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Allelopathic potential of African marigold (*Tagetes erecta*) in sustainable tomato (*Lycopersicon esculentum*) production

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Abstract

The present study was conducted to investigate the efficacy of African marigold (*Tagetes erecta*) root extract in controlling root-knot nematodes *Meloidogyne incognita* species and to determine its effectiveness as an intercropped plant on tomato (*Lycopersicon esculentum*) morphological and quality traits as well as on soil attributes. The results of the *in vivo* study revealed that the highest mortality rate (82.33%) of nematodes was observed in T3 treatment (3:1 marigold root extract in distilled water) after 72 hours while minimum egg hatching (9.33) by nematodes was obtained after 12 hours with a similar concentration. Whereas, the pot experiment of intercropping with tomato showed that after 45 days of transplanting, the maximum plant height (79.14 cm), number of leaves (28.22) and leaf area (24.46 cm^2) in tomato were obtained in the T3 treatment (Tomato 1 + Marigold 2 + Root knot nematodes (RKN) 1). Similarly, maximum fruit TSS (4.90 °brix) and shelf life (8.67 days) were observed with the same treatment. Concerning root-knot nematode infestation, the maximum number of galls (39.33) and eggs (38.66) on the tomato root system were recorded in T4 (Tomato 2 + Marigold 1 + RKN 2). However, the soil analysis of intercropped crops showed that there was no significant impact of marigold and tomato intercropping on soil pH and electrical conductivity, while this intercropping scheme slightly decreased soil nutrient levels.

Key words: Intercropping, marigold, organic, root-knot nematode, tomato

Introduction

Tomato (*Lycopersicon esculentum*) is one of the most economical crops grown across the globe due to its wide adaptability and versatility (Mourvaki *et al*., 2005). It is the second most important crop after potato consumed either fresh or in processed form (Quinet *et al*., 2019). It is highly nutritious and is enriched with minerals (Na, K, Fe), vitamins (retinol and ascorbic acid), and antioxidants (lycopene, salicylate, lutein, and phytoene) (Andres *et al*., 2017; Viuda-Martos *et al*., 2014). However, the crop is affected by various types of pests such as fruit borer, white fly, aphids, hornworms, stink bugs and nematodes (Afreen *et al*., 2017). Among parasites, root-knot nematodes (RKNs) are severe problems in tomato production as they make them susceptible to various other pathogens such as bacteria, fungus and viruses that result in sufficient yield losses (Rivera and Aballay, 2008).

During feeding, RKNs develop massive galls on the tomato root system that affect its biological efficiency and the affected plant exhibits nutrient deficiency symptoms, as the galls on plant roots reduce the capability of plants to absorb nutrients and water (Coyne *et al*., 2007). Synthetic nematicides are highly effective in the management of RKNs (Dubey and Trivedi, 2011; Adegbite and Agbaje, 2007). However, these synthetic products are expensive, hazardous to human health and have environmental safety issues (Rezaei *et al*., 2015). Further, the high demand for organic, nutritious and healthy food in the pandemic conditions of Covid-19 has increased the impact of organic products (Khan *et al*., 2020). Therefore, it is of utmost need to develop alternate sustainable solutions to control nematodes (IFCS, 2004).

Marigold (*Tagetes erecta* L.) is a well-known plant that is grown worldwide for ornamental, medicinal and cosmetic purposes (Fonseca *et al*., 2016). It contains thiophenes in its tissues that suppress various insects, fungi and nematodes (Gómez-Rodríguez *et al*., 2007; Riga *et al*., 2005). The flavonoids such as rutin and quercetin present in marigold flowers have anti-inflammatory properties and their levels should be higher than 0.4% for therapeutic effects (Anvisa, 2011). It is reported that *Tagetes spp.* contains oxygen radicals like a-terthienyl that control nematode populations (Hamaguchi *et al.,* 2019). Further, French marigolds also contain limonene as a major constituent that minimizes white fly performance (Conboy *et al.,* 2019). Therefore, this species can be used as an intercrop or in crop rotation to control fruit fly and nematodes (Natarajan *et al*., 2006).

Intercropping is a sustainable alternative approach to avoid the use of chemicals for plant disease management (Gómez-Rodríguez *et al*., 2007). In the last few decades, it has enhanced the attention of farmers and researchers, mainly due to ecological interactions, potential management and economic feasibility (Fonseca *et al*., 2016). As compared to monoculture, it is an applicable and easily available technique having little environmental impact (Rezende *et al*., 2002). Moreover, this farming system is highly preferred by farmers due to its increased production, diversification, labor resources, and the efficient use of arable land (Montezano and Peil, 2006). Moreover, to preserve soil fertility and to protect crops, integrated approaches are being used to utilize natural processes (Tringovska *et al*., 2015).

Intercropping is a technique of growing two or more crops simultaneously as companion crops. The companion crops are helpful to each other as they maintain microclimatic conditions around the plant canopy, retain soil nutrients and organic matter in the soil and are quite effective in disease management (Bomford, 2009; Gómez-Rodríguez *et al*., 2003). However, the intercrop plants may compete for light and nutrients that can reduce their yield (Borowy, 2012). But these adverse impacts could be minimized by restricting the growth of companion crops, by applying proper fertilizers and irrigation to plants, and by selecting suitable plants as an intercrop (Tringovska *et al*., 2015; Kołota and Adamczewska-Sowinska, 2013; Adamczewska-Sowinska and Kołota, 2008). The intercropping of two crops may enhance the biological efficiency, composition and growth cycles of the associated crop (Sobkowicz and Tendziagolska, 2005). Moreover, it may affect morphological attributes like leaf and stem thickness and may affect the carbon assimilation rate (Taiz and Zeiger, 2002).

The precision of selection and management of intercrops may result in increased production and quality of crops (Mandal and Dash, 2012). The intercropping of tomatoes with marigold has shown less foliar and fruit damage as compared to non-intercropped tomatoes. That might be due to the allelopathic properties of marigold (Gómez-Rodríguez *et al.,* 2003). Several investigations to control nematodes by marigold as intercrops have been conducted that indicate the great interest of researchers in controlling pests through sustainable solutions (Hooks *et al*., 2010; Youssef *et al*., 2011; Tringovska *et al*., 2015). Further, the discouraging behavior of farmers towards chemical nematicides due to their hazardous effects on health and the environment compels them to explore organic strategies for pest control. Effective intercropping promotes biological diversity with improvised utilization of natural resources as it is a promising, environment-friendly technique that controls plant spreading pathogens and improves soil nutritional status (Bomford, 2009).

The study was planned to evaluate the allelopathic effect of marigold root water as it might be a sustainable approach in organic agriculture and there is very little research available on this matter. The research studied the effects of marigolds, as an intercrop, on tomato growth and soil chemistry. As organic products have increased in demand because of their nutritional value, growers may find it beneficial to select an appropriate intercropping system to aid tomato production and plant disease management using the organic approach. This study will examine questions related to intercropping, such as which marigold cropping scheme or pattern provides increased sustainable tomato production and whether intercropping impacts soil nutritional and chemical composition.

Materials and methods

In vitro experiment was conducted at plant pathology laboratory while the greenhouse studies were conducted at the Horticulture Research Area of PMAS-Arid Agriculture University, Rawalpindi, Pakistan during the year, 2017. The tomato (*Lycopersican esculentum*) *cv.* "Grandula" and African marigold (*Tagetes erecta*) were obtained from a government-registered nursery, "Awan Seed garden" located in Islamabad. The selected tomato cultivar was a hybrid having an indeterminate growth habit and is used for fresh consumption.

***In vitro* marigold root extract preparation:** To evaluate the allelopathic effect of marigold on the suppression of nematode infestation, a lab experiment was conducted in the plant pathology laboratory, at PMAS-Arid Agriculture University, Rawalpindi. In this experiment, ten African Marigold plants in their post-flowering stage (70 days old, approximately 100cm tall) were chosen and their roots were dipped in a 1-liter volume of distilled water. Plants were kept in water until they dry out. After the aqueous extract of root, water was filtered out with the help of muslin cloth and three marigold dilutions were prepared in distilled water with the following ratios; T_1 (1:1), T_2 (2:1) and T_3 (3:1), respectively.

Evaluation of juvenile mortality and egg hatching suppression: For studying juvenile mortality, 100 J^2 of RKNs were suspended in 10 mL of aqueous extract and marigold root water and Petri dishes were kept at ambient temperature. After 12, 24, 48, and 72 hours of incubation, all dead and alive 2nd stage juveniles (J^2) were counted with the help of a microscope at 100X magnification. The dead juveniles attained the shape of a straight line and the mortality was censured by touching the juveniles with a fine needle. The ratio of dead nematodes/number of total nematodes expressed the percentage mortality. While for egg hatching suppression, 100 medium size egg masses handpicked from the galls of the tomato plant were placed in each of Petri dishes containing 50 mL of extracts. After 7 days of exposure, the number of juveniles that hatched was counted with the help of a microscope (40X magnification, Model B-50b Optika, Italy).

Planting materials for the *in vivo* experiment: Fifteen days before planting, the planting media soil and farmyard manure (FYM) were placed in open space separately and were covered with polythene sheets for sun sterilization to kill all harmful pathogens (Qadri *et al*., 2018). After solar sterilization, the potting media of soil and FYM were prepared to have 1:3 ratios, respectively. Before filling the pots, all debris (stones, straws, and plant material) was sorted and removed from the media, and the pots were filled with pebbles at the bottom to remove excess water after irrigation and to allow for gaseous exchange. The tomato seedlings were then transplanted into pots with marigold seeds as companion crops. After transplanting, root knot nematodes (RKNs) were drained from a 10 mL volumetric pipette. After counting under a microscope at magnification 100X, the volume of suspension containing the standard 100 J^2 determined to be approximately 0.25 mL. The inoculums were carefully placed around the plant root system of each tomato plant.

The intercropping of tomato and marigold with RKN was in the following treatments, T0 (Tomato 1 + Marigold 0 + RKN 0), T1 (Tomato 1+ Marigold 1 + RKN 0), T2 (Tomato 1 + Marigold 1 + RKN 1), T3 (Tomato 1 + Marigold 2 + RKN 1), T4 (Tomato 2 + Marigold 1 + RKN 2) and T5 (Tomato 1 + Marigold 0 + RKN 1). Each treatment (intercropping level) was grown separately in pots with five replicates.

Plant attributes measured: To evaluate the effect of companion plants on tomato, physical parameters like plant height (cm), number of leaves plant^{-1} and leaf area (cm^2) were measured up to 45 days with a 15 day interval. After fruit harvest, the morphological and biochemical characteristics of the fruit, such as the number of fruit plants, 1, 0, fruit size, fruit color, fruit firmness, fruit shelf life (days), and total soluble solids (TSS), were measured.

Soil parameters measured: To evaluate the effect of intercropping on soil fertility, the soil macronutrients nitrogen (N), phosphorous

(P) and potassium (K) were measured along with soil pH and electrical conductivity (EC).

Nematodes infestation on tomato roots: To estimate the nematode infestation on tomato roots, the number of galls present on tomato roots was measured by the galling index, while the number of egg masses present on each tomato root system was counted by the estimation of the galls present on the tomato root system.

Statistical analysis: The experiment was laid out under a completely randomized design (CRD) with five replications and the data was statistically analyzed by using analysis of variance (ANOVA) and differences among treatments were compared at a 5% level of probability by Tukey's HSD (Abdi and Williams, 2010).

Results

Allelopathic effect of marigold root extract on the juvenile RKNs: Marigold root extract had a significant effect on controlling root-knot nematodes (Table 1). The results show that the highest mortality rate in nematodes (82.33%) was noticed in T3 after 72 hours. While the least mortality (6.67%) was observed in T1 after 12 hours of incubation. Similarly, the results related to egg hatchings also exhibited the significant impact of the marigold root extract in controlling nematode egg hatching (Table 2). The results indicated that the minimum eggs (9.33) were observed in T3 after 12 hours, followed by 48 hours (11) with the same treatment. The overall results of this experiment demonstrate that T3 (3:1 marigold root extract in distilled water) is more effective in controlling egg hatching and the nematode population.

Impact of intercropping on morphological traits of tomato:

Table 1. Effect of marigold root extract on mortality rate of juvenile root knot nematodes

Intervals	Treatments			Means
	T_1	T_2	T_3	
I_1	6.67 i	47.33 g	52.33 f	35.44 D
I_2	7.67 hi	57.33 e	67.33 c	44.11 C
I_3	8.67 hi	62.67 d	73.00 b	48.11 B
I_4	11.33 h	79.00 a	82.33 a	57.56 A
Means	8.58 C	61.58 B	68.75 A	

Means with similar letters indicate non-significant relationship at ($P≤0.05$)

Table 2. Effect of marigold root extract on egg hatch suppression of root knot nematodes

Intervals	Treatments			Means
	T_1	T_2	T_3	
I_1	87.67 a	18.00 d	9.33 e	38.33 C
I_2	89.00 a	18.67 c	11.00 de	39.56 BC
I_3	89.67 a	21.00 bc	11.67 de	40.78 AB
I_4	90.67 a	22.33 b	13.67 d	42.22 A
Means	89.25 A	20.00 B	11.42 C	

Means of similar letters indicate non-significant relationship at ($P≤0.05$)

The intercropping of marigold and RKNs significantly affected the tomato morphological attributes as depicted in Table 3. The results revealed that plant height was significantly improved in all intercropping patterns as compared to non-intercrops. There was a gradual increase observed in plant height from transplant to the final day of harvest. However, the maximum plant height was observed in T3 treatment at day 15 (17.02 cm), day 30 (32.04 cm) and day 45 (79.14 cm), respectively. While the minimum plant height after 45 days of the transplant was observed in T4 (54.94 cm) which was at par with T5 (56.08 cm), respectively. Likewise, a similar trend was observed in the number of leaves and leaf areas. In our findings, after 15 days of transplant, the highest number of leaves (9.22) was observed in T3 treatment, followed by T4 (7.01) and T2 (5.81), respectively. However, after 45 days of transplant, the highest number of leaves (28.22) was observed in T4 followed by T2 (19.22). The leaf area was also found highest (24.46 cm^2) in T3 after 45 days of transplant followed by T2 (17.22 cm^2). However, the lowest leaf area was observed in T1 (13.68 cm^2) which was at par with T4 (14.44 cm^2).

Table 3. The intercropping effect of marigold and root-knot nematodes on morphological traits of tomato

Trait	DAT	T0	T1	T2	T3	T4	T5
Plant height (cm)	15 DAT	13.11d	13.98c	14.72b	17.02a	15.08b	13.40d
	30 DAT	21.82d	25.14b	25.52b	32.04a	24.04c	22.11d
	45 DAT	59.11bc	57.58cd	60.42b	79.14a	54.94d	56.08d
Number of leaves	15 DAT	4.60cd	5.23cd	5.81bc	9.22a	7.01b	4.11d
	30 DAT	7.40cd	8.01cd	9.01bc	15.21a	10.60b	4.01d
	45 DAT	15.01de	16.11cd	19.22b	28.22a	17.81bc	13.23e
Leaf area (cm^2)	15 DAT	4.82b	5.18b	7.18a	8.62a	5.53b	5.22b
	30 DAT	8.71b	7.32b	8.76b	14.6a	8.42b	7.13b
	45 DAT	14.46b	13.68c	17.12b	24.46a	14.44c	11.16d

Means with similar letters indicate non-significant relationship at ($P≤0.05$)

Impact of intercropping on fruit quality traits of tomato: The intercropping of marigold exhibited a significant effect on the fruit quality traits (Table 4). The data revealed that the maximum number of fruits per plant (42.66) was observed in T3 followed by T1 (38.66). While the minimum number of fruits was observed in T0 (30.66). The intercropping significantly improved the fruit size of the tomato as well. The highest fruit size (44.26 mm) was observed in T3 followed by T1 (28.79 mm), while the lowest fruit size was obtained in T5 (9.54 mm). Moreover, the fruit shelf-life was greatly affected by intercropping pattern. The maximum shelf life of tomato fruit (8.67 days) was observed in T3 which was at par with T2 (8.02 days). While the minimum shelf life of tomato fruit (5.33 days) was observed in T5.

Table 4. The intercropping effect of marigold and root-knot nematodes on fruit quality traits of tomato

Treatments	Number of fruits/ plant	Fruit size (mm)	Fruit shelf life (days)	TSS (°Brix)	Fruit firmness (Nmm^{-1})
T_0	30.66 de	26.74 bc	6.12 bc	2.33 e	3.50 c
T_1	38.66 b	28.79 b	6.67 b	3.91 b	4.23 b
T_2	36.33 bc	21.79 c	8.02 a	3.16 cd	4.70 a
T_3	42.66 a	44.26 a	8.67 a	4.90 a	4.86 a
T_4	33.66 c	18.36 d	5.67 b	3.46 c	4.76 a
T_5	31.12 d	9.54 e	5.33 c	3.03 d	3.70 c

Means with similar letters indicate non-significant relationship at (P≤0.05)

Regarding fruit biochemical attributes, the highest total soluble solids (4.90 °brix) were observed in T3 followed by T1 (3.91 °brix) whereas the lowest TSS (2.33 °brix) was observed in T0. For the fruit firmness attribute, the highest fruit firmness (4.86 Nmm^{-1}) was observed in T3 which was at par with T4 (4.76

Nmm^{-1}) and T2 (4.70 Nmm^{-1}), respectively. However, the lowest fruit firmness (3.50 Nmm^{-1}) was recorded in T0.

Nematode infestation on tomato roots: Concerning nematode infestation, a highly significant difference was observed among intercropped treatments (Fig. 1). The results showed that the highest number of galls in tomato roots was observed in T4 (39.33) followed by T5 (34.67), while the minimum gall count was noticed in T0 (24.33). Similarly, the maximum number of nematode eggs (38.66) was observed in T4 followed by T5

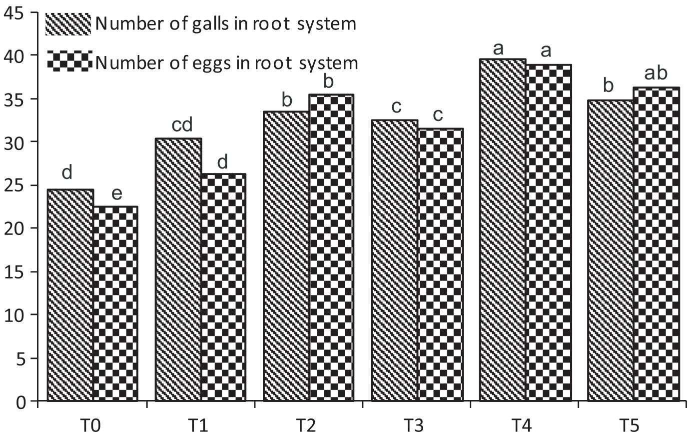

Fig. 1. Effect of marigold intercropping on nematode infestation of tomato roots

(36.01) which showed that there is a positive relationship between the number of galls and eggs. While the minimum number of eggs per plant (22.33) was noted in T0.

Effect of intercropping on soil properties: The soil analysis after the final crop harvest revealed that intercropping and its patterns have a significant impact on soil properties. The results showed that intercropping significantly affects the soil's major nutrients, N, P, and K as shown in Table 5. In this study, the highest amount of nitrogen (2.67 ppm) was obtained in T0 at par with T1 (2.23 ppm), while the least amount of nitrogen (1.11 ppm) was observed in T4. Likewise, the maximum content of phosphorous (4.33 ppm) and potassium (136.3 ppm) in soil was observed in T0. However, the minimum contents of phosphorous (2.75 ppm) and potassium (121.8 ppm) were observed in T5. Moreover, no change in soil pH and electrical conductivity was observed by intercropping.

Table 5. Intercropping effect of marigold and root-knot nematodes on soil fertility attributes

Treat-ments	Nitrogen (ppm)	Phosphorous (ppm)	Potassium (ppm)	pH	Electrical conductivity
T_0	2.67 a	4.33 a	136.3 a	8.01 NS	0.13 NS
T_1	2.23 a	4.11 ab	134.4 a	7.61 NS	0.07 NS
T_2	1.67 b	3.83 ab	133.6 a	7.11 NS	0.14 NS
T_3	1.22 bc	3.86 ab	128.1 ab	7.01 NS	0.13 NS
T_4	1.11 c	3.33 bc	132.5 ab	6.78 NS	0.11 NS
T_5	1.38 bc	2.75 c	121.8 b	7.12 NS	0.10 NS

Means with similar letters indicate non-significant relationship at ($P \leq 0.05$)

Discussion

Nematodes are one of the major concerns in sustainable agricultural production. Integrated pest management has realized that nematicides are not the most sustainable approach to control nematodes due to their adverse effect on the soil and the environment (Wang *et al*., 2002). As a result, there is an urgent need to investigate a long-term solution for nematode control and environmental protection.Marigold is a multipurpose companion crop having antibacterial, antifungal and nematicidal properties that protect plants and soil from harmful pathogens (Li *et al*., 2020). This study revealed that the use of marigolds in tomato crops is highly effective in suppressing nematode populations because of their nematicidal properties. Several studies have reported that the nematode infestations have been controlled by marigold either by using it as an intercrop, cover crop or by applying its aqueous extract to a crop (Xie *et al*., 2017: Hooks *et al*., 2010; Natarajan *et al*., 2006). Conboy *et al*. (2019) encouraged both French and English marigolds as effective companion plants to control pests, either directly due to their volatile chemistry nature (Ben *et al*., 2014), or indirectly by enhancing beneficial arthropods populations (Zhao *et al*., 2017: Blazan, 2017).

In our study, we discovered that intercropping patterns improved plant morphological attributes such as plant height, number of leaves, and leaf area significantly more than monocrop controls. The increase in plant phenotypic traits could be attributed to the shade of marigolds on tomato plants, as shade from intercropped plants increases the physical attributes of the plants (Taiz and Zeiger, 2002; Lin *et al*., 2001).Similar findings were observed by other researchers who found an increase in morphological traits of several horticultural crops when intercropped with marigold cultivation (Fonseca *et al*., 2016; Gómez-Rodríguez *et al*., 2007). However, an appropriate combination of the crop is essential to get the desired traits (Singh *et al.,* 2014).

In this study, the intercropped marigold significantly improved the productivity of the tomato crop. That might be due to the appropriate selection of the crop as there was no competition for nutrients in the plants for their growth and development. Our results agree with the findings of Cecílio and May (2002) who observed an increase in the production of radish roots when intercropped with lettuce. Similar findings were observed by others, who recorded an increase in the yield of several agronomic crops intercropped with marigolds (Agrawal *et al.,* 2010; Prakash *et al.,* 2009; Singh and Datta, 2006). However, the adverse effect of intercropping was observed when white mustard and tomato were intercropped (Hartz *et al*., 2005). The researchers stated that this effect might be due to the selection of inappropriate crops that have increased the competition for nutrients, sunlight and water among them (Bybee-Finley and Ryan, 2018). Therefore, the selection of appropriate plant species is highly essential to increasing production along with environmental factors (Maia *et al*., 2008).

The intercropping of marigold in tomato helps maintain fruit quality, *viz.*, enhanced fruit firmness, total soluble solids and fruit shelf life. Similar findings were observed by Gómez-Rodríguez *et al*. (2007) who stated that the appropriate selection of companion crops for the tomato increases its quality. Besides, environmental factors such as temperature, light, humidity, irrigation, crop maturity stage and its genetics play a major role in developing fruit quality (Akram *et al*., 2020; Luna *et al*., 2013). Further biotic and abiotic factors are also responsible for changes in plant sugars (Gil *et al*., 2012). Gliessman (2002) found intercropping to be extremely beneficial, particularly for farmers with small holdings, because it provides a high yield. Our findings have also a resemblance with other researchers, who got high-quality yield in cherry tomato by using different cover crops (Ambrosano *et al*., 2018; Ambrosano *et al*., 2014).

The soil analysis showed that the intercropping of marigold and tomato is less competitive. The highest decrease in the soil fertility level was observed in T4 (Tomato 2 + Marigold 1 + RKN 2), which might be due to the highest number of plants observed in this combination. The other soil analysis results revealed a minor or non-significant impact of intercropping on the soil. Intercropping is also a sustainable approach to reduce soil erosion as it fixes nitrogen and increases land-use efficiency (Li *et al*., 2013). Moreover, multiple cropping patterns increase the diversity of soil microorganisms and optimize the soil structure that prevents plants from biotic stresses (Tian *et al*., 2019; Wu *et al*., 2018). While the mono-cropping system in agriculture inhibits plants growth and is associated with the development of soil-borne diseases (Li *et al*., 2020; Zhang *et al*., 2013).

The results of the study concluded that the selection of suitable plant species and planting schemes is highly essential in intercropping. Intercropping marigolds and tomatoes is highly recommended because it increases plant height, number of leaves, and leaf area.The intercropping scheme (Tomato 1 + Marigold 2 + RKN 1) greatly affects the fruit quality traits and has increased fruit TSS, firmness, and shelf life. Further, the intercropping of marigold significantly reduces the root-knot infestation on tomato roots and induces a negligible change in soil chemical properties that make them suitable intercrops for sustainable tomato production.

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