Management of *Meloidogyne javanica* in Tomato using Extracts from Asteraceous Plants for Enhanced Productivity and Quality

Tobias Alubi[1], Lucy Kananu Murungi[1*], John Kinyuru[2], Leonard Kiirika[1]
[1]Department of Horticulture and Food Security; [2]Department of Food Science and Technology, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi, Kenya
[*]Corresponding author Email**:** lkananu@jkuat.ac.ke

Abstract

The Javanese root knot nematode, *Meloidogyne javanica* **(Treub) Chitwood, is a major threat to solanaceous plants globally, particularly impacting smallholder tomato farmers in Africa due to its rapid reproduction and resistance to synthetic nematicides. This study assessed the impact of extracts from three asteraceous plants (*Tithonia diversifolia, Bidens pilosa*, and *Tagetes minuta*) on *M. javanica* in tomato cultivation. Various plant parts (roots, stems, flowers, and leaves) were ground and mixed with sterile soil in pots. Tomato plants were grown in these pots and inoculated with second-stage juveniles (J2s) and eggs of *M. javanica* three weeks post-transplanting. The treatments were evaluated for nematode numbers, damage, plant growth, yield, and soil organic carbon and nitrogen content. Results showed that extracts from the three plants had significant effects (P <0.001) on the nematode's reproduction and development. Root extracts of *T. diversifolia* and *B. pilosa* reduced the J2 population by 89.1% and 84.7%, respectively. Moreover, *T. minuta* and *T. diversifolia* root extracts decreased the reproductive potential of *M. javanica* by 69.8% and 72.3%, respectively. A significant (P = 0.001) interaction effect between time and treatment on tomato root growth was observed. The highest yield was achieved with *T. minuta* leaf extracts (0.461±0.039 kg/plant). Ascorbic acid content in tomato varied significantly (P <0.001) with *T . minuta* stem extract treatments showing doubled levels at the mature green stage. Soil analysis revealed that *T. minuta* root extracts significantly (P = 0.001) increased soil organic carbon content to 1.96%, with *B. pilosa* leaf extracts (1.8%) and *B. pilosa* root extracts (1.4%) also contributing positively. *T. diversifolia* stem and leaf extracts had the most significant (0.4%, P < 0.001) impact on soil organic nitrogen content. This study highlights the potential of organic extracts from *T. diversifolia, T. minuta*, and *B. pilosa* in managing RKNs, improving tomato yield, and enhancing soil properties, offering promising avenues for sustainable agriculture.**

Key words: *Bidens pilosa*, Root-knot nematodes, Plant-based treatments, Reproductive fitness, *Tagetes minuta*, *Tithonia diversifolia*

Introduction

Tomato (*Solanum lycopersicum* L.) is a globally cherished vegetable, integral to human diets for its nutritional richness. with essential components such as ascorbic acid, lycopene, and β-carotene (Schweiggert *et al*., 2014) To ensure year-round production of tomato, farmers have turned to polytunnel cultivation, offering the flexibility to grow them in varying climates (Pack and Mehta, 2012). The protective shield of polytunnels presents several advantages, including increased yield and size, superior quality, and consequently, premium pricing (Arah *et al*., 2015). However, despite its promise, the adoption of polytunnel technology for tomato production remains hindered by persistent pests especially where the crop is continuously cultivated. Among these challenges, the presence of root-knot nematodes (RKNs), such as *Meloidogyne incognita* (Kofoid & White, 1919), *Meloidogyne arenaria* (Neal, 1889)

Chitwood, 1949, and *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949 (Anwar and McKenry, 2012), poses a significant threat. These microscopic parasitic organisms infiltrate the plant's root system, exploiting its defense mechanisms for sustenance (Gheysen and Mitchum, 2011). The culmination of this infestation leads to yield reductions, with a staggering annual global economic loss of $157 million (Ali *et al*., 2015). Africa bears the brunt of these losses, exacerbated by farmer unawareness of RKN presence and symptoms (Onkendi and Moleleki, 2013). In Kenya, losses of up to 68% in tomato have been reported (Wanjohi *et al*., 2018).

Past studies suggest that solutions lie in cover crops (Abawi and Widmer, 2000), organic amendments (Ka and Ermiyahu, 2002), and biological control agents (Sikora, 1992) which exhibit varying bioactivity against RKNs. Extracts from *Tagetes erecta*, *Ricinus communis*, and *Allium cepa* have shown promise against RKNs (Tibugari *et al*., 2012). Additionally, the use of augmentative biological control with invertebrates and microbial agents emerges as a sustainable nematode management technique, avoiding pesticide residue and offering improved yields (Van Lenteren *et al*., 2018). Notable examples include *Bacillus thuringiensis* crystal (Li *et al*., 2007) and *Pochonia chlamydosporia* fungus employed in combined crop rotations (Atkins *et al*., 2003). However, gaps persist in understanding the impact of these strategies on *M. javanica* and their systemic effects on tomato yield and quality (Coyne *et al*., 2006). Despite encouraging results, research on mode of action remains limited for certain natural agents like *Tithonia diversifolia* and *Bidens pilosa* (Taba *et al*., 2014; Premachandra and Amarasinghe, 2016). The current study seeks to address these gaps by investigating the effects of organic extracts from Asteraceae plant parts on RKN infestations in 'greenhouse'-grown tomatoes. The study aims to determine the influence of these amendments on both tomato yield and nutritional quality. By bridging these knowledge gaps, the research contributes to a holistic understanding of RKN management, providing actionable insights for sustainable tomato cultivation.

Materials and methods

Plants

Tomato seeds of the cultivar Anna F1, an indeterminate hybrid, were sourced from a local agroseed merchant in Nairobi, Kenya. Before sowing, the trays were cleaned under running tap water, followed by immersion in a diluted mancozeb solution at a concentration of 2 g/l to eliminate potential fungal contaminants on the surface (Landis and Morgan, 2009). The sterile cocopeat, employed as the planting medium, was soaked in water overnight to eliminate excess salts and other compounds before being placed in pre-cleaned trays (Thomas *et al*., 2013). A mixture of sand and red soil in a 1:1 volume ratio was prepared and blended with asteraceae plant extracts from *B. pilosa*, *T. minuta*, and *T. diversifolia*. This mixture was then introduced into plastic sleeves measuring 8'×4'×4'. Following three days from the incorporation of the plant extracts into the mixture, three-week-old tomato seedlings were transplanted into these sleeves. Subsequently, these transplanted seedlings were placed in a screen house, where conditions were set at a temperature of 18/30 °C day/night, with 12 hours of light and a relative humidity range of 60-70%.

Asteraceae plants of *T. minuta*, *T. diversifolia*, and *B. pilosa*, collected from the field in Juja (-1.025540 S, 37.0131930 E; 1521 m asl), Kenya. Plant parts, including roots, stems, leaves, and flowers of respective plants, were chopped into small pieces using a chaff cutter and air-dried in the shade for 21 days. Subsequently, they were packed in labeled khaki bags. The resulting material was ground into powder using a bench-top laboratory

grinding machine and sieved through a 3.5 mm sieve. To ensure purity, the grinder hopper, rotary blades, and internal sieve were cleaned using bristle brushes each time a different plant part from the respective species was pulverized and stored in dry conditions until use.

Nematodes

Root-knot nematode egg masses of *M. javanica*, were sourced from a pure culture carefully maintained on tomato plants at the International Centre of Insect Physiology and Ecology (1.2219° S, 36.8966° E), Kenya. To increase their numbers, the nematodes were introduced onto African nightshade plants in a controlled screen house environment, with conditions set at 27 ± 2 °C, 60-70% relative humidity, and a 12/12 hr. light/dark photoperiod. Inoculation involved placing a single egg mass per pot. The plants were adequately nourished with either CAN or NPK fertilizer at a rate of 10 g per pot after two weeks. After about 60 days following inoculation, plants displaying galled root systems were uprooted for the purpose of egg mass collection. The roots were gently rinsed under running tap water to eliminate soil particles, after which they were subjected to staining with Phloxine B solution (0.15 g/L water) for 15 minutes to enhance the visibility of the egg masses. Subsequently, the stained egg masses were delicately detached from the roots utilizing a fine needle under a stereo microscope. They were then placed in culture plates filled with 2 mL of distilled water and stored in an incubator with a temperature of 28 ± 2 °C for a duration of 3-7 days, allowing the J2s to hatch. After this hatching period, the emerged juveniles (J2s) were collected using a 1 mL plastic transfer pipette and counted on a concave counting dish positioned on a stereomicroscope (x40 magnification). These juveniles were then transferred to 15 mL falcon tubes containing 1 mL of distilled water, where they were stored until their use in subsequent bioassays. Over a seven-day period, the first-stage juveniles molted into the second-stage infective juveniles. Out of these, a total of 200 infective juveniles, along with three egg masses per pot, were introduced in close proximity to the roots of potted tomato seedlings.

Treatments

The experiment comprised seven treatments arranged in a completely randomized design (CRD), with each treatment replicated three times, consisting of 10 plants in each replicate. Plant extracts obtained from the roots (R), stems (S), leaves (L), flowers (F), and the entire plant (W) of three asteraceous plant species - *T. minuta*, *T. diversifolia*, and *B. pilosa* were utilized. The experimental setup also included a negative control (untreated) and a positive control (Ecotech, a garlic concentrate with 99.99% purity). For the incorporation of plant extracts, the powdered form was added to sterilized soil (autoclaved at 121 °C for 20 minutes) at a rate of 2.5 g/kg of soil. Ecotech, on the other hand, was applied at a rate of 25 ml/kg of sterilized soil, following the manufacturer's recommendations. From each treatment group, one plant was selected for subsequent analysis. The experiment was independently replicated thrice.

Nematode counts and damage assessment

Infective juveniles and egg masses were assessed starting from four weeks after transplanting and continued over a period of 10 weeks, with evaluations conducted every two weeks. To acquire samples for egg mass analysis, one plant per treatment was randomly uprooted, placed in khaki bags (440 x 440 mm), and then carefully packed in cooler boxes for transportation to the laboratory for further processing. Concurrently, approximately 200 g of soil was sampled from the corresponding treatment pots and treated in a similar manner for subsequent nematode quantification. The process of extracting infective stage two (J2) juveniles was performed at 4, 6, 8, and 10 weeks post-transplantating using Baermann's technique. This technique involved using a domestic sieve lined with a paper towel, placed atop a plastic plate filled with water to facilitate the

collection of nematodes. The sampled soil from each treatment was positioned on the paper towel. After a 24-hour interval, the nematodes that had migrated from the soil to the water-filled plate were extracted using a 27 µm sieve. These extracted nematodes were then concentrated into a 5 ml falcon tube and quantified utilizing a stereo microscope. The process of evaluating the roots included washing them under running tap water to eliminate adhering soil, followed by staining with Phloxine-B solution (0.15 g/l) for a duration of 20 minutes. Subsequently, the roots were washed again to remove excess stain, and then dipped in clear clean water to facilitate the counting of egg masses. The egg masses were assessed using a scale ranging from 0 to 5: 0 denoting no egg mass; 1 indicating 1-2 egg masses; 2 representing 3-10 egg masses; 3 signifying 11-30 egg masses; 4 denoting 31-100 egg masses; and 5 indicating over 100 egg masses per root system (Sikora et al., 2005).

Plant growth, yield and C/N assessment

Plant growth

For each treatment, a single mature tomato plant was selected at random and uprooted for the purpose of measuring root biomass. This procedure was repeated every two weeks over a span of 10 weeks. Upon performing the destructive root harvesting, the soil within the pots was sifted through a 2 mm sieve and subsequently washed under a stream of running water to dislodge any roots that were adhering to soil particles. The tomato roots were then subjected to oven drying at a temperature of 70 °C for a duration of 48 hours before being weighed. The relative growth rate, denoting the quantity of biomass produced per unit of pre-existing biomass at time (t_1) per unit of time (Radford, 1967) as follows; $RGR = In(W_2-W_1) / t_2-t_1$

where In = Natural log, W_1= Dry weight of plant (g) at time t_1 and W_2 = Dry weight of plant at time t_2.

Yield

Five out of ten plants within each plot were chosen at random and designated with tags for identification. The process of harvesting physiologically mature, ripe, and marketable fruits commenced 50 days after transplanting and persisted for a duration of two months, with the exact quantity varying from plot to plot. The harvested fruits were weighed on a scale that exclusively considered the marketable fruits, thereby excluding pest-damaged fruits and those displaying physiological disorders. To ascertain the average tomato yield per plant, the cumulative weight of marketable fruits was divided by the number of plants within each plot. The resulting value was expressed in kg/plant.

Total organic carbon

For each treatment, one pot was selected for sampling, and the soil was allowed to air-dry for a span of five days. Subsequently, the dried soil was ground using a mortar and pestle, then sieved through a 0.42 mm sieve to achieve a uniform consistency. A 0.1g portion of the ground soil sample was accurately weighed using an electronic scale and then introduced into a 250 ml Erlenmeyer flask. To this flask, 10 ml of 1N Potassium dichromate ($K_2Cr_2O_7$) solution was added. Concurrently, 50 ml of concentrated sulphuric acid (H_2SO4) was introduced into the swirling Erlenmeyer flask to ensure thorough mixing with the reagent. The resulting mixture was subjected to heating on a hot plate for a duration of 10 min, all while continuing to swirl the flask, until a temperature of 135 °C was achieved. Subsequent to heating, the mixture was allowed to cool within a fume chamber for 30 minutes, following which 150 ml of distilled water was incorporated. To the reagent mixture, three drops of a 0.16% diphenylamine indicator were added. Volumetric titration was carried out to determine the excess dichromate that remained unreacted, using hydrated ammonium ferrous sulfate [$Fe(NH4)_2(SO_4)_26H_2O$)] as the titrant.

Throughout the process, distinct color changes were observed, transitioning from a dirty green as the starting point to blue-green and ultimately light green, signifying the endpoint of the titration. In order to standardize the iron sulphate solution, a blank was prepared in the same manner, side by side with the sample analysis, following the established methodology of Walkley and Black (1934). The organic carbon in the sample was calculated from the formula; $2K_2Cr_2O_7 + 3C + 16H^+ + 4Cr^{3+} + 8H_2O + 3CO_2 \uparrow$; Where 1 ml of Potassium dichromate is equivalent to 3 mg of carbon. Finally the percentage carbon was determined from the formula;

Organic carbon (% C) = $\frac{0.003g \text{ X N X } 10 \text{ mL X } (1-T)x100}{ODW} = \frac{3\ (1-T/S}{W}$

Where; N= Normality of $K_2Cr_2O_7$ solution; T= Volume of $FeSO_4$ used in sample titration (ml); S = Volume of $FeSO_4$ used in blank (ml); ODW= Oven-dry sample weight (g)

Total organic nitrogen

At intervals of 4, 6, 8, and 10 weeks, soil samples were collected and subsequently subjected to air drying at ambient room temperature for a period of 48 hours. After air-drying, a 0.3g portion of the soil sample was finely ground and passed through a 2 mm sieve, ensuring the removal of any coarse particles. This sieved soil was then packaged, taking care to shield it from moisture and potential contaminants. For further analysis, a well-sieved soil sample weighing 0.3 g was placed within a digestion tube. A digestion mixture consisting of 420 mL of sulphuric acid, 350 ml of hydrogen peroxide, 0.42 g of selenium powder, and 14 g of Lithium sulphate was added to the tube. Subsequently, the sample was digested at a temperature of 110 °C for 1 hour. After the digestion process, and upon cooling, an additional 50 mL of distilled water was introduced to the mixture. A 10 mL aliquot was then extracted from this solution to facilitate the subsequent distillation process, following the Macro Kjeldahl procedure (Zumbado *et al*. 1998). The percentage Nitrogen (N) in soil was calculates as follows:

% N in the soil sample = $\frac{(a-b)x\ 0.1\ x\ V\ x\ 100}{100\ x\ W\ x\ Al}$

where % N=Estimated nitrogen in the soil sample; a= volume of titrant 0.01NHCL for the sample; V= volume of titrant 0.01N HCL for the blank; b= final volume of digestion; W= weight of the sample taken; Al= Aliquot of the solution taken for analysis

Quality assessment

Nutrition analysis: Quarter pieces were obtained from three representative tomato fruits per treatment, and each quarter weighed approximately 5 g. These segments were carefully ground using a pestle and mortar, and the resulting mixture was homogenized to achieve a fine concentrate. Subsequently, a 2-g portion of the sample concentrate was weighed and subjected to an extraction process involving 0.8% metaphosphoric acid to isolate L-ascorbic acid. To prepare the juice, distilled water was introduced, resulting in a total volume of 20 ml, which was then subjected to centrifugation at a speed of 10,000 revolutions per minute. The supernatant was filtered and subsequently diluted with 10 ml of 0.8% metaphosphoric acid solution, based on the technique employed by Cotruţ and Badulescu (2016). This diluted solution was passed through a 0.45 µm filter before a 20 µl sample was injected into the High Performance Liquid Chromatography (HPLC) system, following the procedure described by Vikram *et al*. (2005). For quantification purposes, a standard calibration graph was employed to determine the concentration of L-ascorbic acid in the samples.

Data analysis

To evaluate the impact of the treatments on the nematode population, a repeated measures analysis of variance (ANOVA) was employed. Data on infective juvenile stage 2 (J2) nematodes collected at fortnightly intervals was square root transformed to ensure

homogeneity of variance (Kalinda *et al*., 2017). Initially, a full model with interaction terms was applied; however, the interaction was found to be non-significant. Consequently, the optimal model without interaction terms, a two-way ANOVA, was adopted. For assessing the influence of respective plant parts on the number of egg masses laid, the count of egg masses at specific intervals post-application of plant extracts was taken into account. To enhance homogeneity of variance, the count of egg masses was square root transformated before being subjected to ANOVA (Garson, 2012). To analyze the impact of plant extracts on root growth, a two-way analysis of variance model devoid of interaction was employed. To study the influence of both harvesting time and plant extract application on the concentration of Ascorbic acid in harvested tomato fruits, the data was subjected to the Shapiro-Wilk test for normality. Subsequently, a two-way analysis model was employed, as described by Shapiro and Martin (1965). To assess the effects of *T. diversifolia*, *T. minuta*, *B. pilosa*, and their respective plant parts on tomato yield, a one-way analysis of variance was utilized. In order to ascertain the effects of plant extracts on soil organic carbon (C) and nitrogen (N) content, in conjunction with the sampling time, a two-way analysis of variance without interaction was employed. All data related to J2 nematode count, egg masses count, total organic carbon and nitrogen content, root growth, and yield were analyzed using STATA software (Stata Corp, 2015).

Results

Nematode count

The count of juvenile stage 2 (J2) nematodes acquired at 4, 6, 8, and 10 weeks subsequent to the initial inoculation demonstrated no statistically significant variations ($F_{3,48}$=1.55, p = 0.2137). However, notable disparities emerged ($F_{16,48}$=4.22, p = 0.0001) across all treatments involving *T. diversifolia*, *T. minuta*, and *B. pilosa* in relation to the J2 nematode count. Remarkably, the outcomes highlighted the efficacy of root extracts from *T. diversifolia* (69.16 ± 62.24), showcasing a significant reduction in the *M. javanica* population. This was followed by root extracts of *B. pilosa* (97.16 ± 68.99) and the entire *B. pilosa* plant (143.91±75.27) when compared to the control, respectively (Figure 1).

Plant growth and yield of Tomato

The outcomes concerning the root growth of tomato under the influence of various plant extracts exhibited significant differences between the time intervals of 4-6, 6-8, and 8-10 weeks ($F_{2,32}$ = 4.70: p = 0.01122). Moreover, a significant interaction effect between time and treatment was identified (p = 0.001) in relation to tomato root growth. A relative growth rate of -0.216 ± 0.06 g d^{-1} was observed in *B. pilosa* root extract, signifying inhibitory and marginal growth effects between 6-8 weeks, along with corresponding stimulatory effects of 0.201 ± 0.049 g d^{-1} between 8-10 weeks. Similarly, *T. minuta* root extracts exhibited a marginal relative growth rate of -0.071 ± 0.02 g d^{-1} between 4-6 weeks, along with an optimal relative growth rate of 0.076±0.02 g d^{-1} between 6-8 weeks when compared to the control (Figure 3). This indicates that the root extracts of *B. pilosa* and *T. minuta* exert both suppressive and promotive effects on the root growth of tomato.

The findings revealed significant differences in the impact of plant extracts on tomato yield ($F_{16,\ 34}$ = 3.29, p= 0.0017) compared to the control. Notably, all the plant extracts derived from *T. diversifolia*, *B. pilosa,* and *T. minuta* exhibited a positive effect on tomato yield. Remarkably, the highest average tomato yield was achieved with *T. minuta* leaf extract (0.461±0.039 kg/plant), closely followed by *B. pilosa* whole plant extract (0.441 ± 0.08 kg/plant). Conversely, the lowest average yield of (0.298± 0.034 kg/plant) was observed from *T. diversifolia* stem extracts (Figure 4).

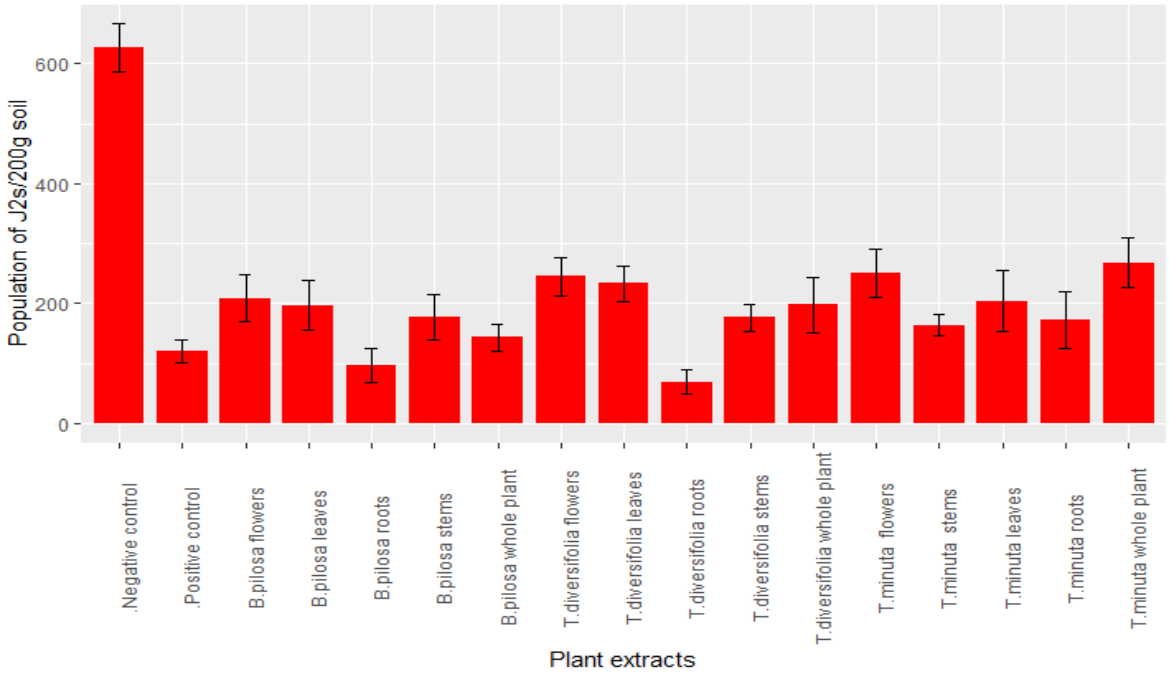

Figure 1: Influenceof *Tithonia diversifolia*, *Tagetes minuta*, and *Bidens pilosa* plant extract applications on the population of *Meloidogyne javanica* in high tunnel-cultivated tomato (Anna F1). Error bars in the graph represent the standard error of the mean. Means with non-overlapping error bars suggest significant differences at the $p < 0.05$ using analysis of variance.

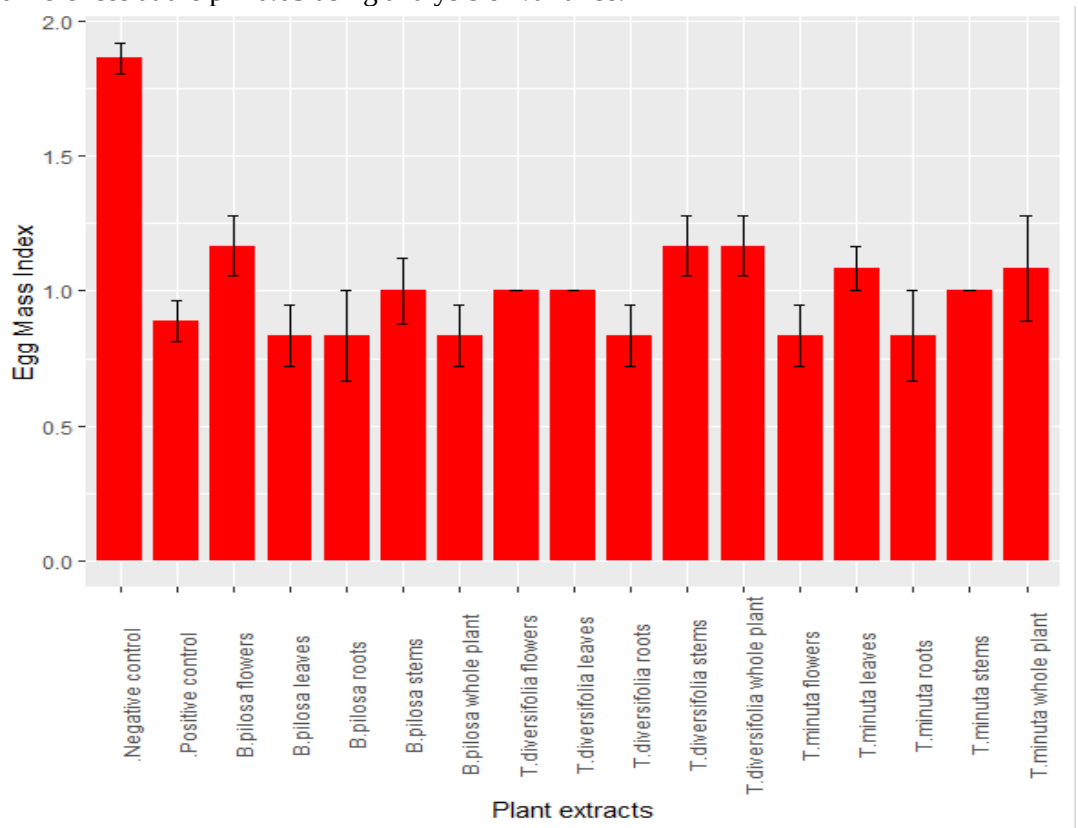

Figure 2: Impact of *Tithonia diversifolia*, *Tagetes minuta*, and *Bidens pilosa* plant extract applications on the reproductive fitness of *Meloidogyne javanica* in high tunnel-cultivated tomato. Error bars in the graph represent the standard error of the mean. Means with non-overlapping error bars suggest significant differences at the $p < 0.05$ level using analysis of variance.

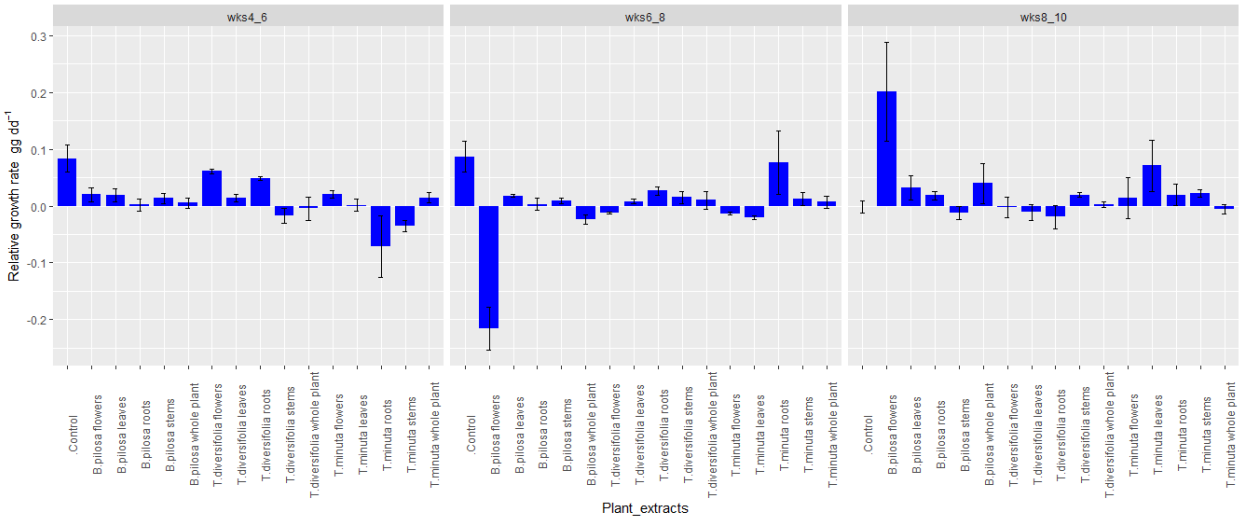

Figure 3: Impactof *Tithonia diversifolia*, *Tagetes minuta*, and *Bidens pilosa* plant extract applications on tomato root growth. Error bars in the graph represent the standard error of the mean. Means with non-overlapping error bars suggest significant differences at the $p < 0.05$ level using analysis of variance.

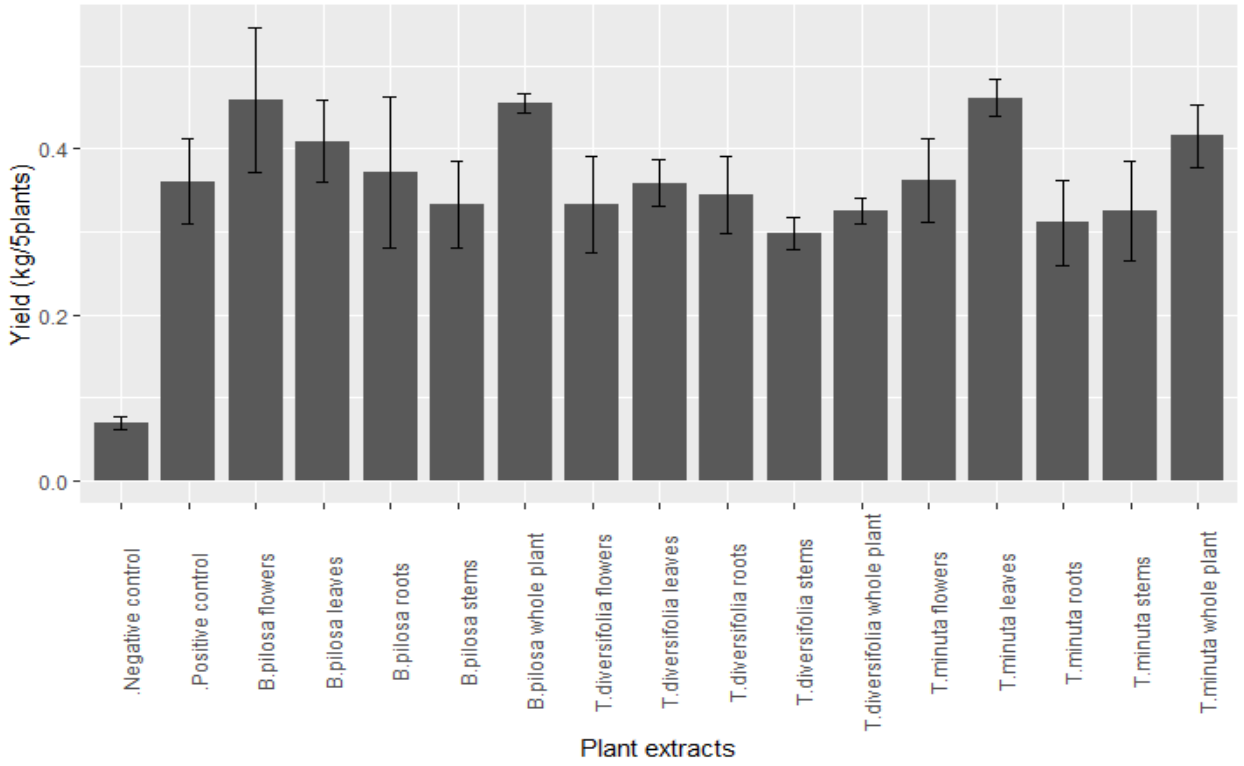

Figure 4: Impact of *Tithonia diversifolia*, *Tagetes minuta*, and *Bidens pilosa* plant extract applications on tomato yield. Error bars in the graph represent the standard error of the mean. Means with non-overlapping error bars suggest significant differences at the $p < 0.05$ level using analysis of variance.

Ascorbic acid content

The obtained results revealed no significant differences in the ascorbic acid content of tomatoes treated with *T. diversifolia, T. minuta,* and *B. pilosa* plant extracts at a significance level of $p<0.05$

However, significant variations in ascorbic acid content emerged during different maturity stages of tomatoes ($F_{2,32}$ = 25.99: pP = <0.001). Higher levels of ascorbic acid were observed in tomatoes treated with *T. minuta* stem extract (13.59 ± 11.50 mg/100g) at the mature green stage. Conversely, the lowest amounts of ascorbic acid were found in *B. pilosa* flower extract (5.12 ± 2.23 mg/100g) at the green stage, *T. diversifolia* stem extracts (7.07 ± 1.75 mg/100g) at the breaker stage, and *T. diversifolia* stem extracts (8.20 ± 0.81 mg/100g) at the mature red stage, in comparison to the control (Table 1).

Table 1: Effect (Mean ±SE) of *Tithonia diversifolia*, *Tagetes minuta*, and *Bidens pilosa* plant extracts application on mean amount of ascorbic acid content on tomato at different maturity stages

	Ascorbic acid (100 g/mg) at different maturity stages		
	Green	Breaker	Red
Bidens pilosa extract			
Flower	5.12±2.23	7.24±1.32	9.74±2.33
Leaf	7.99±2.79	8.94±2.82	11.49±0.88
Root	8.67±2.79	9.34±1.96	11.43±0.96
Stem	5.66±3.90	7.55±4.10	10.84±0.61
Whole plant	8.70±4.08	11.12±2.35	12.57±0.86
Tagetes Minuta extract			
Flower	6.68±4.88	8.69±2.84	11.71±0.56
Leaf	6.08±3.40	8.52±3.00	10.53±1.35
Root	6.79±3.86	8.01±2.35	10.96±1.15
Stem	13.59±11.50	8.07±1.72	10.48±0.08
Whole plant	6.98±1.19	8.50±1.57	11.37±0.65
Tithonia diversifolia extract			
Flower	6.53±0.71	7.41±0.53	10.74±1.85
Leaf	6.26±0.98	7.11±0.70	8.53±0.36
Root	9.35±1.21	10.85±1.94	12.70±0.86
Stem	6.03±2.91	7.07±1.75	8.20±0.81
Whole plant	7.11±0.43	8.22±0.11	9.77±0.08
Control	13.06±2.17	13.02±3.39	14.061±2.34

Note: ANOVA indicates significant differences among groups ($p < 0.001$). Post-hoc tests were not conducted; therefore, specific group differences are not identified.

Organic carbon analysis

The obtained results indicated significant variations in the organic carbon content of soil treated with *Tithonia diversifolia*, *Tagetes minuta*, and *Bidens pilosa* plant extracts ($F_{16,126}$ = 3.31, p = 0.001). The highest organic carbon content in the soil was observed in *T. minuta* root extract treatment (1.960 ± 1.04), followed by *B. pilosa* leaf extract treatment (1.750 ± 0.88) and *B. pilosa* root extract treatment (1.416 ± 0.42), respectively (Figure 5).

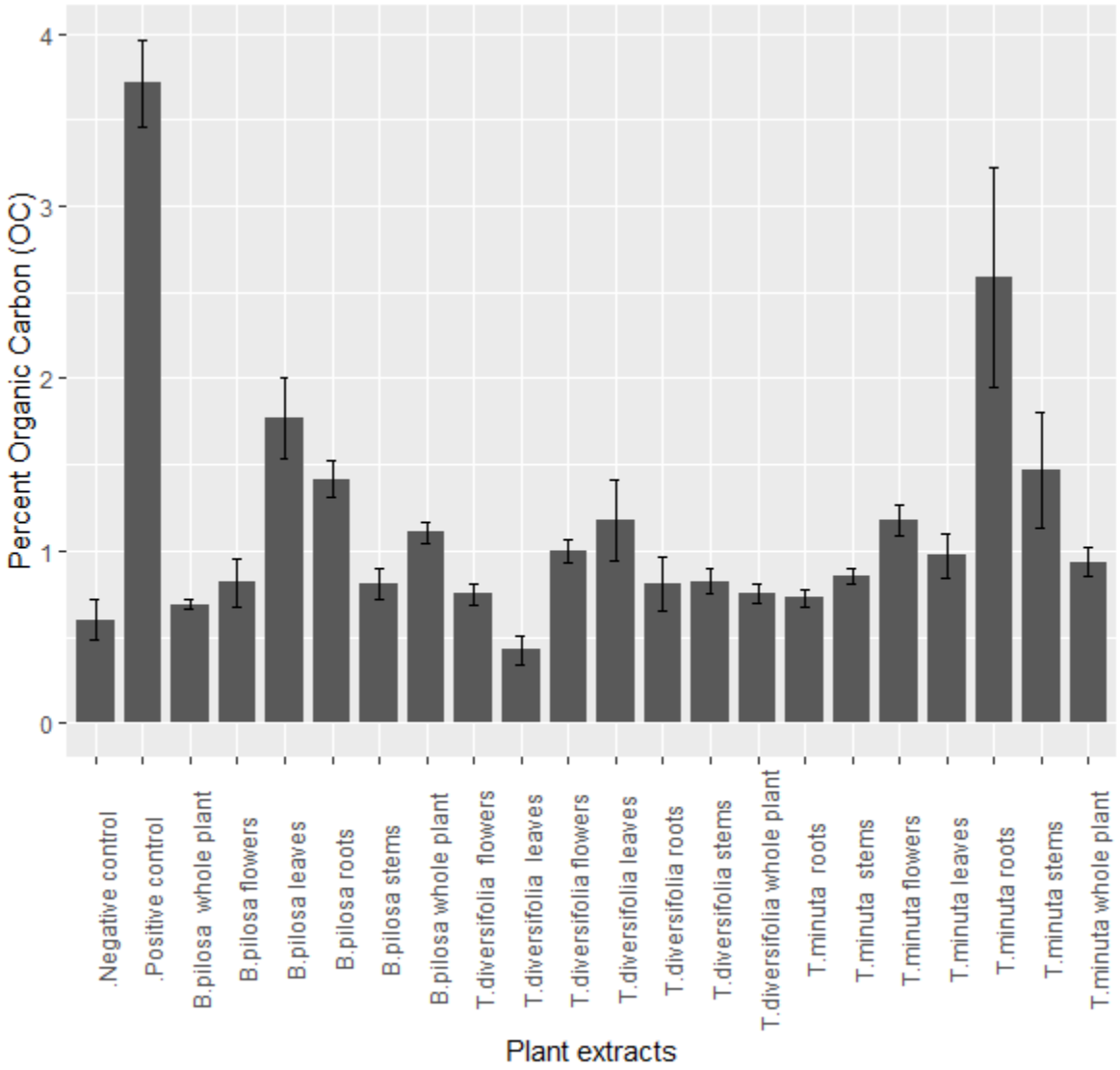

Figure 5: Effects of *Tithonia diversifolia*, *Tagetes minuta*, and *Bidens pilosa* plant extract application on soil organic carbon in *Meloidogyne javanica* infested potted soil planted with tomato under high tunnel conditions. Error bars in the graph represent the standard error of the mean (SEM). Means with non-overlapping error bars suggest significant differences at the $p < 0.05$ level.

Organic Nitrogen analysis

The results obtained indicate statistically significant differences in soil organic nitrogen (N) when *T. diversifolia*, *T. minuta*, and *B. pilosa* plant extracts were applied compared to the control (P = < 0.001). The highest percentage of organic nitrogen was recorded in the soil treated with *T. diversifolia* stem extract (0.3633 ± 0.27) and *T. diversifolia* leaf extract (0.3575 ± 0.13), respectively (Figure 6).

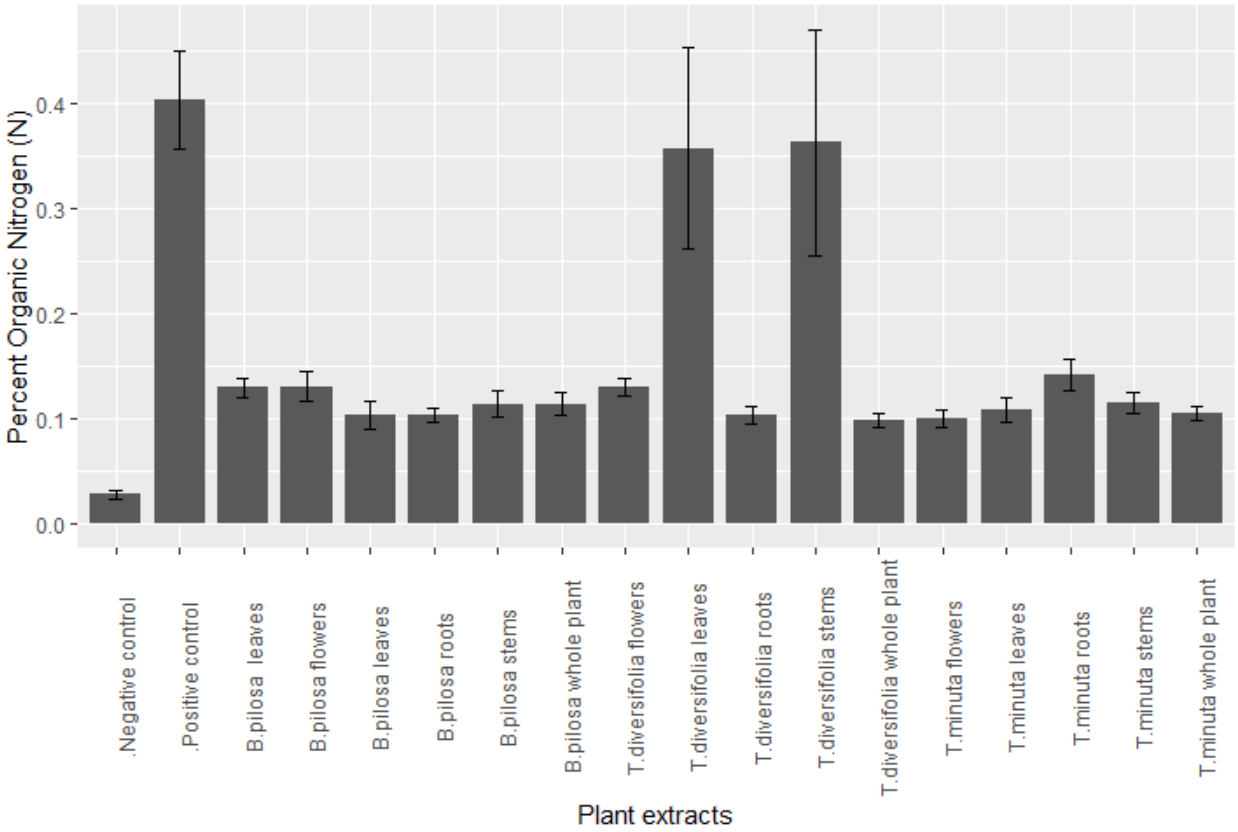

Figure 6: Impact of *Tithonia diversifolia*, *Tagetes minuta*, and *Bidens pilosa* plant extracts on total organic nitrogen in *Meloidogyne javanica* infested potted soil planted with tomato under high tunnel conditions. Error bars in the graph represent the standard error of the mean. Means with non-overlapping error bars suggest significant differences at the $p < 0.05$ level.

Discussion

The results demonstrate that the application of plant extracts from *T. diversifolia*, *B. pilosa*, and *T. minuta* has a significant impact on the reproduction and population of root knot nematodes (RKN), as well as the promotion and suppression of plant growth. Moreover, these plant extracts influence tomato yield and vitamin C content at the full physiological mature ripening stage. Significant suppression of RKN reproduction was observed in the presence of *T. diversifolia* root extract, *B. pilosa* whole plant extract, and *B. pilosa* root extract. The suppression of J2 population by root extracts of *T. diversifolia* and *B. pilosa*, as well as *B. pilosa* whole plant extract, could be attributed to their higher organic nitrogen content and lower carbon-to-nitrogen (C/N) ratio. Previous studies (Jama *et al*., 2000) have highlighted that higher nitrogen and a narrow C/N ratio facilitate the production of toxic ammonia during plant extract decomposition, which can lead to immediate nematode suppression. This process is driven by the release of volatile compounds during active decomposition stages, further hampering RKN populations (Ros *et al*., 2008).

The plant extracts from *T. diversifolia*, *B. pilosa*, and *T. minuta* significantly reduced the reproductive health of RKN, irrespective of the plant part used. These reductions in egg mass index demonstrate the effectiveness of both root and aerial part extracts in managing RKN pests. While root exudates of *T. minuta* have been reported to inhibit *M. javanica* egg hatching (Hooks *et al*., 2010), our findings reveal a novel reduction in egg mass index due to aerial plant extracts. The effectiveness of leaf extracts from *T. diversifolia* could be

attributed to the presence of poisonous compounds (Ploeg, 2002) and active thiophene compounds in *T. minuta* that inhibit egg mass index in *M. javanica* (Schosser *et al*., 2006). Application of *B. pilosa*, *T. minuta*, and *T. diversifolia* plant extracts produced a range of stimulatory and inhibitory effects on tomato root growth. Extracts from *T. diversifolia* (stems, leaves, and flowers), *T. minuta* (stems, flowers, and leaves), and *B. pilosa* (whole plant, flowers, and stems) exhibited inhibitory effects on root development. These results suggest that inhibitory compounds may affect root development at different growth stages, with the rate of compound release depending on the type and concentration of the plant extract. Recent research has demonstrated that *B. pilosa* residues inhibit sprouting in *Cyprus rhodus* (Hsueh *et al*., 2020) due to compounds like caffeic acid (Khanh *et al*., 2009) and phenolic substances, which suppress the root growth of weeds such as *Rotala indica* and *Raphanus sativa* (Deba *et al*., 2007). The inhibitory activity of *T. diversifolia* leaf extracts on root growth in radish (Tongma *et al*., 1998) further supports these findings.

The plant extracts from *B. pilosa* flowers, roots, and stems, as well as *T. minuta* roots, stems, and flowers, demonstrated dual characteristics of stimulatory and inhibitory effects on tomato root growth. This suggests the presence of multiple chemical compounds in varying concentrations that can either promote or suppress root growth without leading to root death. The observed stimulatory and inhibitory behavior in *T. diversifolia* extracts also indicates the presence of allelochemical compounds (Oyerinde *et al*., 2009). While *T. minuta* stimulates root-shoot ratios in tomato seedlings (Darryn *et al*., 2016), the underlying mechanisms remain unclear. The sigmoid growth pattern of *B. pilosa* plant extracts during tomato growth stages requires further investigation. All the plant extracts from *T. minuta*, *B. pilosa*, and *T. diversifolia* resulted in reduced ascorbic acid accumulation in tomatoes at different ripening stages, except for *T. minuta* stem extracts, which did not differ from the control at the mature green stage. This suggests that these plant extracts contain similar compounds that reduce ascorbic acid biosynthesis during tomato ripening stages, or that the outcome is influenced by genotypic variations. While Ilupeju *et al*. (2015) reported increased ascorbic acid content in tomatoes when composite *T. diversifolia* material was used as soil amendment, the difference could be attributed to the state of the plant extracts used in our study.

Plant extracts from *T. diversifolia*, *T. minuta*, and *B. pilosa* led to increased tomato yield per plant. Notably, the negative control showed no progressive increase in yields irrespective of the plant extract used. This indicates that the application of these plant extracts could enhance tomato yield, offering an alternative source of organic fertilizer. Similar findings have been reported for *T. minuta* leaf extracts (Taye *et al*., 2012) and *T. diversifolia* for tomato yield enhancement (Salamil, 2017). The improved yield may stem from reduced soil salinity (Saeed and Ahmad, 2009), increased organic matter, improved soil temperature, and enhanced bulk density. Overall, the findings indicate that all aerial parts of *T. diversifolia*, *B. pilosa*, and *T. minuta* increase the organic carbon content in the soil, except for *T. diversifolia* leaf extracts. This implies that root and stem extracts of *B. pilosa*, flower extracts of *T. diversifolia*, and root and stem extracts of *T. minuta* can serve as good sources of organic carbon, particularly in soils with low organic carbon content. Rapid decomposition of *T. diversifolia* biomass in the soil acts as an enhancer of the organic carbon pool (Hafifah *et al*., 2016), soil improvement agent (Olabode *et al*., 2007), and accelerates C mineralization due to the narrowed C/N ratio (Partey *et al*., 2014). This likely explains the higher organic carbon content in soils treated with the plant extracts. The plant extracts from

T. diversifolia, *T. minuta*, and *B. pilosa* increased the percentage of organic nitrogen in the soil. Leaves and stem extracts of *T. diversifolia*, as well as root extracts of *T. minuta*, contributed the most to the total organic nitrogen content. This suggests that the mineralization rate of organic nitrogen in *T. diversifolia* leaves and stems is faster than in *T. minuta* and *B. pilosa* extracts, making them alternative and cost-effective nitrogen sources for nitrogen-deficient soils. Jama *et al*. (2000) reported that *T. diversifolia* contributes 3.5% nitrogen from leaves on a dry basis and 0.98% nitrogen from fresh leaves and young stems on a fresh weight basis (Shokalu *et al*., 2010), making it a valuable nitrogen supplier to the soil.

In conclusion, the research findings suggest that incorporating plant extracts from *T. diversifolia*, *B. pilosa*, and *T. minuta* has the potential to suppress *M. javanica* population and RKN reproductive health when dried extracts are incorporated into the soil before tomato planting. These plant extracts promote tomato growth, suppress root growth, and increase tomato yields. While these plant extracts' inhibitory action does not lead to root death, they do reduce the average vitamin C content in mature green, breaker, and red ripening stages. The inhibitory action of *B. pilosa* stem, leaf, and root extracts may have potential applications in the development of weed killers.

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