Effect of Interaction of Fungicides and Vesicular - Arbuscular Mycorrhizal Fungi on the Growth of *Acacia nilotica* SSP. *indica*

BY

Anandalakshmi M.

A THESIS SUBMITTED TO THE AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN (DEEMED UNIVERSITY) COIMBATORE - 641 043

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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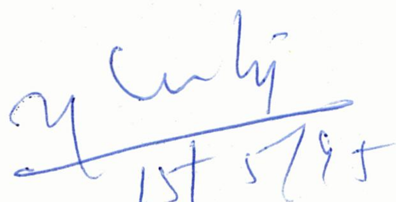

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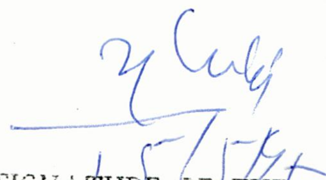

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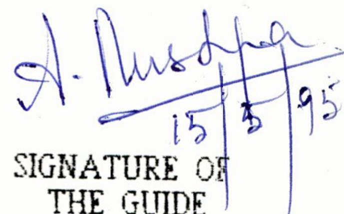

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Introduction

1.0. INTRODUCTION

Vesicular - Arbuscular Mycorrhizal (VAM) fungi are ecologically important and form an integral part of many tropical ecosystems (Osonubi et al., 1991). They are obligate symboints associated with the fine feeder roots of higher plants (Verma, 1994).

Various experimental results have proved the effectiveness of VAM symbiosis in plantation programme on most inhospitable sites and in nurseries (Singh et al., 1993). It is now recognized that inoculation of suitable VAM fungi is a significant factor in producing quality seedlings of forest trees in nurseries (Narayana Bhat, et al., 1993). However, little work has been done to improve seedling quality in forest nurseries by using these fungi (Thapar et al., 1992).

Fortifying tree seedlings with VAM fungi is very important especially in survival, establishment and growth of seedlings after transplating (Khan, 1994). They improve uptake of nutrients especially phosphorus (Padma and Sullia, 1991) and water (Kehri and Chandra, 1990). VAM fungi also increase nodulation, nitrogen fixation (Ginwal, 1993) and biomass (Patterson et al., 1990). Besides, they render increased tolerance to root pathogens (Jalali, 1993), drought (Bagchi, 1992) and salinity (Hosur and Patil, 1994).

It is proved that VAM fungi undoubtedly contribute to the success of plants establishment and survival (Muthukumar et al., 1994). VAM fungus is therefore a good candidate for biofertilizer (Tewari, 1992)Consequently, there is an increasing world-wide interest in VAM symbiosis (Sambandan et al., 1994).

Acacia nilotica ssp. indica commonly called as 'Babul' is an important multipurpose tree of family Leguminosae (Balakrishnan and Toky, 1993). It is an indigenous species selected for social forestry programme due to its adaptability to different agro-climatic zones (Srivastava and Kalyani,1990).Its high resistance to drought (Prasad, 1991), salinity (Sharma et al., 1992) and easy propagation through seeds make the species apt for planting under social forestry schemes (Banerjee and Mandal, 1994).

Babul is an excellent source of fuel-wood, timber, fodder, tannin, gum etc.(Tenjarla and Kavathekar,1990).Almost every part of A.nilotica is useful. It also improves soil fertility by fixing atmospheric nitrogen (Tewari, 1994). Various surveys conducted reveal that acacias are mycorrhizal-dependent (Sankaran, 1994) and A.nilotica is colonized by Glomus fasciculatum (Narayana Bhat et al., 1994).

It is documented that seeds of A.nilotica are often attacked by pathogenic fungi (Dwivedi, 1993). Such

seed - borne pathogens become active upon the germination of infested seeds and cause either seedling mortality or produce disease at a later stage (Bhardwaj, 1994).

As a result the use of fungicides has become a common practice (Mehrotra, 1991). Many studies report that such fungi toxicants may exert deleterious impact on non-target VAM fungi with influence on the host plant (Sukarno et al., 1994). On the other hand, Rajkumar et al. (1993) suggests that fungicides may also stimulate the VAM symbiosis.

The fungicides Bavistin (systemic) and Dithane M-45 (non-systemic) are found to be commonly used in forest nurseries to control seed borne fungal pathogens. However influence of seed-treated fungicides, Bavistin and Dithane M-45 of varying concentrations on *G. fasciculatum* colonizing *A.nilotica* has not yet been considered. A need therefore, has been felt to work out appropriate fungicidal dosages which when applied would not inhibit the beneficial VAM fungi development in plants. This information is also vital for the rational use of fungicides in control.

With these problems and perspectives at background the present study was conducted with the following objectives.

(1) To determine the effect of selected fungicides at varying concentrations on mycorrhizal infection.

(2) To estimate characteristic biochemical parameters with respect to growth and nutrient status in *A.nilotica* due to the combined effect of VAM and fungicides.

(3) To determine the appropriate dosage of the fungicides at which they serve beneficial to both VAM fungi and the plant.

Review of Literature

2.0. REVIEW OF LITERATURE

The review of literature pertaining to the present study entitled "Effect of interaction of fungicides and vesicular - arbuscular mycorrhizal fungi on the growth of *Acacia nilotica* ssp. *indica*" is discussed under the following headings:

2.1. Vesicular - arbuscular mycorrhizae (VAM)

  2.11. Events in root colonization

2.2. Role of VAM in forestry

2.3. Influence of VAM on plant diseases

2.4. Seed-borne diseases and their management by using fungicides

  2.41. Seed treatment

  2.42. Fungicides

    (a) Bavistin

    (b) Dithane M-45

2.5. Interaction between fungicides and VAM

2.1. Vesicular - arbuscular mycorrhizae (VAM)

Vesicular - arbuscular mycorrhizal fungi are exceptioanally common micro-organisms that occur in a wide variety of biomes (Read, 1991). This fungus - plant mutualistic symbiosis, termed mycorrhiza, occurs in 80% of the plant species (Azcon, 1994). VA mycorrhizae are characterised by the formation of branched haustorial structures called 'arbuscules' within certain root cortical

cells, and by terminal and intercalary hyphal swellings called 'vesicles' which occur within or between the cells (Wilcox, 1990).

2.11. Events in root colonization

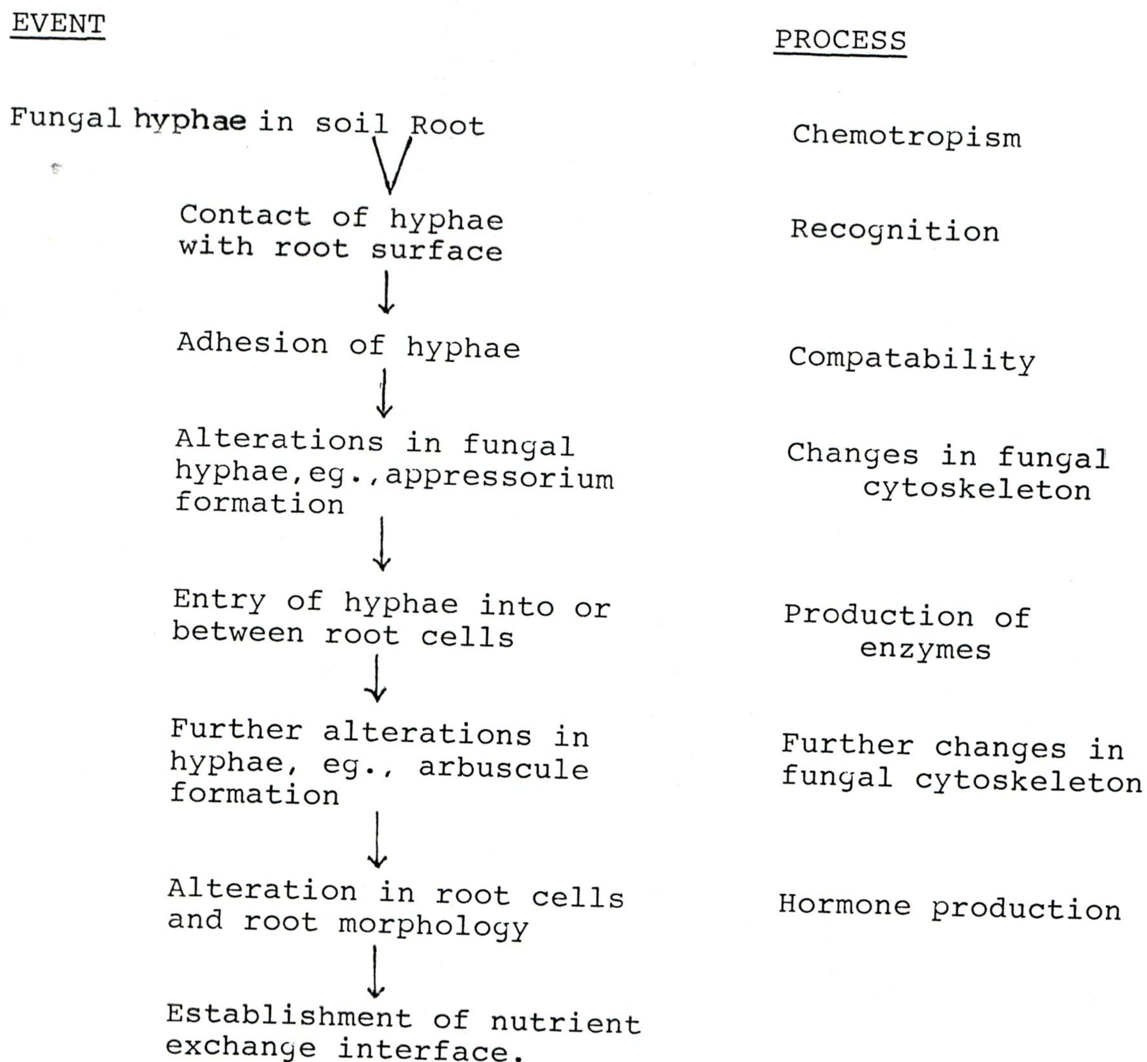
VA mycorrhizal fungi are obligate symbionts and their survival depends on the ability of their hyphae to infect a host plant rapidly and effeciently (Manuela et al., 1993). Spore germination has been reported to be independent of the presence of a host plant (Daniels et al., 1980), though recent reports have shown that both spore germination and hyphal growth, upto the formation of infection structures, could be increased by the presence of a host plant or of its root exudates (Nair et al., 1991).

It has been mentioned by Gianinazzi (1991), a low level of fungal aggressiveness and a weak plant reaction are two key factors for the establishment of the VAM symbiosis. The interaction between the fungus and the plant cell takes place at both an extracellular and intracellular level (Arines et al., 1993).

Mycorrhizal infection is established by a peculiar structure, the appressorium, whose formation is the first and most important indication of fungal recognition of a potential host plant (Stapels and Macko,1980). Thus the interaction between VAM fungi and roots involve a series of

events ultimately leading to an integrated functioning structure (Peterson and Farquhar, 1994).

SCHEME OF EVENTS IN VAM SYMBIOSIS.



2.2. Role of VAM in forestry

The role of VAM symbiosis in forestry is boosting up the growth of seedlings in nursery and establishment of man made forest (Ginwal, 1993). Agroforestry on agricultural land essentially requires the plantation of

tree species and in this the scope of VAM inoculation has far better prospect compared to natural forest regeneration (Verma, 1993).

Acacia nilotica ssp. indica is an important nitrogen fixing multipurpose tree. It is tolerant to high salinity (Yadav, 1989) and may be an important species for reclamation of salt affected soils (Hosur **and Patil, 1994).**

Reena and Bagyaraj (1990) demonstrated that the seedlings of A.nilotica when inoculated with 13 different VAM fungi had shown greater plant height, leaf number, stem girth, biomass and phosphorus and zinc content.

It has been reported by Srinivas (1987) that G.fasciculatum inoculation to A.nilotica, Eucalytus tereticornis and Leucaena leucocephala increased biomass by 168, 332 and 39 per cent respectively over control.

Menge et al., (1980) increased the growth of avocado seedlings by 49 to 254 per cent in sterilized loamy sand by inoculation with two isolates of G.fasciculatum. Plenchette et al., (1981) observed 142 per cent increase in shoot length in unsterilized field soil through VAM inoculation. Mukherjee and Jagpal (1987) observed that VAM inoculated Leucaena leucocephala not only showed better growth performance but also increased the vigour of the plant to overcome the adverse conditions such as salinity,

low fertility and drought in wastelands near Delhi.

2.3. Influence of VAM on plant diseases

Of late, the significance of VAM in the disease resistance of plants is gaining momentum (Mohankumar et al., 1992). VA mycorrhizae are capable of reducing the effects of various fungal pathogens and suppressing the effects of plant parasitic nematodes. In mycorrhizal roots the starch content is diminished but there is an increase in soluble carbohydrates and reducing sugars which is indirectly attributed to provide protection against root pathogens (Schenck, 1981).

The roots colonized by VAM fungus exhibit high chitinolytic activities which are effective against fungal pathogens and protect the plants from root diseases (Boller, 1985). Histochemical studies have shown that mycorrhizal roots contain more phenols than non-mycorrhizal roots (Krishna, 1981). Such increased levels of phenolic compounds also provide protection against root pathogens (Krishna and Bagyaraj, 1983).

Besides, increased production of plant protective phytoalexins such as isoflavonoids is attributed to the symbiosis (Morandi et al., 1984). They viewed this accumulation of isoflavonoids as an indication that mycorrhizal plants respond more quickly to attack by pathogenic fungi and nematodes as compared to non-mycorrhizal plants.

Mehrotra (1991) had decoumented about increased resistance of host to root pathogens due to VAM fungi. Thus the interactions between VA mycorrhizal infections and plant pathogens are clearly complex and may involve,

(a) competition for actual sites of infection on the root
(b) changes in the nutrition of the host plant
(c) enhancement of tolerance of the host plant to infection by the mycorrhizal fungi and
(d) provision of physical barriers to compensate for the damage to roots caused by the pathogens (Rohini Iyer **and Sanal Kumar, 1994).**

Gunjal and Patil (1992) reported that dual inoculation with Frankia and the two mycorrhizal fungi (G.fasciculatum and Gigaspora calospora) afforded maximum protection against Trichosporium vesiculosum wilt and also increased the plant biomass.

2.4. Seed - borne diseases and their management by using fungicides.

Seed - borne diseases of forest plants are incited by different causal agents such as fungi, prokaryotes and viruses. Amongst these, the impact of fungi is considerable and many of them are serious pathogens of maturing seeds and reduce the yield both quantitatively and qualitatively (Bhardwaj et al., 1988).

Seed - borne infections behave in different ways depending on the nature of the pathogen and also on sowing and growing conditions. Severly infected seeds in which the embryo is affected will not germinate. More superficial infections mainly cause pre-emergence decay but do not manifest on the older plants. Slightly infected seeds will, however, germinate to serve as infection centres from which the disease spread to the adjoining trees causing significant losses (Luna, 1989).

The important seed - borne fungal diseases of *A.nilotica* are caused by fungi such as *Aspergillus flavus*, *A.fumigants*, *A.nidulans*, *A.niger*, *A.terreus*, *Drechslera* species, *Fusarium equiseti*, *Rhizopus* species and *Sclerotium bataticola* (Abdalla, 1988).

2.41. Seed treatment

The effectiveness of seed treatment with fungicides in the control of externally as well as internally seed borne pathogens has been proved (Moly Saxena *et al*., 1994). Proper germination of seed is a pre-condition for a good plant (Randhawa *et al*., 1992) and seed treatment with certain fungicides has been found to improve germination (Ua, 1990).

Chemical management of seed borne diseases is the cheapest and easiest method. The seed dressers (fungicide and suitable sticker) may be applied to seeds in small seed

lot using relatively simple seed treaters or rotary seed dresser, in which dry seed treatment is done. Another method ie. wet seed treatment through dip involves preparing fungicide suspension in water, often at field rates, and then dipping the seed for specific time (Bhardwaj, 1994).

2.42. Fungicides

Bavistin and Dithane M-45 were found effective against some of the commonly occurring true seed - borne fungi such as Aspergillus niger, A.Sydowi, Rhizopus oryzae and Trichoderma viride (Mittal and Sharma, 1981).

(a) Bavistin

Bavistin commonly known as 'Carbendazim' is a broad spectrum systemic fungicide. Chemically, it is methyl-2-benzimidazole carbamate (MBC). It is both protective and curative fungicide.It acts by inhibiting development of the germ tubes, the formation of appressoria and the growth of mycelia (Clive, 1994). When applied as dip or seed treatment it is usually translocated throughout the plant system (Vashishta, 1993). Patil (1980) has reported about the control of seed - borne fungi such as Rhizoctonia bataticola, Penicillium species and Alternaria alternata by bavistin seed treatment at the rate of 0.2 per cent.

(b) Dithane M-45

Dithane M-45 commonly known as 'Mancozeb' is a non-systemic fungicide. Chemically it is manganese ethylene

bis (dithiocarbamate) (polymetric) complex with zinc salt. It is a fungicide with protective action and is recommended for seed treatment (**Clive,** 1994). It is effective in eliminating external seed pathogens (Bidari, 1995). It is a fungicide used effectively for controlling the damping-off disease in the nursery seedlings (Sudhakara Reddy and Natarajan, 1994).

2.5. Interaction between fungicides and VAM

In forest nurseries, fungicides are extensively used to control soil - borne and seed- borne fungal pathogens. However, these are not pathogen-specific but also affect a wide range of non-pathogenic fungi (Sukarno et al., 1993). Several investigations have shown that VAM fungi can be affected by the application of fungicides (Fitter et al., 1988). Thus the benefits of fungicides (increased crop growth via destrucion of pathogens) may be diluted by harmful effects on beneficial microbes like mycorrhizal fungi (Trappe et al., 1984).

Studies conducted by Gunasekaran et al., (1987) suggest that only 9 per cent VAM infection was recorded in Benomyl treated cowpea plants at 50 ppm level. Seed treatment with Brassicol, Thiram and Emisan exhibited inhibitory effects on dry weight of shoots, mycorrhizal density and phosphate uptake in mungbean (Jalali, 1987). Application of Captan has been observed to have no effect on

mycorrhizal colonization(Manjunath and Bagyaraj 1984).Nemec (1980) found that Difolatan reduced root infection by VAM fungi, when applied to soil on which citrus plants were growing. However, this fungicide did not affect the spore development of mycorrhizal fungi and on the contrary increased the spore population in some cases. Findings of Ocampo and Hayman (1980) indicate that population of VA mycorrhizal fungi are adversely affected by fungicide, resulting in decreased mycorrhizal infections and spore numbers.

The data now available on the interaction between mycorrhizal fungi and fungicides indicate that fungicides typically delay or reduce vesicular - arbuscular mycorrhizal infection but rarely eliminate it altogether (Menge, 1982).

Experimental Procedure

3.0. EXPERIMENTAL PROCEDURE

The experimental details pertaining to the present study "Effect of interaction of fungicides and vesicular-arbuscular mycorrhizal fungi on the growth of *Acacia nilotica* ssp. *indica*," is discussed as follows:

(a) Collection of biofertilizer

Glomus fasciculatum culture was obtained from Department of Agricultural Microbiology, Tamilnadu Agricultural University, Coimbatore.

(b) Collection of *A.nilotica* seeds

The seeds of *A.nilotica* were obtained from Department of Seed Technology, Institute of Forest Genetics and Tree Breeding, Coimbatore.

(c) Preparation of potting mixture

Unsterilised potting mixture was prepared by mixing red soil and sand in the ratio 1:1. The potting mixture which was found to have pH 9.4 was filled in polythene bags of size 11 x 21 cm.

(d) Seed treatment

The seeds of *A.nilotica* were surface sterilised with 80 per cent ethanol for 1-2 minutes. In order to propagate *A.nilotica* efficiently, the seeds must be

subjected to presowing treatment to ensure not only a high final germination percentage but rapid and uniform germination after sowing (Doran *et al.*, 1983). Therefore the seeds were soaked in cold water for 24 hours. Then the seeds were treated with the selected fungicides.

(e) Fungicidal treatment of seeds

The seeds were divided into 7 seed lots, 3 lots for each fungicide treatment i.e., Bavistin and Dithane M-45 and one lot for control. The fungicides were mixed at concentrations of 0.1, 0.2 and 0.3 per cent with the pretreated seeds and allowed to stand for about 24 hours. Then the seeds were sown in the potting mixture filled in polythene bags.

(f) Method of VAM application

The VAM fungi (*G.fasciculatum*) was applied at the rate of 5g/polythene bag. The inoculum was applied at 2.5 cm below the soil surface at the time of sowing.

The treatment details are as follows:

T_1 - VAM inoculated control

T_2 - VAM + Bavistin 0.1 per cent

T_3 - VAM + Bavistin 0.2 per cent

T_4 - VAM + Bavistin 0.3 per cent

T_5 - VAM + Dithane M-45 0.1 per cent

T_6 - VAM + Dithane M-45 0.2 per cent

T_7 - VAM + Dithane M-45 0.3 per cent

(g) Maintenance of seedlings

Watering : The seedlings were watered once a day upto 90 days

Weeding : Weeds appearing on the soil surface of polythene bags were removed manually.

The samples were collected after 30, 60 and 90 days of sowing. During each sampling 4 plants were removed from each treatment.

The plants were washed in running tap water to remove adhering soil particles and pressed between filter paper folds to remove water droplets. The following observations were recorded.

Biometric analysis

(1) Shoot length

The shoot length was measured from the ground level till the tip in cm.

(2) Root length

The saplings were uprooted gently and the root length was measured in cm.

(3) Root collar diameter

The saplings were uprooted and the root collar diameter was measured at the collar region of the plant and expressed in cm.

(4) Fresh weight

The saplings were uprooted, washed, weighed and the fresh weight was expressed in grams per plant.

(5) Dry weight

The plants were dried in a oven at $70^{\circ}C$ for 24 hours and the dry weight was recorded in grams per plant.

(6) Germination percentage

The number of seeds which were germinated after 10 days of sowing was counted.

Infection studies

(7) Percentage infection

The percentage of *G.fasciculatum* infection in *A.nilotica* roots was estimated by the method of Phillips and Hayman (1970). The details of the method are given in Appendix - I.

Biochemical analysis

(8) Estimation of Chlorophyll

The chlorophyll content of fresh leaves was estimated by Spectrophotometric method (Yoshida *et al*., 1971) as given in Appendix - II.

For the estimation of total nitrogen, phosphorus and potassium content, the saplings were dried in an oven at

$80^{o}C$ for 24 hours and then powdered using mortar and pestle. The dry powder was subjected to analysis of total nitrogen, phosphorus and potassium content.

(9) Estimation of Total Nitrogen

Plant total nitrogen content was estimated by Microkjeldahl method (Humphries, 1956), the details of which are given in Appendix - III.

(10) Estimation of Phosphorus

The phosphorus content was estimated by the method of Jackson (1973) as given in Appendix - IV.

(11) Estimation of Potassium

The potassium content was estimated by using flame photometer (Jackson, 1973). The details of the method are given in Appendix - V.

(12) Esimation of Total Phenols

The total phenol content of roots was estimated by Folin-Ciocalteau method (Mahadevan and Sridhar, 1986). The details of the method are given in Appendix - VI.

(13) Estimation of Total Carbohydrate

The total carbohydrate content of roots was estimated by Anthrone method (Sadasivam and Manickam, 1991) as given in Appendix - VII.

(14) Estimation of Protein

The protein content of shoot was estimated by Spectrophotometric method (Lowry _et al_., 1951) as given in Appendix - VIII.

Soil Analysis

The soil samples used for the growth of _A.nilotica_ saplings were analysed for available nitrogen, phosphorus and potassium levels before and after the experimental period. These soil samples were designated as ST_1 to ST_7.

(15) Estimation of Available Nitrogen

The available nitrogen in the soil was estimated by Alkaline potassium permanganate method (Subbiah and Asija, 1956) as given in Appendix - IX.

(16) Estimation of Available Phosphorus

The available phosphorus in the soil was estimated by Olsen's method (Olsen _et al_., 1954). The details of the method are given in Appendix - X.

(17) Estimation of Available Potassium

The available potassium in the soil was estimated by using flame photometer (Stanford and English, 1949). The details of the method are given in Appendix - XI.

Statistical Analysis

Statistical analysis of the data was carried out as suggested by Panse and Sukhatme (1985). Analysis of variance was conducted for the biometric observations and biochemical parameters to find out the significant difference between various treatments for all the experimental periods.

Results and Discussion

4.0. RESULTS AND DISCUSSION

In the present study entitled "Effect of interaction of fungicides and vesicular-arbuscular mycorrhizal fungi on the growth of *Acacia nilotica* ssp. *indica*", six combinations of vesicular - arbuscular mycorrhizal (VAM) fungi and varying concentrations of selected fungicides with one VAM inoculated control were tried, to assess the effect of VAM and fungicidal interaction on the percentage of VAM colonization in *A.nilotica* seedlings. Characteristic biometric and biochemical parameters which reflect the growth and nutrient status of the seedlings were analysed. Also soil analysis was conducted before and after the growth of *A.nilotica* seedlings.

The different treatments chosen were:

T_1 - VAM inoculated control

T_2 - VAM + Bavistin 0.1 per cent

T_3 - VAM + Bavistin 0.2 per cent

T_4 - VAM + Bavistin 0.3 per cent

T_5 - VAM + Dithane M-45 0.1 per cent

T_6 - VAM + Dithane M-45 0.2 per cent

T_7 - VAM + Dithane M-45 0.3 per cent

The results and discussion pertaining to the present study are dealt under the following headings:

<u>Biometric measurements</u>

(a) Shoot length

(b) Root length

(c) Root collar diameter

(d) Fresh weight

(e) Dry weight

(f) Germination percentage

<u>Infection studies</u>

(g) Percentage infection

<u>Biochemical parameters</u>

(h) Total chlorophyll content

(i) Total nitrogen content

(j) Phosphorus content

(k) Potassium content

(l) Total phenol content

(m) Total carbohydrate content

(n) Protein content

<u>Available nutrients in the Soil</u>

(o) Nitrogen

(p) Phosphorus

(q) Potassium

The biometric observations, infection studies and biochemical analysis were performed at three stages of plant growth namely on 30, 60 and 90 days of sowing. Soil analysis was conducted before sowing seeds and at the end of the experimental period (90 days).

Biometric measurements

(a) Shoot length

Shoot length is one of the variable characters that reflects the growth of the plant.

Table I presents the shoot length of *Acacia nilotica* after 30, 60 and 90 days of treatment with VAM fungi and six different combinations of VAM and selected fungicides at varying dosages.

TABLE I

SHOOT LENGTH OF *Acacia nilotica*

Treatments	Shoot length (cm)		
	30 days	60 days	90 days
T_1	11.50	18.47	36.61
T_2	11.59	18.53	40.60
T_3	10.98	17.95	25.80
T_4	10.67	15.87	20.30
T_5	11.28	18.51	38.50
T_6	11.73	20.93	42.50
T_7	11.03	17.50	24.70

- mean of four replications

S.E.D. = 3.83

C.D. ($P<0.05$) = 8.35

Among the 30th day seedlings, shoot length was found to be maximum in seedlings treated with VAM + Dithane

M-45 0.2 per cent (11.73 cm) and minimum in VAM + Bavistin 0.3 per cent treatment (10.67 cm). T_6 was followed by T_2, T_1, T_5, T_7 and T_3 with respect to shoot length. Highest shoot length (20.93 cm) was registered on 60th day by VAM + Dithane M-45 0.2 per cent treated seedlings and lowest by VAM + Bavistin 0.3 per cent treated seedlings (15.87 cm). T_6 was succeeded by other treatments in the order of T_2, T_5, T_1, T_3 and T_7. Among the shoot lengths recorded on 90 th day, seedlings treated with VAM + Dithane M-45 0.2 per cent showed the highest value of 42.5cm and those treated with VAM + Bavistin 0.3 per cent recorded the lowest value of 20.3 cm. Shoot lengths of other treatments observed on the 90th day followed a similar trend as that of the 60th day.

Both T_2 and T_6 were on par with each other in influencing the shoot length on A.nilotica and it is clear from Table I that VAM + Bavistin 0.1 per cent and VAM + Dithane M-45 0.2 per cent treatments would serve best in improving shoot length of A.nilotica seedlings. Jalali and Chhabra (1991) reported that VAM + Bavistin 0.2 per cent treatment decreased the plant height when compared to VAM inoculated control in pearl millet. This report parallels the findings of the present study.

(b) Root length

Table II and Figure I illustrate the root length of Acacia niotica after 30, 60 and 90 days of treatment with VAM and six different combinations of VAM and fungicides at varying concentrations.

TABLE II

ROOT LENGTH OF *Acacia nilotica*

Treatments	Root length (cm)		
	30 days	60 days	90 days
T_1	39.17	52.17	63.20
T_2	43.00	55.62	70.11
T_3	32.93	48.25	59.37
T_4	29.73	45.80	55.83
T_5	40.73	51.55	65.40
T_6	44.97	54.92	72.90
T_7	34.37	49.25	60.21

- mean of four replications

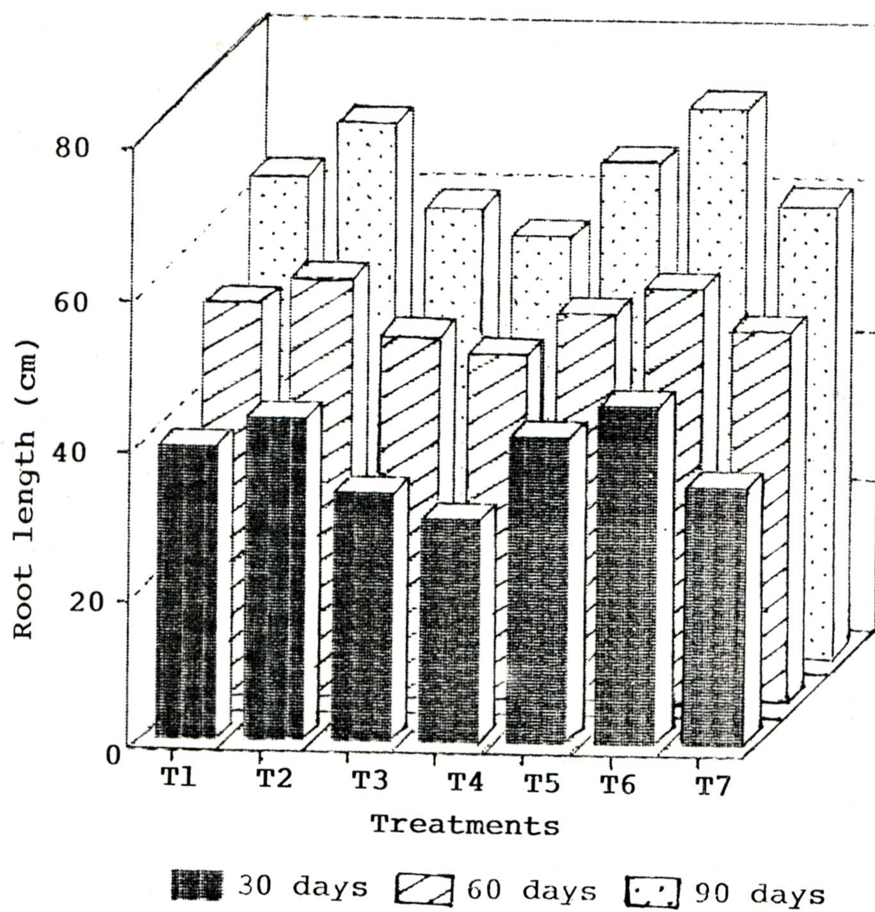
S.E.D. = 1.30

C.D. ($P<0.05$) = 2.83

From the data presented in Table II it is evident that the root length of 30th day seedlings was highest in VAM + Dithane M-45 0.2 per cent treatment (44.97 cm) and lowest in VAM + Bavistin 0.3 per cent treated seedlings (29.73 cm). T_6 was followed by T_2, T_5, T_1, T_7 and T_3 with regard to root length of A.nilotica. On 60th day maximum root length of 55.62 cm was registered by VAM + Bavistin 0.1 per cent treatment and minimum was registered by T_4 (45.80 cm). T_2 was followed T_6, T_1, T_5, T_7 and T_3 showing a gradation in root length. Among 90th day

FIGURE I

ROOT LENGTH OF *Acacia nilotica*



seedlings, highest root length of 72.90 cm was recorded by those subjected to VAM + Dithane M-45 0.2 per cent treatment and lowest value of 55.83 cm was recorded by VAM + Bavistin 0.3 per cent treated seedlings. All other treatments on 90th day showed a similar trend in root length as that of 30th day.

According to the result of the present study, both 0.1 per cent concentration of Bavistin and 0.2 per cent concentration of Dithane M-45 along with VAM application exhibited equally good effects on root length of A.nilotica. Sukarno et al. (1993) observed increased root length in mycorrhizal Allium cepa on Aliette and Ridomil application, while the root length decreased on Benlate application.

The increased root length in the best treatments T_2 and T_6 might be due to high VAM colonization which lead to extensive spread of roots that ramified the soil to absorb nutrients.

(C) Root collar diameter

Table III depicts the root collar diameter of A.nilotica at different time points of study on treatment with VAM and six different combinations of VAM and fungicidal dosages.

TABLE III

ROOT COLLAR DIAMETER OF *Acacia nilotica*

Treatment	Root collar diameter (cm) #		
	30 days	60 days	90 days
T_1	0.60	1.20	1.60
T_2	0.70	1.50	2.80
T_3	0.50	1.00	1.30
T_4	0.40	0.90	1.30
T_5	0.70	1.40	1.70
T_6	0.80	1.50	2.00
T_7	0.50	1.10	1.30

- mean of four replications

S.E.D. = 0.20

C.D. ($P<0.05$) = 0.43

On 30th day, VAM + Dithane M-45 0.2 per cent treated seedlings recorded maximum root collar diameter of 0.8 cm and VAM + Bavistin 0.3 per cent treatment (T_4) showed minimum value of 0.4 cm. Other treatments which influenced root collar diameter were in the order of T_2 = T_5, T_1, T_7 and T_3, preceeded by T_6. After 60 days, both VAM + Dithane M-45 0.2 per cent and VAM + Bavistin 0.1 per cent treated seedlings registered maximum root collar diameter of 1.5 cm, whereas, seedlings subjected to VAM + Bavistin 0.3 per cent treatment registered minimum root collar diameter of 0.9 cm. The treatments T_6 and T_2 were followedby T_5, T_1, T_7 and T_3. Among 90th day seedlings, VAM + Bavistin 0.1 per cent (T_2)

treated seedlings recorded maximum root collar diameter of 2.8 cm and minimum value of 1.3 cm was recorded by three treatments such as T_3, T_4 and T_7. Treatment T_2 was followed by T_6, T_5 and T_1 with respect to root collar diameter.

Thus the results of the present study indicate that both VAM + Bavistin 0.1 per cent (T_2) and VAM + Dithane M-45 0.2 per cent (T_6) were the best treatments to enhance root collar diameter of A.nilotica seedlings.

(d) Fresh weight

Fresh and dry weights are important indices of quantity and quality of the plant as a whole. Besides, these factors contribute to the economic value of the plant to the outside world.

Table IV presents the fresh weight of A.nilotica after 30,60 and 90 days of treatment with VAM and six different combinations of VAM and fungicidal concentrations.

TABLE IV

FRESH WEIGHT OF *Acacia nilotica*

Treatments	Fresh weight (g/plant) #		
	30 days	60 days	90 days
T_1	1.48	2.84	5.65
T_2	1.69	3.41	11.72
T_3	1.30	2.77	4.86
T_4	1.22	2.29	3.73
T_5	1.61	3.08	8.82
T_6	1.74	3.62	13.42
T_7	1.52	2.90	5.97

- mean of four replications

S.E.D. = 1.60

C.D. ($P<0.05$) = 3.48

On 30th day, maximum fresh weight of 1.74 g/plant was registered by VAM +Dithane M-45 0.2 per cent treated seedlings and minimum of 1.22 g/plant was registered by VAM + Bavistin 0.3 per cent treated seedlings. The treatment T_6 was followed by T_2, T_5, T_7, T_1 and T_3 on 30th day with respect to fresh weight of *A.nilotica*. At the other two stages namely, 60 and 90 days also, all the seven treatments followed the same trend as that of 30th day with regard to fresh weight.

No significant differences in the increment of fresh weight of *A.nilotica* was noticed between T_2 and T_6 and these treatments were found to be superior to the rest.

(e) Dry weight

Table V and Figure II depict the dry weight of <u>A.nilotica</u> after 30, 60 and 90 days of treatment with VAM and six different combinations of VAM and selected fungicides at varying dosages.

TABLE V

DRY WEIGHT OF <u>Acacia nilotica</u>

Treatments	Dry weight (g/plant) #		
	30 days	60 days	90 days
T_1	0.46	1.15	2.62
T_2	0.76	1.31	5.15
T_3	0.37	0.99	1.96
T_4	0.31	0.89	1.39
T_5	0.48	1.29	4.66
T_6	0.80	1.45	6.18
T_7	0.51	1.21	2.77

- mean of four replications

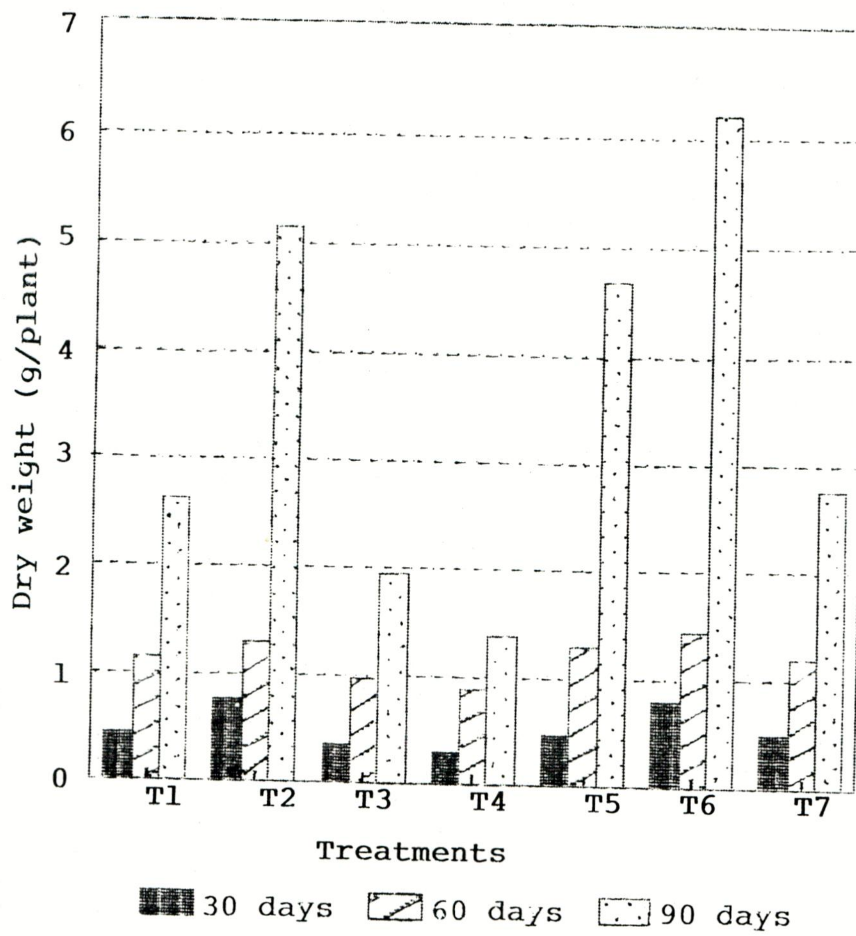
S.E.D. = 0.7

C.D. ($P<0.05$) = 1.67

Among seven treatments, VAM + Dithane M-45 0.2 per cent treated seedlings recorded maximum dry weight of 0.8 g/plant and VAM + Bavistin 0.3 per cent treated seedlings showed minimum value of 0.31 g/plant on 30th day. The treatment T_6 was suceeded by T_2, T_7, T_5, T_1 and T_3 with regard to dry weight of <u>A.nilotica</u>. On 60th and 90th day, seedlings subjected to VAM + Dithane M-45 0.2 per cent

FIGURE II

DRY WEIGHT
OF *Acacia nilotica*



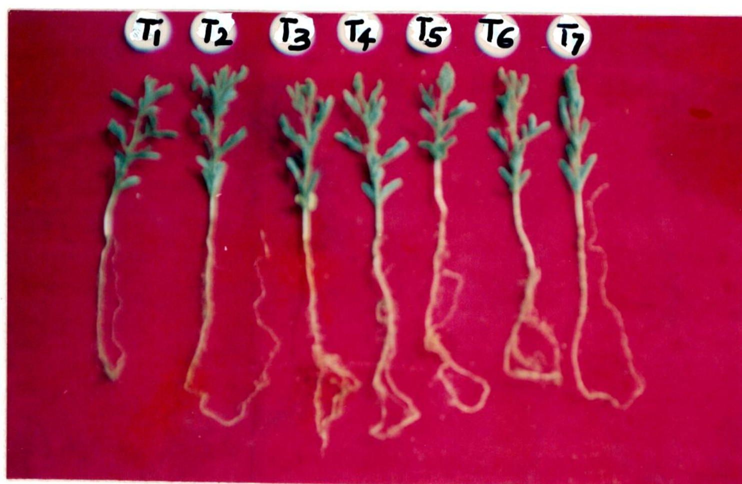
treatment recorded highest dry weights of 1.45 and 6.18 g/plant respectively while the lowest values were recorded by VAM + Bavistin 0.3 per cent treatment with dry weight of 0.89 and 1.39 g/plant respectively. At these two stages all other treatments followed T_6 in order T_2, T_5, T_7, T_1 and T_3.

From Table V it is evident that dry weight of A.nilotica was increased by VAM + Dithane M-45 0.2per cent treatment (T_6). Also VAM + Bavistin 0.1 per cent treatment (T_2) was found to influence dry weight in the same way as that of T_6. Therefore, both T_2 and T_6 could be recommended to elevate the dry weight of A.nilotica seedlings. Studies conducted by Jalali and Sharma (1993) indicated that VAM + Bavistin 0.2 per cent treatment of pearl millet adversely affected the total dry weight of plant when compared to mere VAM treatment. In our study also VAM + Bavistin 0.2 per cent treatment (T_3) was found to reduce the dry weight of A.nilotica.

(f) Germination percentage

The overall germination percentage was calculated by counting the number of seeds that germinated after 10 days of sowing. The germination percentage of A.nilotica in the present study was found to be 84 per cent.

Plate 1 : Effect of interaction of fungicides and VAM fungi on <u>A.nilotica</u> at 30 days



<u>Treatments</u>

T_1 - VAM inoculated contro

T_2 - VAM + Bavistin 0.1%

T_3 - VAM + Bavistin 0.2%

T_4 - VAM + Bavistin 0.3%

T_5 - VAM + Dithane M-45 0.

T_6 - VAM + Dithane M-45 0.

T_7 - VAM + Dithane M-45 0.

Plate 2 : Effect of interaction of fungicides and VAM fungi on <u>A.nilotica</u> at 60 days

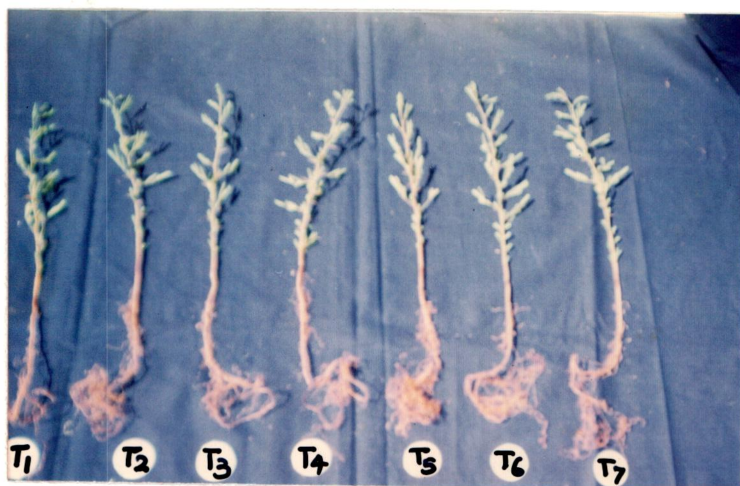


Plate 3 : Effect of interaction of fungicides and VAM fungi on *A.nilotica* at 90 days

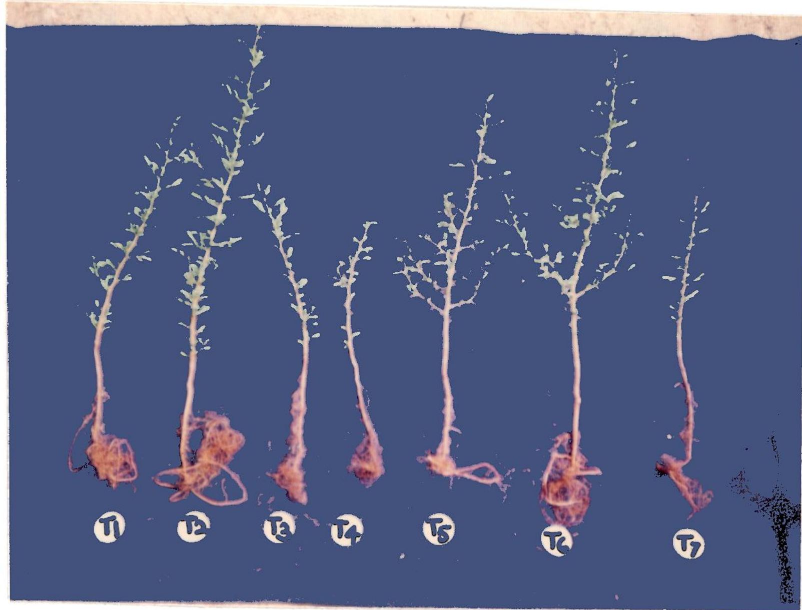
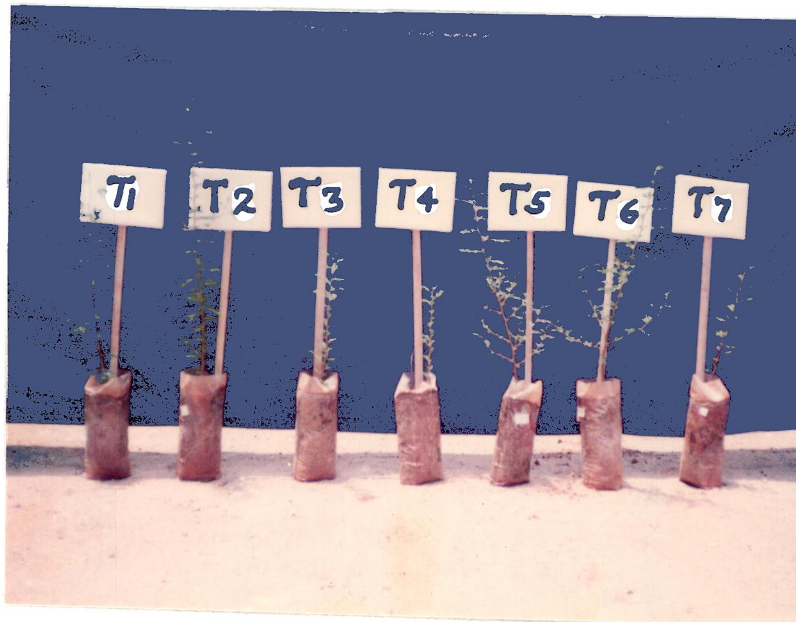


Plate 4 : *A.nilotica* seedlings grown in polythene bags as viewed at the end of the experimental period



Infection studies

The intensity of infection contributes to the measure of ultimate benefits rendered by VAM to the host plant which necessitated this study.

(g) Percentage infection.

Table VI and Figure III illustrate the percentage infection in A.nilotica after 30, 60 and 90 days of treatment with VAM and six different combinations of VAM and varying fungicidal dosages.

TABLE VI

PERCENTAGE INFECTION IN Acacia nilotica

Treatments	Percentage infection (Per cent)					
	30 days	V A	60 days	V A	90 days	V A
T_1	16	- -	24	+ +	35	+ +
T_2	19	- -	28	+ +	40	+ +
T_3	10	- -	18	+ +	24	+ +
T_4	7	- -	15	+ +	21	+ +
T_5	18	- -	26	+ +	38	+ +
T_6	20	- -	30	+ +	41	+ +
T_7	12	- -	20	+ +	29	+ +

V - vesicles

A - arbuscules

According to the data presented in Table VI

FIGURE III

PERCENTAGE INFECTION IN *Acacia nilotica*

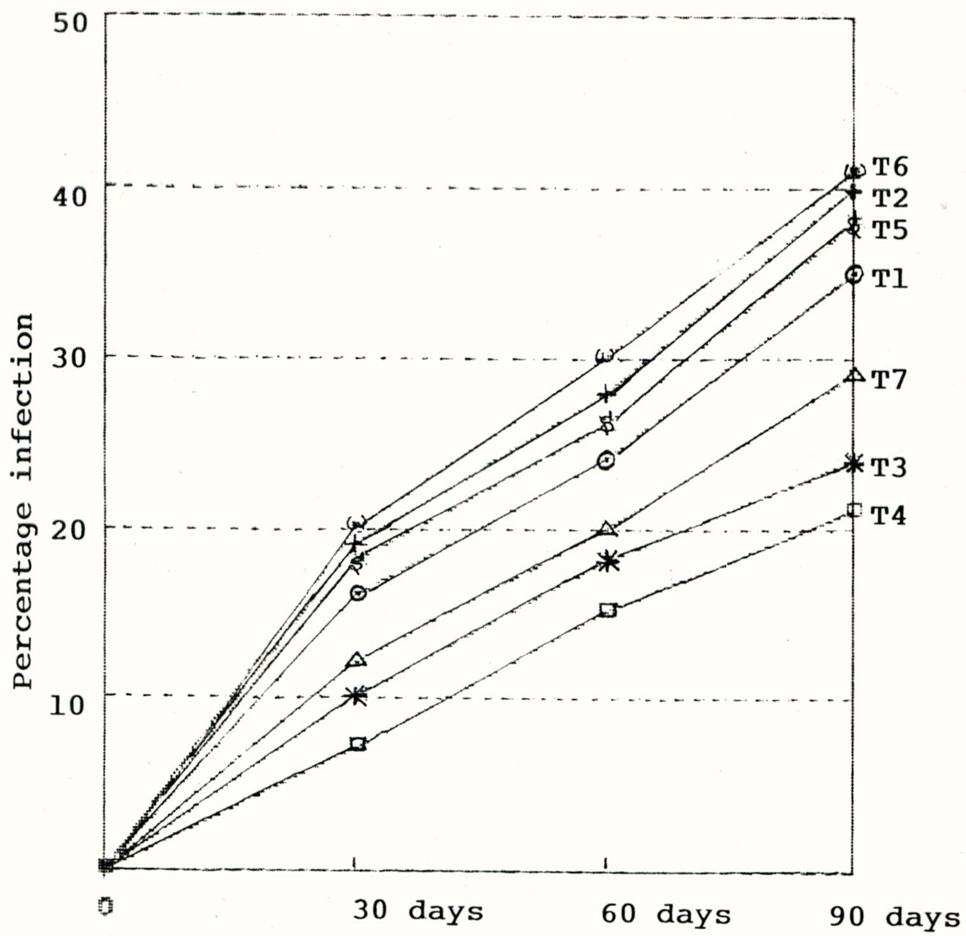
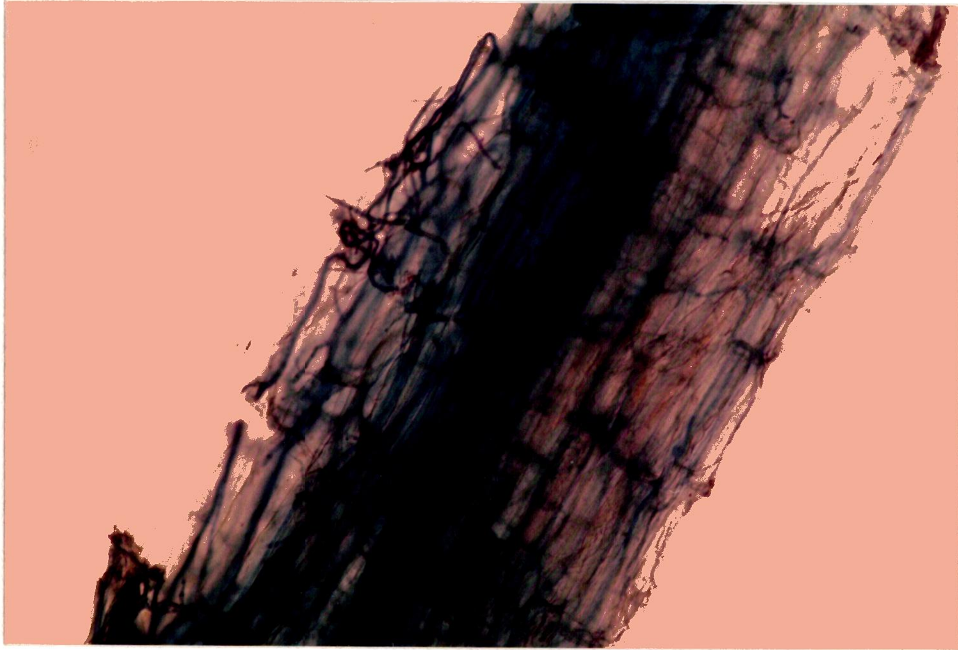


Plate 5 : Microscopic view of a highly VAM infected root of <u>A.nilotica</u> stained with tryphan blue



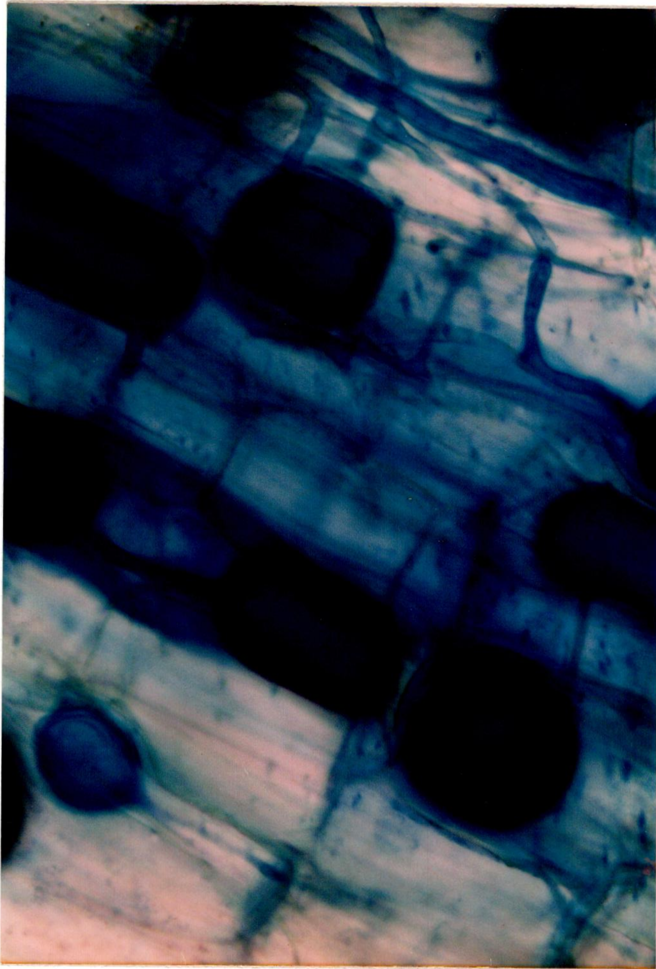


Plate 6: Root cortical cells of A.nilotica with intercalary vesicles and hyphal network

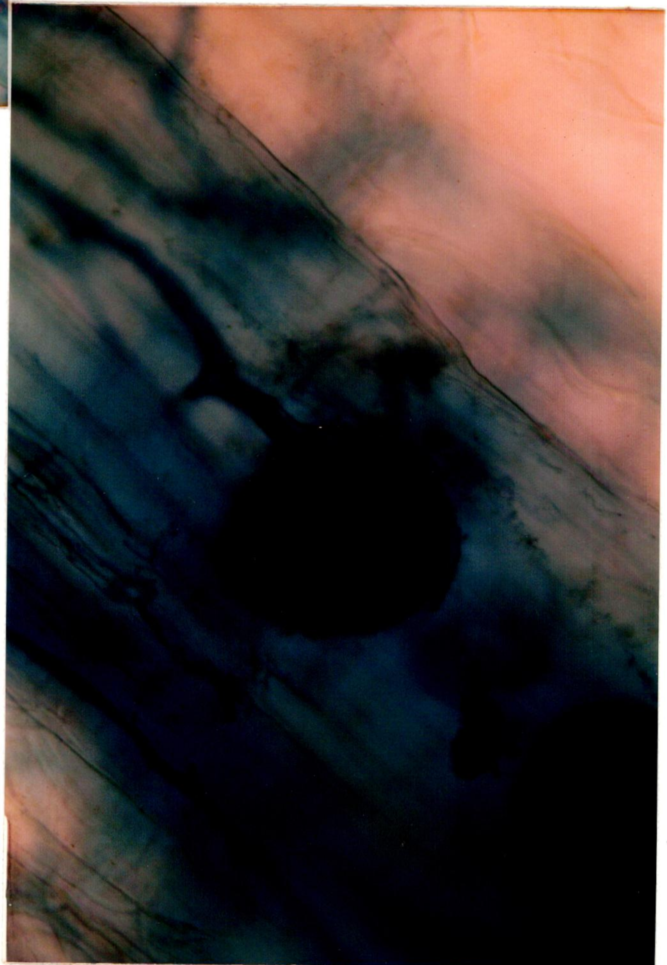


Plate 7: Appearance of a spherical vesicle attached to a hypha

maximum infection per cent was recorded by VAM + Dithane M-45 0.2 per cent (T_6) treated seedlings on 30th, 60th and 90th days with values of 20,30 and 41 per cent respectively. Whereas, VAM + Bavistin 0.3 per cent treatment (T_4) was found to be poorly colonized by VAM at the end of 30, 60 and 90 days with infection values of 7, 15 and 21 per cent respectively. T_6 was followed by T_2, T_5, T_1, T_7 and T_3 with respect to percentage infection at all three time points of study.

From Table VI it is obvious that among Dithane M-45 treatments, 0.2 per cent concentration and among Bavistin treatments, 0.1 per cent concentration were the best to enhance VAM colonization in A.nilotica. Even VAM + Dithane M-45 0.1 per cent treatment(T_5) showed increased VAM colonization than that of VAM inoculated control. This indicated that at certain low concentrations fungicides trigger VAM colonization. This may be attributed to root exudation, an important factor governing mycorrhizal colonization. It was suggested that fungicides may alter quality and quantity of the root exudation (Schwab et al., 1982) such as significant increase in exudation of soluble sugars from mycorrhizal roots (Jabaji et al., 1985). This may favour the multiplication of G.fasciculatum and suppress antagonists to VAM fungi including mycoparasite and predators (Sreenivasa and Bagyaraj, 1989). But in the present study with increase in fungicidal concentrations

ie., 0.2 and 0.3 per cent Bavistin and 0.3 per cent Dithane M-45, VAM colonization was suppressed. This result is supported by observations of Jalali and Chhabra (1991) in which 0.2 per cent Bavistin seed treatment exhibited measurable inhibition in VAM colonization of pearl millet.

Since a large proportion of VA mycorrhizal hyphae are inside the roots it would appear that non-systemic fungicides (Dithane M-45) can affect VAM primarily by inhibiting spore germination and infection processes and so are probably less damaging to mycorrhizal symbiosis than systemic fungicide (Bavistin). It was postulated that non-systemic fungicides may be fungistatic but once the mycorrhizal fungus gains entry to the root these chemicals have little effect and may actually increase spread within the root (Dinesh and Jayashree, 1987). Therefore Dithane M-45 was found to be less damaging to VAM fungi than Bavistin.

Biochemical parameters

(h) Total chlorophyll content

Table VII depicts the total chlorophyll content of *A.nilotica* after 30, 60 and 90 days of treatment with VAM and six different combinations of VAM and selected fungicides.

TABLE VII

TOTAL CHLOROPHYLL CONTENT OF *Acacia nilotica*

Treatments	Chlorophyll content (mg/g)#		
	30 days	60 days	90 days
T_1	1.05	1.37	2.53
T_2	1.24	2.41	4.29
T_3	1.14	1.91	3.80
T_4	1.11	1.79	3.20
T_5	1.19	2.11	3.99
T_6	1.28	2.72	4.42
T_7	1.15	1.97	3.77

- mean of four replications

S.E.D. = 0.25

C.D. ($P<0.05$) = 0.54

After 30, 60 days and 90 days, seedlings subjected to VAM + Dithane M-45 0.2 per cent treatment (T_6) recorded highest chlorophyll contents of 1.28, 2.72 and 4.42 mg/g respectively and VAM inoculated control (T_1) showed minimum values of 1.05, 1.37 and 2.53 mg/g respectively. On 30th, 60th and 90th days T_6 was followed by T_2, T_5, T_7, T_3 and T_4 treatments.

At all three stages VAM + fungicidal treatment recorded increased chlorophyll content compared to non-fungicidal VAM treatment. As increased chlorophyll content

is an indication of good health status of a plant it is amenable to apply VAM in combination with the two selected fungicides to enhance the health status of A.nilotica seedlings. However, our study revealed that correct dosage of fungicide should be applied for sufficient chlorophyll content and consequent good health status of A.nilotica.

(i) Total nitrogen content

Table VIII presents the total nitrogen content of A.nilotica after 30, 60 and 90 days of treatment with VAM and six different combinations of VAM and fungicides at varying dosages.

TABLE VIII

TOTAL NITROGEN CONTENT OF *Acacia nilotica*

Treatments	Nitrogen content (mg/plant) #		
	30 days	60 days	90 days
T_1	0.30	0.73	1.02
T_2	0.35	0.77	1.06
T_3	0.26	0.68	0.95
T_4	0.21	0.62	0.90
T_5	0.31	0.74	1.04
T_6	0.36	0.80	1.09
T_7	0.28	0.66	1.0

- mean of four replications

S.E.D. = 0.01

C.D. ($P<0.05$) = 0.025

The maximum total nitrogen content recorded after 30, 60 and 90 days were 0.36, 0.80, 1.09 mg/plant respectively which were found in VAM + Dithane M-45 0.2 per cent treatment (T_6). Minimum nitrogen contents of 0.21, 0.62 and 0.90 mg/plant on 30th, 60th and 90th days were recorded by VAM + Bavistin 0.3 per cent treatment (T_4). The order of other treatments following T_6 with respect to nitrogen content was found to be T_2, T_5, T_1, T_7 and T_3 on 30th and 90 th days and T_2,T_5, T_1, T_3 and T_7 on 60th day.

From Table VIII it is clear that treatment of mycorrhizal A.nilotica with 0.1 per cent Bavistin and 0.2 per cent Dithane M-45 served equally good in boosting up plant total nitrogen content. Jalali and Chhabra (1991) reported that there was significant increase in total nitrogen content of VAM inoculated pearl millet than those treated with VAM + Bavistin 0.2 per cent combination. A similar result was obtained in our study and this might be because of the high concentration of bavistin (0.2 per cent) which would have inhibited VAM colonization. Besides, high VAM colonization increases nitrogen content of the host by elevating the levels of ammonia assimilating enzymes that participate in nitrogen uptake.

(j) Phosphorus content

Table IX and Figure IV illustrate the phosphorus content of A.nilotica after 30, 60 and 90 days of treatment

with VAM and six different combinations of VAM and selected fungicides at varying concentrations.

TABLE IX

PHOSPHORUS CONTENT OF *Acacia nilotica*

Treatments	Phosphorus content (mg/plant) #		
	30 days	60 days	90 days
T_1	0.06	0.14	0.21
T_2	0.07	0.17	0.24
T_3	0.05	0.12	0.19
T_4	0.03	0.10	0.15
T_5	0.06	0.16	0.22
T_6	0.08	0.18	0.25
T_7	0.05	0.13	0.18

- mean of four replications

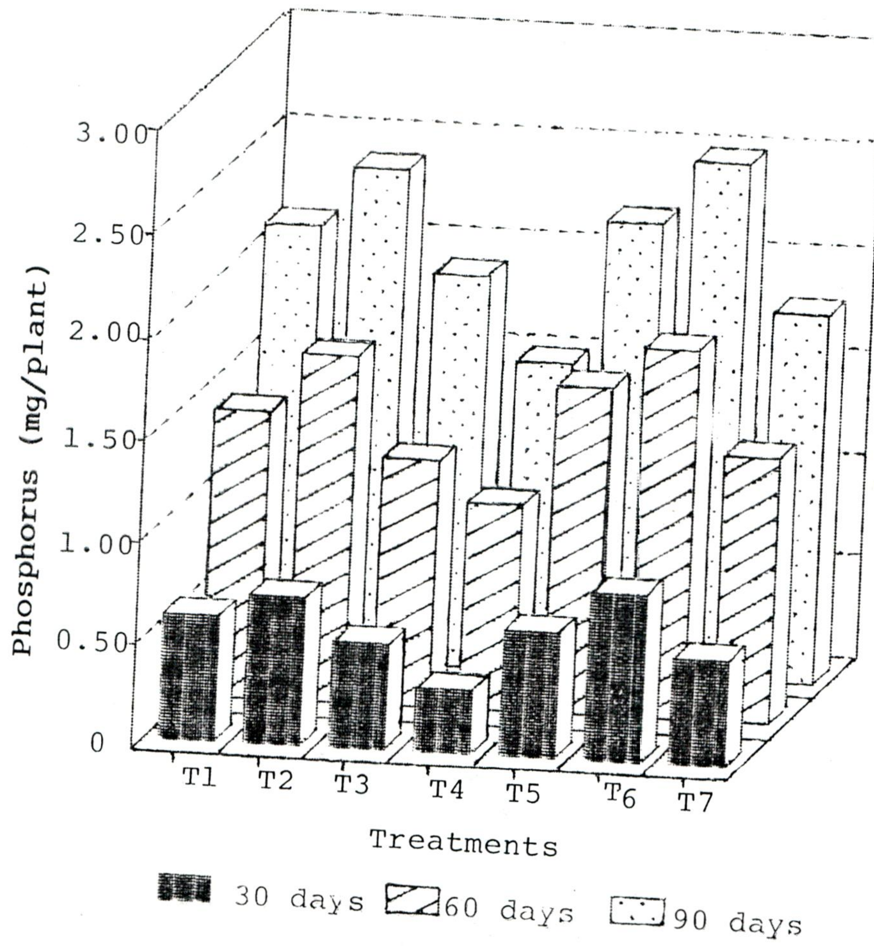
S.E.D. = 0.01

C.D. ($P<0.05$) = 0.02

From Table IX it is evident that seedlings treated with VAM + Dithane M-45 0.2 per cent (T_6) showed maximum phosphorus contents of 0.08, 0.18 and 0.25 mg/plant corresponding to 30, 60 and 90 days of sowing. At these stages VAM + Bavistin 0.3 per cent (T_4) recorded minimum phosphorus contents of 0.03, 0.10 and 0.15 mg/plant respectively. T_6 was followed by T_2, $T_1 = T_5$ and $T_3 = T_7$ on 30 days. On 60th day, treatments T_2, T_5, T_1, T_7 and T_3 were preceeded by

FIGURE IV

PHOSPHORUS CONTENT OF *Acacia nilotica*



T_6 where as on 90th day T_6 was followed by T_2, T_5, T_1, T_3 and T_7 treatments.

According to the results of the present study the plant phosphorus content was found to be very high in T_6 and T_2 compared to mere VAM inoculation. Even the VAM + Dithane M-45 0.1 per cent (T_5) treated seedlings showed significant increase in phosphorus content than VAM inoculated control (T_1). As T_6 and T_2 were on par with each other in influencing phosphorus content of A.nilotica, both these treatments were considered the best with respect to phosphorus content of A.nilotica. It has been reported by Jalali and Chhabra (1991) that the combination VAM + seed treated Bavistin at 0.2 per cent concentration recorded low root and shoot phosphorus contents than that of VAM inoculated control in the case of pearl millet.

The increase in phosphorus content in T_2 and T_6 might be due to increased activity of phosphatases. However, high colonization by VAM was known to increase these enzyme levels resulting in enhanced phosphorus uptake. Therefore increased levels of phosphorus in T_2 and T_6 might be due to rich VAM colonization.

(k) Potassium content

Table X depicts the potassium content of A.nilotica after 30, 60 and 90 days of treatment with VAM and

six different combinations of VAM and varying fungicidal dosages.

TABLE X

POTASSIUM CONTENT OF *Acacia nilotica*

Treatments	Potassium content (mg/plant) #		
	30 days	60 days	90 days
T_1	0.16	0.37	0.66
T_2	0.20	0.41	0.70
T_3	0.12	0.32	0.58
T_4	0.10	0.27	0.51
T_5	0.17	0.39	0.66
T_6	0.22	0.42	0.71
T_7	0.14	0.33	0.60

- mean of four replications

S.E.D.= 0.01

C.D. ($P<0.05$) = 0.03

VAM + Dithane M-45 0.2 per cent (T_6) treated seedlings showed highest potassium levels of 0.22, 0.42 and 0.71 mg/plant on 30, 60 and 90 days respectively. Lowest level of potassium on 30,60 and 90 days was found in VAM + Bavistin 0.3 per cent treatment (T_4) with corresponding values of 0.10, 0.27 and 0.51 mg/plant. All other treatments followed T_6 in the order T_2, T_5, T_1, T_7 and T_3 with respect to potassium content on 30, 60 and 90 days.

From the Table X it is evident that 0.1 per cent dosage of Bavistin and 0.2 per cent dosage of Dithane M-45 did not influence potassium content in markedly different ways. Therefore T_2 and T_6 were the best treatments in boosting up potassium uptake by A.nilotica. On the other hand reports of Jalali and Chhabra (1991) suggested that potassium uptake was not seriously disturbed by seed treated Bavistin (0.2 per cent) and VAM combination in pearl millet.

(1) Total phenol content

Table XI presents the total phenol content of A.nilotica roots after 30, 60 and 90 days of treatment with VAM and six different combinations of VAM and selected fungicides.

TABLE XI

TOTAL PHENOL CONTENT IN ROOTS OF *Acacia nilotica*

Treatments	Root total phenol content (mg/g) #		
	30 days	60 days	90 days
T_1	0.42	0.61	0.70
T_2	0.50	0.68	0.76
T_3	0.40	0.57	0.68
T_4	0.37	0.54	0.65
T_5	0.46	0.62	0.73
T_6	0.51	0.70	0.80
T_7	0.41	0.59	0.70

- mean of four replications

S.E.D. = 0.01

C.D. ($P<0.05$) = 0.01

The 30th, 60th and 90th day experimental results revealed that root total phenol content was highest in VAM + Dithane M-45 0.2 per cent treated seedlings with values of 0.51, 0.70, 0.80 mg/g respectively. At all these three stages lowest phenol content was recorded in VAM + Bavistin 0.3 per cent treatment (T_4) with corresponding values of 0.37, 0.54 and 0.65 mg/g. T_6 was followed by T_2, T_5, T_1, T_7 and T_3 with regard to root phenol content at the three time points of study.

Thus it is clear that both T_2 and T_6 were superior to the rest of the treatments in enriching A.nilotica with high root total phenol content. As phenols are said to offer resistance to disease and pests in plants, these two best treatments could be recommended for increasing disease resistance in nursery grown A.nilotica seedlings.

(m) Total carbohydrate content

Table XII presents the total carbohydrate content of A.nilotica roots after 30 and 90 days of treatment with VAM and fungicides.

TABLE XII

TOTAL CARBOHYDRATE CONTENT IN ROOTS OF *Acacia nilotica*

Treatments	Root total carbohydrate (mg/g) #		
	30 days	60 days	90 days
T_1	0.51	0.60	0.72
T_2	0.55	0.67	0.81
T_3	0.48	0.58	0.70
T_4	0.46	0.55	0.67
T_5	0.52	0.62	0.75
T_6	0.58	0.70	0.82
T_7	0.49	0.59	0.71

- mean of four replications

S.E.D. = 0.01

C.D. ($P<0.05$) = 0.02

Highest root total carbohydrate level was found in T_6 on all 30, 60 and 90 days with values of 0.58, 0.70 and 0.82 mg/g respectively. T_4 recorded lowest carbohydrate levels of 0.46, 0.55 and 0.67 corresponding to 30, 60 and 90 days of sowing. At all three stages of growth T_6 was followed by T_2, T_5, T_1, T_7 and T_3 with regard to root carbohydrate content.

T_6 and T_2 were on par with each other in influencing the total carbohydrate content of *A.nilotica* roots. Therefore it is evident that both T_6 and T_2 were the best treatments to increase root total carbohydrate content of *A.nilotica*. This high level of carbohydrate may be

attributed to the increased photosynthetic activity due to remarkable levels of VAM colonization.

(n) Protein content

Table XIII illustrated the shoot protein content of Acacia nilotica after 30, 60 and 90 days of treatment with VAM and six different combinations VAM and selected fungicides.

TABLE XIII

PROTEIN CONTENT IN SHOOT OF Acacia nilotica

Treatments	Shoot protein content (mg/g) #		
	30 days	60 days	90 days
T_1	0.94	1.12	1.30
T_2	0.98	1.16	1.35
T_3	0.91	1.07	1.23
T_4	0.89	1.03	1.20
T_5	0.96	1.14	1.33
T_6	1.01	1.18	1.38
T_7	0.93	1.09	1.28

- mean of four replications

S.E.D.= 0.01

C.D. ($P<0.05$) = 0.02

Table XIII revealed that T_6 recorded maximum shoot protein levels of 1.01, 1.18 and 1.38 mg/g on 30, 60 and 90 days respectively, whereas at these stages minimum protein values of 0.89, 1.03 and 1.20 mg/g was recorded by T_4. T_6 was followed by T_2, T_5, T_1, T_7 and T_3 on 30th, 60th and 90th days with regard to shoot protein content of A.nilotica.

All treatments influenced shoot protein content of A.nilotica significantly ($P<0.05$) with T_6 being superior to the rest. According to the present experimental results VAM + Dithane M-45 0.2 per cent treatment (T_6) could be safely put into practice in cases where this plant finds importance as a source of animal fodder.

(o) Available nutrients in the Soil

Table XIV shows the soil available nutrients, nitrogen, phosphorus and potassium contents before and after the growth of *Acacia nilotica* seedlings.

TABLE XIV

AVAILABLE NUTRIENTS IN THE SOIL BEFORE AND AFTER THE GROWTH OF *Acacia nilotica*

Soil samples	Nitrogen (Kg/hectare)	Phosphorus (Kg/hectare)	Potassium (Kg/hectare)
Before sowing	75	9	115
After sowing			
ST_1	99	10	138
ST_2	120	12	165
ST_3	108	11	151
ST_4	104	10	147
ST_5	122	11	160
ST_6	136	12	168
ST_7	100	10	155

As is evident from Table XIV all the three soil available nutrient levels increased after 90 days than those registered before the growth of A.nilotica seedlings. Therefore there is no doubt that VAM application increases soil fertility.

Comparison of the results of biometric analysis, infection studies, biochemical estimations and soil analysis declare that VAM + Bavistin 0.1 per cent treatment and VAM + Dithane M-45 0.2 per cent treatment are amenable in nursery practices than mere VAM application, with respect to Acacia nilotica. Seed treatment with 0.1 per cent Bavistin and 0.2 per cent Dithane M-45 could be safely employed, because at these concentrations the respective fungicides have been found non-hazardous to VAM fungi (G.fasciculatum) and infact enhance VAM symbiosis and plant growth.

Summary and Conclusion

5.0. SUMMARY AND CONCLUSION

In the present study entitled "Effect of interaction of fungicides and vesicular-arbuscular mycorrhizal fungi on the growth of Acacia nilotica ssp. indica", investigations were carried out to study the combined effect of varying concentrations of selected fungicides and vesicular-arbuscular mycorrhizal (VAM) fungi on the growth of Acacia nilotica seedlings. Biometric observations, infection studies and biochemical analysis were conducted after 30, 60 and 90 days of sowing. Also soil analysis was done before sowing and after 90 days of experimental period. The various treatments imposed on seeds of A.nilotica were :

T_1 - VAM inoculated control

T_2 - VAM + Bavistin 0.1 per cent

T_3 - VAM + Bavistin 0.2 per cent

T_4 - VAM + Bavistin 0.3 per cent

T_5 - VAM + Dithane M-45 0.1 per cent

T_6 - VAM + Dithane M-45 0.2 per cent

T_7 - VAM + Dithane M-45 0.3 per cent

It was clear from the experimental results that both VAM + Bavistin 0.1 per cent and VAM + Dithane M-45 0.2 per cent were the best treatments to increase shoot length, root length and root collar diameter of A.nilotica, during the study period of 30, 60 and 90 days. Observations at

these three time points revealed that VAM + Bavistin 0.3 per cent treatment showed the least effect on parameters such as shoot length, root length and root collar diameter. The increased root length in the best treatments might be due to high VAM colonization which lead to extensive spread of roots that ramified the soil to absorb nutrients.

Both fresh weight and dry weight of A.nilotica was increased significantly by VAM + Bavistin 0.1 percent treatment and VAM + Dithane M-45 0.2 per cent treatment. Therefore these two treatments could be recommended inorder to elevate the fresh and dry weights of A.nilotica. Regarding these two parameters VAM + Bavistin 0.3 per cent treatment recorded the poorest effect which might be due to suppressed VAM colonization.

The overall germination percentage of A.nilotica during the present study was found to be 84 per cent. The percentage infection of VAM fungi, Glomus **fasciculatum** was studied on 30th 60th and 90th days, after sowing. VAM + Bavistin 0.1 per cent and VAM + Dithane M-45 0.2 per cent treated seedlings recorded maximum VAM colonization and minimum level was registered in VAM + Bavistin 0.3 per cent treatment. The increase or decrease in VAM colonization might be attributed to the fungicidal effect on altering the quality and **quantity** of root exudation. At same concentration (i.e., 0.2 per cent) of Bavistin and Dithane M-45, the former was found to adversely affect VAM symbiosis

and latter was found to enhance VAM colonization. Such an effect might be due to the mode of action of the fungicides. As Bavistin is a systemic fungicide which would be translocated throughout the plant system especially into the roots thus inhibiting the spread of VAM fungi, whereas, Dithane M-45 being a non-systemic fungicide, could not act after the VAM fungus had gained entry to the root. Therefore, Dithane M-45 was found to be less damaging to VAM colonization than Bavistin.

The chlorophyll content was markedly increased in VAM + fungicidal combinations when compared to that of VAM inoculated control. Among the VAM and fungicidal combinations, 0.1 per cent Bavistin and 0.2 per cent Dithane M-45 served the best with regard to chlorophyll content of *A.nilotica*. Increased chlorophyll content is an indication of good health status of a plant. Therefore, it is amenable to apply VAM in combination with the two selected fungicides to improve the growth and health of *A.nilotica*.

The total nitrogen content, phosphorus content and potassium content of *A.nilotica* was elevated to a great extent in both VAM + Bavistin 0.1 per cent treatment and VAM + Dithane M-45 0.2 per cent treatment. On the other hand, these levels were highly decreased in VAM + Bavistin 0.3 per cent treatment. The increment in the levels of nitrogen and phosphorus in T_2 and T_6 might be due to the increase in the levels of ammonia assimilating enyymes and phosphate

solubilizing enzymes respectively, which were again the consequences of remarkable VAM colonization.

Root total phenol and carbohydrate content and shoot protein content was found to be maximum in VAM + Bavistin 0.1 per cent and VAM + Dithane M-45 0.2 per cent treatments of A.nilotica. Minimum values of these three biochemical parameters was registered in VAM + Bavistin 0.3 per cent treated seedlings. As phenols are said to offer resistance to diseases and pests in plants, the best treatments such as VAM + Bavistin 0.1 per cent and VAM + Dithane M-45 0.2 per cent could be recommended for increasing disease resistance in nursery grown A.nilotica seedlings. The increase in total root carbohydrate content might be due to increased photosynthetic activity rendered by VAM symbiosis. Elevation in shoot protein content might be due to remarkable extent of VAM symbiosis which had been already found to increase the total nitrogen content in the best treatments.

The soil analysis performed before and after 90 days of experimental period revealed that soil available nutrients such as nitrogen, phosphorus and potassium contents increased remarkably after the growth of the seedlings. This indicates that VAM inoculation undoubtedly contributes to the improvement of soil fertility.

From the present study it is concluded that both VAM + Bavistin 0.1 per cent treatment and VAM + Dithane M-45 0.2 per cent treatment are amenable in nursery practices than mere VAM inoculation, with respect to *Acacia nilotica*. Seed treatment with 0.1 per cent Bavistin and 0.2 per cent Dithane M-45 could be safely employed because at these concentrations the respective fungicides do not exhibit deleterious effect on *Glomus fasciculatum* and infact enhance VAM symbiosis and plant growth.

The hiking price and pollution hazards of fungicides have lead to the need of using them rationally. As a result it is essential to use minimal quantity of fungicides which would serve the purpose for its application in a very efficient manner.

Recommendation for future studies

Though ample studies have been conducted to study the effect of general VAM culture on plant growth and its interaction with pesticides, fertilizers etc., only a very few data are available with respect to the effect of specific VAM species like *Acaulospora* and *Scutellospora*. Also very little information about genetic aspects of VAM fungi is known. If this need is fulfilled there is no doubt in bringing about new revolutions in the spheres of forestry and agriculture.

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Appendices

APPENDIX I

ESTIMATION OF VAM COLONIZATION

This procedure (Phillips and Hayman, 1970) was adopted for clearing and staining the root segments for rapid assay of mycorrhizal colonization. The root segments were cut into bits of 1 cm length and washed thoroughly in distilled water. They were softened by boiling in 2.5% potassium hydroxide at 90°C for 5-15 minutes depending upon the hardness of the material. Then the root bits were washed in fresh potassium hydroxide solution and 3-4 changes of distilled water. The specimens were acidified with 5N hydrochloric acid for 5 minutes and stained in 0.05 per cent tryphan blue (in lactophenol) for 15-30 minutes. The excess stain if any was removed with clear lactophenol.

Stained root bits were mounted on glass slides in lactophenol and examined through a compound microscope under low power (100x) for the presence of VAM hyphae and vesicles. Portions were observed under high magnification (400-900x) for the occurrence of arbuscules. The per cent root colonization for each plant species was calculated as follows :

$$\text{Percentage root length infection} = \frac{\text{Numberof VAM infected root bits}}{\text{Total number of root bits examined}} \times 100$$

APPENDIX II

ESTIMATION OF TOTAL CHLOROPHYLL

Spectrophotometric method

(Yoshida *et al.*, 1971)

Principle

Chlorophyll pigments present in the sample are extracted using acetone and the green colour of the extract is read in a spectrophotometer at 645 nm and at 663 nm.

Procedure

A sample of 1.0 g of fresh leaves was taken. Cut into small bits and macerated in a glass mortar. Added enough acetone to allow the tissue to be thoroughly homogenised. Homogenisation was continued and then the supernatant was filtered into a 100 ml volumetric flask. By adding 80 per cent acetone to the residue in the mortar, the extraction was repeated. Then the volume was made upto the mark with 80 per cent acetone. The optical density was measured in a spectrophotometer at 663 and 645 nm.

Calculation

$$\text{Total chlorophyll} = \frac{20.2\ A_{645} + 8.02\ A_{663}}{a \times 1000 \times W} \times V \text{ mg g}^{-1} \text{ fresh weight}$$

Where,

a = Path length of the light in the cell (usually 1cm)

V = Volume of the extract in ml

W = Fresh weight of the sample in g

APPENDIX III

ESTIMATION OF NITROGEN

Microkjeldahl method

(Humphries, 1956)

Reagents

(1) Diacid - 4:1 ratio of sulphuric acid and perchloric acid

(2) Mixed indicator

Dissolved 0.5 g bromocresol green and 1g of methyl red in 100 ml ethylalcohol.

(3) N/50 Sulphuric acid

(4) 40 per cent Sodium hydroxide and

(5) 2 per cent Boric acid

Procedure

Ground the plant sample and made it a fine powder. Took 0.5 g of sample in a microkjeldahl flask and 12 ml of diacid. Digested the sample over a sand bath. Made up the volume to 100 ml with distilled water. Pipetted out 10 ml of the aliquot into a microkjeldahl distillation apparatus. Kept at the delivery end 10 ml of 2 per cent boric acid mixed indicator in a 100 ml beaker. Added 10 ml of 40 per cent sodium hydroxide into the microkjeldahl distillation apparatus and steamed the distillate until a blue colour was reached. After distillation, titrated against N/50 sulphuric acid until a red wine colour was got.

APPENDIX IV

ESTIMATION OF PHOSPHORUS

Reagents (Jackson, 1973)

1) Barteu Reagent

Solution A: 25g of Ammonium molybdate was dissolved in 400 ml of warm water.

Solution B: 1.25g Ammonium metavenadate was dissolved in 300 ml of boiling water.

Solution A was added to solution B and the volume was made upto 1000 ml.

Procedure

From the acid extract pipetted out 5 ml of the aliquot into a 25 ml volumetric flask. Introduced a bit of red litmus paper. Neutralised with ammonia solution until litmus paper turned blue. Again acidified it with concetrated nitric acid until litmus paper turned red - Added 5 ml of the Barteu reagent. Made up the volume to 25 ml with distilled water. After 30 minutes the intensity of yellow colour developed was read at 420 nm in a colorimeter. Using the standard phosphorus curve, the phosphorus concentration in the sample was calculated.

Preparation of Standard Phosphorus Curve

Analytical grade of potassium dihydrogen phosphate was dried at 40°C and 0.2195g was dissolved in 400 ml of

distilled water, 25 ml of 7 N sulphuric acid and the volume was made upto 1000 ml. This was 50 ppm of phosphorus. Then 100 ml of this 50 ppm stock solution was diluted to 1000ml to get 5 ppm of phosphorus solution. From this a series of phosphorus standards ranging from 0.01ppm to 1.0 ppm were prepared. Finally 50 ml of this solution was pipetted out into a 25 ml volumetric flask. 5.0 ml of Barteu reagent was added and volume was made upto 25 ml. Intensity of the colour of each standard was measured on the colorimeter and a standard curve was constructed using colorimeter readings and concentrations.

APPENDIX V

ESTIMATION OF POTASSIUM

(Jackson, 1973)

Procedure

5.0 ml of the acid extract was pipetted out into a vial and the transmission was read in a flame photometer. Using a potassium standard curve, the concentration of potassium was determined.

Potassium Standard Curve

1.907 g of Analar grade potassium chloride was dissolved in 100 ml distilled water to get 1000 ppm of potassium 100 ml of 1000 ppm potassium was diluted to one litre to get 100 ppm of potassium. From this a series of potassium standards ranging from 0-100 ppm were prepared and percentage transmission was read on a flame photometer to construct a standard curve.

APPENDIX VI

ESTIMATION OF TOTAL PHENOLS

Folin - Ciocalteau method

(Mahadevan and Sridhar, 1986)

Principle

Estimation of phenols using Folin-Ciocalteau reagent is based on the reaction between phenols and an oxidizing agent phosphomolybdate which results in the formation of a blue complex (Bray and Thorpe, 1954). The intensity of the colour is measured in a colorimeter at 650 nm.

Reagents

(1) Folin - Ciocalteau reagent : Dissolved 100 g sodium tungstate and 25 g sodium molybdatein 700 ml water in one litre flask. Added 50 ml 85 per cent orthophosphoric acid and 100 ml concentrated hydrochloric acid and boiled under reflux gently for 10 hours. Cooled and added 150 g lithium sulphate dissolved in 50 ml water and 4-5 drops of liquid bromine. Boiled the mixture without condensor for 15 minutes to remove the excess bromine, cooled, diluted to volume with water and filtered ; Stored in brown bottle. Diluted the reagent with equal volume of water just before use.

(2) 20 per cent Sodium carbonate

Procedure

1 g of the sample was homogenized using 20 ml of 80 per cent ethanol. The homogenized sample was centrifuged at 10,000 rpm for 20 minutes. The supernatant was collected. The residue was re-extracted with 10 ml of 80 per cent ethanol and centrifuged. The supernatant was collected, pooled and evaporated to dryness. The dry residue obtained was dissolved in a known volume of water (5.0 ml) and 2.0 ml was taken for the experiment.

0.2 to 1.0 ml of the catechol working standard corresponding to 20-100 µg of catechol was pipetted out into a series of test tubes. The volumes were made upto 2.5 ml with distilled water. To all the tubes added 0.5 ml of Folin - Ciocalteau reagent. After 3 minutes added 2.0 ml of 20 per cent sodium carbonate solution to each tube and mixed thoroughly. The tubes were placed in a boiling water bath for exactly one minute. Cooled and measured the absorbance at 650 nm against a reagent blank.

APPENDIX VII

ESTIMATION OF TOTAL CARBOHYDRATE

Anthrone method

(Sadasivam and Manickam, 1991)

Principle

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural . This compound forms with anthrone a green coloured product with an absorption maximum at 630 nm.

Reagents

(1) 2.5 N hydrochloric acid

(2) Anthrone reagent: Dissolved 200 mg anthrone in 100 ml of ice cold 95 per cent sulphuric acid prepared fresh before use.

(3) Standard glucose: Stock - Dissolved 100 mg of glucose in 100 ml of water. Working standard - 10 ml of stock was diluted to 100 ml with distilled water.

Procedure

Weighed 100 mg of the sample into a boiling tube. Hydrolysed by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N hydrochloric acid and cooled to room temperature. Neutralised it with solid sodium carbonate until the effervescence ceased. Made up the volume to 100

ml, and centrifuged, collected the supernatant and took 0.5 and 1 ml aliquots for analysis. Prepared the standards by taking 0.2 to 1 ml of the working standard. Made up the volume to 1 ml in all the tubes including the sample tubes by adding distilled water. Then added 4 ml of anthrone reagent. Heated for eight minutes in a boiling water bath. Cooled rapidly and read the green to dark green colour at 630nm against a reagent blank.

APPENDIX VIII

ESTIMATION OF PROTEIN

Spectrophotometric method

(Lowry *et al.*, 1951)

Principle

The method is based on the principle that different proteins contain different amounts of aromatic residues which react with Folin - Ciocalteau reagent giving a blue colour which is read in a colorimeter at 750 nm.

Reagents

(1) Alkaline copper sulphate reagent

Reagent A - 2 per cent sodium cabonate in 0.1 N sodium hydroxide

Reagent B - 0.5 per cent copper sulphate in 1 per cent sodium potasium tartrate.

Mixed 50 ml of reagent A and 1.0 ml of reagent B freshly before use

(2) 1 N sodium hydroxide

(3) Folin - Ciocalteau reagent.

(4) Protein standard solution : 50 mg of bovine serum albumin was made upto 100 ml with 0.9 per cent saline.

Procedure

Into a series of test tubes pipetted out 0.2 to 1.0 ml of standard albumin solution. Made upto 1.0 ml with

distilled water. To 1.0g of the biological sample added 1.0 ml of 1N sodium hydroxide and heated to 100°C for 5 minutes. Extracted 1.0 ml of this solution for protein estimation.

To the test and standard tubes added 5.0 ml of alkaline copper reagent and allowed the mixture to stand at room temperature for 10 minutes. Added 0.5ml of Folin - Ciocalteau reagent rapidly and mixed immediately. After 30 minutes, measured the absorbance at 750nm in a colorimeter. From the standard graph, calculated the amount of protein present in the sample.

APPENDIX IX

ESTIMATION OF AVAILABLE NITROGEN IN SOIL

Alkaline potassium Permanganate method

(Subbiah and Asija, 1956)

Principle

The amount of soil nitrogen released by the potassium permanagenate oxidation of part of soil organic matter is estimated by distillation with sodium hydroxide. The distillate is collected in boric acid containing double (mixed) indicator and titrated against standard sulphuric acid.

Reagents

(i) 0.32 per cent potassium permanganate

(ii) 2.5 per cent sodium hydroxide

(iii) Double (mixed) indicator: Bromocresol green (0.5g) and methy red (0.1 g) were dissolved in 100 ml of ethyl alcohol

(iv) Standard sulphuric acid (N/50) and

(v) Boric acid 2 per cent

Procedure

Weighed 20 g of soil and transferred to a distillation flask. Added 20 ml of distilled water and 1.0 ml of liquid paraffin. Added a few glass beads (with holes) (to prevent bumping) and then added 100 ml of 0.32 per cent potassium permanganate solution and 100 ml of 2.5 per cent

sodium hydroxide solution. Distilled the contents at a steady rate collecting the liberated ammonia in a 500 ml ice tumbler containing 20 ml of boric acid (mixed) indicator. Continued the distillation for about 30 minutes (or) until 100 ml of distillate was collected in the beaker. Titrated the ammonia collected against the standard acid (N/50) and from the titre value calculated available nitrogen content of the soil.

APPENDIX X

ESTIMATION OF AVAILABLE PHOSPHROUS IN SOIL

Olsen's method

(Olsen *et al*., 1954)

Principle

This method of extraction of available soil phosphorus is suited for calcareous and alkaline soils. The carbonate ions from sodium bicarbonate will react with calcium ions and calcium carbonate is precipitated, thus allowing the phosphorus to come into the solution. The amount of phosphorus extracted was determined colorimetrically.

Reagents

(i) 0.5 M sodium bicarbonate (pH adjusted to 8.5)

(ii) Activated carbon (made phosphorus free by washing with 0.5 M sodium bicarbonate)

(iii) Reagent A: 12 g of ammonium molybdate was dissolved in 250 ml of distilled water 0.291 g of antimony potassium **tartrate** was dissolved in 100 ml of distilled water. Both these solutions were added to 1000 ml of approximately 5 N sulphuric acid. This solution was mixed thoroughly and made upto 2 litres with distilled water.

Regent B: 1.056 g of ascorbic acid was dissolved in 200 ml of reagent A. This solution was prepared freshly as and when required.

Procedure

Weighed 5 g of soil and transferred to a 100 ml polythene shaking bottle (run a blank side by side). Added a pinch of activated carbon (to make the extracted solution colourless). Added 50 ml 0.5 M sodium bicarbonate and shook in a reciprocating mechanical shaker for 30 minutes. Filtered through Whatman No.40 dry filter paper, collecting the filtrate in a clean dry breaker (the filtrate should be clear and colourless). Pipetted out 5 ml of the filtrate in a 25 ml volumetric flask. Diluted the solution in the 25 ml volumetric flask to about 20 ml with distilled water. Added 4.0 ml of reagent B and made up the volume to 25 ml. Waited for 10 minutes for the colour to develop and thereafter measured the intensity of the blue colour in a photoelectric colorimeter using a red filter (660 nm wave length) and adjusting the meter to 100 per cent transmittance with the blank. From the standard curve for phosphorus, determined the concentration of phosphorus (ppm) in the solution against the per cent transmittance observed in the above step.

Preparation of standard curve for available phosphorus estimation.

Dissolved 0.4390 g of potassium dihydrogen phosphate in water in an one litre volumetric flask and made up the volume to the mark. This gives a 100 ppm phosphorus solution. Pipetted out 10 ml of this solution into 100 ml

volumetric flask and made up to the mark. This gives a 10 ppm phosphorus solution. Then prepared a series of concentrations of phosphorus solutions by transferring into 25 ml volumetric flasks, 0.5, 1.0, 1.5, 2.0 and 2.5 ml of 10 ppm phosphorus solution which on dilution to 25 ml gave 0.2, 0.4, 0.6, 0.8 and 1.0 ppm respectively. After pipetting the above aliquotes of 10 ppm solution in the respective, 25 ml volumetric flasks, added 5 ml of Olsen's reagent (0.5 M sodium bicarbonate, pH adjusted to 8.5). Developed the colour and measured its intensity in the colorimeter. Plotted the values of absorbance against concentration of phosphorus in the soil extract.

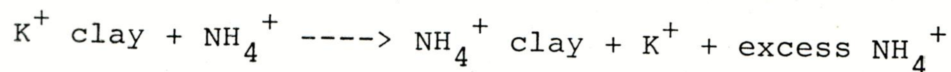
APPENDIX XI

ESTIMATION OF AVAILABLE POTASSIUM IN SOIL

(Stanford and English, 1949)

Principle

The potassium ions in the exchange sites are replaced with ammonium ions and leached from the soil. The reaction may be illustrated as follows:



$$K^{+} \text{ clay} + NH_4^{+} \text{ ----> } NH_4^{+} \text{ clay} + K^{+} + \text{excess } NH_4^{+}$$

The potassium ions in solution is then determined with the flame photometer.

Reagents

(1) Neutral normal ammonium acetate

(2) Standard potassium solution

Procedure

Weighed 50 g soil and transferred it to a 100 ml polythene shaking bottle. Added 25 ml of neutral normal ammonium acetate and shook in a mechanical reciprocating shaker for 5 minutes. Filtered through dry Whatman No.40 filter paper collecting the filtrate in a dry test tube or beaker or injection vial. Measured the amount of potassium in the filtrate in the flame photometer.