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Management of root knot nematode in tomato through *Trichoderma harzianum* and moringa leaf extract

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To assess the impact of Moringa and *T. harzianum* on managing Root Knot Nematode (*M. incognita*) in tomato and to ascertain the impact of combining Moringa and *T. harzianum* on (*M. incognita*) management in tomato, an experiment was carried out at the Ambo Agricultural Research Center in a greenhouse. Combinations of *Moringa oleifera* and *T. harzianum* were examined on plant parameters and *M. incognita* parameters on tomato plants at various concentrations of moringa plant extracts at(100%, 50%, 25%, 10%) and ($1*10^4$, $1*10^6$, $1*10^8$, $1*10^{10}$), respectively. The results showed that were significant variations (P0.05) on number of galls per plant, the number of egg masses per plant, final nematode population density per pot, and the reproduction factor among the treatments in terms of nematode population. Combined application of aqueous moringa plant extracts at S (100 percent) and *T. harzianum* at $1*10^{10}$spore/ml resulst showed the highest plant height 67.5 cm. The outcome showed those pots treated with aqueous moringa plant extracts S and *T. harzianum* $1*10^{10}$Spore/ml had the lowest mean reproduction factor (1.79) and population density (3588) compared to control. Pots treated with aqueous moringa plant extracts S/10 (10%) and *T.harzianum* $1*10^4$Spore/ml had the highest mean reproduction factor and nematodes population density compared to the control. As a result, *T. harzianum* and *M. oleifera* could be utilized to combat *M. incognita* in the field. The findings of this study showed that test plants can lower nematode populations below economic thresholds and are easily

accessible to farmers at no cost. Additional research is required to find new classes of bio-pesticides derived from natural plants that can take the place of the synthetic chemicals now in use.

Key words: *aqueous, bio-pesticide, moringa, root knot nematode, Trichoderma harzianum*

INTRODUCTION

In the world, one of the most valuable agricultural crops is the tomato (*Solanum lycopersicum*) (de Carvalho et al., 2015). It is a widely grown vegetable crop, with Ethiopia producing the eighth-highest amount of it annually (CSA, 2016). For a well-balanced human diet, it provides a rich source of micronutrients such as minerals, vitamins, and antioxidants (Falak et al., 2011). Numerous parasites and illnesses reduce tomato production's quality and output. Plant parasitic nematodes (PPNs), which encompass 4100 species and are prominent in the nematode world in terms of illness (Jones et al., 2013). RKN (*Meloidogyne* spp.), one of the PPNs, are commercially the most significant infections that affect agricultural output and quality (Javed et al., 2006).They are widespread worldwide, polyphagous obligate parasites that are known to parasitize almost all kinds of higher plants, exposing the host to secondary infection (Zhou et al. 2016, Khan & Sharma, 2020). Additionally, it modifies the physiology of the host, and severe infestations can completely destroy the tomato plant (Kamran et al., 2010). They obstruct the uptake of water and nutrients, which has a significant negative impact on photosynthesis (Anwar et al., 2010).This is likely to be a considerable underestimation of the true figure because many farmers in impoverished countries are unaware of PPNs, even while PPN causes severe damage, the loss remains hidden because of various biotic and abiotic pressures and other limiting factors occur simultaneously (Jones et al., 2013). Crop losses caused by phyto nematodes to fruit crops are very high, with an average annual yield losses estimated at about 20–40% worldwide(Kumar et al., 2020). Growing crop output can be accomplished, in part; by reducing crop losses brought on by nematodes. RKNs were managed using a variety of techniques, including chemical control, organic amendments, resistant cultivars, soil solarization, and biological control (Agrios, 2005). Users are discouraged from using chemicals due to their high costs, lack of availability when needed, and risks they represent to humans and non-target creatures (Wachira et al., 2009). The majority of nematicides are now banded, including aldicarb, methyl bromide, and carbofuran. To reduce environmental pollution and maintain more cost-effective management practices, several soil additions such compost, fungi, and botanicals will be evaluated against RKN on tomato. Organic elements, such as bacteria, fungi, and nematode species, lower the incidence of disease brought on by a variety of plant pathogens and plant pests (Abawi & Widmer, 2000; Bailey & Lazarovits, 2003). Increased activity of naturally occurring antagonists (such as bacteria, fungi, and predatory nematodes) may indirectly induce the nematode suppression (Akhtar & Malik 2000; Oka 2010). directly influence the health of plants and crop productivity (Akthar & Malik, 2000).The remarkable *Meloidogyne* spp. suppressant properties of moringa leaf powder may have contributed to treated plants growing larger (Claudius-Cole et al., 2010). Numerous studies have been conducted on *T. harzianum* as a biological control agent against a variety of soil-borne diseases, including RKN, and as a plant growth promoter (Saifullah and Thomas, 1996; Sharon et al., 2001). *Trichoderma* conidia parasitize on nematode cuticle or egg shell by adhering to them (Sharon et al., 2007). According to Chet and Baker (1981), *Trichoderma* spp. creates chitinase in the culture, which may aid in preventing egg hatching. In general, bio-control agents and soil organic amendments play a significant effect in reducing PPNs increasing crop productivity. Despite RKN's economic significance to the nation's tomato industry, research into biocontrol agents and organic soil additives to combat it is still lacking. To evaluate the impact of *Moringa oleifera* and

T. *harzianum* on tomato against *meloidogyne incognita* multiplication is the goal of this study.

MATERIALS AND METHODS

Inoculum preparation

The pure cultures of *M. incognita* were raised from single egg mass and maintain on tomato roots in wire house. Infected plants then uprooted from soil and the entire root system was dipped in water and washed gently to remove adhering soil particles. Egg masses of nematodes were picked up and kept it in small sieves. Then the sieves were placed in sterilized plastic plates and pour the water up to neck of the sieves and kept in the laboratory at room temperature. After 2 to 7 days, eggs were hatched and active juveniles cross the sieve and settle down in plastic plates. The J2 juveniles were collected and then counted by using eelworm nematode counting dish for experimental study. Population densities of J2 were determined from one ml aliquot of an inoculum suspension. 2000 J2 of root knot nematode were used for each treatment in vivo culture study for each treatment.

Treatment preparation

Table 1.Treatment combinations

Treatment number	Treatment combinations
T1	Moringa (S)*_T.harzianum_(1*10^4 spore/ml)
T2	Moringa(S/2)*_T.harzianum_(1*10^4 spore/ml)
T3	Moringa(S/4) *_T.harzianum_(1*10^4 spore/ml)
T4	Moringa(S/10)*_T.harzianum_(1*10^4 spore/ml)
T5	Moringa(S)*_T.harzianum_ (1*10^6 spore/ml)
T6	Moringa(S/2)*_T.harzianum_(1*10^6 spore/ml)
T7	Moringa(S/4)*_T.harzianum_(1*10^6 spore/ml)
T8	Moringa(S/10)*_T.harzianum_(1*10^6 spore/ml)
T9	Moringa(S)*_T.harzianum_(1*10^8 spore/ml)
T10	Moringa(S/2)*_T.harzianum_(1*10^8 spore/ml)
T11	Moringa(S/4)*_T.harzianum_(1*10^8 spore/ml)
T12	Moringa(S/10)*_T.harzianum_(1*10^8 spore/ml)
T13	Moringa(S)*_T.harzianum_(1*10^{10} spore/ml)
T14	Moringa(S/2)*_T.harzianum_(1*10^{10} spore/ml)
T15	Moringa(S/4)*_T.harzianum_(1*10^{10} spore/ml)
T16	Moringa(S/10)*_T.harzianum_(1*10^{10} spore/ml)
T17	Control (Only nematode)

Moringa leaves were harvested, washed, dried in the shade, and ground them. Leaves were washed to get rid of any dust before drying. Water extracts of the tested plant were made by grinding 25g of moringa leaves in 250ml of sterilized water for 24 hours in a 500 ml Erlenmeyer flask (Adegbite and Adesiyan, 2005). The mixture was then filtered through muslin cloth to obtain the clear extract, which was used as the standard "S" concentration. Concentrations of S/2, S/4, and S/10 were also made. The filtrate was suitable for use after a 10-minute centrifugation at 2000 rpm.Finally, S (100 percent), S/2 (50 percent), S/4 (25 percent), and S/10 (10 percent) concentrations were prepared. *T. harzianum* was sourced from the Ambo Agricultural Research Center. 1x10^4, 1x10^6, 1x10^8, and 1x10^{10}spores/ml were the four concentration levels that were produced. The different levels of the two parts were then combined. The treatments were then added to each pot in 5ml solutions.

Raising seedling, nematode inoculation and treatment application

Melkasalsa cultivar seeds were planted in plastic pots under a greenhouse on sterilized soil. Seedlings were transplanted into sixty eight plastic pots (15 cm dia.) with 2 kg of sterilized soil that contained 1:2:3 ratios of sand, compost, and clay when they reached the stage of three leaves. There was only one tomato seedling per pot. Fresh tomato roots were supplied to the Plant Pathology Laboratory from pure culture grown in the wire house. Egg masses were removed using sterile forceps and a dissecting needle, placed in a Petri plate with sterile water, and then kept there until hatching was complete. A suitable nematode suspension was made in a beaker, and 3 ml of the whole suspension was collected and put on a counting dish. The number of juveniles in the suspension was then counted under a stereomicroscope at a magnification of 50x.The population of nematode per ml was calculated from one ml aliquot of an inoculum suspension for in vivo experiments. Finally, seedlings of tomato were inoculated with the 2 ml suspension of *M. incognita* at 2000 juveniles/pot after one week of transplanting. For inoculation, 1-2 cm of top soil was separated out and nematode suspension was poured around the plant. Each treatment was replicated four times and the pots were arranged in a randomized complete design. Inoculated control set of plants were served as control treatment..

RESULTS AND DISCUSSION

Effect of aqueous moringa plant extracts and Trichoderma harzianum on growth parameters on tomato.

Plant height

Tomato plant height was significantly affected by combined treatments of aqueous moringa plant extracts and *Trichoderma harzianum* (P0.05) (Table 2).The highest plant height was recorded by aqueous moringa plant extracts at S and *T. harzianum* at $1*10^{10}$spore/ml, whereas, the lowest plant height was recorded by aqueous moringa plant extracts at S/10 and *T. harzianum* at $1*10^{4}$spore/ml. The findings showed that the combined application of aqueous moringa plant extracts at S and *T. harzianum* at $1*10^{10}$spore/ml produced the tallest plants (67.5 cm) compared to inoculated control (45.50cm). This combination increased plant height by 48% over the inoculated control. However, pots treated with aqueous moringa plant extracts at S/10 and *T. harzianum* at $1*10^{4}$spore/ml showed the lowest plant height (50.5 cm) compared to control (45.50cm). This shows the increament of plant height by 11% over the inoculated control. The pots treated with aqueous Moringa plant extract from S and *T. harzianum* ($1*10^{10}$ spore/ml) had the longest roots (22.43 cm), compared to inoculated control pots (11.08 cm). (Belay et al., 2019) have reported that leaf extracts of *Lantana camara* with *T. harzianum* enhance plant growth over the inoculated control. In similar study (Jinfa et al., 2006*)* reported that, root-knot nematode cause height reduction in plant.

Fresh shoot, root and dry root weight

The results showed that there is a significant difference (P0.05) between treatments on fresh shoot, fresh root, and dried root weight of tomato. The pots treated with aqueous moringa plant extracts at S and *T. harzianum* at $1*10^{10}$spore/ml had the highest shoot weight followed by pots treated with S/2 and *T.harzianum* at $1*10^{10}$spore/ml compared to

Table 2. Effect of aqueous moringa plant extracts and *Trichoderma harzianum* on growth parameters on tomato

Treatments	Length(cm)				Fresh weight (gm)				Dry weight (gm)			
	Root	Y**	Shoot	Y**	Shoot	Y**	Root	Y**	Shoot	Y**	Root	Y**
Moringa (S)*T.harzianum* ($1*10^4$ spore/ml)	17.05^{gh}	54	52.75^{ghi}	16	40.00^{g}	65	9.75^{j}	144	10.03^{f}	46	7.43^{c}	254
Moringa(S/2)*T.harzianum* ($1*10^4$ spore/ml)	16.98^{gh}	53	51.75^{hij}	14	37.75^{h}	56	8.50^{k}	112	9.17^{g}	34	7.34^{c}	249
Moringa(S/4) *T.harzianum* ($1*10^4$ spore/ml)	16.85^{gh}	52	51.25^{ij}	13	35.25^{i}	45	8.00^{k}	100	8.23^{h}	20	7.32^{c}	248
Moringa(S/10)*T.harzianum* ($1*10^4$ spore/ml)	16.23^{h}	46	50.50^{j}	11	34.00^{i}	40	7.25^{l}	81	8.03^{h}	17	7.29^{c}	9
Moringa(S)*T.harzianum* ($1*10^6$ spore/ml)	17.80^{efg}	61	54.50^{g}	20	41.75^{ef}	72	12.75^{g}	219	11.05^{e}	61	8.03^{c}	282
Moringa(S/2)*T.harzianum* ($1*10^6$ spore/ml)	17.53^{fgh}	58	54.25^{g}	19	40.50^{fg}	67	12.00^{h}	200	11.04^{e}	61	8.02^{c}	282
Moringa(S/4)*T.harzianum* ($1*10^6$ spore/ml)	17.25^{gh}	56	54.00^{g}	19	40.25^{fg}	66	11.75^{h}	194	10.95^{e}	60	8.01^{c}	281
Moringa(S/10)*T.harzianum* ($1*10^6$ spore/ml)	17.13^{gh}	55	53.50^{gh}	17	40.13^{g}	65	10.50^{i}	162	10.86^{e}	58	7.96^{c}	279
Moringa(S)*T.harzianum* ($1*10^8$ spore/ml)	19.78^{bcd}	78	62.75^{cde}	38	47.50^{bc}	96	17.50^{c}	327	15.22^{c}	122	14.24^{b}	578
Moringa(S/2)*T.harzianum* ($1*10^8$ spore/ml)	19.55^{bcd}	76	62.25^{edf}	37	46.25^{c}	91	16.88^{d}	322	15.01^{cd}	119	14.23^{b}	578
Moringa(S/4)*T.harzianum*($1*10^8$ spore/ml)	19.25^{cde}	74	61.50^{ef}	35	43.75^{d}	80	16.00^{e}	300	14.94^{cd}	118	14.17^{b}	575
Moringa(S/10)*T.harzianum* ($1*10^8$ spore/ml)	18.88^{def}	70	60.25^{f}	32	42.50^{de}	75	15.25^{f}	281	14.56^{d}	112	14.02^{b}	568
Moringa(S)*T.harzianum* ($1*10^{10}$ spore/ml)	22.43^{a}	102	67.50^{a}	48	50.75^{a}	109	19.00^{a}	375	18.01^{a}	162	16.42^{a}	682
Moringa(S/2)*T.harzianum* ($1*10^{10}$ spore/ml)	20.80^{b}	82	65.25^{b}	43	48.75^{b}	101	18.25^{b}	356	18.00^{a}	162	16.37^{a}	679
Moringa(S/4)*T.harzianum* ($1*10^{10}$ spore/ml)	20.63^{bc}	86	64.50^{bc}	42	48.00^{b}	98	18.25^{b}	356	17.89^{a}	161	15.97^{a}	660
Moringa(S/10)*T.harzianum* ($1*10^{10}$ spore/ml)	20.38^{bcd}	84	63.75^{bcd}	40	47.75^{bc}	97	18.00^{bc}	350	16.94^{b}	147	15.92^{a}	658
Control(Only nematode)	11.08^{i}		45.50^{k}		24.25^{j}		4.00^{m}		6.86^{i}		2.10^{d}	

Note: Means in column with the same letter are not significantly different ($p< 0.05$) DMRT.

Y** increase over inoculated control in percent

Table 3. Effect of aqueous moringa plant extracts and *Trichoderma harzianum* on root knot nematode on tomato

Treatments	No. of galls/plant	Y**	No. of egg mass/plant	Y**	Indices scale[1-5]		Population density/pot	Y**	Reproduction factor
					Gall	Egg-mass			
Moringa (S)**T.harzianum*(1*10^4 spore/ml)	121.00d	54	106bc	57	5.00^a	5.00^a	15722.00^c	35	7.86^c
Moringa(S/2)**T.harzianum*(1*10^4 spore/ml)	127.00c	52	106bc	57	5.00a	4.75^a	15726.00^c	35	7.86^c
Moringa(S/4) **T.harzianum*(1*10^4 spore/ml)	127.00c	52	110bc	55	5.00^a	5.00^a	19058.00^b	22	9.53^b
Moringa(S/10)**T.harzianum*(1*10^4 spore/ml)	135.00b	49	114b	53	5.00^a	5.00^a	20329.00^b	16	10.16^b
Moringa(S)**T.harzianum* (1*10^6 spore/ml)	97.00^f	63	34e	86	4.30cd	4.00^b	12958.00^d	47	6.48^d
Moringa(S/2)**T.harzianum*(1*10^6 spore/ml)	99.00^f	62	90d	63	4.50bc	4.00^b	13097.00^d	46	6.55^d
Moringa(S/4)**T.harzianum*(1*10^6 spore/ml)	107.00e	59	102c	58	4.70ab	4.75a	14376.00^{cd}	41	7.19^{dc}
Moringa(S/10)**T.harzianum*(1*10^6 spore/ml)	117.00d	56	105bc	57	5.00^a	5.00^a	14977.00^c	39	7.49^c
Moringa(S)**T.harzianum*(1*10^8 spore/ml)	47.00ij	82	44.00gh	82	4.00^d	3.75b	9422.00^e	61	4.71^e
Moringa(S/2)**T.harzianum*(1*10^8 spore/ml)	51.00i	81	49.00fg	80	4.00^d	4.00^b	10656.00^e	56	5.33^e
Moringa(S/4)**T.harzianum*(1*10^8 spore/ml)	60.00h	77	53.00f	78	4.00^d	4.00^b	10685.00^e	56	5.34^e
Moringa(S/10)**T.harzianum*(1*10^8 spore/ml)	69.00g	74	56.00f	77	4.00^d	4.00^b	11031.00^e	55	5.52^e
Moringa(S)**T.harzianum*(1*10^{10} spore/ml)	11.00k	96	7.00i	97	2.50e	1.75c	3588.00^g	85	1.79^g
Moringa(S/2)**T.harzianum*(1*10^{10} spore/ml)	44.00^j	83	40.00h	84	4.00^d	4.00^b	6241.00^f	74	3.12^f
Moringa(S/4)**T.harzianum*(1*10^{10} spore/ml)	44.00^j	83	41.00gh	83	4.00^d	4.00^b	6838.00^f	72	3.42^f
Moringa(S/10)**T.harzianum*(1*10^{10} spore/ml)	45.00^j	83	42.00gh	83	4.00^d	4.00^b	6847.00^f	72	3.42^f
Control(Only nematode)	264.00a		244.00a		5.00^a	5.00^a	24399.00^a		12.20^a

Note: Means in column with the same letter are not significantly different ($p < 0.05$) DMRT.
Y** Reduction over inoculated control in percent

inoculated control. Aqueous moringa plant extracts at S and *T. harzianum* at $1*10^{10}$spore/ml have increased the fresh shoot weight of tomato by 109% over the inoculated control. Pots treated with aqueous moringa plant extracts at S/10 and *T. harzianum* at $1*10^{4}$spore/ml, records the lowest shoot weight compared to inoculated control.This resulted 40% increase on plant shoot weight over the inoculated control (Table 2).

Fresh root weight obtained from the control treatment shows lower weight than other treatments. However, female root knot nematodes cause holes in the roots of infested plants, which cause the roots to lose water and moisture content. As a result, the weight of the infected plants' roots is lower than that of the uninfected plants. This might have caused the fresh root weight of the control treatment to decline. The dry root weight of the pots treated with S * *T. harzianum* $1* 10^{10}$ spore/ml, S/2 * *T. harzianum* $1* 10^{10}$ spore/ml, S/4 * *T. harzianum* $1* 10^{10}$ spore/ml, and S/10 * *T. harzianum* $1* 10^{10}$ spore/ml aqueous moringa plant extract were not statistically different from that of the other treatments. Aqueous moringa plant extracts at S and *T. harzianum* at $1*10^{10}$spore/ml has showed 682% increment on dry root weight over the inoculated control. In line with current study, (Muhammad et al., 2014) have recorded that the highest shoot weight of 9.5gm from combined applications of moringa and *T. harzianum* over inoculated control (4.9gm).

Effects of aqueous moringa leave extracts and Trichoderma harzianum against root knot nematode (M. incognita) on tomato

The results showed that the treatments differed significantly (P 0.05) in terms of the average number of galls per plant, the number of eggs per plant, the final nematode population density per pot, and the reproduction factor (Table 3). The pots treated with aqueous moringa plant extracts S and *T. harzianum* $1*10^{10}$Spore/ml resulted the lowest mean number of gall (11), while the pots treated with aqueous moringa plant extracts S/10 and *T. harzianum* $1*10^{4}$Spore/ml shows the maximum (135). Pot treated with aqueous moringa plant extracts S and *T. harzianum* $1*10^{10}$Spore/ml have shown 96% gall reduction over the inoculated control.

The aqueous moringa plant extracts S and *T. harzianum* $1*10^{10}$Spore/ml had the lowest mean number of egg-mass/plant (7), while S/10 and *T. harzianum* $1*10^{4}$Spore/ml had the highest mean value (114) .However, as compared to all treatments the highest mean number of egg-mass per pot (244) was observed with an inoculated control (Table 3). Pot treated with aqueous moringa plant extracts S and *T. harzianum* $1*10^{10}$Spore/ml has reduces the number of egg-mass by 97% as compared with inoculated control. The pots treated with aqueous moringa plant extracts S and *T. harzianum* $1*10^{10}$Spore/ml had the lowest mean population density (3588) and reproduction factor (1.79) compared to control. The highest mean population density (20,329) was, recorded in pots treated in aqueous extracts of the moringa plant S/10 and *T*. *harzianum* $1*10^{4}$Spore/ml.While the pots treated with aqueous moringa plant extracts S/10 and *T. harzianum* $1*10^{4}$Spore/ml showed the highest mean reproduction factor (10.2). Inoculated control treatment has induced the highest mean population (24399) density and reproduction factor (12.2) over the other treatments. The nematode population density on pots treated with aqueous moringa plant extracts S and *T. harzianum* $1*10^{10}$Spore/ml has decreased by 85% over the inoculated control.

In comparison to the control, the root and soil populations of the root knot nematode as well as the root gall index significantly decreased as a result of the population decline caused by the organic amendment introduced into the soil. Similar to this, it has been noted

(Zawam et al., 2003) that aqueous extract of the Moringa plant reduced gall formation and improved plant growth characteristics.

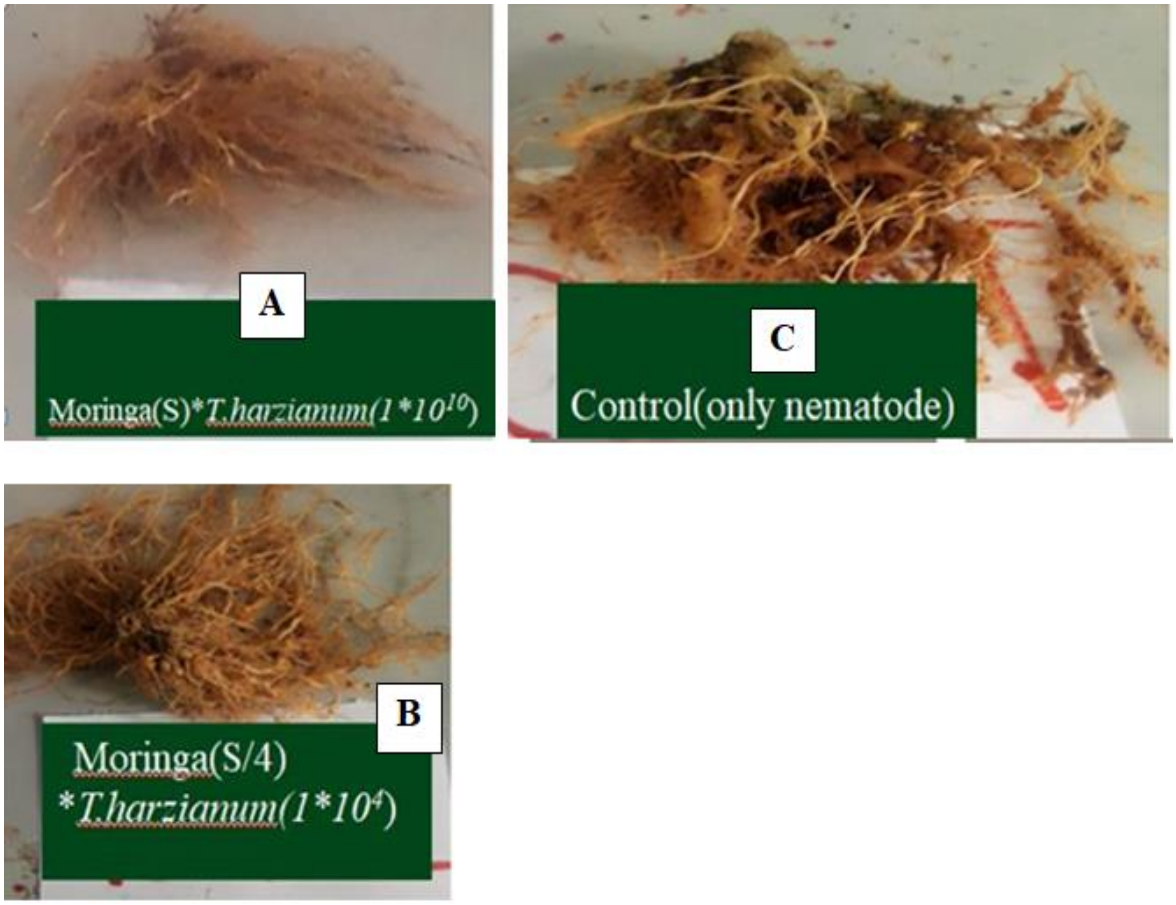

Figure 1. Gall symptom of RKN on tomato roots treated with *moringa* and *T.harzianum,* Where; Best treatment (A), Poor treatment (B) and inoculated Control treatment(C)

Trichoderma harzianum and vermicompost were used to suppress root knot nematodes and boost tomato plant growth, according to Heidari & Olia (2016). In contrast to *Meloidogyne incognita*, which feeds on a variety of fungi, including saprophytic and beneficial microbial organisms, fungivorous nematodes grew in numbers in soils supplemented with various organic substrates, according to a prior study (Neher et al., 2005).

CONCLUSION

Severe pests known as root-knot nematodes (RKN) harm a wide range of crops. Organic additions are acceptable for the environment, but the high quantities needed per area make the method unworkable in big-scale farming operations. The results of the current study indicated that *M. incognita* reproduction was decreased in addition to plant growth characteristics being greatly improved. It was shown that root weight was directly correlated with the lowest number of galls in control plants. As a result of the current research, it has been determined that the combination of *T. harzianum* and *M. oleifera* proved to be quite successful in controlling *M. incognita.* This study showed that the test plants which are easily available to farmers without paying any cost have the ability to

reduce nematode frequency below economic threshold level.. Therefore, finding novel strategies to replace dangerous chemical nematicides could be successful in managing root knot nematodes. Farther research should be conducted under field condition to confirm the greenhouse result.

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AUTHOR CONTRIBUTIONS

The research concept and the work proposal were created by Belay Feyisa. The experiment was carried out by Belay Feyisa, Gemechu Kebede, and Fikeremariam Yimer. They also analyzed the data and published the manuscript. The last draft was created by Belay Feyisa.

COMPETING INTERESTS

The authors have declared that no conflict of interest exists.

ETHICS APPROVAL

Not applicable

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