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# Effect of inorganic phosphatic fertilizers on the efficacy of an arbuscular mycorrhiza fungus against a root-knot nematode on pyrethrum

(*Keywords:* Glomus sp., *Meloidogyne hapla*, inorganic phosphatic fertilizers, root-knot nematode, pyrethrum)

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**Abstract.** Effects of triple super phosphate (TSP) and single super phosphate (SSP) fertilizers on efficacy of a *Glomus* sp. (isolate KS 14) against *Meloidogyne hapla* were determined. The fertilizers were applied at 150 and 300 kg $ha^{-1}$ at the time of fungus inoculation. Two months later, plants were inoculated with the nematodes. Plant growth and nematode disease parameters were determined 2 months after nematode inoculation. The fertilizers at both levels improved plant growth in all treatments. In general, the fungus improved plant growth on its own or in the presence of nematodes, but not in the presence of fertilizers. Both fertilizers at both levels were more effective in improving plant growth than the fungus. The fungus showed sensitivity to inorganic P fertilizers in that the fertilizers significantly reduced fungal root colonization and its pyrethrum growth stimulative effects. The fungus suppressed nematode disease severity unlike the fertilizers. The suppressive effects of the fungus on the nematodes were in most cases reduced by the fertilizers. The nematodes, unlike the fertilizers, did not have any significant effects on root colonization by the fungus or on its ability to improve pyrethrum growth. The presence of nematodes in fertilizer or fertilizer–fungus-treated plants, however, significantly reduced pyrethrum growth.



## 1. Introduction

Pyrethrum (*Chrysanthemum cinerariefolium* Vis.), a perennial shrub that produces white flowers, earns Kenya millions of shillings through exportation of its dried flowers. In 1991, for example, Kenya earned US$10 million through exportation of over 10 000 tons of dried flowers (Wanjala 1991). Dried flowers are a source of pyrethrins, which are active ingredients of some natural insecticides (Parlevliet and Brewer 1971). Pyrethrum marc (ground flowers from which pyrethrins have been extracted) has a high content of protein and is used as an animal feed. Pyrethrum is propagated vegetatively through splits (Clones) from mature plants or tissue cultured materials and generatively using seeds. The splits are multiplied in nurseries for 4 months before splitting them again and transplanting them. Seeds are raised in nursery beds for 5–6 months before transplanting. With public pressure against use of poorly biodegradable and persistent agrochemicals, the demand for pyrethrin-based formulations has been on the increase.

Although Kenya supplies 67–80% of the world's pyrethrum requirements (Wanjala 1992), the production is by small-scale farmers with low per capita income. Soil nutrient depletion without external inputs, poor agronomic practices, pests and diseases hamper increased pyrethrum production to meet the increasing demand (Warui *et al.* 1991). *Meloidogyne hapla* Chitwood, a root-knot nematode, is a major pest of pyrethrum that accounts for 95% of the plant-parasitic nematode populations associated with this crop in Kenya (Parlevliet and Brewer 1971). The nematode alone has been associated with 20–30% pyrethrum yield losses (Warui *et al.* 1991). The nematode, in addition, causes chlorosis, a decrease in flower size and pyrethrin content, stunting in young seedlings and predisposes infected plants to infection by root-rot and wilt fungal pathogens (Warui *et al.* 1991). The cost, and environmental and health hazards posed by the use of nematicides in the management of *M. hapla*, the long recommended rotational periods (Wanjala 1992) and the breakdown of resistance in resistant clones through development of new pathogenic races (Triantaphyllou 1985) make the search for alternative *M. hapla* management strategies imperative.

Components of a viable control programme should be cost-effective and environmentally safe. Arbuscular mycorrhiza fungi (AMF) are obligate endophytic symbionts that have the potential of suppressing nematodes (Sikora 1979, Kellam and Schenck 1980, Waceke *et al.* 2001a, b) and would, therefore, provide such an alternative. Waceke *et al.* (2001a) revealed that a *Glomus* sp. (Isolate KS14), the fungal isolate used in this study, suppressed *M. hapla* on pyrethrum by up to 75% and improved pyrethrum growth by 47%. Besides, AMF enhance plant growth and yield, plant water relations, soil aggregation and structure, and ameliorate aluminium and iron toxicity (Harley and Smith 1983). Agricultural practices such as change in land use, cropping systems, edaphic and host factors have, however, profound effects on AMF efficacy to improve growth and suppress disease through their influence on fungus survival, spore production, inoculum density, inoculum potential and on plant colonization (Sieverding 1990). Inorganic fertilizers, for example, either increase or decrease the efficacy and functioning of AMF depending on AMF species, soil fertility, organic matter, balance of nutrients and mycorrhiza dependency of the crop species (Gryndler *et al.* 1990, Vivekanandan and Fixen 1991). In general, however, high levels of phosphorus are reported to reduce significantly root colonization and sporulation

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through alteration of root exudates, in particular the soluble carbohydrates (Hayman 1975, Menge *et al*. 1978).

In this study, therefore, the effects of inorganic P fertilizers (TSP and SSP) recommended for pyrethrum production in Kenya on *Glomus* sp. (isolate KS14) were determined.

## 2. Materials and methods

The fertilizers were incorporated at two different rates (150 and 300 kg $ha^{-1}$) into sterilized soil contained in 15-cm-diameter pots and thoroughly mixed. The soil (clay, pH 5.2) used was obtained from the field where the fungus was obtained. The soil was mixed with sand (3:1) before autoclaving it for two separate 1-h periods at 121°C. A mineral content analysis of the soil done at the National Agricultural Research Laboratories (NARL), Kenya, revealed that the soil was deficient in plant available P (P = 9 ppm). After fertilizer application, the plants were inoculated with a 20 g mixed fungal inoculum obtained from a 3-month-old culture.

### 2.1. Test plant

Pyrethrum variety P4, recommended for its high flower yield and pyrethrin content (Ikahu and Ngugi 1989) was used as the test plant. The pyrethrum seeds, procured from Pyrethrum Board of Kenya (PBK), were germinated in a sterile sand–soil (1:4) mixture before transplanting. The plants were watered as required and supplied with 0.3% Wuxal nutrient solution (12% N, 4% $P_2O_5$, 6% $K_2O$, 0.02% boron and 0.01% copper) monthly.

### 2.2. Inocula preparation and inoculation procedure

*2.2.1. Arbuscular mycorrhiza fungi*. The fungal isolate used was isolated from the rhizosphere of pyrethrum plants growing in Kenyenya, Kisii (1960 m, altitude 35°000′E, 0°38′S), a major low-altitude pyrethrum growing area in Kenya. The fungus was isolated using a combination of wet sieving (Gerdemann and Nicolson 1963) and sucrose centrifugation techniques (Jenkins 1964). The fungus was identified by a mycorrhizologist, Professor Morton of the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM), West Virginia University, VA, USA. The fungus was placed in the genus *Glomus*. The identification to species level is still in progress at INVAM. The fungus was initially cultured and maintained on *Pueraria phaseoloides* Benth. (tropical kudzu) and later on pyrethrum (Mason *et al*. 1991). Healthy spores were placed on root tips of freshly germinated tropical kudzu seedlings. Before spore placement, the seedlings were placed in a 3-cm depression made in sterilized soil contained in a plastic pot. After spore placement, the depression was covered with soil. The cultures were checked after 6 weeks for sporulation. Thereafter, the cultures were maintained on pyrethrum. The fungus is currently being maintained as KE114 at INVAM and KS14 at Kenyatta University, Botany Department.

Pyrethrum roots from 3-month-old cultures were cut into 1-cm-long segments and thoroughly mixed with the growth medium. Of the mixed fungal inoculum, 20 g was placed in a 3-cm-depression made in sterilized soil–fertilizer mixture contained in a 15-cm-diameter plastic pot. The inoculum consisted of the growth medium, spores, external mycelia and colonized root segments. The pots were then planted with 6-week-old pyrethrum seedlings so that the inoculum was directly below the pyrethrum root systems. Untreated plants received an equivalent amount of growth medium from non-mycorrhized plants.

*2.2.2.* Meloidogyne hapla. The *Meloidogyne hapla* used was obtained from galled pyrethrum roots sampled from where the fungus was obtained. The nematodes were maintained on tomato (*Lycopersicon esculentum* Mill. cv. Moneymaker) growing in a 2:1 ratio of sterile sand–soil mixture in the greenhouse.

Nematode inoculum was prepared by extracting *M. hapla* eggs from the galled tomato roots using the sodium hypochlorite (NaOCl) technique (Hussey and Barker 1973). The egg suspension was aerated for 10–14 days at room temperature to facilitate hatching of eggs. Plants were inoculated with a nematode suspension containing 6000 second-stage juveniles (J-2), 3 months after inoculating with the fungus. Inoculation involved dispensing the nematode suspension onto a 3-cm-depression made around the root system. The depression was then covered with soil. Pots in which no fertilizers, nematodes or fungus were added served as controls.

The 5 × 2 × 2 factorial experiment was arranged in a split–split plot design where nematodes represented the main, fungus the sub-plot and fertilizers the sub-sub-plot factors. The treatments were replicated six times. The plants were watered appropriately.

### 2.3. Data collection

*2.3.1. Plant performance*. Dry shoot and fresh root weights were obtained at the end of the experiments, 5 months after fungal inoculation. Shoot systems were dried at 80°C for 48 h before obtaining their weights. Roots were thoroughly but gently cleaned using tap water, blotted dry and weighed.

*2.3.2. Root colonization by the fungi*. Roots from each replicate were cut into 1-cm-long segments (after assessing the gall index). Roots were thoroughly mixed before taking five 1-g fresh weight sub-samples of fine roots for fungal colonization assessment. The roots were cleared and stained using Walkers (unpublished data) Cold Staining technique, a modification of Phillip and Hayman's (1970) technique. Roots were cleared in 2.5% KOH for 72 h at room temperature. The KOH was changed after every 24 h. The roots were thoroughly rinsed in running tap water before acidifying them in 1% HCl for 12 h at room temperature. The roots were stained in 0.05% trypan blue for 24 h at room temperature and destained in acidified glycerol. The roots were then assessed for colonization with the aid of a dissecting microscope (×40) using a Grid-line Intersect method (Giovannetti and Mosse 1980). Colonized root length was expressed as a percentage of the total root length.

*2.3.3. Nematode disease assessment*. To assess nematode damage on pyrethrum, the following parameters were obtained.

- Galling indices as a measure of disease severity: roots were gently washed and rated for galling using a 0–4 galling scale where 0 = no galls, 1 = 1–25%, 2 = 26–50%, 3 = 51–75% and 4 = 76–100% of root system galled (Krusberg and Nelson 1958).

- Number of females within the roots: a 1-g fresh root subsample per replicate was obtained, cleared and stained using the NaOCl-acid fuchsin technique (Byrd *et al.* 1983). The number of females within the root segments was determined using a dissecting microscope (×40).
- Number of eggs within the roots: eggs were extracted using Hussey and Barker's (1973) technique. The egg suspension was adjusted to 50 ml and 1 ml of the suspension was taken and the number of eggs enumerated in a Hawksley's slide counter.
- Number of J-2 in 100 $cm^3$ of thoroughly mixed growth medium: the J-2s were extracted using Jenkins' (1964) centrifuge–flotation technique and enumerated in a Hawksley's counter.

### 2.4. *Data analysis*

Treatment effects were assessed by a Multiway ANOVA using Genstat 5 Release 3.2. Treatment means were separated using Least Significant Difference (LSD).

## 3. Results

### 3.1. *Effects of fertilizers on plant growth* (table 1)

There were highly significant differences ($p < 0.001$) in dry shoot ($F = 70.6$, d.f. = 4) and fresh root weights ($F = 110.5$, d.f. = 4). Control plants had significantly the least dry shoot and fresh root biomasses. Both TSP and SSP fertilizers at both rates and the fungus improved plant growth significantly compared to the controls. Fertilizers, $TSP_1$, $TSP_2$, $SSP_1$, $SSP_2$ and the fungus improved pyrethrum's top biomass by 210, 195, 162, 217 and 109%, respectively. In addition, the treatments significantly improved root weights by up to 291% ($TSP_1 = 82\%$, $TSP_2 = 174\%$, $SSP_1 = 147\%$, $SSP_2 = 291\%$ and fungus = 66%).

### 3.2. *Effects of fertilizers and fungus on plant growth* (table 1)

Generally, plants treated with both the fertilizers and the fungus had significantly ($p < 0.05$) heavier dry shoots and fresh roots than those treated with the fungus alone. Fertilizers improved shoot and root growth of fungus-treated plants by up to 70% ($TSP_1 = 52\%$, $TSP_2 = 70\%$, $SSP_1 = 41\%$ and $SSP_2 = 52\%$) and 135% ($TSP_1 = 39\%$, $TSP_2 = 135\%$, $SSP_1 = 75\%$ and $SSP_2 = 120\%$), respectively. The fungus–fertilizer-treated plants, on the other hand, were not significantly different from those treated with the respective fertilizers except for $TSP_2$–fungus-treated plants which were significantly heavier than those treated with $TSP_2$ alone. In addition, $TSP_1$– or $TSP_2$–fungus-treated plants had significantly heavier fresh roots than plants treated with the respective fertilizer levels alone. Although the fungus, in most cases, had no significant effects on shoot and root weights of fertilizer-treated plants, it improved dry shoot weights of $TSP_2$-treated plants by 21% and fresh root weights of $TSP_1$- and $TSP_2$-treated plants by respective 26 and 42%).

### 3.3. *Effects of fertilizers and nematodes on plant growth* (table 1)

Plants treated with fertilizers and nematodes had significantly ($p < 0.001$) heavier dry shoots ($F = 13.27$, d.f. = 4) and fresh roots ($F = 10.74$, d.f. = 4) than those treated with the nematodes alone. Plants treated with $TSP_1$ and nematodes, however, did not differ significantly in fresh root weights from nematode-treated plants. Fertilizers, $TSP_1$, $TSP_2$, $SSP_1$ and $SSP_2$ improved dry top biomass of nematode-treated pyrethrum by 45, 57, 52 and 69%, respectively. The fertilizers improved root weights by up to 34% ($TSP_2 = 33\%$, $SSP_1 = 20\%$ and $SSP_2 = 34\%$).

### 3.4. *Effects of fertilizers on plant growth in the presence of fungus and nematodes* (table 1)

Fertilizers significantly improved root and shoot growth of pyrethrum in the presence of both the nematodes and the fungus. This was revealed by significantly heavier roots and shoots of plants treated with the three treatments than those of plants treated with both the fungus and the nematodes. In the triple treatments, $TSP_1$, $TSP_2$, $SSP_1$ and $SSP_2$ improved shoot weights by 20, 30, 19 and 27%, respectively. The fertilizers

*Table 1. Mean dry shoot weights (DSW), fresh root weights (FRW) and percent root colonization (%RC) of pyrethrum treated with a* Glomus *sp., inorganic P fertilizers and* M. hapla *(MH)*

| | DSW (g) | | | | FRW (g) | | | | %RC | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No fungus | | Fungus present | | No fungus | | Fungus present | | Fungus present | |
| | No MH | MH present | No MH | MH present | No MH | MH present | No MH | MH present | No MH | MH present |
| No fertilizer | 2.37 | 3.36 | 4.96 | 4.74 | 5.58 | 16.42 | 9.24 | 8.78 | 57.66 | 52.73 |
| $SSP_1$ | 6.2 | 5.12 | 7 | 5.62 | 13.77 | 19.63 | 16.21 | 14.82 | 17.19 | 14.83 |
| $SSP_2$ | 7.52 | 5.69 | 7.54 | 6.04 | 21.84 | 21.94 | 20.37 | 17.03 | 18.44 | 15.98 |
| $TSP_1$ | 7.36 | 4.8 | 7.52 | 5.69 | 10.17 | 17.49 | 12.82 | 13.14 | 12.64 | 11.44 |
| $TSP_2$ | 6.99 | 5.26 | 8.42 | 6.15 | 15.29 | 21.81 | 21.74 | 17.44 | 7.48 | 7.31 |
| $LSD_{0.05}$ | 0.42 | 0.59 | 0.59 | 0.83 | 1.24 | 1.76 | 1.76 | 2.49 | 2.9 | 4.1 |
| SE | 0.21 | 0.3 | 0.3 | 0.42 | 0.63 | 0.89 | 0.89 | 1.25 | 1.44 | 2.04 |

Data are means of six replicates.

improved root weights of fungus–nematode-treated plants by up to 99%. Although fertilizers improved shoot and root weights of nematode–fungus-treated plants, it was by a lower percentage than when either the fungus or the nematode was present.

### 3.5. *Effects of fungus on plant growth in the presence of nematodes and fertilizers* (table 1)

Plants treated with both the nematodes and the fungus had significantly ($p < 0.05$) heavier dry shoots ($F = 17.9$, d.f. = 1) and lighter fresh roots ($F = 13.27$, d.f. = 1) than those treated with nematodes alone. The fungus improved shoot weight and reduced root weight of nematode-treated plants by 41 and 47%, respectively. The fungus, in the presence of both the nematodes and TSP fertilizers, also significantly improved shoot biomasses of the plants relative to those treated with both TSP fertilizers and nematodes. In the presence of TSP fertilizers and nematodes, the fungus improved dry shoot weights by 19 and 17%, respectively. The fungus, however, reduced root weights of plants receiving the three treatments by up to 25%.

### 3.6. *Effects of nematodes on plant growth in the presence of fertilizers and fungus* (table 1)

The presence of nematodes significantly ($p < 0.001$, $F = 195.5$, d.f. = 1) reduced dry shoot weights of fertilizer-treated plants. This was revealed by the significantly lower dry shoot weights of plants treated with both the nematodes and fertilizers than those treated with the respective fertilizers. The nematodes reduced dry shoot weights of $TSP_1$-, $TSP_2$-, $SSP_1$- and $SSP_2$-treated plants by 35, 25, 17 and 23%, respectively. On the other hand, root weights of $TSP_1$-, $TSP_2$- and $SSP_1$-nematode-treated plants were significantly ($p < 0.05$, $F = 138$, d.f. = 1) higher than those of plants treated with fertilizers alone. The presence of the nematodes in $TSP_1$-, $TSP_2$-, $SSP_1$-treated plants caused an increase in root weight of up to 72%.

Unlike in fertilizer-treated plants, nematodes did not have a significant effect on dry shoot or fresh root weights of fungus-treated plants. In fertilizer–fungus-treated plants, the nematodes significantly reduced their shoot biomasses. The reduction was greatest in $TSP_2$-fungus-treated plants (27%). In $TSP_1$-, $SSP_1$- and $SSP_2$-fungus-treated plants the reduction was by 24, 20 and 20%, respectively. Root weights of $TSP_2$- or $SSP_2$-fungus-treated plants were also significantly reduced by the presence of the nematodes. The reduction was 20 and 16% for $TSP_2$- and $SSP_2$-fungus-treated plants, respectively.

### 3.7. *Effects of fertilizers and nematodes on root colonization* (table 1)

There were significant differences ($p < 0.05$), in per cent root colonization among treatments. Plants treated with the fungus alone or with the fungus plus the nematodes had significantly greater root colonization than plants treated with all the three treatments. Nematodes alone or in the presence of fertilizers did not have any significant effect on root colonization by the fungus. The fertilizers, on the other hand, significantly ($p < 0.001$, $F = 329.7$, d.f. = 4) reduced per cent root colonization by the fungus. Fertilizers, $TSP_1$, $TSP_2$, $SSP_1$ and $SSP_2$ reduced root colonization by isolate KS14 by 78, 87, 70 and 68%, respectively. In the triple treatment, the fertilizers reduced root colonization by up to 86%.

### 3.8. *Effects of fertilizers and fungus on nematode disease development* (table 2)

There were highly significant differences ($p < 0.001$) in gall indices (disease severity) ($F = 27.26$, d.f. = 4), number of eggs ($F = 84.2$, d.f. = 4), females ($F = 32.1$, d.f. = 4) and J-2 ($F = 40.69$, d.f. = 4) among treatments. Plants treated with both the fungus and the nematodes had significantly the fewest galls, eggs, females and J-2. The fungus reduced nematode disease severity, number of eggs, females and J-2 by 71, 78, 71 and 77%, respectively. The presence of the fungus, in addition, significantly ($p < 0.001$, $F = 77.3$, d.f. = 1) reduced disease severity and number of J-2 in fertilizer–nematode-treated plants. The fungus reduced disease severity and J-2 of $TSP_1$-, $TSP_2$-, $SSP_1$- and $SSP_2$-nematode-treated plants by up to 35, 41, 50 and 37%, respectively. Except in $SSP_1$-treated plants, the presence of the fungus in fertilizer-nematode-treated plants also significantly ($p < 0.001$, $F = 147.55$, d.f. = 1) reduced egg production by up to 21%. The presence of the fungus had no significant effects on number of females in roots of fertilizer-nematode-treated plants.

Gall indices of plants treated with both the fertilizers and the nematodes though lower than those of nematode-treated plants

*Table 2. Mean gall indices (GI), number of eggs, females and second stage juvenile (J-2) in pyrethrum treated with P fertilizers and Glomus* sp.

| | GI† | | Eggs ml$^{-1}$ | | Females g$^{-1}$ | | J2/100 ml | |
|---|---|---|---|---|---|---|---|---|
| | No fungus | Fungus present | No fungus | Fungus present | No fungus | Fungus present | No fungus | Fungus present |
| No fertilizer | 3.5 | 1 | 1350 | 292 | 144 | 41.3 | 3204 | 743 |
| $SSP_1$ | 3 | 1.5 | 621 | 522 | 61.2 | 53.8 | 2169 | 1207 |
| $SSP_2$ | 3.17 | 2 | 851 | 730 | 83 | 73.7 | 2019 | 1617 |
| $TSP_1$ | 2.83 | 1.83 | 863 | 682 | 77.2 | 71.7 | 2378 | 1931 |
| $TSP_2$ | 2.83 | 1.67 | 977 | 858 | 88.5 | 85.2 | 2494 | 1948 |
| $LSD_{0.05}$ | 0.52 | 0.73 | 70.6 | 99.8 | 11.6 | 16.42 | 188.4 | 266.4 |
| SE | 0.26 | 0.36 | 35 | 49.5 | 5.76 | 8.15 | 93.5 | 132.3 |

Data are means of six replicates.

†Gall indices based on a 0–4 gall rating scale, where 0=no galls, 1=1–25%, 2=26–50%, 3=51–75% and 4=76–100% of root system galled.

did not differ significantly. Fertilizers, however, significantly reduced number of eggs, females and J-2. Fertilizers, $TSP_1$, $TSP_2$, $SSP_1$ and $SSP_2$ lowered number of eggs, females and J-2 by up to 46, 39, 58 and 42%, respectively. Except for the number of eggs and females where significant differences among rates of respective fertilizers were noted, gall indices and number of J-2 were not significant.

The presence of fertilizers in fungus-nematode-treated plants significantly ($p < 0.001$) increased egg production and nematode soil infestation by up to 193.8%. In addition, $TSP_1$ and $SSP_2$ significantly increased disease severity by 83 and 100%, respectively. Fertilizers, $TSP_1$, $TSP_2$ and $SSP_2$ significantly increased number of females in fungus-nematode-treated plants by 74, 79 and 106%, respectively.

## 4. Discussion

Inorganic P fertilizers at both levels significantly improved dry shoot and fresh root weights of non-mycorrhized, mycorrhized, nematode-treated and mycorrhized-nematode-treated pyrethrum plants. This confirms earlier reports by Ngugi and Ikahu (1989) and Mwakha (1979) that vegetative growth and flower yield of pyrethrum increased with increasing rates of TSP. In most pyrethrum growing areas in a Kenya, TSP or SSP applications are recommended at the rates of 150–200 and 300–400 kg $ha^{-1}$, respectively (Ngugi and Ikahu 1989). Sastry and Singh (1990), in addition, revealed that pyrethrum response to P applications is greater in infertile acid soils than in fertile soils. Sastry and Singh further reported that pyrethrum growing in soils with 22 ppm of P do not require any P additions. An analysis of available P in the soil used for the study revealed that it was deficient in P ($p$ <18 ppm).

The fungus improved plant growth on its own and in the presence of TSP and/or nematodes. The fungus, however, was less efficient in improving plant growth than both fertilizers at both levels. The fact that the fungus has to establish a symbiotic relationship with the plant before the plant can derive any nutritional benefits from it might explain the relatively poor responses of pyrethrum to fungal inoculation as compared to fertilizer applications. In addition, the nutrients once taken up by the fungal extra matrical hyphae must be translocated to the plant through the internal hyphae to the arbuscules before assimilation by the plant. This in effect increases the path through which the nutrients must travel before they can be of benefit to the plant. Fertilizers, on the other hand, once in soil solution are directly taken up by plant roots and assimilated depending on the soil pH among other factors (Tisdale *et al.* 1990)

A combination of both the fungus and fertilizers significantly improved growth of pyrethrum's shoots and roots relative to pyrethrum treated with the fungus alone. The addition of the fungus to SSP-treated plants unlike TSP-treated plants had no significant effects. It is apparent that the enhanced pyrethrum growth by a combination of the fungus and the fertilizers is largely due to the fertilizers and not the fungus. It might also be that the addition of SSP fertilizer enhanced pyrethrum growth optimally so that the addition of fungus had no further effects on growth. By the time the fungus established a symbiotic relationship with the plant, the plant might have already taken up sufficient P and hence the non-significant effect of the fungus on SSP-treated plants. In addition, the fertilizers appear to act independently in improving growth and not via the fungus. Besides, the fungus showed sensitivity to addition of inorganic P fertilizers. The fact that the fertilizers significantly reduced root colonization by the fungus and subsequently its growth stimulatory ability supports this speculation. Yield responses of mycorrhized plants to increasing P levels have been reported to depend on AMF tolerance or sensitivity to P. Salina *et al.* (1985), for example, reported that yield responses of a pasture grass and a pasture legume depended heavily on the presence of P tolerant and functionally effective AMF species. The effect of fertilizer on the fungus is not unexpected because the site from which the fungus was isolated had low P levels (P = 9 ppm) with no external P inputs (Wanjala 1992). The fungus might have enhanced growth of TSP-treated plants through enhanced uptake of other mineral nutrients that might have become limiting to pyrethrum growth due to high P. The speculations, however, need to be confirmed by further work.

The presence of nematodes in fertilizer-treated or fertilizer-fungus-treated plants significantly reduced pyrethrum growth. Nematodes reduced shoot weights of fertilizer-treated plants by up to 35% and increased root weights by up to 72%. The fertilizers, in most cases, improved dry shoot and root weights of nematode-treated plants by up to 70 and 34%, respectively. The fertilizers and the nematodes seemed to be mutually inhibitory where fertilizer application minimized effects of nematodes on pyrethrum probably through disease escape or improved pyrethrum resistance and the presence of nematodes reduced growth responses that were due to fertilizer application. This might be because the nematodes affect the functioning of roots (mineral and water uptake and translocation). The nematode-infected plants might not have been able to benefit from fertilizer applications. The increase in root weights in dually treated plants might be due to the weight of nematode induced galls.

The nematodes did not reduce the fungal ability to improve plant growth as was revealed by the non-significant differences between shoot and root weights of fungus and fungus-nematode-treated plants. This confirms work by Waceke *et al.* (2001a) that revealed that the nematodes had no significant effect on fungal ability to improve pyrethrum growth.

The presence of fertilizers alone or in addition to nematodes significantly reduced root colonization of pyrethrum by the fungus. High P fertilizer levels have been reported to negatively influence AMF root colonization and spore production (Menge *et al.* 1978, Salina *et al.* 1985). Inverse relationships have, for example, been reported between increasing P supply and fungal root colonization of maize (*Zea mays* L.) (Hayman 1975) and subterranean clover (*Trifolium subteranean* L.) (Same *et al.* 1983). Adecrease in the supply of soluble carbohydrates to the fungus in fertilizer-treated plants might in part explain the reduced root colonization (Ratynayake *et al.* 1978, Graham *et al.* 1981). High P levels reduced levels of soluble carbohydrates in roots colonized by *Gigaspora calospora* or *Glomus fasciculatum* (Mosse 1973, Mosse *et al.* 1973, Jasper *et al.* 1979, Thomson *et al.* 1986, 1990). Further tests, however, need to be carried out to verify the above-speculated mechanisms of suppressed fungal root colonization by the fertilizers.

The presence of the nematodes alone, on the other hand, did not affect root colonization by the fungus as was revealed by the non-significant differences in root colonization of fungus-

treated plants and nematode-fungus-treated plants. The non-significant differences in root colonization of fungus-fertilizer-treated plants and those treated with the three treatments further reveal that the presence of nematodes had no effects on root colonization by the fungus. This confirms a previous report by Waceke *et al*. (2001a) that *M. hapla* had no significant effects on root colonization by the fungus. Kellam and Schenck (1980) and Strobel *et al*. (1982), for example, reported that *M. incognita* had no effects on *Glomus macrocarpus* sporulation and root colonization on soybean and, on *G. margarita* and *G. etunicatum* on peach. *Meloidogyne hapla* was also reported to have no effects on spore production and root colonization *by G. fasciculatum* on onions (MacGuidwin *et al*. 1985). Nematodes might have reduced uptake and availability of both macro and micronutrients by the plants so that the fungus perceived an environment of lower mineral nutrient concentration. This might have enhanced fungal colonization and might explain the nematodes' non-effect on fungal colonization of roots.

The presence of the fungus in nematode-treated plants significantly reduced disease severity, egg, female and J-2 production. This confirms earlier reports which revealed that the fungus suppressed the nematodes by up to 75% (Waceke *et al*. 2001a). The fungus might have enhanced plant resistance against the nematode through improved nutrient uptake and/or phytohormone production or altered pyrethrum's attractiveness to the nematode thereby reducing penetration (Smith 1988). The suppressive effects of the fungus on nematodes were, in most cases, reduced by the fertilizers.

Fertilizers significantly reduced egg and female production and J-2 but not gall development. The suppressive effects of P fertilizers on nematodes and the subsequent growth improvement of nematode-treated plants by the fertilizers might be due to improved plant nutritional status. Enhanced nutritional status of pyrethrum might have led to disease escape or improved pyrethrum resistance to the nematodes. The fertilizers, in addition, might have improved plant growth of nematode-treated plants by compensating for the nematode-induced nutritional sink on pyrethrum. Without further experimentation, however, it is difficult to explain the increases in gall indices, number of eggs, females and J-2 by the fertilizers in the presence of the fungus.



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