EFFICACY OF *TAGETES MINUTA* AND *TITHONIA DIVERSIFOLIA* FORMULATIONS AGAINST *MELOIDOGYNE INCOGNITA* USING A NOVEL RELEASE APPLICATION TECHNIQUE IN TOMATO

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Abstract

Tomato (*Solanum lycopersicum*) is an economically important crop in East Africa and is produced largely by small-scale farmers. The root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood, causes serious constraints in tomato production in the African continent. Organic additives of plant origin have been known to control nematodes. The efficacy of different formulations of these additives dispensed as a slow-release in filter bags against *M. incognita* remains unknown. This study investigated the efficacy of two formulations; powder, and pellet from leaves and stems (above parts) and roots (below parts) of *Tithonia diversifolia* and *Tagetes minuta* on *M. incognita* in tomato. The filter bags were made of non-woven interfacing fusible fabric, and they contained the extracts used in the experiments. The experiment was laid out in a completely randomized design with 18 treatments and replicated four times. Tomato seedlings were inoculated with about 1500 freshly hatched second stage juveniles of *M. incognita* five days after seedling transplant. Data was collected on root galling, number of egg masses per root, root-knot nematode second-stage juveniles in the soil, and tomato yield and subjected to analysis of variance (ANOVA). Treatment means were compared using the Tukey Studentized Range Test at a 5% probability level (P = 0.05). Results showed that all formulations significantly (P= <0.001) reduced nematode populations relative to the untreated control. However, untagged (without filter bag) formulations were about five times better than the tagged (with filter bag) in both the preventive and curative trials at 42- and 84-days post-inoculation (DPI). No significant differences were observed at 126 DPI. Powder formulations of *T. minuta* roots (79%) and *T. diversifolia* leaves (78%) significantly (P = <0.001) reduced *M. incognita* juvenile populations followed by pellet formulations of *T. minuta* leaves (74%) and *T. diversifolia* roots (72%) relative to the positive control (70%) Bionematon® Powder formulations (79%) reduced RKN populations better than the pellet formulations (73%) but the yield was higher (70.7 t/ha) in the latter. Tagged powder formulations of *T. diversifolia* roots recorded a 7% decrease in yield in the preventive trial compared to the curative trial. However, within the same trial, tagged pellets of *T. minuta* roots and the positive control recorded more than 10% increase in yield relative to the curative trial. These findings indicate that formulations of *T. minuta* and *T. diversifolia* incorporated in filter bags can be used for management of RKNs in tomato and other vegetable crops.

Keywords: Filter bags, Mexican marigold, Mexican sunflower, management, plant parasitic nematodes

Introduction

Tomato, *Solanum lycopersicum* L., (Solanaceae) is the second most important cultivated vegetable in the world after potato. It is a rich source of minerals and vitamins (Naz *et al.*, 2012). In Kenya, tomato is an important fruit vegetable with a half a million annual turnover (Geoffrey *et al.*, 2014; FAO,2020). In addition, it is an essential source of income for smallholder farmers, contributing to 7% of the total horticultural produce and a further 14% of all the vegetable produce (Ochilo *et al*., 2019). Despite its economic importance, tomato production is hampered by plant-parasitic nematodes especially root-knot nematodes (*Meloidogyne* spp.) whose infection causes about 80-100% yield loss (Birithia *et al*., 2012; Onkendi *et al*., 2014). At least 97 *Meloidogyne* species including *Meloidogyne incognita* Chitwood, 1949, *M. javanica* Chitwood, 1949, *M. arenaria* Chitwood, 1949 and *M. hapla* Chitwood, 1949 are the most common species affecting tomato (Kafikavalci, 2007; Naz *et al*., 2012 ; Brennan *et al*., 2020). Out of these, *M. incognita* is considered the most destructive since it is capable of causing total crop failure (Asif *et al.,* 2016).

Root knot nematodes are motile and thus seek a new host during the second stage juvenile (J2) (infective stage) which penetrates suitable host roots intracellularly, locates the vascular cylinder, and initiates nutrient sinks resulting in the formation of knots within the root which severely restrict absorption of water and other nutrients. The wounds created during root entry act as a pathway for easy entrance of other disease causing microorganisms (Belay *et al*., 2013; Jones *et al.*, 2013; Ireri *et al.*, 2019). The resulting symptoms include leaf discoloration, wilting and stunted growth which are often confused with other biotic and abiotic effects. As such, farmers result in the indiscriminate use of pesticides and fertilizers increasing the cost of production. Farmers with the knowledge of RKNs tend to use synthetic nematicides which are highly harmful to the environment and human health (Kepenekçi *et al.*, 2016; Kepenekci *et al.,* 2017). Other management strategies include the use of resistant plants, crop rotation among others. However, there has been unreliable results from crop rotation systems thus necessitating the need for safer and sustainable management strategies (Osei *et al.*, 2011; Metwally, 2019).

About 57 plant families have nematicidal effects including the Asteraceae where the Mexican sunflower (*Tithonia diversifolia* (Hemsl.) and Mexican marigold *(Tagetes minuta)* belong (Tibugari *et al.*, 2012). Plant botanicals have been used in various forms either as plant extracts, essential oils, powders, oil cakes, plant latex or as organic amendments (Manju and Meena, 2015). The utilization of organic amendments in the soil has been reported to reduce *M. incognita* densities while significantly increasing crop yields (Akhtar and Malik, 2000; Hadian *et al*., 2011; Njenga *et al.,* 2019). Previous studies have shown that leaf extracts from *T. diversifolia* reduced the reproduction and galling of *M. incognita* on yam (*Discordia rotundata*) (Odeyemi and Adewale, 2011). Moreover, marigold roots exudates were reported to have nematicidal effects by suppressing the hatching of nematode eggs (Wanzala *et al.*, 2016; Bhattacharyya, 2017). In addition, D'Addabo *et al.*, (2009) reported that soil amendment with pelleted *Medicago sativa* L. meal increased tomato crop yield and reduced soil population densities and root galling of *M. incognita* under field conditions. Neem powder was also reported to effectively reduce the number of juveniles in the soil and increase pod yield of Bambara groundnuts (Kankam and Adomako, 2014).

Constraints associated with the practical use of essential oils or organic amendments to control RKNs such as difficulty in their incorporation into soil and the need for large biomass has led to the need for more effective ways of utilizing these plants (Pérez *et al.*, 2003). Biofumigant meals and pellets from *Brassica juncea* have been found to release isothiocyanate moderately over time (Lazzeri *et al.*, 2013). Further studies to

investigate the encapsulation of essential oils as a potential controlled release vehicle with site-specific delivery so as to maximize the properties of the oils have been reported (Ntalli and Caboni, 2012). The current study hypothesized that a controlled-release method of either powdered or pelleted extracts of *T. minuta* and *T. diversifolia* incorporated in biodegradable filter bags may influence the reproduction of RKNs in tomato. Asteraceae plants have proven to produce repellent volatiles to *M. incognita* (Mwamba *et al.*, 2021). This research evaluated the use of powder and pellet formulations from roots, leaves and stems of the two asteraceous plants against *M. incognita* on tomato in screen house trials.

MATERIALS AND METHODS

Experimental site

Two screen house experiments were conducted at the Jomo Kenyatta University of Agriculture and Technology (JKUAT), Juja, Kenya (latitude 0°10′48″ S, longitude 37°7′12″ E; altitude 1525 m a.s.l.) from September to December, 2018 and March to August 2019 (Temperature: 27 ± 2 °C and RH 60-70%).

Raising of tomato seedlings

Tomato of cv. 'Cal J' was used in this experiment because the seeds are affordable and the cultivar is susceptible to *M. incognita*. Certified seeds purchased from a local agrochemical store, were raised in a screen house (ambient temperature 27 ± 2 °C; RH 60-70%) in seedling trays pre-filled with peat moss media. Seedlings were watered after every two days. A starter fertilizer containing NPK (19:19:0) was applied at emergence at the rate of 1 gl^{-1} once a week for three weeks. Three-week-old seedlings were transplanted into polythene sleeves (8 cm x 14 cm) pre-filled with autoclaved red soil.

Source of *T. minuta* and *T. diversifolia*

About 100 kg of flowering Mexican marigold (*Tagetes minuta*) and Mexican sunflower (*Tithonia diversifolia*) whole plants were field collected for four weeks within the JKUAT (1°5′36″S: 37°0′51″E, altitude 1530 m above sea level). After every weekly collection, respective whole plants were bulked in a screen house (27 ± 2°C) whereby the above-ground parts (stems, leaves, and flowers) were separated individually from the roots using a sharpened machete. Subsequently, respective plant parts were chopped into smaller units using a chaff cutter and dried on-screen house benches for 21 days until they attained 20% moisture content.

Preparation of formulations and filter bags

Dried plant parts were pulverized into powder using an electric mill (Model; ZF500: Nyagah Mechanical Engineering Limited) for preparing pellets. Pellets were prepared by mixing 1 part of corn starch with 2 parts of the respective pulverized plant (w/w) in 1.5 L of distilled water. The resultant paste was then fed into a pelletizer (Model; ZF800, Nyagah Mechanical Engineering Limited). The resulting 5 mm pellets were placed on a tray and dried on a screen house bench at 27 ± 2°C. To prepare filter bags, non-woven interfacing fusible khaki was purchased from a local tailoring shop in Nairobi, Kenya. The fabric was cut, folded, and sewn to make bags (12 cm length x 7 cm width), with a drawstring inserted at the top edge (Figure 1). About 20 g (weighing scale model: LIBROR EB-3200D) of either pelleted or powdered parts from respective plant species were filled into the filter bags and sealed using the drawstring. The material in infused filter bags was then transferred into khaki bags (165 mm x 100 mm x 295 mm) and stored at room temperature (21 ± 2°C) until use.

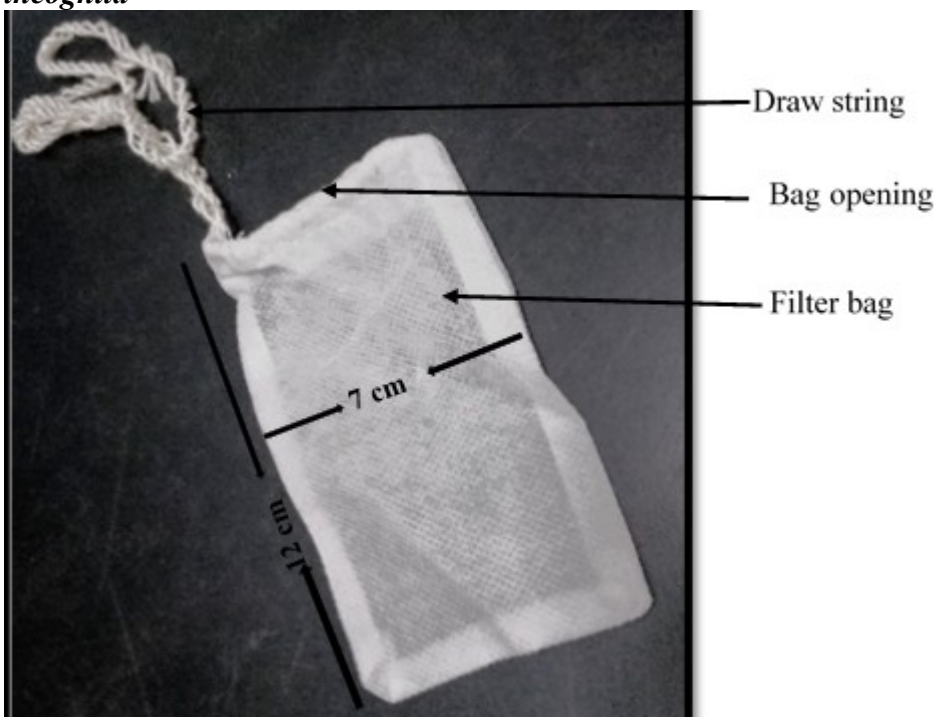


Figure 1: A photo of a filter bag

Extraction of root-knot nematodes

Populations of *M. incognita* were obtained from infected roots of the broad-leaved African nightshade plants (*Solanum scabrum* Mill), maintained in a screen house (27 ± 2 °C, 60-70% RH, with 12:12 hr. L: D photoperiod) at the International Centre of Insect Physiology and Ecology (*icipe*), Duduville Campus, Nairobi (01°13′ 25.3″ S, 36° 53′ 49.2″ E; 1600 m a.s.l). Infected nightshade plants were uprooted, rinsed free of sand debris in running tap water 3-4 times. Galled root segments, were placed on a Petri dish with some distilled water, and egg masses were removed using sterilized fine tweezers under the dissection microscope (Coyne and Ross, 2014). About 10 egg masses /well were placed in a 24-well culture plate, filled with 2 mL of distilled water, and incubated in the dark for 2-4 days to allow hatching. On day 4, hatched juveniles were collected into a measuring cylinder and the nematode number was determined by counting under a stereomicroscope (Leica M125, Leica 8 microsystems, USA). The inoculum was standardized in such a way that 5 mL suspension contained 1500 freshly hatched juveniles and thereafter transferred into 8 mL falcon tubes containing distilled water until use.

Experimental design

The study was conducted in a screenhouse in polythene sleeves (8 inch x 14 inch) filled with 5 kg autoclaved soil in the ratio of 2:1w/w (red soil: sand). Experiments were laid out in a completely randomized design (CRD) with 18 treatments and four replicates per treatment (Figure 2). The treatments included *T. diversifolia* and *T. minuta* pellet and powder of above parts and below parts applied with a filter bag or without a filter bag. Untreated inoculated plants and Bionematon® (*Paeciliomyces lilacinus*) (Osho Chemical Industries Ltd, Nairobi, Kenya) (10 mL/pot) served as the negative and positive control respectively.

Rep I	TaPeU	TiPwT	TaPwU	TiPeU	Cntr
	TaPeT	TiPwU	TaPwT	TiPeT	Bi
Rep II	TiPwT	TaPeU	Cntr	TaPwU	TiPeT
	TiPeU	TaPwT	TiPwU	Bi	TaPeT
Rep III	TiPwU	Cntr	TiPwT	TaPeT	Bi
	TaPwT	TaPwU	TaPeU	TiPeT	TiPeU
Rep IV	TaPwU	TaPeT	TiPeT	TiPwT	TaPwT
	Bi	TiPeU	TiPwU	Cntr	TaPeU

Figure 1: Arrangement of treatments in the screen house during preventive and curative trial. TiPeT = *T. diversifolia* pellets tagged; TiPwT =*T. diversifolia* powder tagged); TaPeT = T. *minuta* pellets tagged; TaPwT = *T. minuta* powder tagged; TiPeU = *T. diversifolia* pellets untagged; TiPwU (*T. diversifolia* powder untagged; TaPeU = *T. minuta* pellets untagged; TaPwU = *T. minuta* powder untagged; Bi= Binematon® (positive (+) control) and negative (-) control (no treatment applied).

Application of treatments

Curative and preventive trials were conducted from September to December 2018 and March to August 2019. The curative trial involved amending the soil with about 20 g of powder or pellets of respective plant parts 15 days after transplanting while in the preventive trial the soil was amended 15 days before transplanting. Powdered or pelleted treatments were applied either loose or contained in filter bags. The powder treatments were applied in bands near the plant roots in the curative trial and conversely mixed with soil in the preventive trial. For treatments in filter bags, they were placed 15 cm deep in the soil and covered in the preventive trial, but in the curative trial they were placed1cm from the root. In both trials, pots were watered thrice per week after the application of different treatments to ease the decomposition of the organic additives (Tariq *et al*., 2018). The positive control was applied concurrently with the other treatments in the curative trial and three days after inoculation in the preventive trial (Terefe *et al*., 2009; Metwally, 2019).

Inoculation of tomato seedlings

Three-week-old tomato seedlings cv. 'Cal J' were transplanted in the respective treatments and maintained for five days to ensure proper seedling establishment before inoculation. About 1,500 infective juveniles (J2) of *M. incognita* were inoculated by making three holes 1 cm from the plant roots to prevent physical damage and pipetted into each hole and covered with soil (Hadian *et al*., 2011; Coyne and Ross, 2014).

Nematode damage assessment

Assessment for root-knot damage was done at 42, 84- and 126-days post inoculation (DPI). Three plants per treatment were randomly uprooted, the root system excised and washed under running water to remove soil debris. The roots were visually examined for root-knot gall formation on each root system and the gall severity was rated on a 0-5 scale described by Taylor & Sasser (1978), where; 0= no galls; 1= 1-2 galls; 2= 3-10 galls; 3 = 11-30 galls; 4= 31-100 galls; and 5 more than 100 galls. The number of egg-masses formed on each root system was examined. To enhance egg mass visibility, the roots were submerged in Phloxine B 0.1% for 15 min to stain them pink then rinsed in water. Roots were placed on a white tray and the stained egg masses per root system were counted with naked eyes. The egg masses were also scored similarly to the galls. The Juveniles (J2) in the soil were

extracted from the growth media using the following modified extraction tray method described by Coyne (2014). About 200 g potting media was collected from 3 pots per treatment. The samples were thoroughly mixed and 100 g sub-sample was placed on a double layer of filter paper tissue supported by a sieve. The sieves were then placed in a shallow dish and water was added to a level where it just touched the soil so that the soil layer looked wet. After 24 h, the sieves were carefully removed and the nematode suspension concentrated by passing through a series of 45 µm aperture sieves, and the juveniles were collected in 25 mL bottles from each of the sieves. One mL aliquots of a well agitated nematode suspension were then pipetted into a counting slide and observed under a light microscope. Counting was repeated for four aliquots and the average was calculated. The number of juveniles (J2) was expressed per 100 g of soil.

The reduction Percentage in nematode parameters was calculated according to Bakr (2018).

$$\text{Reduction percentage} = \frac{Control\ (negative\ ) - Treatment}{Control\ (negative\ )} \times 100$$

Plant yield

Tomato fruits were harvested weekly at the pink to the red-ripe stage. Five plants per treatment were sampled and fruits from each treatment were separately weighed using a weighing balance (LIBROR EB-3200D) to determine the weight in kg plant^{-1}. Only marketable fruits were weighed. The weight of individual harvests for each treatment was summed up after the last harvest to obtain the total yield in terms of weight for marketable fruits and expressed in tonnes/hectare (t/ha) for each treatment.

Statistical analysis

Data on root galling, number of egg masses per root, root-knot nematode second-stage juveniles in the soil, and tomato yield were subjected to analysis of variance (ANOVA). Treatment means were compared using the Tukey Studentized Range Test at a 5% probability level ($\alpha = 0.05$). Data collected on yield was pooled before subjection to an analysis of variance (ANOVA). All data were analyzed using the Genstat statistical program (Muiru *et al.*, 2017).

RESULTS

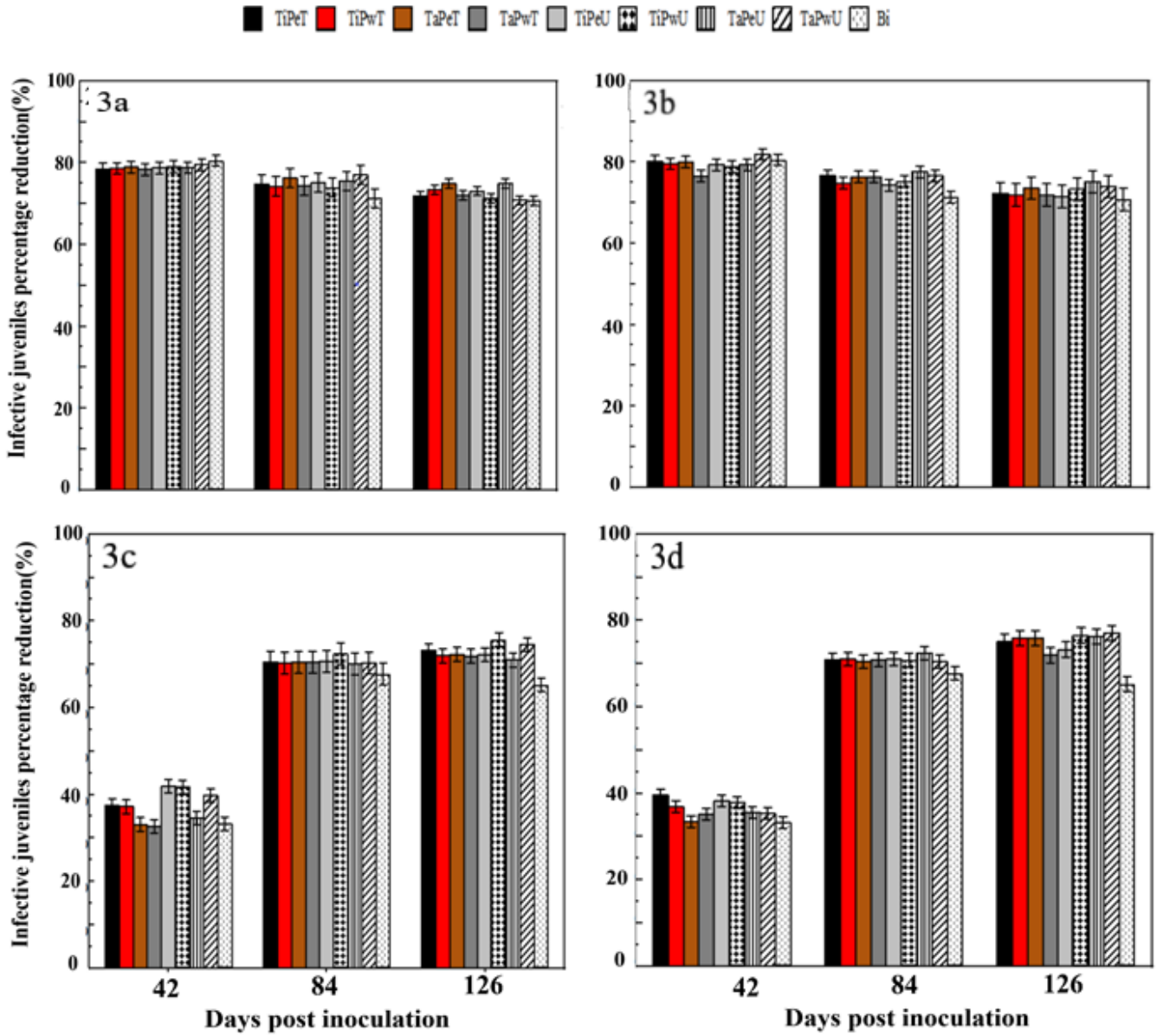
Efficacy of *T. diversifolia* and *T. minuta* formulations on infective juvenile populations

Nematode populations in the preventive trial

Results showed that untagged pellets (without filter bags) of *T. minuta* significantly reduced J2's by about 5 % more relative to the powder untagged formulation (Figure 3a) at 126 days post inoculation (DPI). However, the untagged powder formulation had a significantly ($P < 0.001$) higher nematode reduction relative to the positive control 84 DPI. All the other treatments showed no significant differences (Figure 3a). In addition, tagged pellet formulations of *T. minuta* roots (below-ground) significantly ($p < 0.001$) reduced J2s by about 4-5 % more relative to the powder at 42 DPI. All formulations were significantly ($P < 0.001$) more effective than the positive control at 84 DPI. At 126 DPI, no significant differences ($P = 0.001$) were observed between the tagged and untagged treatments (Figure 3b).

Nematode populations in the curative trial

At 42 DPI untagged pellet and powder formulations of *T. diversifolia* were 21% more effective than the positive control followed by untagged powder formulations of *T. minuta* leaves and stems at 16%. Moreover, the untagged formulations were about six times better than the tagged (Figure 3c). At 126DPI, all treatments were significantly ($P < 0.001$) more effective than the positive control by about 8-13%. No significant differences were detected across all treatments 84 DPI (Figure 3c). Tagged and untagged roots of *T. diversifolia* pellet formulations were 13-15% more effective than the positive control at 42 DPI. At 126 DPI, all treatments were significantly 8-16 % more effective than the positive control. No significant difference was observed between



the tagged and untagged formulations (Figure 3d).

Figure 2: Infective juveniles' reduction (%) in tomato plants after treatment with *Tagetes minuta* and *Tithonia diversifolia* formulations. Preventive trial using above ground parts (leaves and stems) (3a) and belowground parts (roots) (3b). Curative trial using above ground parts and (3c) and below ground parts (3d). TiPeT = *T. diversifolia* pellets tagged; TiPwT = *T. diversifolia* powder tagged; TaPeT = *T. minuta* pellets tagged; TaPwT = *T. minuta* powder tagged; TiPeU = *T. diversifolia* pellets untagged; TiPwU =*T. diversifolia* powder untagged; TaPeU = *T. minuta* pellets untagged; TaPwU = *T. minuta* powder untagged and Bi (positive (+) control) = Binematon®. ($P < 0.05$, Tukey's test). Vertical error bars represent standard errors of differences of means.

Efficacy of *T. diversifolia* and *T. minuta* formulations on nematode egg mass

Egg masses in the preventive trial

All formulations had a significantly higher egg mass reduction of about 43% more relative to the positive control at 42 DPI, with the tagged (with filter bag) powder of T. *minuta* and *T. diversifolia* causing an 8-13% better egg masses reduction than the untagged (without filter bags) formulation. Moreover, untagged pellets of *T. minuta* leaves and stems (above parts) significantly reduced egg masses by about 25% more relative to the tagged (with filter bags) pellets at 126DPI (Figure 4a). Further, at 42 DPI untagged *T. minuta* roots (below

ground) powder significantly reduced egg masses by more than 50% as compared to other treatments. However, the positive control reduced egg masses by about 30% more relative to tagged pellets of *T. diversifolia* (Figure 4b). All formulations significantly ($P < 0.001$) reduced egg masses relative to the positive control at 84 DPI (Figure 4b). At 126 DPI, the untagged formulations reduced egg masses about 5 times better than the tagged formulations except for untagged *T. minuta* pellets which had no significant difference with the tagged counterpart (figure 4b).

Egg mass in the curative trial

At 42 DPI, tagged pellets and powder of *T. diversifolia* were 5-7 times better than its untagged counterpart. The positive control had a 17% egg mass reduction relative to untagged *T. minuta* pellets (Figure 4c). At 84 DPI tagged powder of *T. minuta* leaves and stems were more than 5 times better than other treatments while recording a 45% egg mass reduction relative to the positive control. There was no significant difference between the positive control and tagged *T. minuta* pellets at 126 DPI. There was also no significant ($P > 0.001$) difference between the tagged and untagged treatments (Figure 4c). At 42 DPI untagged pellets of *T. diversifolia* roots and the positive control were 40% more effective than its powder counterpart. At 84 and 126 DPI all treatments were significantly 45-50% more effective than the positive control, except untagged powder of *T. minuta* roots which had no significant difference with the positive control. The untagged treatments were more than six times better than the tagged at 42,84 and 126 DPI (figure 4d)

Efficacy of *T. diversifolia* and *T. minuta* formulations on nematode galling

Gall reduction in the preventive trial

Results showed tagged pellets of *T. minuta* had a higher gall reduction relative to the untagged powder formulation at 42 DPI. No significant difference was observed between other tagged and untagged formulations (Figure 5a). At 84 DPI there was no significant difference between tagged and untagged *T. diversifolia* and *T. minuta* powder respectively. However, the two formulations were more than 7% more effective relative to the other formulations. All untagged formulations had a higher gall reduction than the tagged formulation except for untagged *T. diversifolia* powder which had a similar gall reduction to tagged *T. minuta* pellets at 126 DPI (figure 5a). All formulations significantly reduced galling relative to the positive control at all days post inoculation. Tagged powder formulation of *T. minuta* roots had the highest gall reduction relative to other formulations at 42 DPI (Figure 5b). No significant difference was observed between the tagged and untagged formulations at 42 and 84 DPI except for untagged *T. minuta* pellets which had the least gall reduction at 42 DPI. The untagged formulations had a higher gall reduction relative to the tagged formulations at 126 DPI except for tagged and untagged pellets of *T. minuta* which showed no significant differences (Figure 5b).

Gall reduction in the curative trial

At 42 DPI tagged powder formulation of *T. minuta* leaves and stems reduced galling by about 35-50% relative to other treatments. However, gall reduction in the positive control was 17% better than in untagged and tagged pellets of *T. diversifolia*. Tagged pellets of *T. minuta* significantly reduced galling by more than 10% relative to other formulations at 84 DPI (Figure 5c). At 126 DPI all formulations significantly reduced galling by more than 60% relative to the positive control, there was no significant difference between the tagged and untagged treatments except for untagged *T. minuta* powder which was more than 7 times better than other formulations (Figure 5c). All root formulations significantly reduced galling by more than 20% relative to the positive control at 42 DPI. At 84 DPI, tagged pellets of *T. diversifolia* and *T. minuta* roots were30-50% more effective than other formulations. At 126 DPI all formulations were significantly different relative to the positive control. No significant difference

was observed between the tagged and untagged formulations at 84 and 126 DPI (Figure 5d)

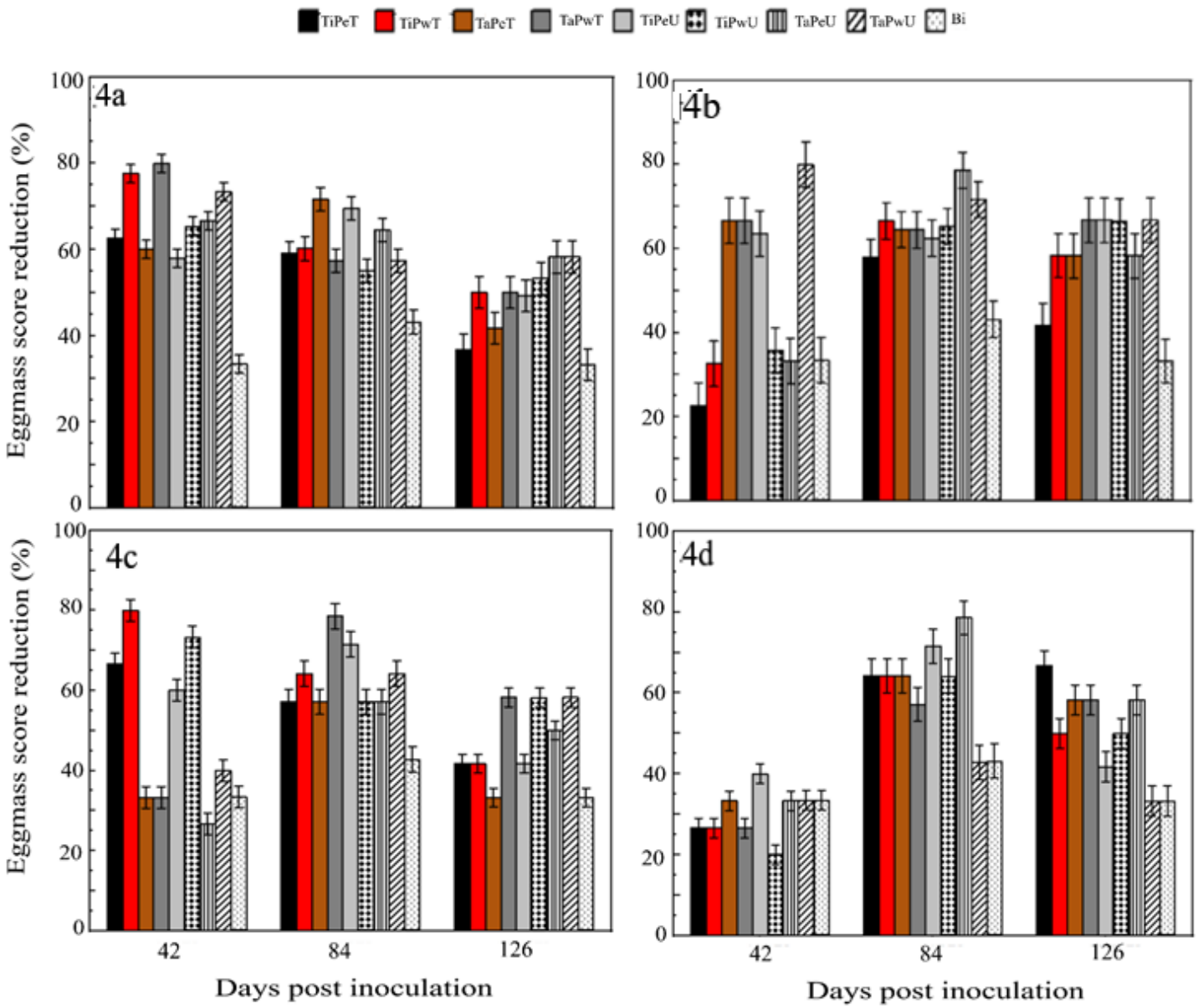


Figure 3: Reduction (%) in the egg mass score on tomato plants after treatment with *Tagetes minuta* and *Tithonia diversifolia* formulations. Preventive trial using above ground parts (leaves and stems) (4a) and belowground parts(roots) (4b). Curative trial using above ground parts and below ground parts (4d). TiPeT = *T. diversifolia* pellets tagged; TiPwT = *T. diversifolia* powder tagged; TaPeT = *T. minuta* pellets tagged; TaPwT = *T. minuta* powder tagged; TiPeU = *T. diversifolia* pellets untagged; TiPwU =*T. diversifolia* powder untagged; TaPeU = *T. minuta* pellets untagged; TaPwU = *T. minuta* powder untagged and Bi (positive (+) control) = Binematon®. ($P < 0.05$, Tukey's test). Vertical error bars represent standard errors of differences of means.

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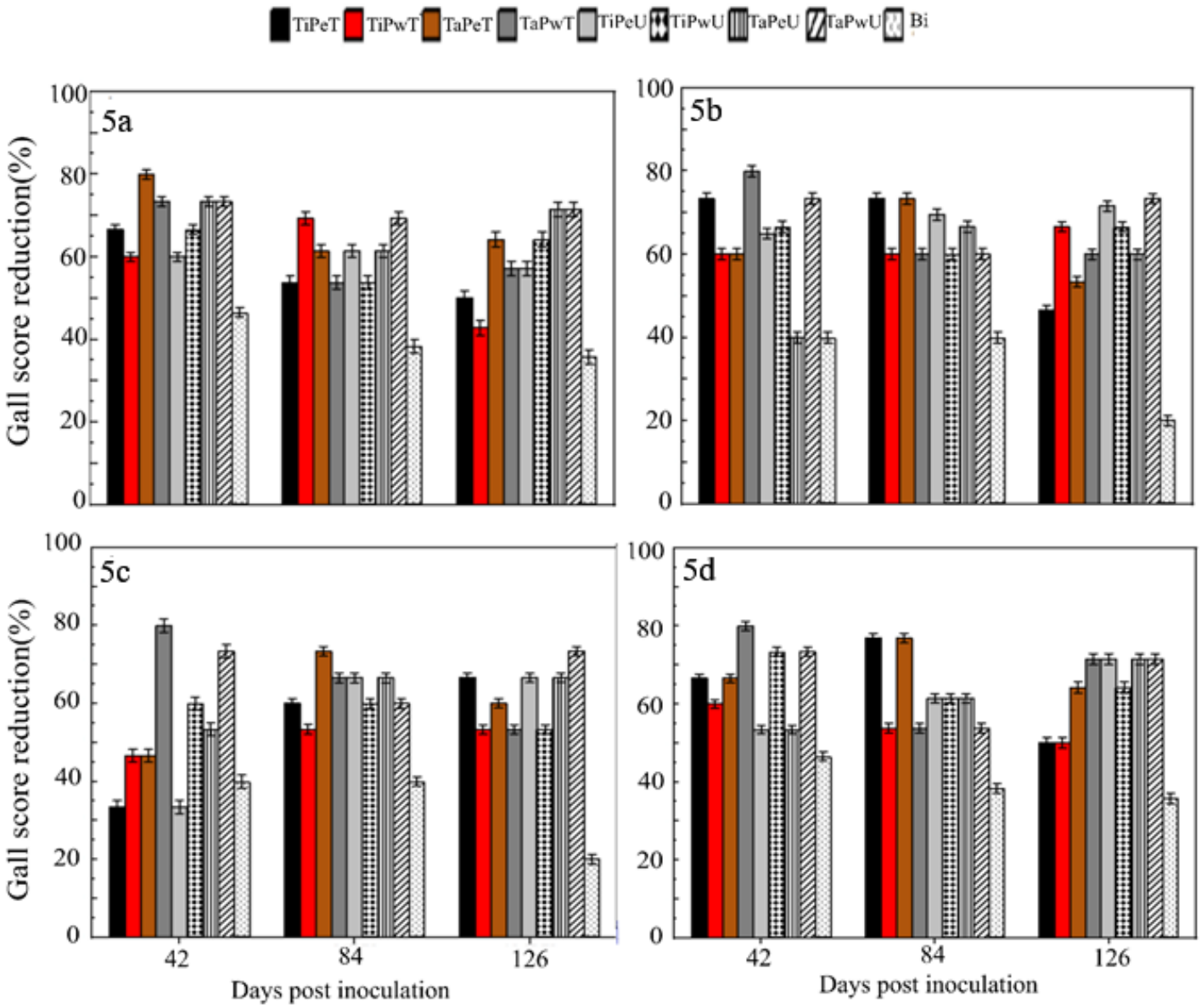

Figure 4: Reduction (%) in the gall score on tomato plants after treatment with *Tagetes minuta* and *Tithonia diversifolia* formulations. Preventive trial using above ground parts (leaves and stems) (5a) and belowground parts(roots) (5b). Curative trial using above ground parts (5c) and below ground parts (5d). TiPeT = *T. diversifolia* pellets tagged; TiPwT = *T. diversifolia* powder tagged; TaPeT = *T. minuta* pellets tagged; TaPwT = *T. minuta* powder tagged; TiPeU = *T. diversifolia* pellets untagged; TiPwU =*T. diversifolia* powder untagged; TaPeU = *T. minuta* pellets untagged; TaPwU = *T. minuta* powder untagged and Bi (positive (+) control) = Binematon®. ($P < 0.05$, Tukey's test). Vertical error bars represent standard errors of differences of means.

Tomato yield

Application of the different formulations on tomato plants significantly influenced tomato yield per hectare. Untagged pellets *T. minuta* above parts recorded the highest yield in both preventive and curative trials (Figure 6a). There was no significant difference in yield among the treatments in the two trials except for Tagged powder of *T. diversifolia* whose yield was higher in the curative trial (65.2t/ha) than in the preventive trial (60.6 t/ha) (Figure 6a). Untagged pellets of *T. minuta* roots had the highest yield (70.7t/ha and 68.1t/ha) in both trials (Figure 6b).

Further, the tagged powder of *T. diversifolia* roots recorded a 7% decrease in yield in the preventive trial as compared to the curative trial. However, within the same trial tagged pellets of *T. minuta* roots and the positive control recorded more than a 10% increase in yield relative to the curative trial (Figure 6b).

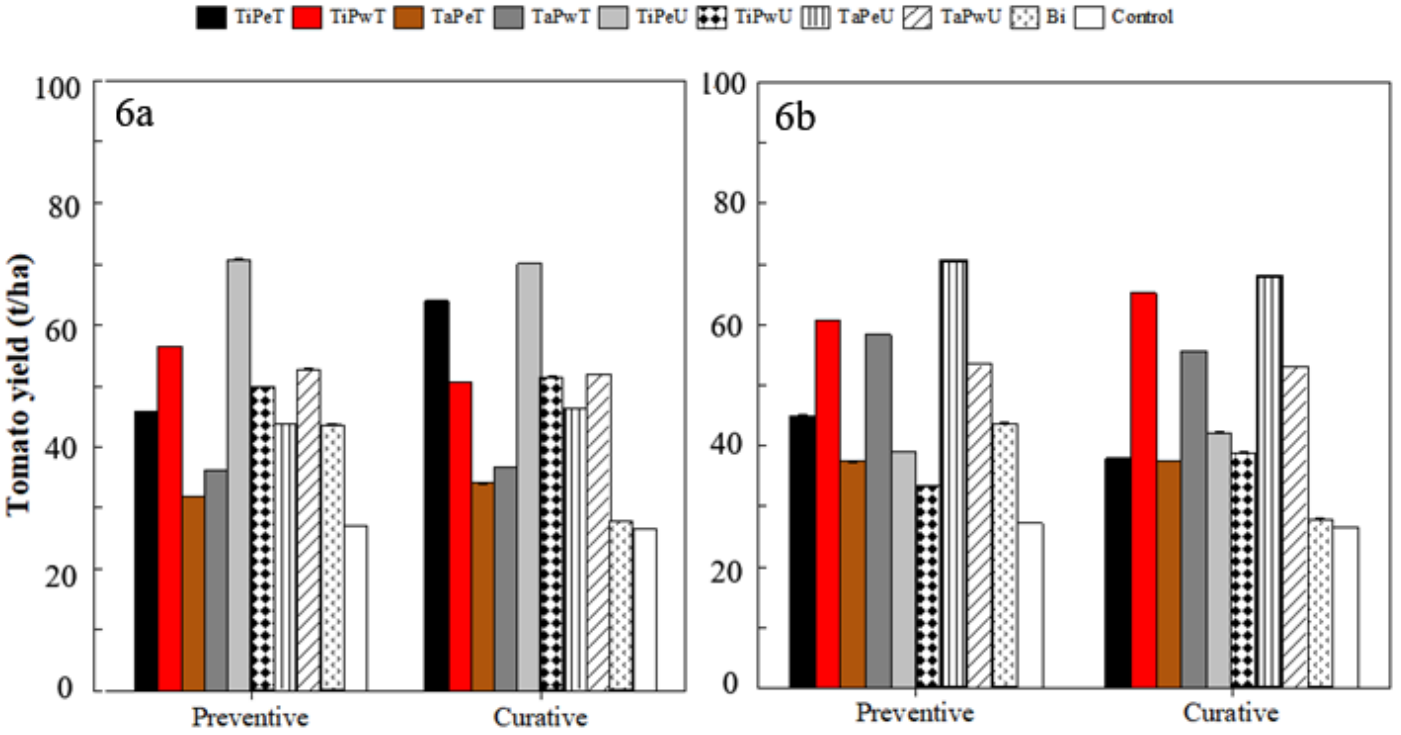

Figure 5: Yield (t/ha) of tomato plants pre- and post- treated with *Tagetes minuta* and *Tithonia diversifolia* formulations. (6a) above ground parts (leaves and stems) and (6b) belowground parts(roots). TiPeT = *T. diversifolia* pellets tagged; TiPwT =*T. diversifolia* powder tagged); TaPeT = T. *minuta* pellets tagged; TaPwT = *T. minuta* powder tagged; TiPeU = *T. diversifolia* pellets untagged; TiPwU (*T.* d*iversifolia* powder untagged; TaPeU = *T. minuta* pellets untagged; TaPwU = *T. minuta* powder untagged; Bi= Binematon® (positive (+) control) and negative (-) control (no treatment applied) ($P < 0.05$, Tukey's test).

Discussion

This study showed that application of powder and pellet formulations of *T. diversifolia* and *T. minuta* either in a tagged or untagged form greatly influenced the soil nematode population density leading to improved yield when compared to the untreated control. Application of *T. minuta* hampers the growth and activity of nematodes in the soil through production of alpha-terthienyl and flavonoids while *T. diversifolia* produces alkaloids which suppress egg hatching of *M. incognita* (Ntalli and Caboni, 2012) In the preventive trial, tagged and untagged formulations of above and below ground parts had no significant difference in infective juvenile reduction at 42 and 84 DPI but a 5% drop in reduction was observed at 126 DPI (Figure 3a and 3b). This scenario is perhaps associated with a reduction in nematoxic substances over time. A previous study by Sowley *et al*,. (2018) showed that application of moringa leaf powder after planting resulted in a significant reduction in the number of second stage juveniles of *Meloidogyne* spp. compared to the application at planting. When the treatments were applied after nematode inoculation, a better reduction in nematode populations was observed in the

first 84 days relative to 126 days. This corroborates with previous studies that showed that application of Bionematon and other biofumigants was best done before and at the time of transplanting compared to the delayed applications (Belay *et al*. 2013; Kruger *et al*. 2013). This is based on the fact that infective juveniles might have already invaded tomato roots before the formulations had released the nematoxic substances. This may explain further why formulations in the preventive trial were more effective than in the curative trial.

Powder formulations of above ground parts (leaves and stems) of *T. minuta* and *T. diversifolia* were better than the pellets at reducing the egg mass score at 42 DPI however the pellets performed better at 126 DPI. The root formulations also significantly reduced the egg mass score with the untagged formulations outdoing the tagged. The better performance of the untagged powder formulations could be attributed to the ease in decomposition hence a faster release of nutrients and other components into the soil compared to those in filter bags. This is in agreement with the findings of Tariq and Dawar, (2015), that showed that pellet and powder amendments in soil easily breakdown and release nutrients and toxicants into the soil relative to the capsule formulations, which has a covering of gelatin and takes time to disintegrate and release the powder in soil. This suggests that the use of filter bags led to the slow release of nematoxic substances resulting in their availability in the soil over a longer period. In addition, the findings of Odeyemi and Adewale, (2011) showed that *Tithonia* residue treatment significantly suppressed the number of galls, and juveniles per root system on yam plants inoculated with *M. incognita* while egg hatching was reduced by 98%.

Both powder and pellet formulations resulted in an increase in yield compared to the untreated control. This confirms a previous report by Srivastava, (2019) who reported that application of root exudates of marigold (*Tagetes erecta*) resulted in effective gall control and a subsequent increase in chilli and brinjal yield. The highest yield in both the curative and preventive trial was observed in untagged pellets of *T. diversifolia* leaves and stems (above parts) and untagged pellets of *T. minuta* roots. Previous reports by Tariq and Dawar, (2015) showed that application of mangroves (*Avicennia marina* and *Rhizophora mucronata*) pellets, enhanced plant length and weight of okra and mung bean while the powder amendment greatly suppressed *M. javanica* gall formation. In other studies, the growth and yield of cowpea was significantly improved by *Tithonia* and Chromolaena soil amendments while *M. incognita* infectivity (the number of galls, nematode population, reproduction) was significantly reduced (Odeyemi *et al.*, 2014). Further, Akinyemi *et al*., (2009) reported that *T. diversifolia* leaves used as mulch on plantain reduced nematode infestation and increased the plantain bunch yield. However for maximum benefit, application of the amendments should coincide with the most vulnerable stage of *M. incognita* preferably at seed planting or seedling transplant (Brennan *et al.*, 2020).

Conclusion

Based on these findings formulations of *T. diversifolia* and *T. minuta* offer a dual role as an excellent alternative to the use of synthetic nematicides and inorganic fertilizers, due to their impact on soil fertility. Smallholder farmers could adopt these formulations in powder or pellet formulation that are space friendly as opposed to companion cropping with these cosmopolitan weeds. Untagged and tagged formulations may be recommended for annual and perennial crops respectively. These formulations further offer an easy form of utilizing plant botanicals in organic farming as well as in large conventional farms. A field trial would be necessary to further evaluate the effect of these formulations on tomato as well as other crops on a large scale basis.

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