphur

[3] NPK: Nitrogen: Phosphorus : Potassium

[4] S/A: Sulphate of Ammonia

[5] DAP: Diammonium phosphate

5.0. Experiment 3

Effects of soil amended with coffee pulp and husks in a ratio of 10:1 of different ages on the proliferation of *Trichoderma* spp. were investigated. Age was the period of decomposition after pulping the coffee.

5.1. *Trichoderma* population

Population of *Trichoderma* spp. per gram of soil were significantly (P=0.05) different at most of the bioassay times (Table 17). Fresh and one-month-old coffee pulp and husks significantly (P=0.05) enhanced the population build-up of *Trichoderma* spp. in the soil more than the other age categories and the control (Table 17). One-month-old coffee pulp and husks supported the highest *Trichoderma* spp. throughout the experimental duration (Table 17 and Figure 8). The lowest and second lowest populations of *Trichoderma* spp. were obtained from non-amended (control) and four-month-old coffee pulp amended soil, respectively, throughout the experimental time (Table 17 and Fig. 8).

The proliferation of *Trichoderma* spp. in soils amended with coffee pulp of different ages was significantly (P=0.05) influenced by the carbon content of the pulp in most cases (Table 21). The influence was quadratic (Table 21). The influence of nitrogen content and carbon : nitrogen ratio on proliferation of *Trichoderma* spp. was not significant (Table 21).

Soil pH had a non-significant effect on the proliferation of *Trichoderma* (Table 22).

Table 17: *Trichoderma* population in soils amended with coffee pulp and husks in a 10:1 ratio at different decomposition ages.

	Trichoderma population x 10^5/g of soil						
	Time of Bioassay						
Age of pulp	1st week	1st month	2nd month	3rd month	4th month	5th month	6th month
Fresh	1.0	3.7[a1]	4.0[a]	3.7[a]	4.0[a]	6.6[a]	5.7[a]
One month	1.3	5.7[b]	5.7[a]	4.7[a]	6.0[b]	7.3[a]	6.0[a]
Two months	1.7	2.7[ac]	2.7[b]	2.0[b]	2.3[c]	2.0[b]	2.3[b]
Three months	2.3	2.3[ac]	2.0[bc]	1.3[bc]	1.7[cd]	1.3[bc]	1.7[bc]
Four months	1.7	1.7[c]	1.0[c]	1.0[bc]	1.0[cd]	1.0[bc]	0.7[bc]
Control	1.3	1.0[c]	0.7[c]	0.7[c]	0.7[d]	0.3[c]	0.3[c]
	NS[2]						

[1] Means followed by the same letter are not significantly different from each other (P=0.05) according to Duncan's Multiple Range test.

[2] Not significant

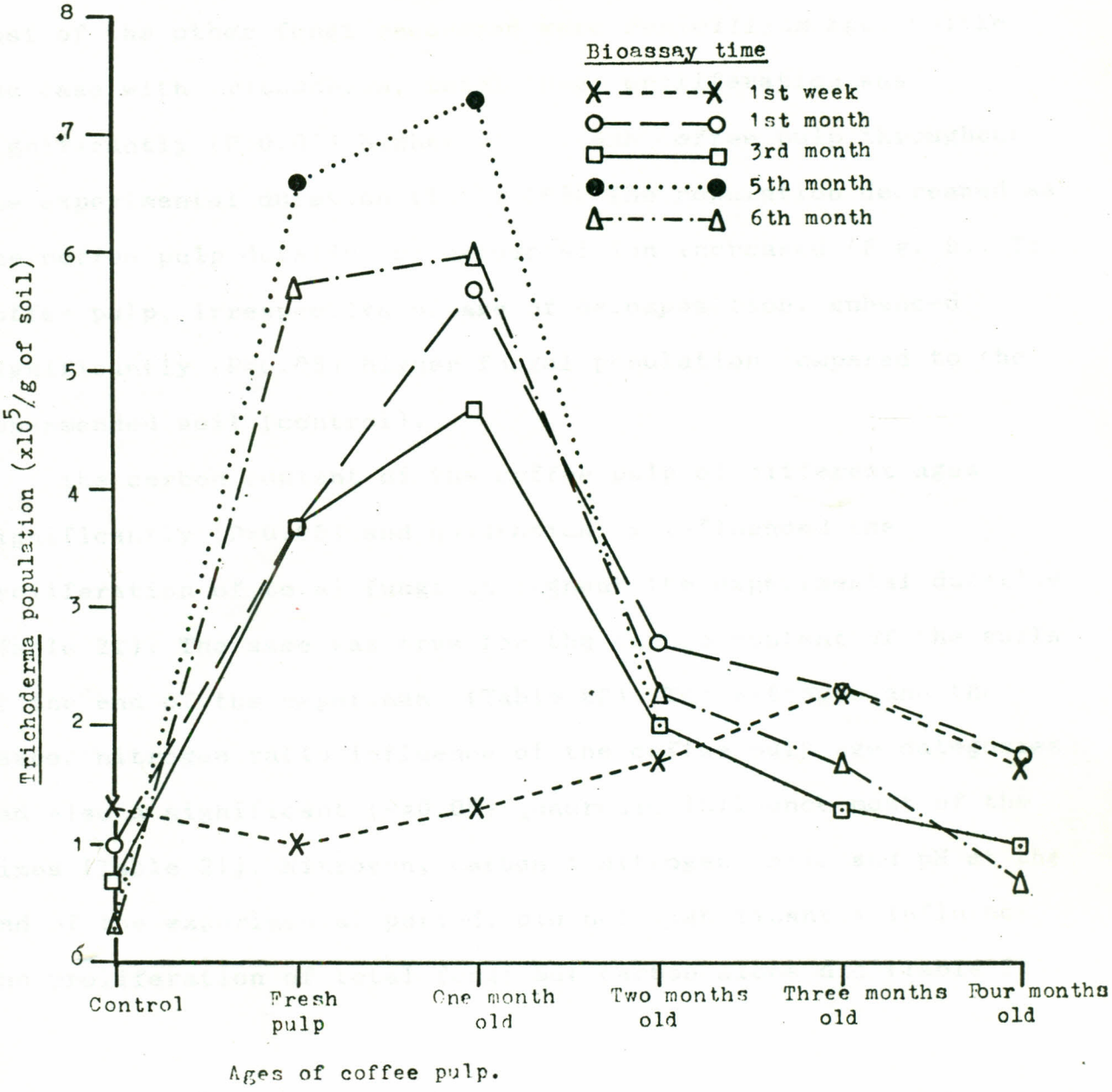


Fig. 8 : Age of coffee pulp and husks and Trichoderma population with time.

5.2 Total fungal population

Most of the other fungi recovered were *Penicillium* spp. Unlike the case with *Trichoderma*, total fungi proliferation was significantly (P=0.05) higher with fresh coffee pulp throughout the experimental duration (Table 18). The population decreased as the coffee pulp duration of decomposition increased (Fig. 9). The coffee pulp, irrespective of age of decomposition, enhanced significantly (P=0.05) higher fungal population compared to the non-amended soil (control).

The carbon content of the coffee pulp of different ages significantly (P=0.05) and quadratically influenced the proliferation of total fungi throughout the experimental duration (Table 21). The same was true for the carbon content of the soils at the end of the experiment (Table 22). The nitrogen and the carbon:nitrogen ratio influence of the coffee pulp age categories had also a significant (P=0.05) quadratic influence most of the times (Table 21). Nitrogen, carbon : nitrogen ratio and pH at the end of the experimental period, did not significantly influence the proliferation of total fungi but carbon alone did (Table 22).

Table 18: Total fungi populations in soil amended with coffee pulp and husks in a 10:1 ratio at different decomposition ages

	Total fungal population x 10^5/g of soil						
	Time of Bioassay						
Age of pulp	1st week	1st mth	2nd mth	3rd mth	4th mth	5th mth	6th mth
Fresh	8.7	160.3[a]	180.0[a]	172.3[a]	161.0[a]	118.7[a]	100.6[a]
One month	10.7	141.0[b]	106.0[b]	95.0[b]	82.7[b]	68.3[b]	57.3[b]
Two months	14.0	103.3[c]	84.0[c]	62.3[c]	62.7[b]	59.3[b]	53.7[b]
Three months	14.0	83.3[d]	60.7[c]	58.7[c]	61.7[b]	49.7c	33.7[c]
Four months	20:3	46.7[e]	25.7[e]	32.7[d]	30.7[c]	29.3d	19.3[d]
Control	9.3	4.7[f]	4.7[f]	3.0[e]	3.0[d]	3.3e	3.3[e]
	NS[2]						

[1] Means followed by the same letter are not significantly different from each other (P=0.05) according to Duncan's Multiple Range test.

[2] Not significant

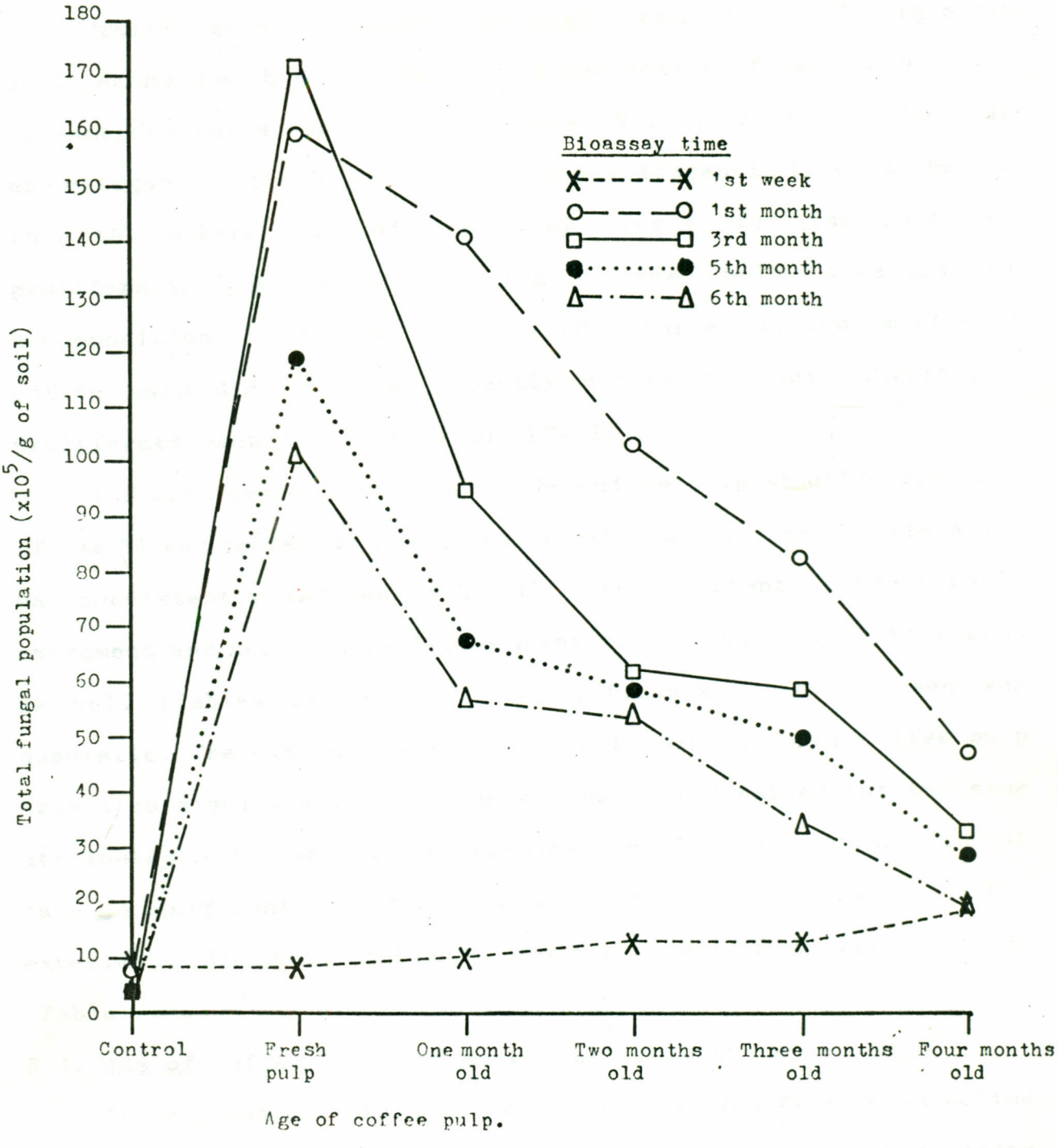


Fig. 9 : Age of coffee pulp and husks and total fungal population with time.

5.3. Total bacterial population

Proliferation of bacteria was significantly (P=0.05) higher in soils amended with the coffee pulp irrespective of age, than in the non-amended soils, the control (Table 19). Within the coffee pulp age categories, fresh coffee pulp was significantly (P=0.05) better than the others most of the times (Table 19). The bacterial proliferation decreased as the coffee pulp duration of decomposition increased (Figure 10). Three and four-month-old coffee pulp did not significantly differ in their ability to proliferate bacterial population (Table 19).

The chemical composition of the coffee pulp at different ages (Table 3) and bacterial proliferation showed that the proliferation was consistently influenced by the carbon content of the organic amendment and the soil carbon content at the end of the experiment as well (Tables 21 and 22). The influence in both cases was quadratic. The nitrogen and carbon: nitrogen ratio of coffee pulp ages also significantly influenced the proliferation but the same attributes in the soil at the termination of the experiment did not have a significant influence. The soil pH at the termination of the experiment did not influence proliferation of bacteria either (Table 22).

5.4. Age of coffee pulp and microbial activity (A 490 nm)

The highest microbial activity was realized from fresh coffee pulp (Fig. 11). The activity decreased as the coffee pulp duration of decomposition increased (Table 20). Coffee pulp amendment, irrespective of its age significantly (P=0.05) enhanced higher microbial activity than the control (Table 20).

Table 19: Total bacterial population in soil amended with coffee pulp and husks in a 10:1 ratio at different decomposition ages.

Age of pulp	Bacterial population x 10^7/g of soil — Time of Bioassay						
	1st week	1st mth	2nd mth	3rd mth	4th mth	5th mth	6th mth
Fresh	45.3[a1]	259.0[a]	70.0[a]	43.0[a]	45.0[a]	48.3[a]	36.7[a]
One month	33.0[b]	206.3[b]	61.7[a]	30.7[b]	33.7[b]	31.3[b]	27.0[b]
Two months	29.0[b]	183.7[c]	36.0[b]	27.3[bc]	26.7[b]	24.0[bc]	21.0[bc]
Three months	24.7[b]	115.0[d]	31.3[b]	22.7[bc]	22.7[bc]	21.3[c]	17.3[c]
Four months	24.0[b]	101.7[d]	21.3[b]	19.3[c]	18.0[c]	16.7[c]	15.7[c]
Control	4.3[c]	6.7[e]	4.3[c]	4.0[d]	4.0[d]	4.0[d]	3.7[d]

[1] Means followed by the same letter are not significantly different from each other (P=0.05) according to Duncan's Multiple Range test.

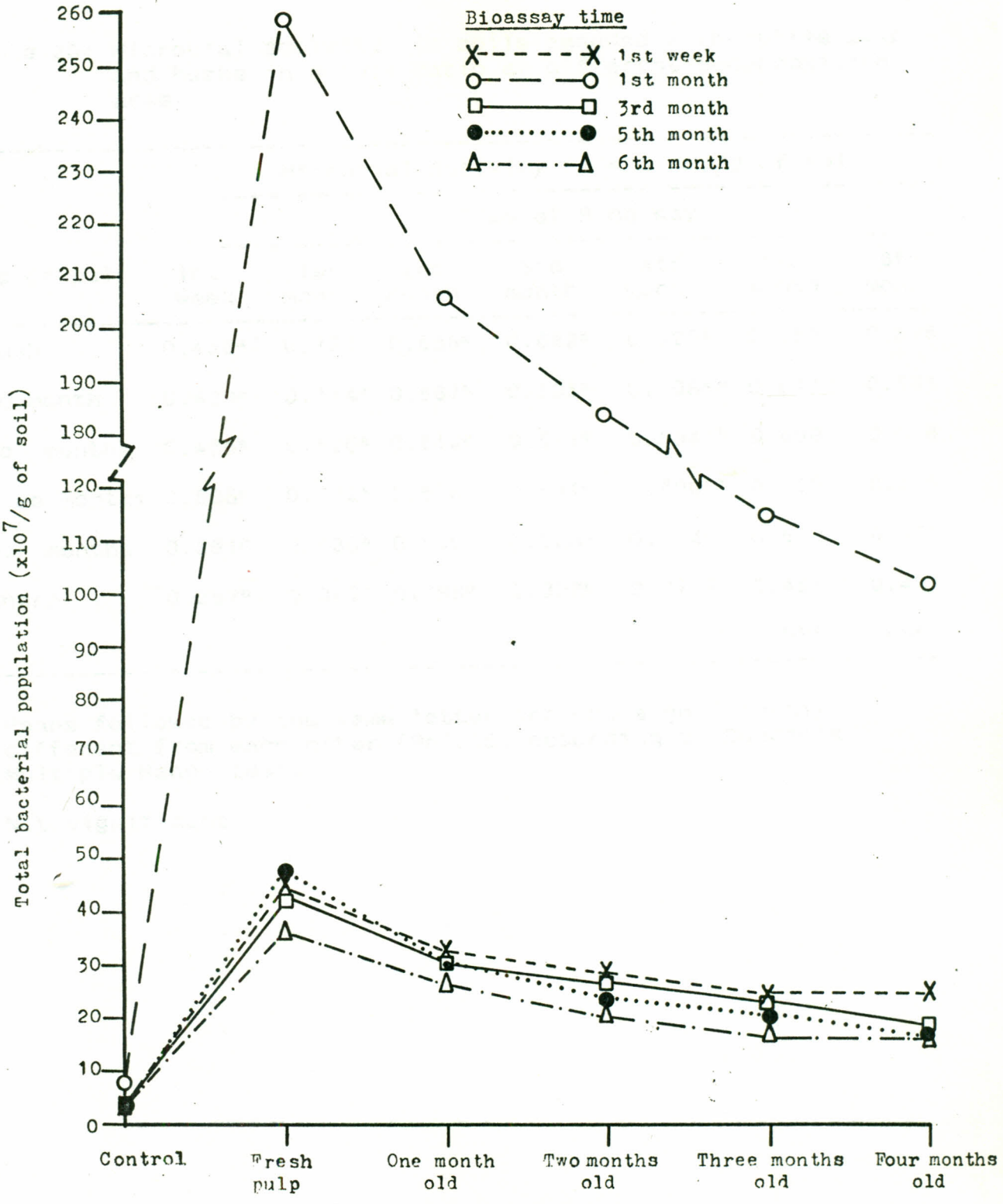


Fig. 10 : Age of coffee pulp and husks and total bacterial population with time.

Table 20: Microbial activity in soils amended with coffee pulp and husks in a 10:1 ratio at different decomposition ages

Age of pulp	Microbial activity (A 490 nm)/g of soil						
	Time of Bioassay						
	1st week	1st month	2nd month	3rd month	4th month	5th month	6th month
Fresh	0.452[a 1]	0.733	0.685[a]	0.682[a]	0.622[a]	0.668	0.576
One month	0.429[a]	0.714[a]	0.667[a]	0.635[a]	0.608[a b]	0.641	0.531
Two months	0.455[a]	0.690[a]	0.614[a]	0.561[a]	0.594[a b]	0.598	0.518
Three months	0.505[b]	0.682[a]	0.598[a]	0.561[a]	0.590[a b]	0.563	0.516
Four months	0.361[c]	0.630[a]	0.590[a]	0.528[a]	0.504[b]	0.518	0.473
Control	0.267[d]	0.347[b]	0.299[b]	0.302[b]	0.271[c]	0.455	0.433
						NS[2]	NS[2]

[1] Means followed by the same letter are not significantly different from each other (P=0.05) according to Duncan's Multiple Range test.

[2] Not significant

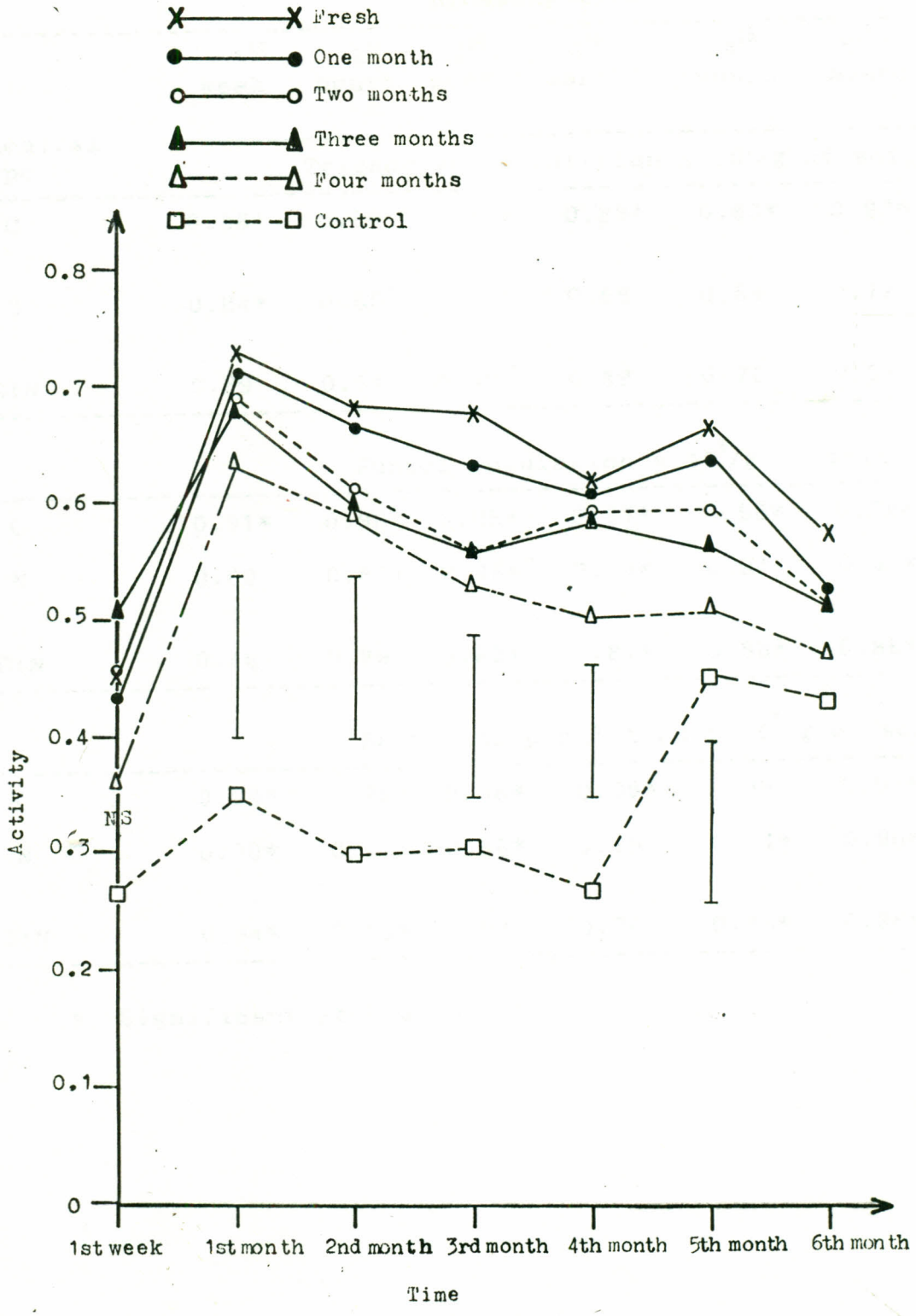


Fig. 11 : Age of pulp and microbial activity (A 490 nm) per gram of soil with time (months).

Table 21: Correlation coefficients (r) of a regression analysis between microbial populations and carbon (c), nitrogen (n) contents and C:N ratios of the ages of coffee pulp

Chemical type	Bioassay time						
	1st week	1st month	2nd month	3rd month	4th month	5th month	6th month
	Trichoderma population x 10^5/g of soil						
C	0.86*	0.80	0.85*	0.89*	0.83*	0.92*	0.94*
N	0.84*	0.60	0.61	0.66	0.58	0.72	0.74
C:N	0.69	0.54	0.52	0.69	0.76	0.57	0.60
	Fungal population x 10^5/g of soil						
C	0.91*	0.98*	0.98*	0.97*	0.96*	0.98*	0.98*
N	0.69	0.87*	0.92*	0.94*	0.94*	0.94*	0.93*
C:N	0.46	0.79	0.82*	0.87*	0.88*	0.88*	0.85*
	Bacterial population x 10^7/g of soil						
C	0.99*	0.99*	0.98*	0.99*	0.99*	0.98*	0.99*
N	0.98*	0.92*	0.86*	0.96*	0.94*	0.96*	0.96*
C:N	0.94*	0.86*	0.67	0.76	0.88*	0.86*	0.89*

* Significant at P=0.05

Table 22: Correlation coefficients (r) of a regression analysis between microbial populations and carbon (c), nitrogen (n) contents, C:N ratio and pH from soil amended with different ages of coffee pulp as at the end of the experiment.

	Bioassay time						
	1st week	1st month	2nd month	3rd month	4th month	5th month	6th month
Chemical type	*Trichoderma* population x 10^5/g of soil						
C	0.93*	0.89*	0.91*	0.96*	0.92*	0.98*	0.97*
N	0.34	0.57	0.41	0.45	0.40	0.45	0.45
C:N	0.89*	0.60	0.56	0.59	0.56	0.55	0.53
pH	0.11	0.74	0.71	0.63	0.71	0.51	0.50
	Fungal population x 10^5/g of soil						
C	0.82*	0.95*	0.89*	0.89*	0.86*	0.88*	0.88*
N	0.63	0.63	0.71	0.62	0.64	0.69	0.73
C:N	0.67	0.57	0.45	0.40	0.37	0.45	0.50
pH	0.36	0.43	0.25	0.13	0.14	0.25	0.32
	Bacterial population x 10^7/g of soil						
C	0.96*	0.96*	0.97*	0.94*	0.95*	0.92*	0.96*
N	0.77	0.77	0.59	0.74	0.69	0.68	0.71
C:N	0.64	0.67	0.55	0.59	0.57	0.49	0.59
pH	0.32	0.47	0.37	0.33	0.31	0.21	0.29

* Significant at P=0.05

6.0. Experiment 4

Effects of soil amended with normal coffee pulp and husks, ground coffee pulp and husks and filter press cake on the proliferation of *Trichoderma* spp. and other soil microorganisms were investigated in this experiment.

6.1 *Trichoderma* population

Trichoderma populations were significantly (P=0.05) different throughout the period of the experiment (Table 23).

Ground coffee pulp significantly (P=0.05) enhanced *Trichoderma* population build-up in the first week more than the filter press cake and control. Although ground coffee pulp amended soil had a higher population of the fungus than that supported by normal coffee pulp amended soil in the first week, the difference was not significant (Table 23). Normal coffee pulp amended soil significantly (P=0.05) enhanced proliferation of *Trichoderma* more than the ground coffee pulp amended soil, except for the first week (Fig. 12). Although the normal coffee pulp amended soil enhanced *Trichoderma* population build-up more than filter press cake, the difference was not significant (Table 23).

Table 23: *Trichoderma* populations obtained from organic amended and non-amended soils.

Treatments	*Trichoderma* population x 10^5/g of soil			
	Time of Bioassay			
	1st week	1st mnth	2nd mnth	3rd mnth
Ground coffee pulp	3.3[a1]	3.0[a]	3.0[a]	2.3[a]
Filter press cake	1.8[bc]	3.5[ab]	4.5[b]	3.3[ab]
Normal coffee pulp	2.7[ca]	4.3[b]	5.3[b]	4.0[b]
Control	1.5[b]	1.1[c]	1.0[c]	0.8[c]

[1] Means followed by the same letter are not significantly different from each other (P=0.05) according to Duncan's Multiple Range test.

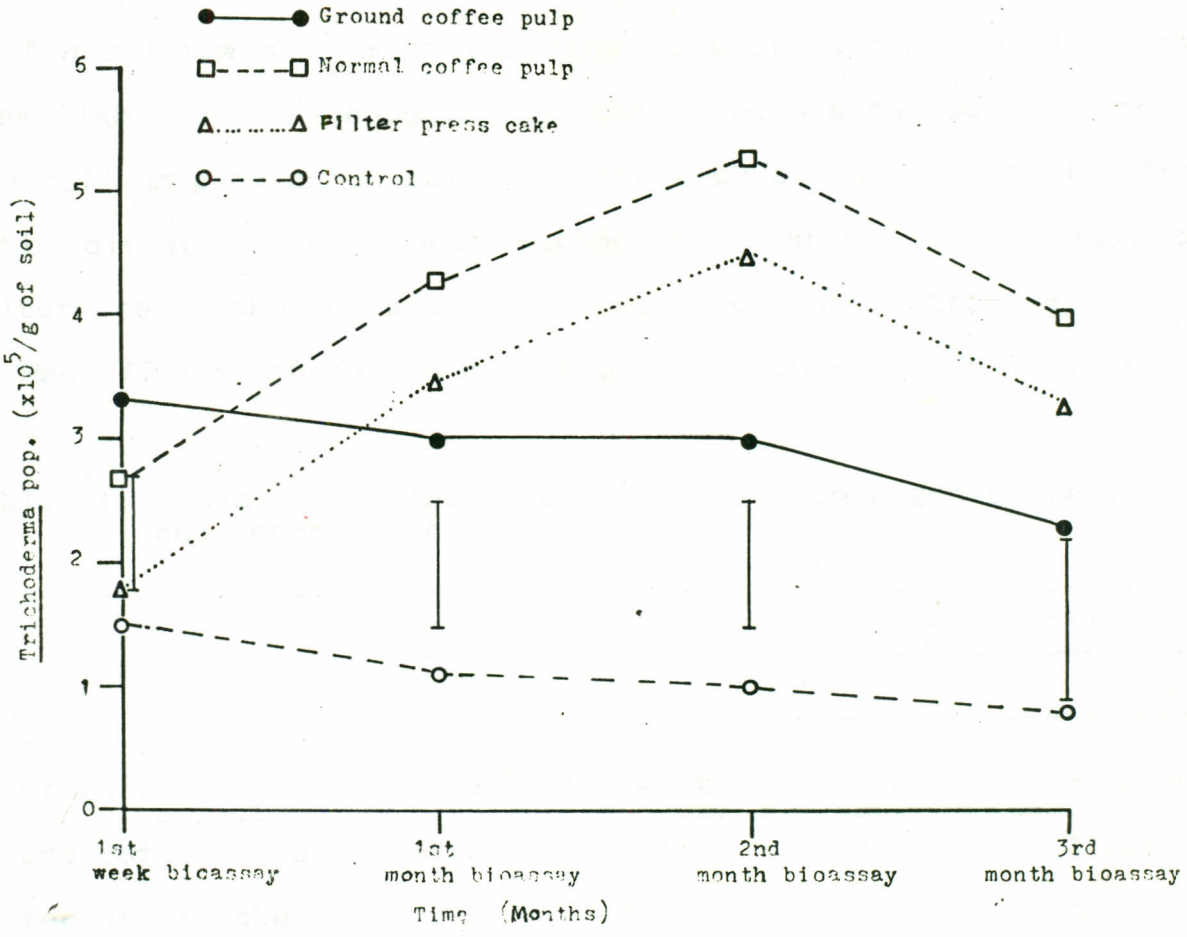


Fig. 12 : Effect of normal, ground coffee pulp and husks and filter press cake amendments on Trichoderma population.

The verticle bars denote least significant differences (LSD) bars.

6.2 *Penicillium* population

All treatments significantly (P=0.05) enhanced proliferation of *Penicillium* spp. more than the control, except in the first week (Table 24). Although normal coffee pulp enhanced the highest *Penicillium* population build-up, the population was not significantly different from those supported by ground coffee pulp or filter press cake amended soil. Except for the control, a decline in *Penicillium* population occurred by the third month (Fig. 13).

Table 24: *Penicillium* population obtained from organic amended and non-amended soils.

	Penicillium population x 10^5/g of soil			
	Time of Bioassay			
Treatments	1st week	1st month	2nd month	3rd month
Ground coffee pulp	5.5	61.0[a]	60.8[a]	21.0[a]
Filter press cake	3.3	70.8[a]	70.8[a]	24.3[a b]
Normal coffee pulp	3.5	74.5[a]	72.0[a]	25.3[a]
Control	4.5	3.8[b]	4.0[b]	6.0[b]
	NS[2]			

[1] Means followed by the same letter are not significantly different from each other (P=0.05) according to Duncan's Multiple Range test.

[2] Not significant

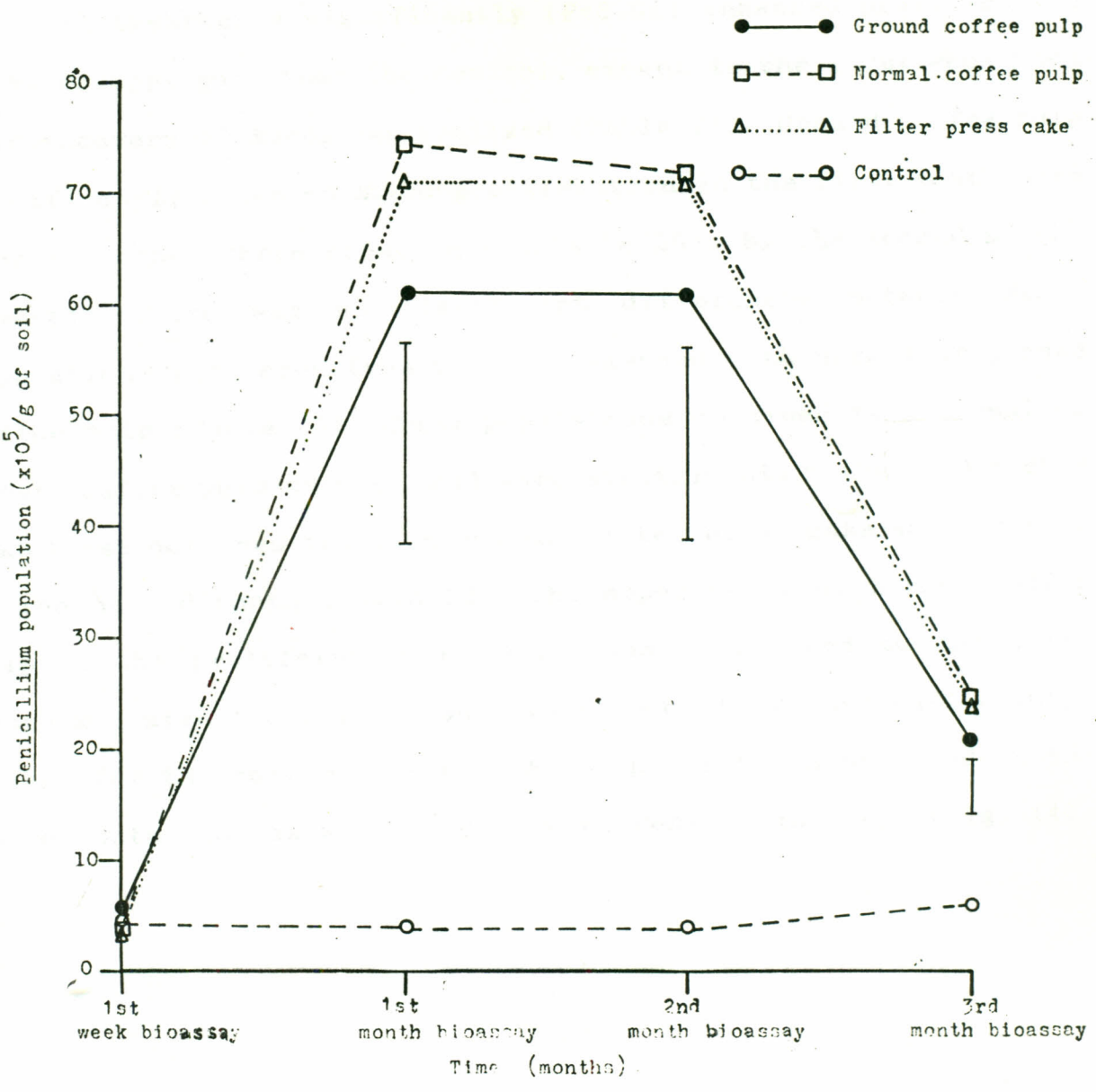


Fig. 13 : Effect of normal, ground coffee pulp and husks and filter press cake amendments on Penicillium population.

The verticle bars denote LSD bars.

6.3 *Mucor* population

All treatments significantly (P=0.05) enhanced proliferation of *Mucor* spp. more than the control, except in the first week when zero recovery of *Mucor* was realized (Table 25). Ground coffee pulp significantly enhanced *Mucor* proliferation in the first month more than the other three treatments (Table 25). By the second month, however, there was no significant differences between *Mucor* populations recovered from the soil amended with normal or ground coffee pulp (Table 25). *Mucor* populations obtained from normal or ground coffee pulp amended soil were significantly (P=0.05) higher than those obtained from control and filter press cake amended soil by the second month (Table 25). The superiority of normal coffee pulp in the proliferation of *Mucor* spp. persisted to the third month and significantly so only in respect to ground coffee pulp. Except for the control, drop in *Mucor* populations occurred in the third month just as was the case with *Penicillium* spp. (Fig. 14).

Table 25: *Mucor* population obtained from organic amended and non-amended soils.

Treatments	*Mucor* population x 10^5/g of soil			
	Time of bioassay			
	1st week	1st month	2nd month	3rd month
Ground coffee pulp	0.0[1]	10.8[a 2]	10.3a	1.3[a]
Filter press cake	0.0	6.3[b]	6.3[b]	1.8[a b]
Normal coffee pulp	0.0	5.5[b]	9.8[a]	2.0[b]
Control	0.0	0.0[c]	0.0[c]	0.0[c]

[1] For analysis of variance 0.0 was substituted with 0.1

[2] Means followed by the same letter are not significantly different from each other (P=0.05) according to Duncan's Multiple Range test.

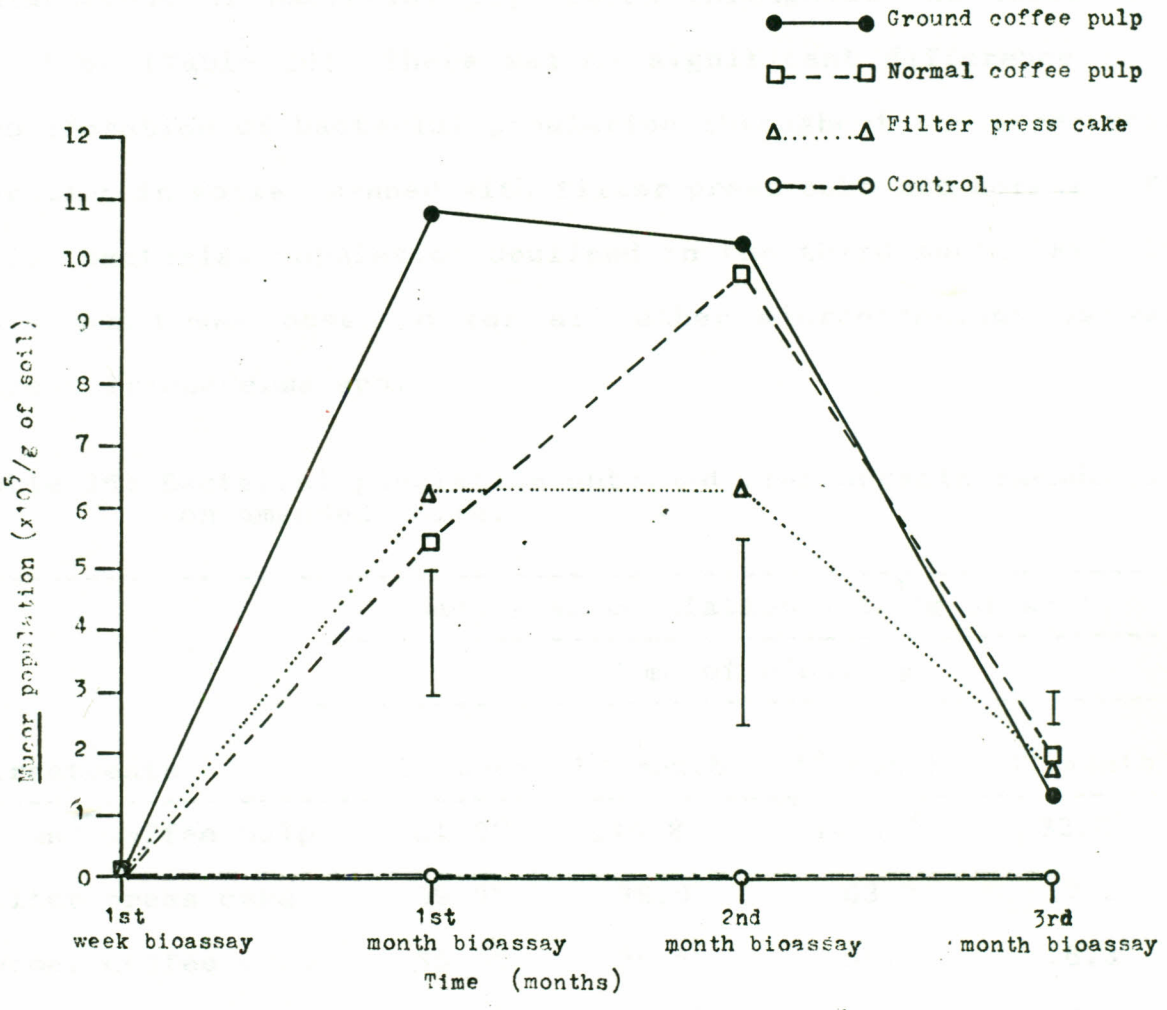


Fig. 14 : Effect of normal, ground coffee pulp and husks and filter press cake amendments on Mucor population.

The vertical bars denote LSD bars.

6.4. Total bacterial population

All amendments significantly (P=0.05) enhanced proliferation of bacteria more than the control throughout the experimental duration (Table 26). Ground coffee pulp was significantly (P=0.05) superior to filter press cake and normal coffee pulp in the enhancement of bacterial population throughout the experimental duration (Table 26). There was no significant difference in the proliferation of bacterial population throughout the experimental duration in soils amended with filter press cake and normal coffee pulp. Bacterial population declined in the third month (Fig. 15). This trend was observed for all other microorganisms as well, except *Trichoderma* spp.

Table 26: Bacterial population obtained from organic amended and non-amended soils.

	Bacterial population x 10^7/g of soil			
	Time of Bioassay			
Treatments	1st week	1st month	2nd month	3rd month
Ground coffee pulp	61.5[a1]	140.8[a]	140.0[a]	32.0[a]
Filter press cake	39.0[b]	38.0[b]	63.3[b]	17.3[b]
Normal coffee pulp	35.3[b]	36.5[b]	55.0[b]	16.3[b]
Control	4.8[c]	4.3[c]	4.3[c]	5.3[c]

[1] Means followed by the same letter are not significantly different from each other (P=0.05) according to Duncan's Multiple Range test.

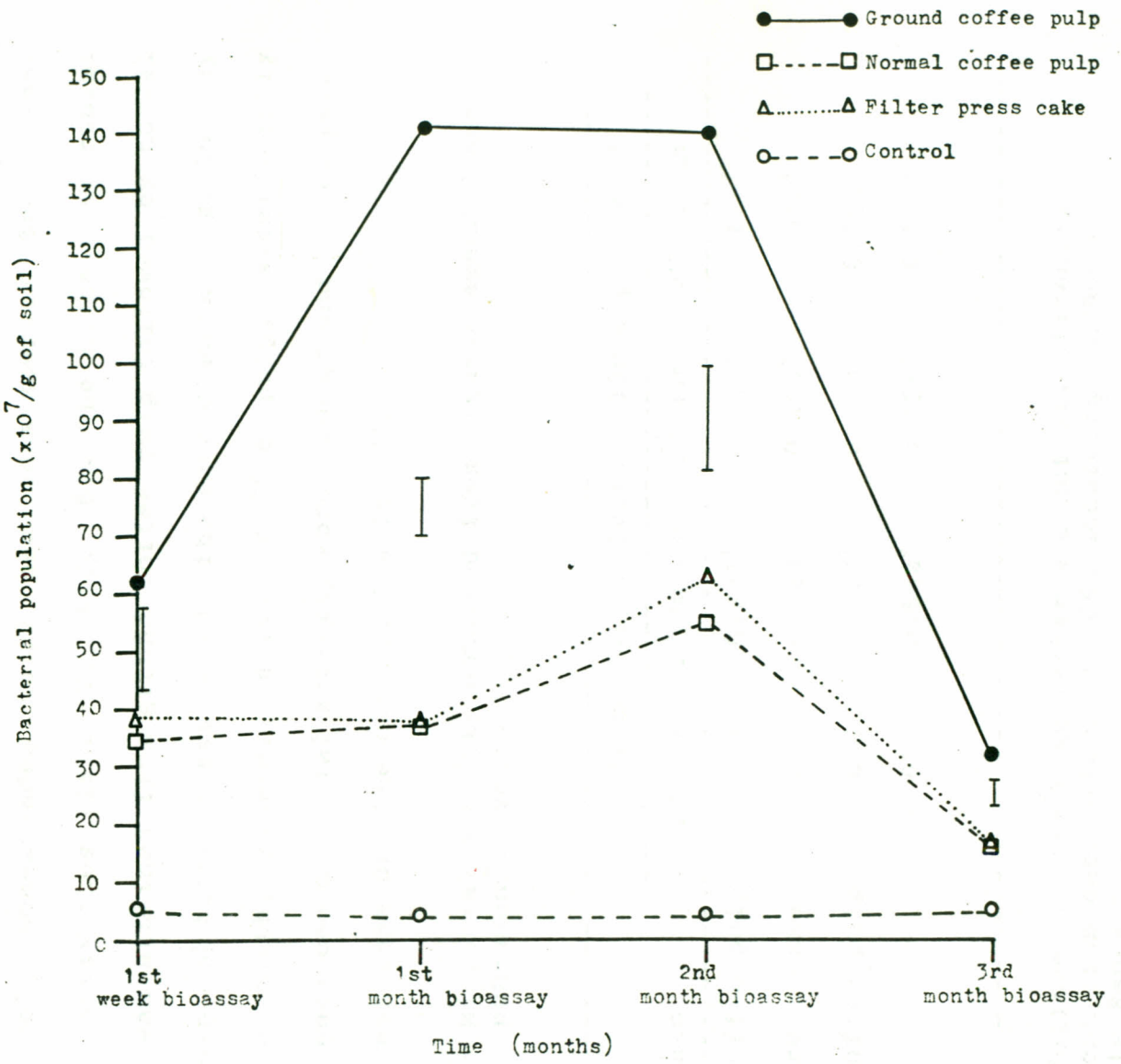


Fig. 15 : Effect of normal, ground coffee pulp and husks and filter press cake on bacterial population.

The vertical bars denote LSD bars.

6.5 Microbial activity (A 490 nm)/gram of soil due to amending soil with normal and ground coffee pulp and filter press cake

Significant (P=0.05) differences were detected in the microbial activities throughout the experimental period (Table 27). The control (non-amended soil) had consistently the lowest microbial activities (Table 27, Fig. 16). The highest microbial activity was attained from ground coffee pulp followed by normal coffee pulp and filter press cake by the second month (Fig. 16). By the third month, however, normal coffee pulp, significantly (P=0.05) enhanced microbial activity more than ground coffee pulp, filter press cake and the control (Table 27).

Table 27: Microbial activity obtained from organic amended and non-amended soils.

Treatments	Microbial activity A 490nm/g of soil			
	1st week	1st month	2nd month	3rd month
Ground coffee pulp	0.842	0.801[a]	0.543[a]	0.666[ab]
Filter press cake	0.830	0.723[a]	0.404[a]	0.515[a]
Normal coffee pulp	0.838	0.778[a]	0.480[a]	0.700[b]
Control	0.670	0.662[b]	0.328[b]	0.507[a]
	NS[2]			

[1] Means followed by the same letter are not significantly different from each other (P=0.05) according to Duncan's Multiple Range test.

[2] Non significant

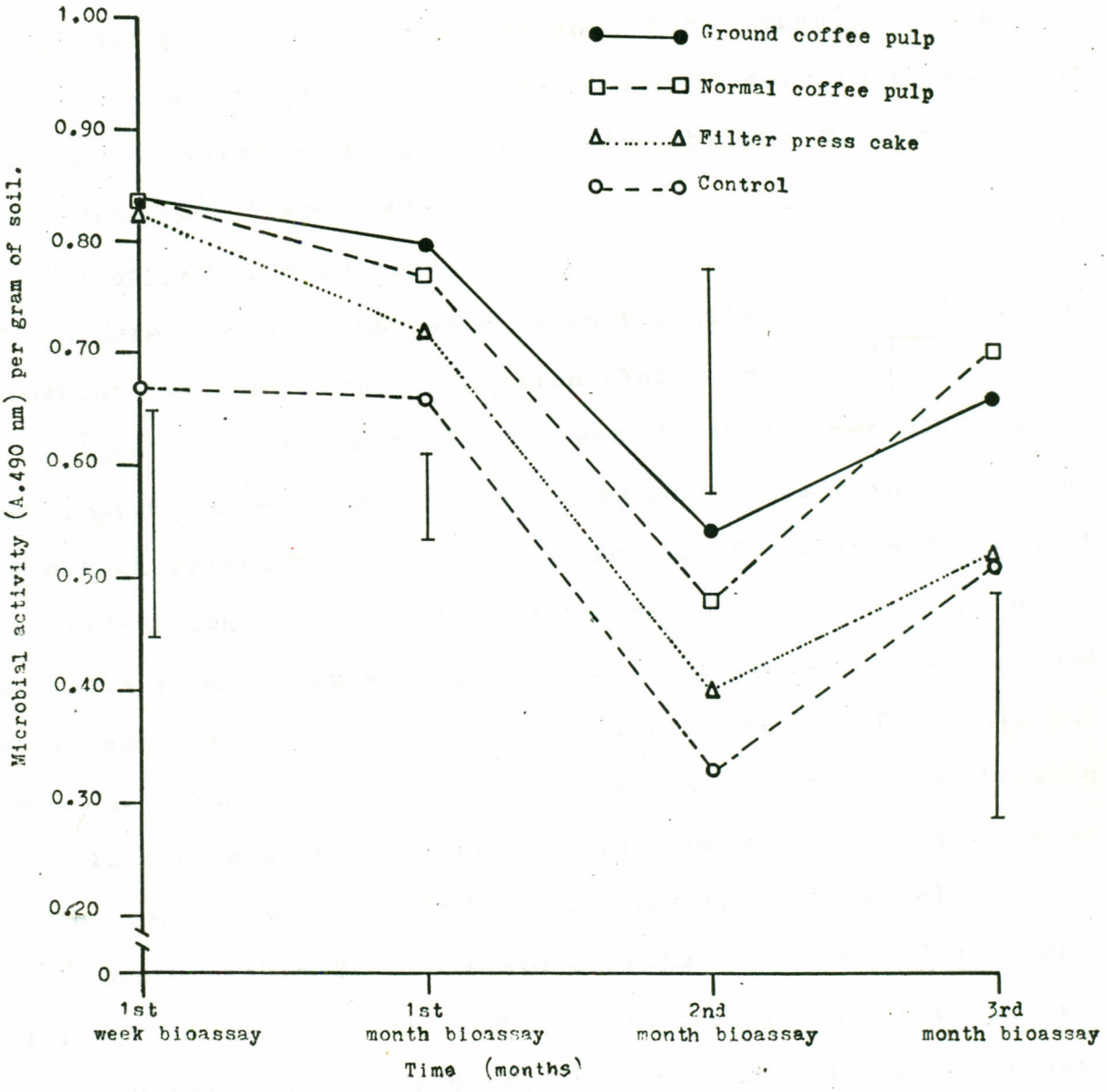


Fig. 16 : Effect of normal, ground coffee pulp and husks and filter press cake. on microbial activity.

The vertical bars denote LSD bars.

7.0. Experiment 5

Effects of sub-lethal doses of a soil fumigant (basamid) on the proliferation of *Trichoderma* spp. and other soil microorganisms were investigated in this experiment. For comparison purposes, coffee pulp and husks in a 10:1 ratio and filter press cake were included as treatments due to their ability to proliferate *Trichoderma* spp. (Experiment 1).

7.1. *Trichoderma* populations

Trichoderma populations were significantly (P=0.05) different throughout the experimental duration (Table 28).

Coffee pulp enhanced the highest *Trichoderma* proliferation throughout the experimental duration as was the case in experiment 1. The high *Trichoderma* populations from coffee pulp amended soil were significantly (P=0.05) different from those of other treatments in most cases (Table 28). Filter press cake was second best in enhancing proliferation of *Trichoderma* to coffee pulp but it was significantly (P=0.05) inferior most of the times. Basamid at the lowest rate (0.1g) enhanced *Trichoderma* proliferation more than the control and attained significant (P=0.05) levels only in the 5^{th} month (Table 28). Basamid at 0.2g suppressed *Trichoderma* proliferation until the 4^{th} month. A general decline in population of *Trichoderma* occurred from the 3^{rd} month for all the treatments except for basamid at both rates. Significant population increase occurred in basamid treated soil by the 5^{th} month compared to the control (Fig. 17).

Table 28: *Trichoderma* population in soils treated with sub-lethal doses of basamid and some organic materials

Treatments	*Trichoderma* population x 10^5/g of soil					
	Time of bioassay					
	1st day	1st month	2nd month	3rd month	4th month	5th month
Coffee pulp	0.0[2]	9.0[a1]	7.8[a]	7.8[a]	6.0[a]	5.3[a]
Filter press cake	0.0	5.3[b]	6.5[b]	6.5[b]	5.5[a]	3.8[b]
Basamid 0.2g	0.0	1.3[c]	0.8[e]	0.5[c]	1.0[b]	3.3[b]
Basamid 0.1g	0.0	2.7[d]	2.5[d]	2.8[d]	2.3[b]	3.0[b]
Control	0.0	2.0[d]	1.3[d]	2.0[e]	1.3[b]	1.0[c]
	NS[3]					

[1] Means followed by the same letter are not significantly (P=0.05) different from each other according to Duncan's Multiple Range test.

[2] For analysis of variance, zero was substituted with 0.1.

[3] Non significant.

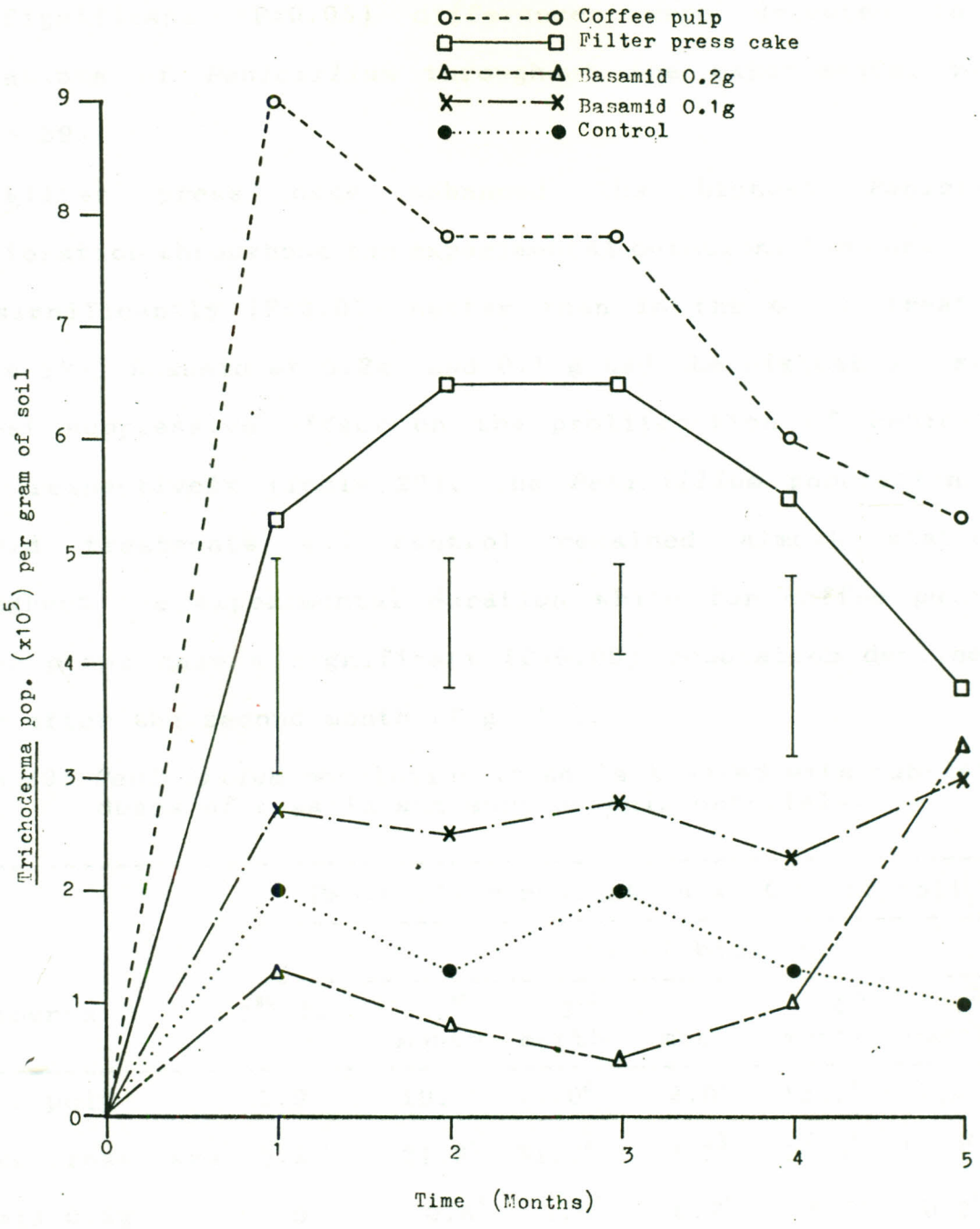


Fig. 17 : Sub-lethal doses of a soil sterilant (Basamid) and organic amendments on Trichoderma population ($x10^5$) per gram of soil.

Vertical bars denote LSD bars.

7.2. *Penicillium* population

Significant (P=0.05) differences were detected in the populations of *Penicillium* throughout the experimental period (Table 29).

Filter press cake enhanced the highest *Penicillium* proliferation throughout the experimental duration. The enhancement was significantly (P=0.05) better than in the other treatments (Table 29). Basamid at 0.2g and 0.1 g had the highest and second highest suppressive effect on the proliferation of *Penicillium* spp., respectively (Table 29). The *Penicillium* population from Basamid treatments and control remained almost stationary throughout the experimental duration while for coffee pulp and filter press cake a significant (P=0.05) population decline was noted after the second month (Fig. 18).

Table 29: *Penicillium* population in soils treated with sub-lethal doses of basamid and some organic materials.

Treatments	*Penicillium* population x 10^5/g of soil					
	Time of bioassay					
	1st day	1st month	2nd month	3rd month	4th month	5th month
Coffee pulp	1.9	19.5[a]	14.0[a]	12.0[a]	13.0[a]	7.8[a]
Filter press cake	0.8	21.8[a]	31.3[b]	17.5[b]	17.8[b]	13.3[b]
Basamid 0.2g	0.0	0.8[b]	0.5[c]	0.8[c]	0.8[c]	0.3[cd]
Basamid 0.1g	0.0	1.0[b]	1.0[c]	2.8[c]	1.0[c]	0.8[cd]
Control	1.7	3.8[b]	3.0[c]	3.5[c]	2.5[c]	2.0[d]
	NS[2]					

[1] Means followed by the same letter are not significantly (P=0.05) different from each other according to Duncan's Multiple Range test.
[2] Non significant

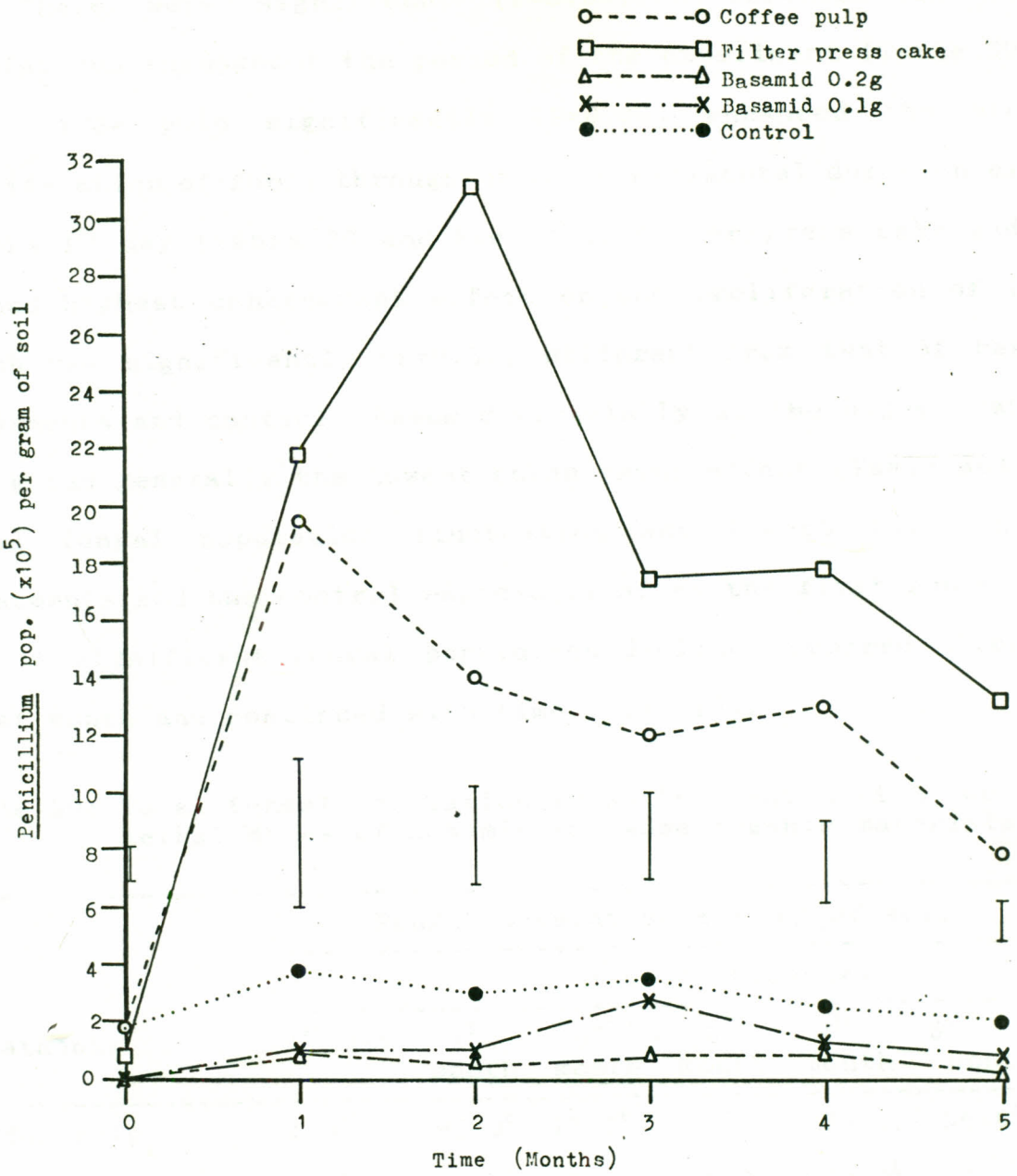


Fig.18 : Sub-lethal doses of a soil sterilant (Basamid) and organic amendments on Penicillium population ($x10^5$) per gram of soil.

Vertical bars denote LSD bars.

7.3. Total fungal population

There were significant (P=0.05) differences in fungal populations throughout the period of the experiment (Table 30).

Coffee pulp significantly (P=0.05) enhanced the highest proliferation of fungi throughout the experimental duration except on the 1st day (Table 30 and Fig. 19). Filter press cake had the second highest enhancement effect on the proliferation of fungi which was significantly (P=0.05) different from that of basamid treatments and control. Basamid especially at the higher rate of 0.2 g had generally the lowest enhancement effect (Table 30). The total fungal population fluctuation was lowest with basamid treatments and the control especially after the first month (Fig. 19). A significant fungal population decline occurred after the first month and continued with time (Fig. 19).

Table 30: Total fungal population in soils treated with sub-lethal doses of basamid and some organic materials

Treatments	Fungal population x 10^5/g of soil					
	Time of bioassay					
	1st day	1st month	2nd month	3rd month	4th month	5th month
Coffee pulp	1.9[a1]	92.0[a]	71.0[a]	66.3[a]	64.5[a]	56.8[a]
Filter press cake	0.8[b]	70.5[b]	48.5[b]	43.8[b]	43.0[b]	29.0[b]
Basamid 0.2g	0.0[b]	7.3[c]	6.5[c]	7.3[c]	5.3[e]	5.8[c]
Basamid 0.1g	0.0[b]	15.5[c]	8.5[c]	12.3[d]	8.3[c]	7.8[c]
Control	1.7[a]	19.5[c]	12.0[c]	12.8[d]	7.8[c]	7.8[c]

[1] Means followed by the same letter are not significantly (P=0.05) different from each other according to Duncan's Multiple Range test.

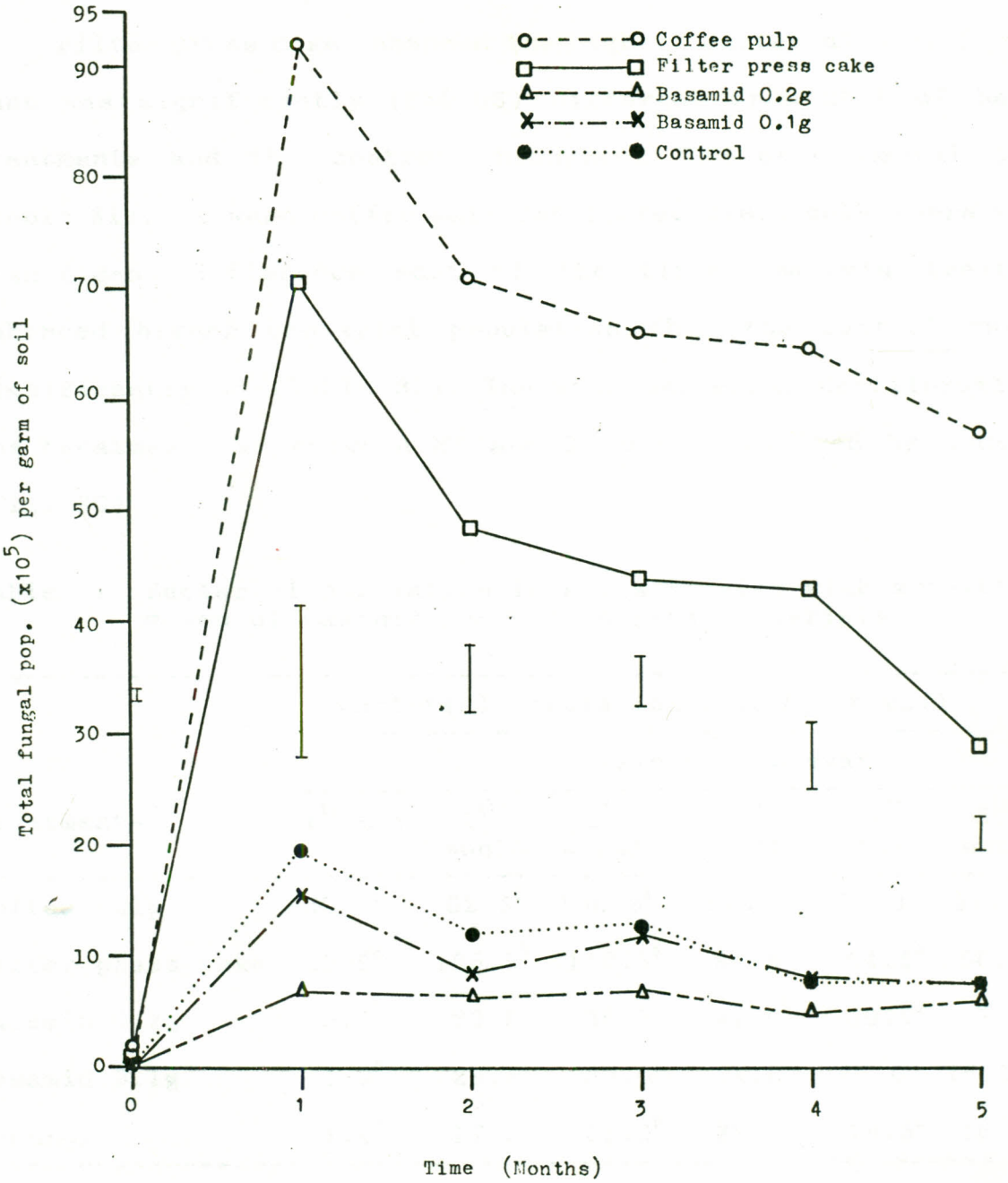


Fig. 19: Sub-lethal doses of a soil sterilant (Basamid) and organic amendments on total fungal population ($x10^5$) per gram of soil. Vertical bars denote LSD bars.

7.4. Total bacterial population

There were significant (P=0.05) differences in bacterial populations throughout the experimental period (Table 31).

Filter press cake enhanced the highest bacterial proliferation that was significantly (P=0.05) different from that of basamid treatments and the control throughout the experimental period (Table 31). Between coffee pulp and filter press cake there was no significant difference most of the times. Basamid treatments enhanced higher bacterial population than the control but not significantly so (Table 31). The peak bacterial proliferation of the treatment was between 2nd and 3rd month followed by a decline (Fig. 20).

Table 31: Bacterial population in soils treated with sub-lethal doses of basamid and some organic materials.

Treatments	Bacterial population x 10^7/g of soil					
	Time of bioassay					
	1st day	1st month	2nd month	3rd month	4th month	5th month
Coffee pulp	30.0[a1]	62.5[a]	106.8[a]	98.0[a]	78.0[a]	51.0[a]
Filter press cake	40.9[a]	106.8[b]	110.3[a]	84.5[a]	64.5[a]	50.3[a]
Basamid 0.2g	0.7[b]	23.8[c]	36.0[b]	42.0[b]	30.0[b]	27.8[b]
Basamid 0.1g	4.9[b]	28.5[c]	30.0[b]	34.8[bc]	26.0[bc]	15.3[c]
Control	4.4[b]	17.0[c]	23.0[b]	22.5[c]	18.5[c]	16.0[c]

[1] Means followed by the same letter are not significantly (P=0.05) different from each other according to Duncan's Multiple Range test.

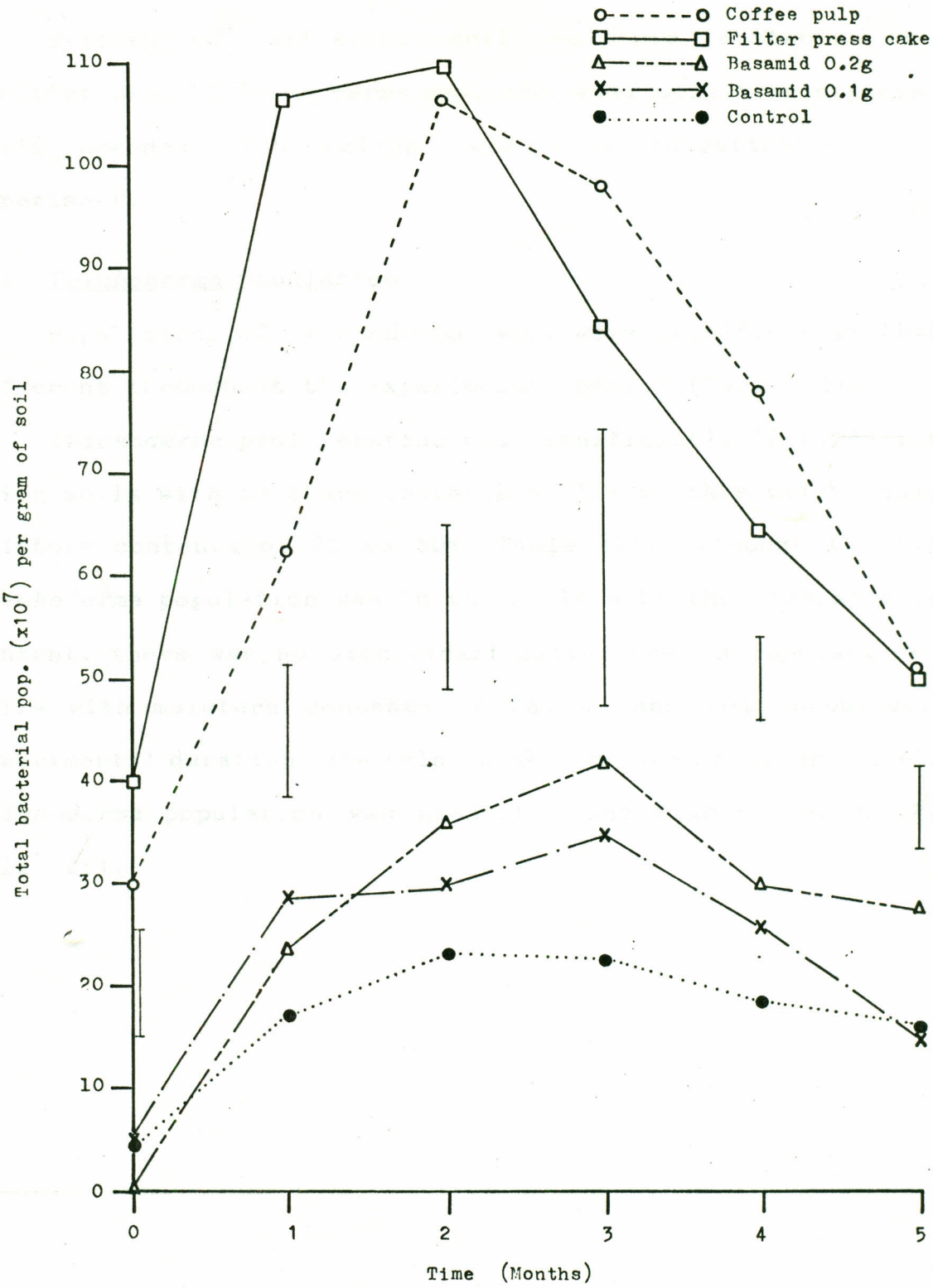


Fig. 20 : Sub-lethal doses of a soil sterilant (Basamid) and organic amendments on total bacterial population ($x10^7$) per gram of soil

Vertical bars denote LSD bars.

8.0. Experiment 6

Effects of different soil moisture contents on the proliferation of *Trichoderma* spp. and other soil microorganisms in soils amended with coffee pulp were investigated in this experiment.

8.1. *Trichoderma* population

Populations of *Trichoderma* spp. were significantly (P=0.05) different throughout the experimental period (Table 32).

Trichoderma proliferation was significantly (P=0.05) less in drier soils with moisture contents of 15-25% than wetter soils of moisture contents of 35 to 50% (Table 32). Although the highest *Trichoderma* population was in the soils with the highest moisture content, there was no significant difference in populations from soils with moisture contents of 35, 40 and 50% throughout the experimental duration. The relationship between moisture levels and *Trichoderma* population was quadratic and significant at P=0.01 (Fig. 21).

Table 32: *Trichoderma* population recovered from coffee pulp amended soil at different moisture contents.

Moisture content (%)	*Trichoderma* x 10^5/g of soil			
	1st week	1st month	2nd month	3rd month
15	0.0[a1]	0.0[a]	0.3[a]	0.0[a]
25	0.7[ab]	0.7[a]	0.7[a]	1.0[b]
35	1.0[bc]	4.0[b]	3.7[b]	2.3[c]
40	1.0[bc]	4.0[b]	3.7[b]	2.3[c]
50	1.3[c]	5.0[b]	4.0[b]	3.0[c]

[1] Means followed by the same letter are not significantly different from each other at P=0.05 according to Duncan's Multiple Range test.

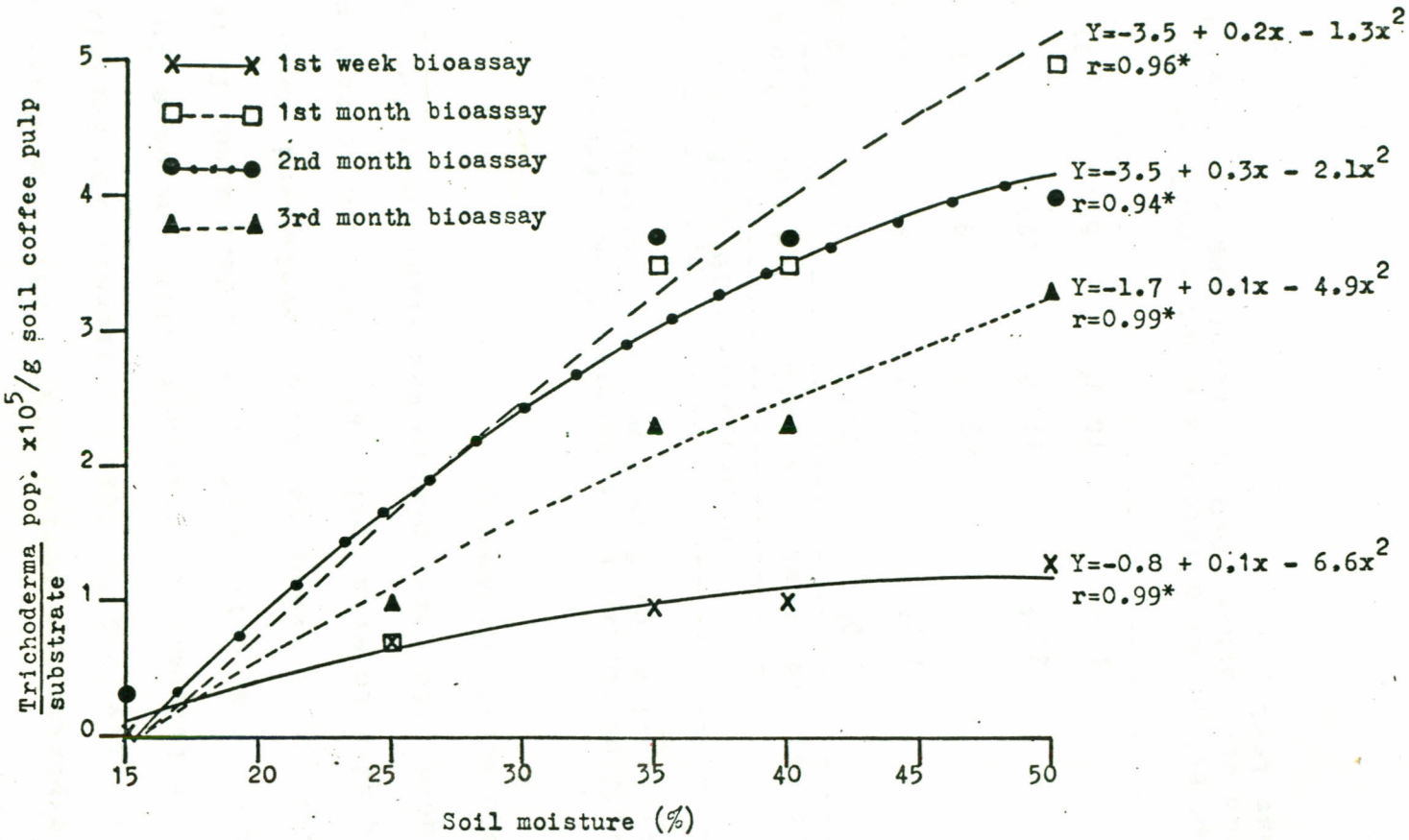


Fig.21 : Regression between soil moisture levels in soils amended with coffee pulp substrate and Trichoderma population.

8.2. *Penicillium* population

Penicillium populations were significantly (P=0.05) different throughout the experimental period (Table 33). The drier soils supported significantly (P=0.05) less proliferation of *Penicillium*. The population proliferation increased with increase in soil moisture content up to an optimum of 40% then decreased (Table 33).

The relationship between moisture levels and Penicillium population was quadratic with optima at a moisture content of 40% (Fig. 22). The quadratic relationship was significant (P=0.05) at the 1st and second month bioassays.

Table 33: *Penicillium* population recovered from coffee pulp amended soil at different moisture contents.

	Penicillium x 10^5/g of soil			
Moisture content (%)	1st week	1st mth	2nd mth	3rd mth
15	1.0^{a1}	0.7^{a}	2.7^{a}	2.3^{a}
25	4.3^{b}	37.7^{a}	81.3^{b}	21.0^{b}
35	2.7^{c}	87.3^{b}	91.7^{bc}	34.3^{c}
40	2.7^{c}	141.0^{c}	127.3^{c}	73.3^{d}
50	1.0^{a}	70.7^{b}	61.3^{b}	21.3^{b}

[1] Means followed by the same letter are not significantly different from each other (P=0.05) according to Duncan's Multiple Range test.

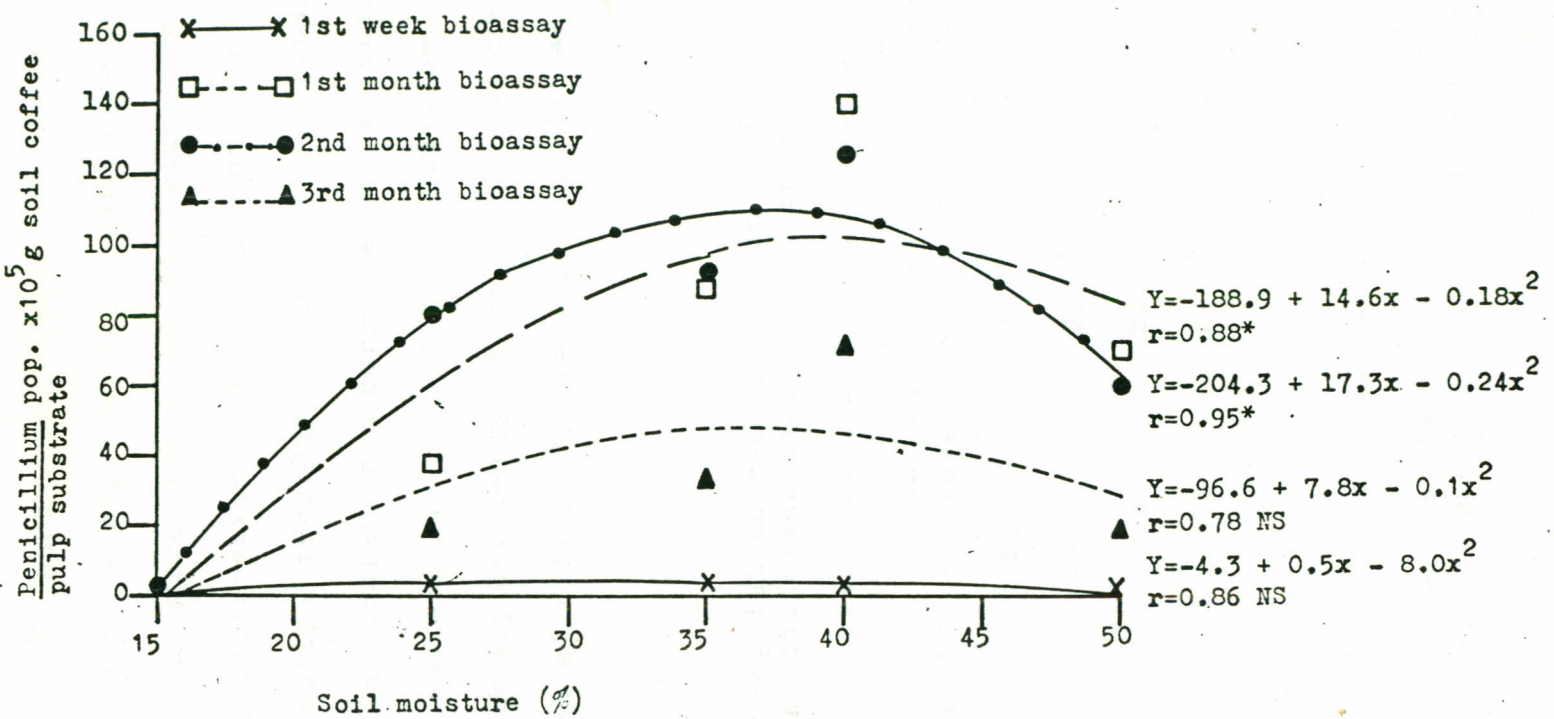


Fig.22. : Regression between soil moisture levels in soils amended with coffee pulp substrate and *Penicillium* population.

8.3 *Mucor* population

Populations of *Mucor* spp. were significantly (P=0.05) different throughout the experimental period (Table 34).

The lowest moisture level of 15% significantly (P=0.05) supported less proliferation of *Mucor* spp. Moisture content of 25 to 40% significantly (P=0.05) enhanced *Mucor* population (Table 34). A decline in population occurred at a moisture content of 40%. This trend was similar to that of *Penicillium*.

The relationship between moisture levels and *Mucor* population was quadratic and significantly (P=0.05) so, with an optima at 40% (Fig. 23).

Table 34: *Mucor* population recovered from coffee pulp amended soil at different moisture contents.

	Mucor x 10^5/g of soil			
Moisture content (%)	1st week	1st month	2nd month	3rd month
15	0.0^{a1}	0.0^{a}	0.0^{a}	0.0
25	18.0^{b}	22.3^{a}	15.7^{b}	8.7
35	26.3^{b}	22.7^{b}	17.3^{bc}	8.3
40	26.7^{b}	16.0^{cd}	15.7^{b}	7.0
50	23.3^{b}	12.3^{d}	8.7^{c}	5.3
				NS^{2}

[1] Means followed by the same letter are not significantly different from each other (P=0.05) according to Duncan's Multiple Range test.

[2] Non significant

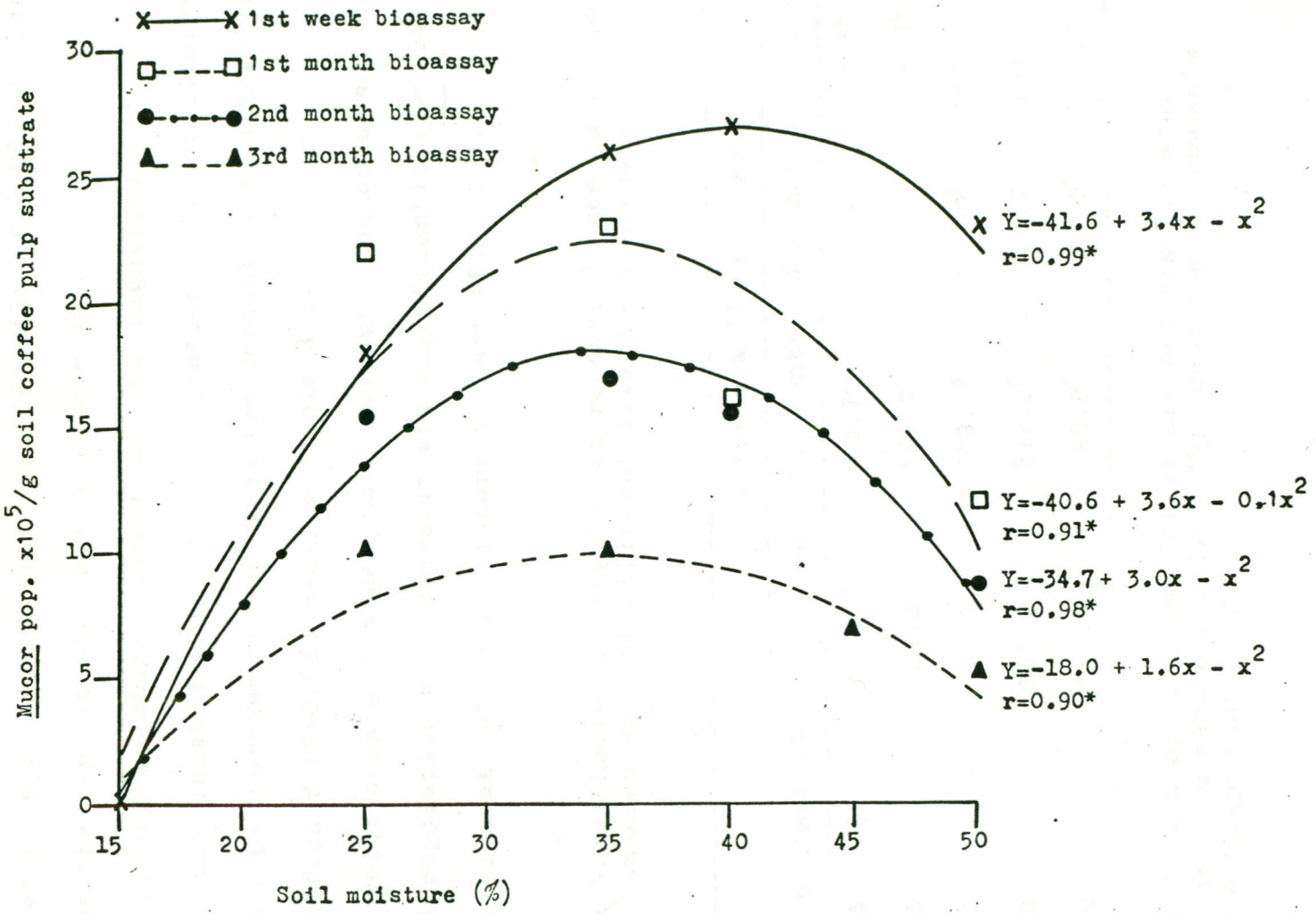


Fig. 23: Regression between soil moisture levels in soils amended with coffee pulp substrate and Mucor population with time.

8.4. Bacterial population

Bacterial populations were significantly (P=0.05) different throughout the experimental period (Table 35).

The lowest moisture content of 15% significantly (P=0.05) supported the least bacterial proliferation. The proliferation increased with increase in soil moisture content up to 40% followed by a significant (P=0.05) decline (Table 35).

The relationship between soil moisture contents and total bacterial population was quadratic and significantly (P=0.05) so, with an optima at 35% soil moisture content (Fig. 24).

Table 35: Total bacterial population recovered from coffee pulp amended soil at different moisture contents.

	Bacteria x 10^7/g of soil			
Moisture content (%)	1st week	1st month	2nd month	3rd month
15	3.0^{a1}	0.7^{a}	1.0^{a}	2.3^{a}
25	50.0^{b}	169.7^{b}	128.7^{b}	126.3^{b}
35	91.3^{c}	203.0^{bc}	145.0^{b}	151.3^{b}
40	107.0^{c}	242.0^{c}	145.7^{b}	154.7^{b}
50	100.3^{c}	82.0^{d}	52.0^{c}	56.7^{c}

[1] Means followed by the same letter are not significantly different from each other (P=0.05) according to Duncan's Multiple Range test.

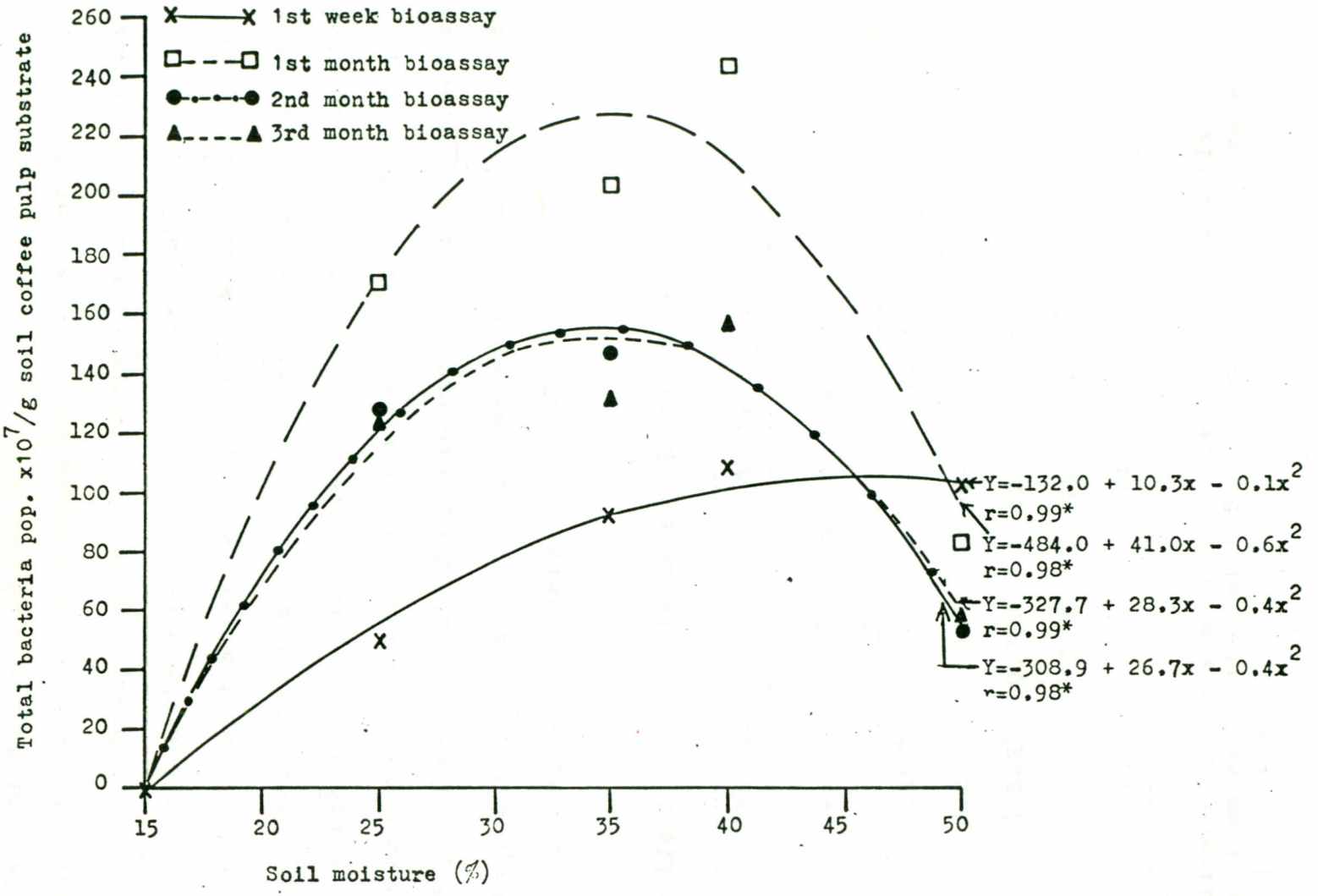


Fig. 24 : Regression between soil moisture levels in soils amended with coffee pulp substrate and total bacterial population with time.

8.5. Effects of moisture levels in soils amended with coffee pulp on microbial activity (A 490 nm)

Microbial activity as determined by hydrolysis of Fluorescein Diacetate (FDA) through microbial enzymes was significantly different throughout the experimental duration (Table 36).

Soil at the lowest moisture content of 15% had significantly (P=0.05) the lowest microbial activity throughout the experimental period. The microbial activity increased with increase in moisture levels except for a reduction in activity at 50% moisture content from the 1st month to the end of the experiment. For the second and third months, microbial activity optima occurred at 35 and 40% moisture contents, respectively (Table 36).

Table 36: Effect of moisture levels on microbial activity

Moisture content (%)	Microbial activity (A 490 nm)/g of soil			
	1st week	1st month	2nd month	3rd month
15	0.207[a1]	0.154[a]	0.161[a]	0.120[a]
25	0.262[a]	0.656[b]	0.584[b]	0.309[b]
35	0.562[b]	0.703[b]	0.628[b]	0.356[bc]
40	0.679[bc]	0.779[b]	0.579[bc]	0.434[c]
50	0.736[c]	0.750[b]	0.474[c]	0.423[c]

[1] Means followed by the same letter are not significantly different from each other at P=0.05 according to Duncan's Multiple Range test.

The regression analysis between total microbial population and microbial activity in relation to soil moisture contents was quadratic and highly significant (P=0.01) as indicated by the correlation coefficient (r) values (Table 37).

Table 37: Regression between microbial population ($x10^7$) per gram of soil and microbial activity (A 490 nm) under different moisture levels

	1st week		1st mth		2nd mth		3rd mth	
Moisture levels (%)	Pop.	Activ.	Pop.	Activ.	Pop.	Activ.	Pop.	Activ.
15	0.03	0.207	0.72	0.154	1.1	0.161	2.3	0.120
25	50.30	0.262	171.00	0.656	130.1	0.584	127.1	0.309
35	91.60	0.562	205.00	0.703	146.6	0.628	132.3	0.356
40	107.30	0.679	444.20	0.779	147.6	0.579	156.0	0.434
50	100.60	0.736	83.4	0.750	53.1	0.474	57.4	0.423
Correl. coeff. (r)	0.98**		0.86**		0.99**		0.81**	

* Significant at P=0.05

** Significant at P=0.01

9.0. Experiment 7

Antibiotic activities of *Trichoderma* isolates (Table 38) to *A. mellea* were investigated in this experiment.

9.1. Table 38: *Trichoderma* isolates tested for antibiotic activities against *A. mellea.*

Isolate number	*IMI number	Species name
1	339493	*Trichoderma koningii* Oudem
2	339494	*Trichoderma koningii* Oudem
3	339495	*Trichoderma longibrachiatum* Rifai
4	339496	*Trichoderma harzianum* Rifai
5	339497	*Trichoderma harzianum* Rifai
6	342180	*Trichoderma koningii* Oudem
7	342181	*Trichoderma koningii* Oudem
8	342182	*Trichoderma koningii* Oudem
9	342183	*Trichoderma koningii* Oudem
10	342184	*Trichoderma harzianum* Rifai
11	342185	*Trichoderma harzianum* Rifai

*IMI CAB International Mycological Institute, Kew, U.K.

9.2. *Trichoderma - Armillaria mellea* vegetative interactions

Trichoderma isolates significantly (P=0.05) reduced radial vegetative growth of *A. mellea* isolates M (Table 39) and N (Table 40). The percentage growth of *A. mellea* in the presence of *Trichoderma* isolates was significantly (P=0.05) less than that of the control (Tables 39 and 40). The *Trichoderma* isolates inhibited

radial growth of *A. mellea* to different extents. *Trichoderma koningii* (isolate 9). *T. longibrachiatum* (isolate 3) and *T. harzianum* (isolate 4) were significantly (P=0.05) better inhibitors of *A. mellea* growth than the other isolates (Tables 39 and 40).

Table 39: Radial growth of *Armillaria mellea* (isolate M) as a result of *Trichoderma* - *A. mellea* vegetative interaction.

Trichoderma isolates		Radial growth of *A. mellea* (mm) Duration of growth (days) 7	12	16	20	% growth from the 7th day
T. koningii	9	15.0[a1]	15.0[a]	15.5[a]	15.5[a]	3.4[a]
T. longibrachiatum	3	15.0[a]	15.0[a]	15.5[a]	15.5[a]	3.5[a]
T. harzianum	4	15.0[a]	15.3[a]	15.5[a]	15.5[a]	3.4[a]
T. koningii	2	17.0[a]	17.8[b]	18.8[b]	18.8[b]	10.5[b]
T. koningii	1	16.8[a]	18.0[b]	18.3[b]	18.5[b]	10.6[b]
T. koningii	6	16.5[a]	18.3[b]	19.3[bc]	19.3[bc]	16.8[b]
T. harzianum	5	16.3[a]	18.3[b]	19.3[bc]	19.3[bc]	18.6[b]
T. koningii	7	15.8[a]	17.8[b]	20.0[c]	21.3[c]	35.0[c]
T. koningii	8	15.8[a]	18.5[b]	20.5[c]	21.8[c]	38.3[cd]
T. harzianum	11	15.8[a]	18.3[b]	20.8[c]	22.3[c]	41.4[d]
Control		17.3[b]	22.3[c]	28.3[d]	33.0[d]	91.3[e]

[1]Means followed by the same letter are not significantly (P=0.05) different from each other according to Duncan's Multiple range test.

Table 40: Radial growth of *Armillaria mellea* (isolate N) as a result of *Trichoderma* - *A. mellea* vegetative interaction.

Trichoderma isolates		Radial growth of *A. mellea* (mm)				
		Duration of growth (days)				
		7	12	16	20	% growth from the 7th day
T. harzianum	4	12.3[a1]	12.3[a]	12.3[a]	12.5[a]	2.2[a]
T. koningii	9	11.8[a]	12.0[a]	12.0[a]	12.0[a]	2.4[a]
T. longibrachiatum	3	12.0[a]	12.0[a]	12.0[a]	12.3[a]	2.4[a]
T. koningii	2	13.5[b]	14.3[b]	15.5[b]	15.5[b]	14.8[b]
T. koningii	1	13.5[b]	14.8[b]	15.3[b]	15.5[b]	15.4[b]
T. koningii	6	13.3[b]	14.5[b]	15.5[b]	15.8[bc]	18.9[b]
T. harzianum	5	13.0[b]	14.8[b]	15.8[b]	16.0[b]	23.5[b]
T. koningii	7	12.5[b]	14.3[b]	16.5[bc]	17.5[bc]	40.1[bc]
T. koningii	8	12.3[ba]	15.0[b]	16.8[c]	17.3[c]	42.8[c]
T. harzianum	11	12.3[ba]	15.3[b]	16.5[c]	17.5[c]	43.1[c]
Control		13.8[b]	18.8[c]	25.8[d]	29.5[d]	115.0[d]

[1]Means followed by the same letter are not significantly (P=0.05) different from each other according to Duncan's Multiple range test.

Re-isolations of *A. mellea* isolates M and N from *T. koningii* 9, *T. longibrachiatum* 3, and *T. harzianum* 4, were unsuccessful (Table 41). However, for the other *Trichoderma* isolates, re-isolation success ranged between 25% and 50%.

Table 41: Re-isolation of *A. mellea* M and N after 25 days of vegetative interaction with *Trichoderma* isolates

Trichoderma isolates		Re-isolation of *A. mellea*/Replicates[1]			
		I	II	III	IV
T. harzianum	4	-	-	-	-
T. koningii	9	-	-	-	-
T. longibrachiatum	3	-	-	-	-
T. koningii	2	+	+	-	-
T. koningii	1	+	-	-	-
T. koningii	6	+	-	-	-
T. harzianum	5	+	+	-	-
T. koningii	7	+	+	-	-
T. koningii	8	+	+	-	-
T. harzianum	11	+	+	-	-
Control		+	+	+	+

[1] Each replicate constitutes two plates
+ denotes successful re-isolation
- denotes unsuccessful re-isolation

9.3. Effect of secondary metabolites of *Trichoderma* isolates on *A. mellea* isolates M and N.

Metabolites of *Trichoderma* isolates inhibited mycelial and rhizomorphal growth of *A. mellea* isolates M and N (Tables 42 and 43). Growth of *A. mellea* in presence of *Trichoderma* metabolites was significantly (P=0.05) lower than the growth of the fungus in absence of the *Trichoderma* metabolites in most cases (Tables 42 and 43; Plates 6-9). The inhibition of *A. mellea* by metabolites of *Trichoderma* isolates varied from isolate to isolate. Metabolites of *T. koningii* 9, *T. longibrachiatum* 3 *and T. harzianum* 4 had the highest inhibiting effect (Tables 42 and 43; Plates 7 and 9).

Table 42: Effect of *Trichoderma* secondary metabolites on *Armillaria mellea*

Source of metabolites	Zero week	1st week		2nd week		3rd week	
	Radial mycelial growth of *Armillaria* isolates M and N (mm)						
	M & N	M	N	M	N	M	N
T. koningii 1	*	15.6[a1]	16.3[a]	21.0[ab]	23.3[ab]	**	41.3[ab]
T. koningii 2		15.3[a]	16.3[a]	20.0[a]	22.3[a]		44.3[ab]
T. koningii 6		14.5[a]	16.0[a]	19.5[a]	22.7[a]		40.0[a]
T. koningii 7		18.3[b]	19.3[b]	25.0[c]	27.7[b]		44.8[b]
T. koningii 8		19.3[b]	19.7[b]	25.5[c]	28.0[b]		40.8[a]
T. koningii 9		11.5[b]	12.3[c]	16.3[d]	18.7[c]		29.3[c]
T. longibrachiatum 3		11.8[c]	12.0[c]	16.3[d]	18.0[c]		29.3[c]
T. harzianum 4		10.8[c]	11.7[c]	15.0[d]	17.3[c]		30.0[c]
T. harzianum 5		14.5[a]	14.0[d]	19.5[a]	20.7[a]		36.0[a]
T. harzianum 10		18.8[b]	19.3[b]	25.0[e]	27.7[b]		37.7[a]
T. harzianum 11		16.5a	17.3[a]	22.3[b]	24.7[b]		40.3[a]
Mixture of isolates		14.0a	15.0[ad]	19.0[a]	22.3[a]		36.0[a]
Control		21.8d	22.6[e]	29.0[f]	32.3[d]	84.0	49.8[d]

[1]Means followed by the same letter are not significantly (P=0.05) different from each other according to Duncan's Multiple Range test.

* Inoculation block size - 3 mm

** Measurements were stopped as soon as the control plates covered the available space.

Table 43: Effect of *Trichoderma* secondary metabolites on *Armillaria mellea*

Source of metabolites		Radial rhizomorphal growth of *Armillaria* isolates M and N (mm)					
		Zero week	1st week		2nd week	3rd week	
		M & N	M	N	M	N	M	N
T. koningii	1	*	30.0[a]	16.0[a]	62.0[ab]	31.3[a]	**	40.3[ab]
T. koningii	2		26.0[b]	14.3[a]	56.3[bc]	28.0[a]		46.2[ad]
T. koningii	6		31.0[a]	15.0[a]	61.3[a]	29.3[a]		36.3[b]
T. koningii	7		29.5[a]	16.0[a]	59.3[ab]	29.7[a]		43.0[a]
T. koningii	8		28.5[a]	16.0[a]	51.0[bc]	31.3[a]		36.0[a]
T. koningii	9		18.5[c]	9.0[b]	49.3[c]	18.0[b]		22.0[c]
T. longibrachiatum	3		19.5[c]	8.6[b]	47.5[c]	19.7[b]		23.7[c]
T. harzianum	4		20.5[c]	9.0[b]	48.8[c]	18.3[b]		23.8[c]
T. harzianum	5		29.5[a]	11.7[c]	60.5[a]	23.7[b]		30.7[b]
T. harzianum	10		22.0[d]	18.0[d]	46.8[c]	32.0[a]		34.8[b]
T. harzianum	11		28.0[a]	14.0[a]	51.5[bc]	26.0[a]		34.3[b]
Mixture of isolates			26.0[b]	17.0[d]	53.8[bc]	28.3[a]		31.0[b]
Control			48.0[e]	18.0[d]	84.0[d]	38.3c	84.0	49.7[d]

[1] Means followed by the same letter are not significantly different from each other according to Duncan's Multiple Range test.

* Inoculation block size - 3 mm

** Measurements were stopped as soon as the control plates covered the available space.

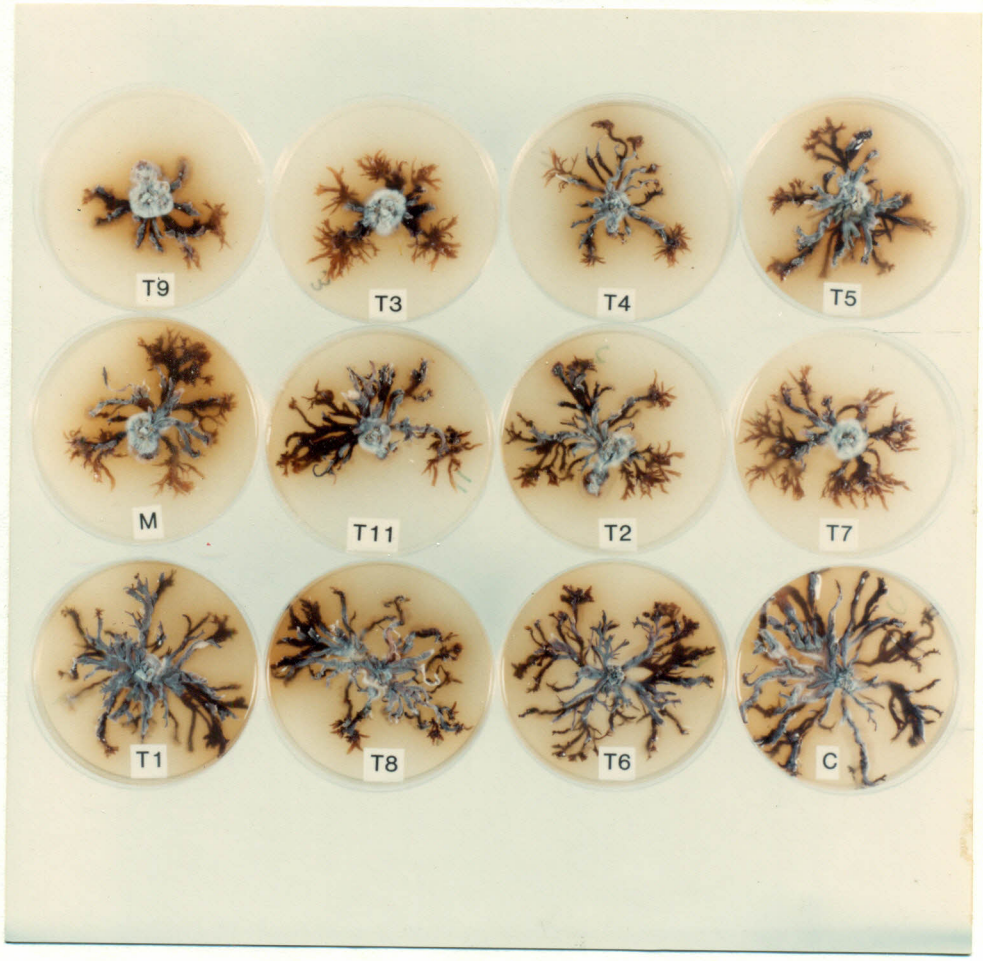


Plate 6: Effect of secondary metabolites of different *Trichoderma* isolates on *Armillaria mellea* isolate M

Trichoderma koningii 10 not included on the plate due to congestion problem.

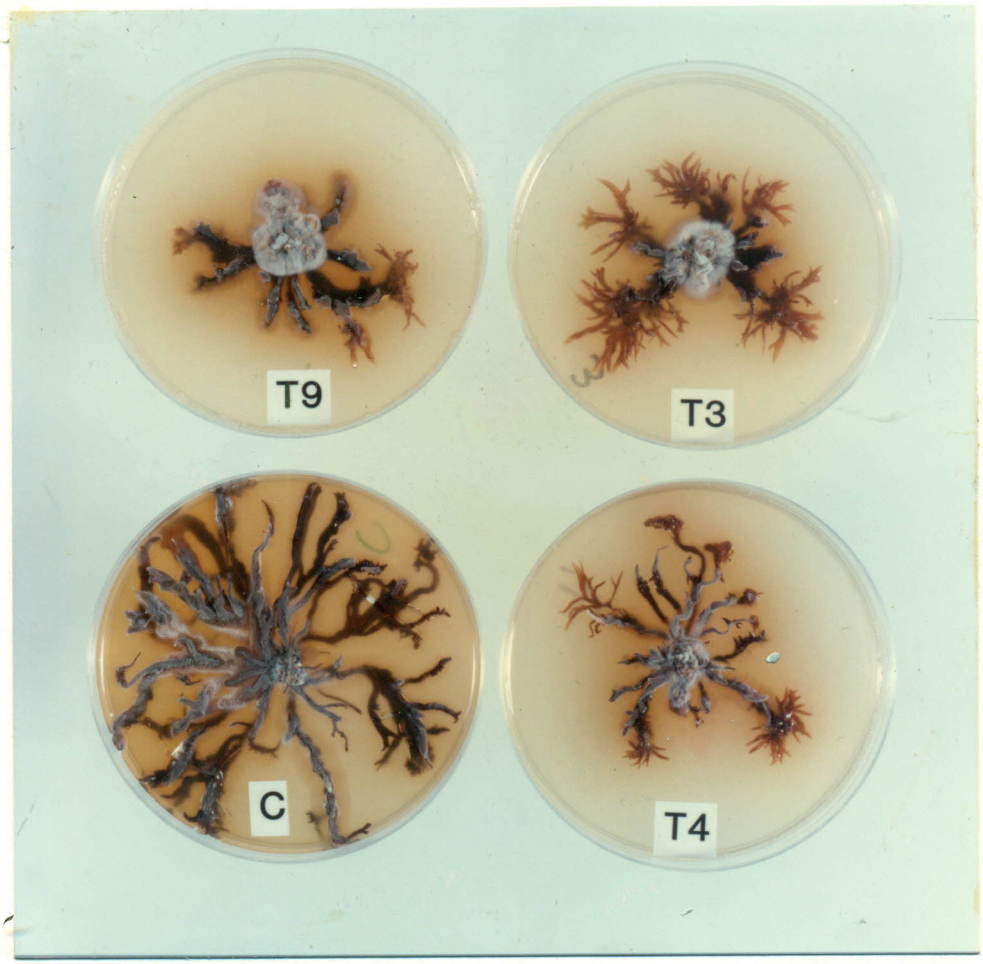


Plate 7: A close-up of the most biologically active *Trichoderma* isolates and the control (c) on *A. mellea* isolate M

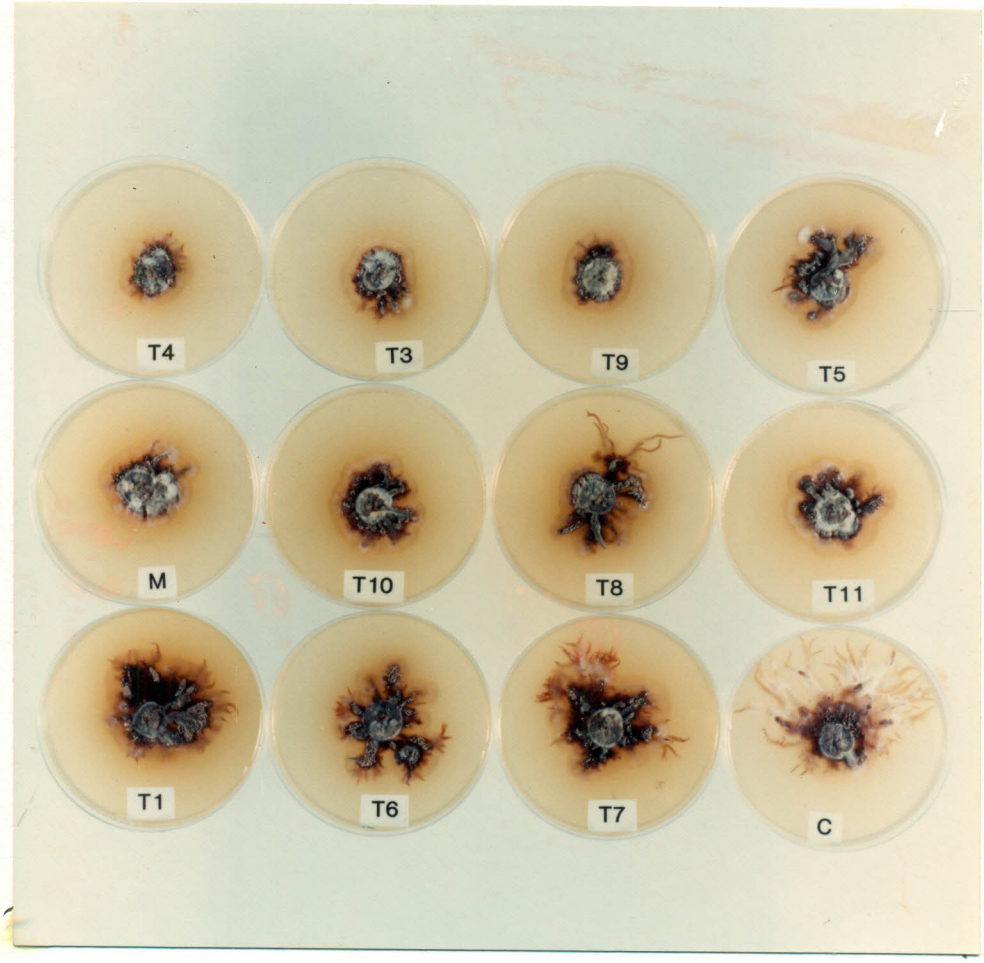


Plate 8 Effect of secondary metabolites of different *Trichoderma* isolates on *Armillaria mellea* isolate N

Trichoderma koningii 2 not included on the plate due to congestion problem.

9.4. Effect of secondary metabolites of *Trichoderma* isolates on biomass accumulation of *A. mellea* isolates M and N

Metabolites of *Trichoderma* hampered significantly (P=0.05) biomass accumulation of *A. mellea* isolates M and N. For all *Trichoderma* isolates except one in each case the effect was significant (Table 44). Metabolites from *T. koningii* 9, *T. longibrachiatum* 3 and *T. harzianum* 4 had the highest inhibiting effect on *A. mellea* biomass accumulation. Metabolites of *T. koningii* 1 and *T. harzianum* 10, did not significantly reduce biomass accumulation of *A. mellea* isolates M and N respectively (Table 44).

Table 44: Effect of *Trichoderma* metabolites on *Armillaria mellea* isolates M and N biomass accumulation

Source of metabolites	Dry Weights (grams) M	N
T. koningii 1	0.280[ad]	0.169[a]
T. koningii 2	0.210[b]	0.168[a]
T. koningii 6	0.213[b]	0.133[b]
T. koningii 7	0.203[b]	0.171[a]
T. koningii 8	0.193[b]	0.162[a]
T. koningii 9	0.153[c]	0.095[c]
T. longibrachiatum 3	0.160[c]	0.081[c]
T. harzianum 4	0.143[c]	0.078[c]
T. harzianum 5	0.193[b]	0.121[b]
T. harzianum 10	0.230[b]	0.193[d]
T. harzianum 11	0.163[c]	0.149[a]
Mixture of isolates	0.173[c]	0.124[b]
Control	0.300[d]	0.197[d]

Means followed by the same letter are not significantly (P=0.05) different from each other according to Duncan's Multiple Range test.

The correlation between the pH nutrient broth at *Trichoderma* harvest and *A. mellea* biomass accumulation (Table 44) showed a non-significant relationship as shown by r-values of 0.11 and 0.12, respectively (Table 45). Because inter-replication variation of pH and *A. mellea* biomass accumulation were very low with equally low covariance of variation (CV) their means were used in the

regression analysis. The *Trichoderma* isolates that produced pigments to the medium, tended to exhibit highest antibiotic activities to *A. mellea* (Table 45).

Table 45: Other parameters taken at *Trichoderma* harvest and a regression analysis between broth pH and biomass accumulation of *A. mellea* M and N

Trichoderma isolates	Pigment Production	*Trichoderma* biomass (gm)	Broth pH
T. koningii 1	None	0.255	4.3
T. koningii 2	None	0.238	5.1
T. koningii 6	None	0.217	4.0
T. koningii 7	None	0.248	4.5
T. koningii 8	None	0.213	4.3
T. koningii 9	Yellowish	0.400	3.9
T. longibrachiatum 3	Greenish	0.227	5.2
T. harzianum 4	Yellow	0.313	5.2
T. harzianum 5	None	0.206	5.1
T. harzianum 10	Greenish	0.228	5.2
T. harzianum 11	None	0.231	4.1
Mixture of isolates	Yellowish	0.227	4.8
Control	None	0.000	4.4
Correlation coefficients (r)	*A. mellea* M		0.11
	A. mellea N		0.12

10.0. Experiment 8

Effects of soil amended with coffee pulp and husks or basamid treated soil on *Trichoderma* population and other microorganisms, incidence of *Armillaria* root rot of tea and tea plant performance were investigated in this experiment. Coffee pulp was used as an organic amendment because of its ability to enhance proliferation of *Trichoderma* population as was revealed in Experiment 1.

10.1. Effect of coffee pulp and husks and basamid on *Trichoderma*

Coffee pulp and husks significantly (P=0.05) enhanced the population build-up of *Trichoderma* in 1988, 1989 and 1990. The population was highest at the initial months after application of coffee pulp and husks and tended to decline with time (Table 46). *Trichoderma* populations in basamid treated soils were not significantly (P=0.05) different from those in the non-treated soils (control) except in the 7th month in 1990 (Table 46).

Table 46: *Trichoderma* population after soil amendment in 1988, 1989 and 1990.

	Trichoderma population x 10^5/g of soil					
	Time of bioassay (months)					
Year	1	3	5	7	9	11
1988*						
Basamid	2^{a1}	2^{a}	4^{a}	3^{a}	2^{a}	4^{a}
Coffee pulp	11^{b}	11^{b}	14^{b}	8^{b}	5^{b}	6^{a}
Control	2^{a}	2^{a}	7^{a}	1^{a}	2^{a}	3^{a}
1989**						
Basamid	5^{a}	4^{a}	5^{a}	5^{a}	3^{a}	3^{a}
Coffee pulp	23^{b}	19^{b}	20^{b}	13^{b}	11^{b}	19^{b}
Control	3^{a}	3^{a}	7^{a}	6^{a}	3^{a}	5^{a}
1990***						
Basamid	3^{a}	2^{a}	4^{0a}	5^{a}	4^{a}	2^{a}
Coffee pulp	20^{b}	10^{b}	17^{b}	18^{b}	17^{b}	23^{b}
Control	5^{a}	4a	7^{a}	8^{a}	4^{a}	2^{a}

[1] Means followed by the same letter are not significantly (P=0.05) different from each other according to Duncan's Multiple Range test

* The application of coffee pulp and basamid was done on 20th June 1987.

** Second application of coffee pulp only was done on 16th December 1988.

*** Third application of coffee pulp was done on 6th August 1990.

10.2. Effect of coffee pulp and husks and basamid on fungal populations.

Coffee pulp and husks significantly (P=0.05) enhanced the population build-up of fungi in 1988, 1989 and 1990 most times (Table 47). Between basamid and the control, no significant differences in fungal proliferation occurred throughout the experimental duration.

10.3. Effect of coffee pulp and husks and basamid on bacterial population.

Coffee pulp and husks significantly (P=0.05) enhanced the population build-up of bacteria in 1988, 1989 and 1990 most times (Table 48). The enhancement was, however, non-significant in the months of January , May and November for 1988 and May and July for 1990 (Table 48). Between basamid and control no significant differences in bacterial proliferation occurred throughout the experimental duration (Table 48).

Table 47: Total fungal population after soil amendment in 1988, 1989 and 1990.

	Fungal population x 10^5/g of soil					
	Time of bioassay (months)					
Year	1	3	5	7	9	11
1988*						
Basamid	25[a1]	19[a]	32[a]	23[a]	19[a]	18[a]
Coffee pulp	29[a]	46[b]	47[b]	28[b]	31[b]	23[a]
Control	15[a]	20[a]	31[a]	15[a]	19[a]	12[a]
1989**						
Basamid	54[a]	40[a]	37[a]	35[a]	19[a]	75[a]
Coffee pulp	146[b]	88[b]	92[b]	107[b]	42[b]	153[b]
Control	44[a]	35[a]	44[a]	56[a]	23[a]	68[a]
1990***						
Basamid	63[a]	59[a]	59[a]	58[a]	23[a]	32[a]
Coffee pulp	133[b]	89[b]	108[b]	126[b]	87[b]	93[b]
Control	60[a]	51a	51[a]	48[a]	17[a]	25[a]

[1] Means followed by the same letter are not significantly (P=0.05) different from each other according to Duncan's Multiple Range test

* The application of coffee pulp and basamid was done on 20th June 1987.

** The second application of coffee pulp only was done on 16th December 1988.

*** The third application of coffee pulp only was done on 6th August 1990.

Table 48: Total bacterial population after soil amendment in 1988, 1989 and 1990

	Bacterial population x 10^7/g of soil					
	Time of bioassay (months)					
Year	1	3	5	7	9	11
1988*						
Basamid	128[a1]	30[a]	81[a]	49[a]	47[a]	11[a]
Coffee pulp	120[a]	55[b]	78[b]	83[b]	104[b]	22[a]
Control	104[a]	46[a]	60[a]	63[a]	48[a]	15[a]
1989**						
Basamid	30[a]	28[a]	54[a]	42[a]	31[a]	59[a]
Coffee pulp	49[b]	43[a]	79[b]	63[b]	57[b]	168[b]
Control	37[a]	34[a]	61[a]	29[c]	40[a]	62[a]
1990***						
Basamid	51[a]	126[a]	129[a]	127[a]	17[a]	32[a]
Coffee pulp	78[a]	134[b]	134[a]	149[a]	24[b]	49[b]
Control	40[a]	123a	114[a]	129[a]	12[a]	31[a]

[1] Means followed by the same letter are not significantly (P=0.05) different from each other according to Duncan's Multiple Range test

* The application of coffee pulp and basamid was done on 20th June 1987.

** The second application of coffee pulp only was done on 16th December 1988.

*** The third application of coffee pulp only was done on 6th August 1990.

10.4. The tea crop performance and *Armillaria* root rot incidence

The coffee pulp and husks in a 10:1 ratio significantly (P=0.05) improved tea yield in 1989 and 1990 (Table 49). Between basamid and control, there was no significant yield difference (Table 49). No disease incidence was noted in the basamid and coffee pulp treated plots. However, a percentage disease incidence of 11.1% was recorded from the control plots (Table 49). Each replicate of the control plots and the guard rows showed some incidence of *Armillaria* root rot of tea.

Table 49: Effect of coffee pulp and basamid on tea yield for 1988, 1989 and 1990 and disease incidence for 1991.

	Tea yield kg mt/ha			
	Time (years)			% disease incidence
Treatment	1988[2]	1989	1990[3]	1991
Basamid	202	876[a]	282[a]	0.0
Coffee pulp	243	1416[b]	453[b]	0.0
Control	188	908[a]	312[a]	11.1
	NS[4]			

[1] Means followed by the same letter are not significantly (P=0.05) different from each other according to Duncan's Multiple Range test

[2] The 1988 yield data is only for three months because this was the first year of the crop.

[3] The 1990 yield data is only for three months because for the early part of the year tea was pruned.

[4] Non significant

10.5. Effect of coffee pulp and basamid on other crop performance attributes

Performance of the tea plants as assessed by stem girths, bush circumference and number of branches was higher in coffee pulp amended soil than that of plants grown in basamid-treated or untreated soil (Table 50). Control plants had significantly (P=0.05) thinner stems, fewer branches and least bush circumference than those of plants grown on treated soils. Bush circumference of plants grown in basamid treated soils were not significantly different from those of control plants (Table 50).

Table 50: Stem girths, bush circumference and number of branches after soil amendment.

Treatments	Stem Girths (cm)	Bush Circumference (cm)	Number of Branches
Basamid	5.8[a 1]	320.4[a]	6.7[a]
Coffee pulp	6.1[a]	365.7[b]	7.0[a]
Control	5.1[b]	300.1[a]	6.0[b]

[1]Means followed by the same letter are not significantly (P=0.05) different from each other according to Duncan's Multiple Range test

10.6. Soil bulk density (gm/cm^3) organic matter content (%), soil pH, soil (ppm) and plant (%) nitrogen after coffee pulp and basamid application

There were no significant (P=0.05) differences in bulk density due to treatments (Table 51). Bulk density was, however, higher at 50-60 cm than at 10-15 cm depth. The organic matter content was highest in coffee pulp amended soil but the differences were not significant (Table 51). Soil pH was significantly (P=0.05) higher in coffee pulp treated soils than in the other treatments (Table 51). The treatments did not significantly influence both soil and leaf nitrogen contents (Table 51).

Table 51: Effect of amendments on soil bulk density, organic matter, pH, nitrogen (N) and leaf N

Treatments	Bulk density 10-15cm	Bulk density 50-60cm	Organic matter content	pH	Soil N	leaf N
Basamid	0.63^{a1}	0.71^{a}	9.7^{a}	3.9^{a}	446^{a}	3.3^{a}
Coffee pulp	0.66^{a}	0.70^{a}	10.9^{a}	4.7^{b}	435^{a}	3.3^{a}
Control	0.62^{a}	0.69^{a}	9.5^{a}	3.9^{a}	414^{a}	3.4^{a}

[1] Means followed by the same letter are not significantly (P=0.05) different from each other according to Duncan's Multiple Range test

10.7. Correlation of tea yield with soil pH, organic matter content, bulk density and *Trichoderma* population

Tea yield was significantly (P=0.05) influenced by soil pH, organic matter content and *Trichoderma* population throughout the experimental period (Tables 52 and 53). But soil bulk density up to

10-15cm depth only significantly (P=0.05) influenced the yield for the years 1988 and 1989. (Tables 52 and 53).

Table 52: Correlation coefficients (r) in a regression analysis between soil physical and chemical properties as a result of coffee pulp and basamid amendments and tea yield (kg mt/ha/year).

	Correlation coefficients (r)			
Yield/ year	Bulk density 10-15 cm	Bulk density 50-60 cm	Organic matter content	Soil pH
1988	0.99*	0.24	0.99*	0.97*
1989	0.96*	0.05	0.98*	0.99*
1990	0.92	0.16	0.96*	0.99*

* Significant at (P=0.05)

Table 53: Correlation coefficients (r) in a regression analysis between *Trichoderma* populations as a result of coffee pulp and basamid amendments and tea yield kg mt/ha.

Yield/year	Month	Correlation coefficients (r) Trichoderma population
1988	1	0.97*
	3	0.97*
	5	0.86
	7	0.99*
	9	0.97*
	11	0.99*
1989	1	0.99*
	3	0.96*
	5	0.99*
	7	0.99*
	9	0.99*
	11	0.99*
1990	1	0.99*
	3	0.99*
	5	0.99*
	7	0.98*
	9	0.99*
	11	0.99*

* Significant at (P=0.05).

CHAPTER 5

DISCUSSION

11.0. Experiment 1

The significantly (P=0.01) different levels of microbial proliferation including those of *Trichoderma* spp. in soils amended with saw dust, coffee pulp and husks, filter press cake, cow manure and goat manure indicated that some of the organic materials could be used to maintain high populations of *Trichoderma* spp. that may be of value in biocontrol of *Armillaria* root rot of tea. The highest and significant (P=0.01) proliferation of *Trichoderma* spp. in coffee pulp amended soils is indicative of the role this amendment can play in boosting the biocontrol potential of a soil against *Armillaria* root rot of tea. The antibiotic activities of *Trichoderma* spp. against *A. mellea* of tea have been demonstrated in this thesis (Expt. 7). The interaction of *A. mellea* on citrus roots with *Trichoderma* spp. as modified by methyl bromide showed an inverse relationship (Ohr *et al*, 1973). The enhancement of higher *Trichoderma* proliferation by the coffee pulp at 16 and 22 g carbon rates than the higher rate of 36 g of carbon might have been due to mineralization that resulted in toxic levels of inorganic elements in the latter (Gilpatrick, 1969; Papavizas and Lewis, 1971; Smith, 1973 and 1976). microbial antagonism, competition, hyperparasitism and/or antibiosis (Alexander, 1977; Baker and Cook, 1974; Clark, 1968; Mankau, 1980; Gottlieb and Shaw, 1970). It is also possible that sub-optimal nutrient supply resulted into the poor *Trichoderma* proliferation in the coffee pulp amended soil at 8 g carbon. These

possibilities, however, need to be investigated. The *Trichoderma* population decline in coffee pulp amended soils after the fifth month implies that for a successful use of coffee pulp in managing *Armillaria* root rot of tea, two to three applications at an interval of five months will be required to maintain high populations of *Trichoderma* spp.

The C:N ratio of the organic amendment used was a better indicator of *Trichoderma* proliferation than either carbon or nitrogen. This could be due to the fact that C:N ratio influences the balance between mineralization and leaching of nutrients from decomposing organic materials (Alexander, 1977; Tisdale *et al*, 1985).

The significantly low microbial populations including those of *Trichoderma* spp. recovered from soils treated with Basamid were a result of the toxic effect of the fumigant (Ohr, 1973). The rather high bacterial recovery from the 5th month observed in Table 10 for Basamid treated soils was attributed to contamination and in absence of competition, the bacteria multiplied fast.

11.1. Experiment 2

The significantly higher enhancement effect of the coffee pulp and husks to that of inorganic fertilizers on the microbial proliferation including that of *Trichoderma* spp., further documents the superiority of this amendment as an enhancer of *Trichoderma* proliferation. The results of this experiment further demonstrate that nitrogen or carbon content *per se* is not as important as carbon:nitrogen ratio in the proliferation of *Trichoderma* spp. (Expt. 1). The effect of N fertilization on soil microorganisms

have given inconsistent results (Soderstrom *et al*, 1983). While an increase in microbial activity has been detected in some studies (Roberge, 1976; Kelly and Henderson, 1978), a decrease has been reported in others (Roberge, 1976; Foster, 1980). The significantly higher enhancement effect of the coffee pulp and husks on the microbial proliferation might have been due to availability of both carbon and nitrogen and/or high pH of soils amended with this material (Alexander, 1977). High soil pH levels have been associated with enhanced microbial proliferation (Alexander, 1977).

11.2. Experiment 3

The significant (P=0.05) differences in *Trichoderma* populations in soils amended with coffee pulp and husks of different ages indicate that the enhancement effect was influenced by the age of the amendment. The decline of *Trichoderma* population after one month of decomposition of coffee pulp could possibly be attributed to reduction in water soluble constituents, proteins and minerals and percentage abundance of celluloses, hemicelluloses and lining (Alexander, 1977). This finding is, however, at variance with the view that *Trichoderma* and other antagonists thrive in substrates amended with mature or well decomposed organic matter (Papavizas, 1985; Hoitink, and Fahy, 1986). This variance could be attributed to the nature of composts in use. A lot of work involving use of organic material or composts for biological control purposes have tended to concentrate on animal manures, hardwood bark composts, crop residues (Chung, 1989; Baker, 1962; Huber and Watson, 1970; Lewis and Papavizas, 1975; Nelson *et al*,

1983; Nelson and Hoitink, 1983), but very little attention has been given to crop processing by-products like coffee pulp.

Coffee husks which are predominantly cellulolitic decompose at a much slower rate than the coffee pulp, a phenomenon that was possibly responsible for increase in carbon content with increase in duration of decomposition of the coffee pulp and husks (Table 3). A similar situation was observed with saw dust, an equally cellulolitic organic material (Expt. 1). It can, therefore, be inferred from these results that cellulose is a poor substrate for *Trichoderma* spp.

The implication of these results is that, if coffee pulp and husks in a 10:1 ratio was to be used for enhancement of the proliferation of *Trichoderma* spp. as biological control agents of *Armillaria* root rot of tea, then one-month-old coffee pulp would give the highest biocontrol potential.

The fact that bacterial and other fungal proliferation was highest and significantly (P=0.05) so with fresh coffee pulp implies that use of one-month-old coffee pulp and husks in a 10:1 ratio preferentially support *Trichoderma* proliferation under reduced competition from other soil microorganisms. The organic matter turn-over as determined by microbial activity (Schnurer and Rosswall, 1982) decreased as the period of coffee pulp decomposition duration increased. This trend was therefore identical to that of non-*Trichoderma* microorganisms in the soil.

11.3. Experiment 4

The short-lived increase and subsequent decrease in *Trichoderma* populations in soils amended with ground coffee pulp and husks indicated that increase in surface area of the amendment had a short-lived enhancement effect of *Trichoderma*. This might have been due to toxicity as a result of fast mineralization (Gilpatrick, 1969; Papavizas and Lewis, 1971; Smith, 1976), microbial antagonism from other microorganisms (Baker, 1977; Mankau, 1980). It is also possible that increased surface area of coffee pulp could have easily resulted in a situation like in Experiment 1, where the highest carbon rate of coffee pulp of 36 g, supported significantly less *Trichoderma* population.

These results are inconsistent with findings of Hoitink and Fahy 1986, who used tree bark composts in their studies, implying that the treatment of organic materials for biological control purpose should be specific to the material. Ground coffee pulp and husks are therefore not a better alternative than the non-ground one in the enhancement and sustenance of *Trichoderma* spp., biocontrol agents of *Armillaria* root rot. The non viability of the ground coffee pulp is further compounded by the fact that, for it to be as viable as non-ground one, the frequency of application would be too high to be practically and even economically sustainable. It is evident therefore, that normal coffee pulp after pulping in the coffee factories would be a better option for use.

11.4. Experiment 5

The higher *Trichoderma* populations obtained from soils treated with the sub-lethal dose of basamid of 0.1 g than those in controls, were possibly because of relatively high tolerance of the fungus to the fumigant and/or reduced antagonism as a result of toxic effects of the fumigant to other soil microorganisms (Munnecke *et al*, 1973; Ohr, 1973). Sub-lethal doses of fumigants also interfere with defense mechanisms of resting structures such as *sclerotia* making them more biologically degradable (Merriman, 1976), hence reduction in microbial competition. But, because the *Trichoderma* populations supported by the sub-lethal dose (0.1g) of basamid were still significantly lower than those supported by coffee pulp and even filter press cake, sub-lethal dose of the fumigant is not a better alternative in the proliferation of *Trichoderma* hence biocontrol of *Armillaria* root rot. However, lethal doses of the fumigant are effective in the direct chemical management of *Armillaria* root rot (Expt. 8). The coffee pulp and husks have the added advantage of enhancing proliferation of other soil microorganisms with possible antagonistic and/or antibiotic activities against *A. mellea*. There is evidence that mixtures of bacterial and fungal antagonists are more effective than single antagonists in inducing suppression of soil-borne plant pathogens (Hoitink and Fahy, 1986; Alexander, 1977; Tisdale, 1985; Mankau, 1980).

11.5. Experiment 6

The adverse effect of drier soils at 15 to 25% moisture content on proliferation of *Trichoderma* spp. confirms previous reports (Cheu and Griffin, 1966; Davet, 1979 and 1981). Also the enhanced *Trichoderma* proliferation in wetter soils (35 to 50% moisture content) documented in this study has been reported before (Danielson and Davey, 1973; Bhatt, 1970; Christensen and Whittingham, 1965; Latter *et al*, 1967; Lee and Baker, 1973; Liu and Baker, 1980). The enhanced proliferation of this fungus in wet soils with high CO_2 levels is indicative of the ability of *Trichoderma* to tolerate anaerobiosis (Bunges and Fenton, 1953; Pugh and Van Emden, 1969).

The implication of these results is that if coffee pulp was to be used for the purposes of *Trichoderma* proliferation and hence biological control of *Armillaria* root rot of tea, the amendment would be more beneficial, if the application was confined to very wet months of the year.

The fact that bacterial and non-*Trichoderma* fungal proliferation was significantly hampered at the wettest moisture content, could be a possible method of preferentially enhancing *Trichoderma* population for biological control purposes, if a situation of non-synergism when *Trichoderma* is in operation with other microorganisms arose.

These results further showed the highest organic matter turnover as determined by microbial activity to be at 40% moisture content throughout the experimental duration. The microbial activity significantly and quadratically correlated to microbial

population. For the purposes of competition of non-*Trichoderma* microorganisms against *Armillaria* for food and air, mediumly moist soils, that is, soils slightly lower than and up to field capacity would be ideal.

11.6. Experiment 7

The significant (P=0.05) differences in antibiotic activities of *Trichoderma* isolates (Tables 42 and 43) even within a species is in agreement with previous reports on variation of antagonistic activity among isolates with very similar cultural-morphological characters (Kamyshko, 1961; Dennis and Webster, 1971[a] and 1971[b]). The variation of antibiotic potency of isolates within species could be attributed to the fact that the taxonomy of *Trichoderma* is exclusively based on morphology (Rifai, 1969). The failure to re-isolate *A. mellea* where its interaction with *T. koningii* (isolate 9), *T. longibrachiatum* (isolate 3) and *T. harzianum* (isolate 4) took place further indicated the possible involvement of antibiosis, lysis or mycoparasitism (Ayers and Adams, 1981; Cook and Baker, 1983). The fact that the filtrates of the three *Trichoderma* isolates significantly (P=0.05) reduced radial growth and biomass accumulation relative to the other *Trichoderma* isolates further demonstrates the production of higher potency metabolites to the media that were toxic to *A. mellea* hence favouring the antibiosis phenomenon. The ability of *Trichoderma* spp. to produce toxic metabolites has been documented (Weindling, 1941; Howell and Stipanovic, 1983; Papavizas, 1985). *T. viride* has been associated with reduction of *Armillaria* in a tea plantation (Venkata Ram and

Devasahayam Joseph, 1974). Although the mechanism of reduction of *Armillaria* by *T. viride* was not elucidated, the fungus is known to produce mycolytic enzymes consisting mainly of ß-1, 3-glucanase and chitinase at an optimum pH and temperature of 5.0 and 45°C, respectively (Nobuo and Ogawa, 1968). The importance of ß-(1-3)-glucanase and chitinase as key enzymes responsible for fungal cell sclerotial wall lysis and degradation has been stressed by other workers (Elad *et al*, 1983; Elad *et al*, 1982[b]; Jones *et al*, 1974). Many isolates of *Trichoderma* isolates indicated a non-synergistic effect. The non-synergistic action could be attributed to competition among the isolates. This area requires specific experimentation to establish more facts because the mode of utilization of *Trichoderma* for biological control may be influenced by the outcome.

For the Kenyan tea situation, since most of the *Trichoderma* isolates significantly reduced radial growth and biomass accumulation of *A. mellea* compared to the control, enhancement of *Trichoderma* spp. population in the tea soils by modifying the soil properties using organic soil amendments especially coffee pulp and husks, might be the first step towards attempts to biologically manage *A. mellea* of tea. Subsequent effort might be to look for ways and means of selective enhancement of the most active *Trichoderma* isolates against *A. mellea*.

11.7. Experiment 8

The significant (P=0.05) enhancement of and the adverse effects on *Trichoderma* proliferation by coffee pulp and husks and

basamid under field conditions respectively, adds more credibility to the greenhouse studies (Experiments 1, 2 and 5). The significantly high tea yield and large circumference of tea bushes and absence of disease on the tea plants grown in soils amended with the coffee pulp and husks, are further evidences that the amendments proliferative ability of *Trichoderma* spp. can contribute towards management of *Armillaria* root rot of tea.

Although organic amendments influence population dynamics of soil microorganisms (Gilpatrick, 1969; Chung, 1989; Cook, 1990; Hoitink and Fahy, 1986), these results have revealed that the type of influence depends on the type of material used as a soil amendment. This study has established that coffee pulp and husks have a high enhancing effect on *Trichoderma* proliferation which was as effective as basamid in the management of *Armillaria* root rot of tea. The efficacy of soil fumigants in the management of *Armillaria* root rot is well established (Rykowski, 1974; Filip and Roth, 1977; Bliss, 1951; Venkata Ram and Devasahayam Joseph, 1974). Coffee husks (hulls) have also been shown to be effective in the control of Fusarium yellow of beans, *Fusarium oxysporum* F. sp. *phaseolic* in the greenhouse (Mutitu *et al*, 1985), perhaps as a result of enhanced mycoparasitism.

A number of *Trichoderma* spp. have been associated with mycoparasitism of a wide range of soil-borne plant pathogens such as *Rhizoctonia solani, Sclerotinia sclerotiorum, Fusarium oxysporum* (Elad *et al*, 1980; Lewis and Papavizas, 1987; Trufman and Keane, 1990; Sivan *et al*, 1987). Suppressiveness in soils to soil-borne plant pathogens has also been attributed to some *Trichoderma* spp.

(Henis *et al*, 1979; Chet and Baker, 1981; Liu and Baker, 1980; Hornby, 1983). Other probable mechanisms are as follows:

Coffee pulp. being an organic amendment could have during composting exposed *A. mellea* to sub-lethal or lethal temperatures thus reducing its inoculum in the soil. A similar phenomenon has been observed on *Rhizoctonia solani, Verticillium albo-atrum, Verticillium dahliae, Phytophthora cinnamomi, Phytophthora cryptogea, Botrytis cinerrea, Sclerotium rolfisii* (Hoitink *et al*, 1976; Yuen and Raabe, 1984; Sterne *et al*, 1979; Wijnene *et al*, 1983; Ylimaki *et al*, 1983). The same has also been observed in the case of *Armillaria mellea* as well (Yuen and Raabe, 1984).

During decomposition of coffee pulp in the soil, toxic products such as ammonia, ethylene, carbon dioxide organic acids, dimethyl sulphide could have been produced in high enough concentrations to cause fungitoxic effects to *A. mellea* and hence subsequent reduction of its inoculum (Gilpatrick, 1969; Sonoda, 1977; Papavizas, and Lewis, 1971; Schippers and Bouman, 1973; Smith, 1976).

Besides *Trichoderma*, coffee pulp enhanced proliferations of other fungi and bacteria. The microbial proliferation could be due to the increased supply of nutrients other than nitrogen (Alexander, 1977; Nakasaki *et al*, 1985; Tisdale *et al*, 1985). The increased non-*Trichoderma* microbial proliferation could have promoted antagonism (Alexander, 1977; Baker and Cook, 1974; Shipton, 1977), competition for food and air (Clark, 1968), hyperparasitism (Mankau, 1980; Lockwood, 1977; Hunter *et al*, 1977), predation (Baker and Cook, 1974), lysis (Lockwood, 1977; Lamana

and Malette, 1965), and antibiosis (Gottlieb and Shaw, 1970) to *A. mellea*. All these, however, need to be investigated.

The significantly (P=0.05) higher tea yields in the coffee pulp amended plots relative to basamid and the control plots, implies added benefits of the amendment above those of disease management. It is evident that change in the soil physical and chemical properties due to coffee pulp amendment also contributed to the yield enhancement as demonstrated by correlations (r=0.92 to 0.99) between soil organic matter content, soil bulk density at 10-15cm depth and soil pH and tea yield. Indeed coffee pulp increased the soil pH to 4.7 which is closer to the pH of 5.0 to 5.6 where tea thrives best (Anon, 1986).

The non-significant (P=0.05) differences in the soil and leaf nitrogen contents due to treatments, implies lack of direct nutritional benefit due to coffee pulp amendment. However, indirect nutritional benefit due to the improved soil pH cannot be ruled out (Fox and Kamprath, 1971; Franklin and Reisenauer, 1960).

The significant (P=0.05) correlation between *Trichoderma* population and tea yield, implies that *Trichoderma* also positively influenced tea yield. Plant growth stimulation by *Trichoderma* spp. has been reported in various floricultural and horticultural plants (Lynch and Whipps, 1989; Baker *et al*, 1984; Chang *et al*, 1986).

CONCLUSIONS AND RECOMMENDATIONS

The results from this study revealed that coffee pulp and husks in a 10:1 ratio, used as an organic amendment enhanced proliferation of *Trichoderma* spp., most of which had antibiotic activities to *Armillaria mellea*. For coffee pulp to sustain high populations of *Trichoderma* for biological control of *Armillaria* root rot, an application interval of five months was found to be ideal. The age of decomposition of the coffee pulp influenced its proliferative ability to *Trichoderma* with one-month-old pulp being the most proliferative. For maximum proliferative ability of coffee pulp to *Trichoderma*, its application during wet months of the year was best.

These results also showed that sub-lethal doses of a soil fumigant (basamid) was inferior to coffee pulp in the ability to enhance proliferation of *Trichoderma*.

The results also showed that coffee pulp was as effective as basamid in the field management of *Armillaria* root rot of tea. It had an added advantage over basamid, in that it improved tea yields as well. The improved tea yield as a result of coffee pulp amendment was attributed to disease management and improved soil physical and chemical properties such as bulk density and pH.

The future work should concentrate on identification of non-*Trichoderma* antagonists to *Armillaria mellea* in the rhizosphere and rhizoplane of tea roots and methods of enhancing their proliferation sought. Ways and means of manipulating the tea root rhizosphere to selectively enhance the population of the most potent *Trichoderma* isolates such as *T. koningii* (isolate 9), *T.*

longibrachiatum (isolate 3) and *T. harzianum* (isolate 4) should be investigated further.

The success of using organic amendment will be influenced by the mode of application. Some work, therefore, needs to be done to establish the best mode of application so that the highest population of antagonists is confined to the rhizosphere and rhizoplane of tea roots which are the target for *Armillaria mellea*.

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