



## Management of Root-Knot Nematode (*Meloidogyne* spp.) on Kiwifruit Seedlings using Different Plant Extracts, Biocontrol Agents, and Chemical Nematicides

Kapil Simkhada<sup>1,a,\*</sup>, Srijana Bhandari<sup>2,b</sup>, Chiranjivi Sharma<sup>3,c</sup>

<sup>1</sup>Faculty of Agriculture, Agriculture and Forestry University, Rampur, Chitwan, Nepal

<sup>2</sup>Warm Temperate Horticulture Center, Kirtipur, Kathmandu, Nepal

<sup>3</sup>Department of Entomology, Louisiana State University, USA

\*Corresponding author

### ARTICLE INFO

### ABSTRACT

#### Research Article

Received : 14.02.2024

Accepted : 15.04.2024

#### Keywords:

Root-knot nematodes  
Management  
Biocontrol agents  
Botanical control  
Nematicide

Root-knot nematodes (RKN), (*Meloidogyne* spp.), are the major biotic factor responsible for the limiting production of Kiwifruit in Nepal including Kiwifruit orchard of Warm Temperate Horticulture Center, Nepal. Hence, there is a pressing demand for nematicides that are both easily accessible and cost-effective while being environmentally friendly. A greenhouse experiment was conducted in the Summer of 2023 with an objective to evaluate the effects of different plant extracts, bio-control agents, and chemical nematicides against RKN on Kiwifruit seedlings. The experiment was set up in a Completely Randomized Design with three replications and eight treatments which include the extracts of *Allium sativum* and *Lantana camara*, *Trichoderma viride*, *Pseudomonas fluorescens*, Cartap hydrochloride, Fosthiazate, Inoculated control and Uninoculated control. The results revealed that *Trichoderma viride* proved to be the most effective in reducing the nematode population, displaying a low root gall index of 3.11, a minimal reproductive factor of 0.24, and a high percentage of nematode control at 91.71%. It was also found to be efficient in promoting the growth parameters of Kiwifruit seedlings. Additionally, regression analysis exhibited a significantly positive interaction between root gall index and reproductive factor, while indicating a negative interaction between reproductive factor and growth parameters. Therefore, *T. viride* (@ 20 gm per 2000 cm<sup>3</sup> of soil) should be soil drenched before the seedlings are transplanted into the main field for effective and sustainable management of RKN. Nevertheless, further research is needed to determine the efficacy of *T. viride* in infested roots of Kiwifruit trees in field condition of Kiwifruit orchard.

<sup>a</sup> [kapilsimkhada2057@gmail.com](mailto:kapilsimkhada2057@gmail.com)

<sup>b</sup> <https://orcid.org/0000-0002-6870-5002>

<sup>b</sup> [bsrijana15@gmail.com](mailto:bsrijana15@gmail.com) <sup>c</sup> <https://orcid.org/0000-0003-4982-787X>

<sup>c</sup> [sharma.chiranjivi123@gmail.com](mailto:sharma.chiranjivi123@gmail.com)

<sup>c</sup> <https://orcid.org/0000-0002-1752-8560>



This work is licensed under Creative Commons Attribution 4.0 International License

## Introduction

Kiwifruit is an emerging fruit crop which is popular and suitable to the mid hills and high hills of Nepal at an altitude of 1200 to 2500 masl (Sharma et al., 2020). Though introduced in 1986 AD, commercial kiwifruit cultivation started in Nepal only in 2009 AD (Atreya et al., 2020). It has a tremendous potential for processed value-added products besides fresh fruit, such as jam, jelly, candy, marmalade, wine, juice, etc. which shows huge prospects to increase the production of Kiwifruit in Nepal for uplifting the livelihood of Nepalese farmers and commercializing agriculture industry of Nepal (Sharma et al., 2020). While the cultivation area is expanding, the increase in production and productivity has not been entirely satisfactory. The unregulated cultivation of kiwifruit seedlings by Nepalese farmers from unknown

sources without adequate phytosanitary measures has led to an escalation in the infestation of root-knot nematodes (RKN) (*Meloidogyne* spp.) in kiwifruit orchards which is considered as a primary contributing factor to the decline in kiwifruit production in Nepal.

It is reported that, more than 40-50 % of Kiwifruit orchard in Nepal including Kiwifruit orchard of Warm Temperate Horticulture Center (WTHC), Kirtipur, Kathmandu is infested with RKN (APR, 2022). Its damage results in poor growth, reduces productive year, quality, and yield, and decreases the resistance of crop against drought and diseases (Subedi et al., 2020). Moreover, they are destructive endoparasite causing average crop losses to 15-20 % annually in the tropical and sub-tropical countries like Nepal (Terefe, 2015; Chhetri, 2019). Kepenekci et al.

(2017) reported that 21-35% annual production loss in Kiwifruit is caused by this notorious pest. In fact, it is difficult to control the soil borne pathogen like RKNs having a wide range of host plants and ability to damage multiple agricultural crops without proper investigation and repeated trials (Muthulakshmi et al., 2010). In addition, there exist a dearth of research and innovation in Nepal to find out the effective, and sustainable control methods against RKN infestation on Kiwifruit.

WTHC, a government-owned fruits' seedlings distributor in Nepal, holds a mandate to produce disease free seedlings. Otherwise, chances of spreading diseases from nurseries to the main field will increase. Given the substantial potential for Kiwifruit to establish itself as a key fruit in Nepal, this study will aid WTHC in identifying an effective control strategy against RKN, enabling the fulfillment of its mandate to provide farmers with healthy seedlings.

## Materials and Methods

### Research Site

The research was conducted in the Warm Temperate Horticulture Centre (WTHC), Kirtipur, Kathmandu, Nepal located at 27°40'27" N Latitude, 85°17'20" E Longitude and at an altitude of 1303 masl under screenhouse conditions during summer season of 2023 from March to July. WTHC is a government owned fruits seedlings' producer and distributor.

### Disease Confirmation and Pathogen Identification

The Kiwifruit trees exhibiting visible symptoms above ground, such as wilting and stunted growth (Hafiza et al., 2016), were chosen from the WTHC fruit orchard. To verify the presence of RKN infestation, the roots of the Kiwifruit were gathered and brought to the Plant Pathology Laboratory at WTHC. The examination revealed the definite presence of root-knots, as depicted in Figure 1.

Juveniles of nematode were extracted from soil following the methods described by Hooper et al. (2005) and Baidya et al. (2023) with some modifications. At first, soil from the vicinity of the Kiwifruit roots was obtained and transported to the laboratory which was then pulverized finely with sterilized hand. A tissue paper was spread inside a plastic sieve placed on an extraction plastic tray. 100 grams of soil sample was spread over the tissue paper and clean tap water was meticulously added from one side of the extraction tray until the soil layer was completely moist. The extraction sets were left undisturbed for 24 hours, allowing the juveniles to migrate from the soil to the extraction tray. The soil-filled plastic sieves were lifted after 24 hours to collect water into the extraction tray. The water with nematodes from the extraction tray was poured into a labelled beaker. The tray was rinsed using a wash bottle, and water was added to the beaker to generate a stock solution, volume of 250 millilitres. After thoroughly stirring, 10 ml of suspension was pipetted out and placed into a petri plate. This small amount of the suspension was examined under a stereomicroscope to aid in identification. The whole set up of the juvenile extraction is shown in the Figure 2. Under the stereomicroscope, juvenile of RKN was observed as shown in the Figure 3.



Figure 1. Sample of Kiwifruit roots showing the formation of root-knots

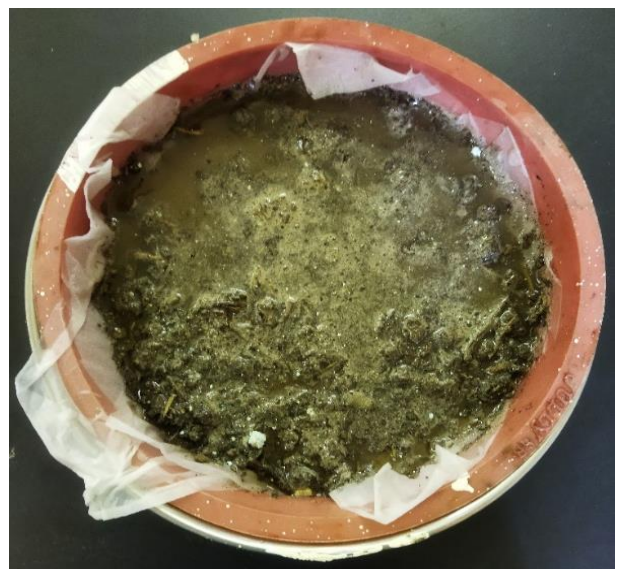


Figure 2. Set up for the extraction of juveniles from the soil using sieve, muslin cloth, and extraction tray



Figure 3. Second stage juvenile (J2) of RKN as observed under stereomicroscope (scale: 10X)

### Seedlings Preparation and Transplantation

The seeds were extracted from the Kiwifruit and collected in tissue paper. These seeds were washed to make free from the pulp and the preliminary selection of the seeds were based on their potential viability done by placing the seeds in water for priming for 24 hours. The viable primed seeds were then treated with gibberellic acid @ 500 ppm for 24 hours to promote germination followed by air drying for 24 hours (Lawes & Anderson, 1980). Seedlings were prepared in the germination tray. One-third portion of vermi-compost and coco-peat were mixed with two-third portion of sand. This mixture was filled in a germination tray followed by sowing of sterilized air-dried seeds at the depth of 2 cm. The germination tray was then covered with jute bag and kept in a screen house maintained at the temperature of  $30 \pm 2$  °C. Watering was done once in two days.

Soil collected from the field was sterilized in an autoclave for 30 min at 15 psi and 120 °C for the potting mixture, which was prepared by adding sterilized soil mixture with vermicompost in the ratio of 3:1. This mixture of volume 2000 cm<sup>3</sup> was then filled in a plastic pot having inner diameter of 17 cm, where 120 seedlings of the four-leaf stage Kiwifruit seedlings at 60 days after sowing were transplanted @ one plant per pot.

### Egg Mass Extraction and Eggs Collection

A heavily knotted roots of the Kiwifruit trees from WTHC, Kirtipur, were used for the extraction of eggs. The technique used for the extraction of eggs from the roots follows the procedure described by Hussey & Barker (1973) and McClure et al. (1973) with some adjustments.

At first, the knotted root sample was cleaned in tap water to remove soil and detached from plant with scissors. 10 gm of sample knotted roots were measured with the help of digital weighing balance which were later chopped into small pieces. The chopped knotted roots were vigorously shaken in magnetic stirrer in 250 ml conical flask containing 1 % sodium hypochlorite solution for 4 min to dissolve the gelatinous matrix of egg sac present in the knotted roots. The resulting suspension of eggs and root debris were poured through 100 and 500-mesh sieves to retain eggs on the 500-mesh sieve. The eggs collected on the 500-mesh sieve were thoroughly washed with distilled water and poured from the sieve into the beaker making a 250 ml solution for the eggs collection. Eggs which escaped through 500-mesh sieve were also recovered by repeated sieving and rinsing. The suspension was stirred, and one ml of aliquot was placed in a nematode counter with the help of a pipette. The eggs were then observed under a stereomicroscope and counted. The counted eggs were kept in refrigerator at 10<sup>0</sup> C for one day to prevent hatching before inoculation.

### Inoculation of RKN Eggs

After seven days of seedlings transplantation, 2-3 cm deep holes were made close to each plant with a plastic stick and the plants were inoculated with nematode eggs @ 1500 eggs per pot, except in an uninoculated control (T<sub>8</sub>) which was nematode free, using a pipette and plastic syringe. After inoculation, holes around the plants were covered with the adjacent soil.

### Experimental Design

The experiment was laid out in a completely randomized design with three replications and eight treatments. There were five pots per treatment in each replication, making 40 pots per replication and 120 as total number of pots used in the research.

### Treatments Detail and Methods of Application

Various treatments were evaluated in the nursery condition of Kiwifruit against RKN caused by *Meloidogyne* spp. The treatments were applied after 7 days of the RKN eggs inoculation in their respective doses, shown in the Table 1, to reduce the impact of RKN on kiwifruit seedlings after its infestation. The two botanical extracts, two biological agents, and two chemical nematicides were compared and evaluated for the management of RKN. *Allium sativum* cloves extract and *Lantana camara* leaves extract were used as a botanical agent. The extracts were prepared by grinding the 100 gm of botanicals in the presence of 100 ml of water in an electric blender followed by filtration through muslin cloth and filter paper. The obtained extracts of 100 % concentration were diluted with distilled water to make different concentrations of 5 %, 10 % and 15 %. The laboratory trial of these concentrations showed the highest mortality rate (65 %) of RKN Juveniles at 15 % concentration. In addition, 75 % of eggs were prevented from hatching, thus plant extracts of 15 % concentration were drenched in the soil @ 40 ml per pot following the research conducted by Subedi (2022) on tomato where 40 ml for the 2500 cm<sup>3</sup> volume of soil was used.

The commercial powdered form of biological agents, *Trichoderma viride* and *Pseudomonas fluorescens* were applied directly on soil based on their recommended dose @ 20 gm per 2000 cm<sup>3</sup> of soil. Moreover, soil was treated with synthetic chemical, Cartap hydrochloride in the form of granule @ 5 gm per volume of soil used in each pot which was its recommended dose and another chemical nematicide, Fosthiazate was first diluted @ 1ml in 1 litre of distilled water to make 30 ml of diluted solution which was then drenched on the soil based on its recommended dose of 30 ml per 2000-2500 cm<sup>3</sup> of soil.

In inoculated control, nematode eggs were inoculated in the soil, but no treatments were used for the control. In uninoculated control, neither nematode eggs were inoculated, nor any treatments were used for the RKN control, i.e., seedlings were grown in a sterilized soil. During the time of treatments application, pots with inoculated control and uninoculated control were soil drenched with 100 ml of distilled water to make uniform application of the treatment.

### Observation Parameters and Data Collection

After two and half months of nematode eggs inoculation, sampled pots from the greenhouse were cautiously carried to the Plant Pathology laboratory of WTHC, Kirtipur, where kiwifruit seedlings were carefully uprooted, washed with tap water, and different observation parameters were carried out.

### Gall Formation Quantity at Roots

All the soil debris attached with the roots were carefully removed by washing gently with tap water and symptomatic assessment of root gall intensity was carried out based on a scale (0-10) given by Bridge & Page (1980). The different scale based on the level of infection by RKN is depicted in Table 2.

Table 1. Treatments details used for management of RKN (*Meloidogyne* spp.) at WTHC, Kirtipur, Kathmandu, Nepal, 2023

S.N.	Name of treatments	Symbolic representation	Application dose per pot
1.	Allium sativum extract	T <sub>1</sub>	40 ml solution of 15 % conc <sup>n</sup>
2.	Lantana Camara extract	T <sub>2</sub>	40 ml solution of 15% conc <sup>n</sup>
3.	Trichoderma viride	T <sub>3</sub>	20 gm powder
4.	Pseudomonas fluorescens	T <sub>4</sub>	20 gm powder
5.	Cartap hydrochloride 4% GR	T <sub>5</sub>	5 gm granules
6.	Fosthiazate 20% EW	T <sub>6</sub>	30 ml of solution
7.	Inoculated control	T <sub>7</sub>	100 ml of distilled water
8.	Uninoculated control	T <sub>8</sub>	100 ml of distilled water

Table 2. A scale for determining root gall index caused by *Meloidogyne* spp. based on a Bridge & Page (1980) explanation

Root gall index (0-10)	Explanation of rating
0	Healthy plant root system without knot formation
1	Very few small knots, difficult to find and can only be detected on close inspection
2	Small knots only, but clearly visible, main roots clean
3	Some larger knots visible, main roots free
4	Larger knots predominate but main roots free
5	50 % of roots are infested. Knotting on parts of main roots, reduced root system
6	More knotting on main roots
7	Majority of main roots knotted
8	All main roots knotted. Few clean roots visible
9	All roots severely knotted and plant usually dying
10	All roots severely knotted. No root system. Plant usually dead.

All secondary roots were separated from primary root by carefully cutting with scissors. Knots on each primary and secondary root of each plant of each treatment were counted with naked eyes. Mean number of galls per plant per treatment was calculated. The same roots were used for the extraction of eggs.

#### Egg count

The same roots which were used for the assessment of gall formation index were used for the extraction of eggs. 10 gm sample of Knotted portion of roots were chopped with the help of scissors and were subjected to the procedure as described in section 2.4. The eggs thus obtained were counted in the nematode counter with the help of stereomicroscope. Total number of eggs present in the root system was calculated by multiplying the eggs present in 10 gm sampled knotted roots to the total weight of knots present in a root system.

#### Juvenile count

Total volume of soil present in each pot was used for the juvenile extraction. The whole extraction process of juveniles follows the description as given in section 3.2 based on Hooper et al. (2005) and Baidya et al. (2023) with some modifications. After stock solution was prepared as mentioned in the section 3.2, 10 ml of suspension was pipetted out and placed into counting plates for the juvenile count. The total number of juveniles present in 10 ml of suspension was counted, and using this number, the total number of nematodes present in the 250 ml of the stock solution (corresponding to 100 grams of soil) was determined.

#### Reproductive factor (Rf)

After counting of nematode eggs present in the knotted roots of Kiwifruit seedlings and juveniles present in the soil of each pot, final population of root knot nematode was computed by adding mean number of eggs per plant and mean number of juveniles per 100 gm soil in each treatment. Rf of *Meloidogyne* spp. was calculated by dividing the final population (Rf) with the initial population (Pi). Thus, Rf is number of nematodes (eggs

and juveniles) produced from one egg, inoculated at the beginning of the experiment.

$$\text{Reproductive factor (R}_f\text{)} = \frac{\text{Final population (Pf)}}{\text{Initial Population (Pi)}}$$

It is believed that when Rf (= Pf/Pi) is  $\geq 1$ , nematodes can grow and develop a population. On the other hand, when the ratio is  $\leq 1$ , nematodes neither grow nor develop a population in agro-ecosystem, hence cannot impose a damage to the culture plants (Ferris & Noling, 1987).

#### Plant height and shoot weight

After the Kiwifruit seedlings were carefully uprooted, they underwent a thorough washing with tap water at the Plant Pathology Laboratory of WTHC. Using scissors, the above-ground section was separated from the roots. The length of the above-ground portion was measured using a 30 cm scale to ascertain the plant height. From each replication, 3 out of 5 randomly selected plants per treatment were chosen for sample measurement. The same above ground portion was used to determine the shoot weight which was measured using digital weighing balance.

#### Root length and root biomass

The roots which were separated from the shoot portion were cleaned gently using tap water and left for some time so that the excess water present in the roots evaporates. Root length was measured on primary root, using a 30 cm length scale. 3 sample plants per treatment per replication were selected to measure root length. The same roots were used the measurement of root biomass using digital weighing balance.

#### Percentage nematode control

It was calculated using the formula given by Davis et al. (2009) with some modifications.

$$\text{Percentage Nematode Control} = \frac{A-B}{A} \times 100 \%$$

Where, A = Nematode population at inoculated control  
B = Nematode population at treatment

**Statistical Analysis**

The gathered data was organized and tabulated into MS-Excel. Analysis of variance was conducted for all the parameters utilizing the statistical software R-Studio, version: 2023.06.2+561. In addition, the Duncan Multiple Range Test (DMRT) was employed for distinguishing means using the same version of R-studio. Regression analysis was performed using XLSTAT.

**Results**

**Root Gall Index and Number of Root Galls Per Plant**

A significant variation was observed between various treatments (at  $p \leq 0.001$ ) in terms of both root gall index and number of root galls per plant as shown in the table 3. *Trichoderma viride* had the lowest root gall index (3.11) and number of root galls per plant (18.00) followed by Fosthiazate with root gall index (4.00) and number of root galls per plant (34.00), while inoculated control had the highest root gall index (7.44) and number of root galls per plant (132.00).

**Egg Count, Juvenile Count, and Reproductive Factor (Rf)**

There was significant difference between different treatments at 0.1% level of significance in terms of egg count, juvenile count and reproductive factor as shown in

the table 4. The lowest value of egg count and juvenile count was obtained in *Trichoderma viride* (333.33 and 32.67) followed by chemical Fosthiazate (533.33 and 70.67), and *Pseudomonas fluorescens* (763.33 and 109.33) respectively. Contrarily, the highest egg count and juvenile count was obtained in inoculated control (4120.00 and 299.00) followed by *Allium sativum* extract (1516.67 and 167.00) and *Lantana camara* extract (1333.33 and 140.33) respectively.

*Trichoderma viride* had the lowest reproductive factor (0.24) which was ahead of Fosthiazate (0.402) and *Pseudomonas fluorescens* (0.58) whereas the highest reproductive factor was obtained in Inoculated control (2.95) followed by *Allium sativum* extract (1.12).

**Effect on Plant Height and Shoot Weight**

Uninoculated control had the highest plant height (6.00 cm) followed by *Trichoderma viride* (4.88 cm), whilst lowest value was observed in inoculated control (2.93 cm) as shown in the table 5. In addition, the highest shoot weight was obtained in uninoculated control (3.70 gm) followed by *Trichoderma viride* (2.80 gm) and *Pseudomonas fluorescens* (1.99 gm). Lowest shoot weight was obtained in inoculated control (1.40 gm) followed by Cartab hydrochloride (1.52 gm).

Table 3. Root gall formation evaluation of different plant extracts, biocontrol agents, and chemical pesticides against RKN (*Meloidogyne* spp.) on Kiwifruit seedlings at WTHC, Nepal

S.N.	Treatments	Root Gall Index (0-10)	Number of galls per plant
1	<i>Allium sativum</i> extract	5.44 <sup>bc</sup>	79.67 <sup>b</sup>
2	<i>Lantana camara</i> extract	4.89 <sup>cd</sup>	58.33 <sup>c</sup>
3	<i>Trichoderma viride</i>	3.11 <sup>f</sup>	18.00 <sup>e</sup>
4	<i>Pseudomonas fluorescens</i>	4.45 <sup>de</sup>	49.33 <sup>c</sup>
5	Cartap Hydrochloride 4 % GR	5.78 <sup>b</sup>	76.67 <sup>b</sup>
6	Fosthiazate 20 % EW	4.00 <sup>e</sup>	34.00 <sup>d</sup>
7	Inoculated Control	7.44 <sup>a</sup>	132.00 <sup>a</sup>
	SEm (±)	0.37	4.51
	F-test	***	***
	CV (%)	9.05	8.62
	LSD (0.05)	0.79	9.67
	Grand Mean	5.01	64

Means followed by common letter(s) within a column do not differ significantly at  $\leq 5$  % level of significance by DMRT; LSD = Least significant difference; significance codes \*\*\*at  $p \leq 0.001$ ; \*\*at  $p \leq 0.01$ ; \*at  $p \leq 0.05$ ; SEm= Standard error of mean, CV = Coefficient of variation

Table 4. Effect of different plant extracts, biocontrol agents, and chemical pesticides on the reproductive performance of RKN (*Meloidogyne* spp.) on Kiwifruit seedlings at WTHC, Kirtipur, Kathmandu

S.N.	Treatments	Final Population (No.)		Reproductive Factor (Rf)
		Eggs	Juveniles	
1	<i>Allium sativum</i> extract	1516.67 <sup>b</sup>	167.00 <sup>b</sup>	1.12 <sup>b</sup>
2	<i>Lantana camara</i> extract	1333.33 <sup>c</sup>	140.33 <sup>c</sup>	0.98 <sup>c</sup>
3	<i>Trichoderma viride</i>	333.33 <sup>g</sup>	32.67 <sup>f</sup>	0.24 <sup>g</sup>
4	<i>Pseudomonas fluorescens</i>	763.33 <sup>e</sup>	109.33 <sup>d</sup>	0.58 <sup>e</sup>
5	Cartap Hydrochloride 4 % GR	1133.33 <sup>d</sup>	156.67 <sup>b</sup>	0.86 <sup>d</sup>
6	Fosthiazate 20 % EW	533.33 <sup>f</sup>	70.67 <sup>e</sup>	0.40 <sup>f</sup>
7	Inoculated Control	4120.00 <sup>a</sup>	299.00 <sup>a</sup>	2.95 <sup>a</sup>
	SEm (±)	72.97	5.94	0.05
	F-test	***	***	***
	CV (%)	6.43	5.22	5.80
	LSD (0.05)	156.50	12.74	0.10
	Grand mean	1390.48	139.38	1.02

Means followed by common letter(s) within a column do not differ significantly at  $\leq 5$  % level of significance by DMRT; LSD = Least significant difference; significance codes \*\*\*at  $p \leq 0.001$ ; \*\*at  $p \leq 0.01$ ; \*at  $p \leq 0.05$ ; Sem= Standard error of mean, CV = Coefficient of variation



Table 5. Evaluation of different plant extracts, biocontrol agents, and chemical pesticides on the growth parameters of Kiwifruit seedlings caused by RKN (*Meloidogyne* spp.) at WTHC, Kirtipur, Kathmandu, 2023

S.N.	Treatments	Plant height (cm)	Shoot weight (gram)	Root length (cm)	Root weight (gram)
1	Allium sativum extract	4.11 <sup>c</sup>	1.88 <sup>c</sup>	6.74 <sup>bc</sup>	1.25 <sup>c</sup>
2	Lantana camara extract	3.77 <sup>c</sup>	1.59 <sup>c</sup>	4.84 <sup>c</sup>	1.03 <sup>c</sup>
3	Trichoderma viride	4.88 <sup>b</sup>	2.80 <sup>b</sup>	8.29 <sup>ab</sup>	1.87 <sup>b</sup>
4	Pseudomonas fluorescens	3.71 <sup>c</sup>	1.99 <sup>c</sup>	7.31 <sup>b</sup>	1.39 <sup>c</sup>
5	Cartap Hydrochloride 4 % GR	4.01 <sup>c</sup>	1.62 <sup>c</sup>	6.28 <sup>bc</sup>	1.13 <sup>c</sup>
6	Fosthiazate 20 % EW	3.65 <sup>c</sup>	1.52 <sup>c</sup>	6.99 <sup>bc</sup>	1.22 <sup>c</sup>
7	Inoculated Control	2.93 <sup>d</sup>	1.40 <sup>c</sup>	4.79 <sup>c</sup>	1.02 <sup>c</sup>
8	Un-inoculated Control	6.00 <sup>a</sup>	3.70 <sup>a</sup>	9.64 <sup>a</sup>	2.57 <sup>a</sup>
	SEm (±)	0.30	0.31	1.004	0.18
	F-test	***	***	**	***
	CV (%)	8.90	18.13	17.92	15.75
	LSD (0.05)	0.63	0.65	2.12	0.39
	Grand mean	4.13	2.06	6.85	1.43

Means followed by common letter(s) within a column do not differ significantly at  $\leq 5$  % level of significance by DMRT; LSD = Least significant difference; significance codes \*\*\*at  $p \leq 0.001$ ; \*\*at  $p \leq 0.01$ ; \*at  $p \leq 0.05$ ; SEm= Standard error of mean, CV = Coefficient of variation

Table 6. Effect of treatments on percentage nematode control of Kiwifruit seedlings at WTHC, Kirtipur, Kathmandu, 2023

S.N.	Treatments	Percentage nematode control (%)
1	Allium sativum extract	61.86 <sup>f</sup>
2	Lantana camara extract	66.66 <sup>e</sup>
3	Trichoderma viride	91.71 <sup>a</sup>
4	Pseudomonas fluorescens	80.25 <sup>c</sup>
5	Cartap Hydrochloride 4 % GR	70.80 <sup>d</sup>
6	Fosthiazate 20 % EW	86.33 <sup>b</sup>
	SEm (±)	1.69
	F-test	***
	CV (%)	2.72
	LSD (0.05)	3.69
	Grand mean	76.27

Means followed by common letter(s) within a column do not differ significantly at  $\leq 5$  % level of significance by DMRT; LSD = Least significant difference; significance codes \*\*\*at  $p \leq 0.001$ ; \*\*at  $p \leq 0.01$ ; \*at  $p \leq 0.05$ ; SEm= Standard error of mean, CV = Coefficient of variation

#### Effect on Root Length and Root Biomass

A significant difference was observed between treatments in terms of root length ( $p \leq 0.01$ ) and root weight ( $p \leq 0.001$ ) as shown in the table 5. Uninoculated control had the highest root length (9.64 cm) followed by *Trichoderma viride* (8.29 cm). Contrarily, the lowest root length was obtained in inoculated control (4.79) followed by *Lantana camara* extract (4.83 cm) and Cartap hydrochloride (6.28 cm).

In addition, Uninoculated control had the highest root biomass (2.57 gm) followed by *Trichoderma viride* (1.87 gm) and *Pseudomonas fluorescens* (1.39 gm). In contrast, Inoculated control had the lowest root biomass (1.02 gm) which was statistically at par with other treatments except *T. viride* and uninoculated control.

#### Percentage Nematode Control

Percentage nematode control was significantly higher in *Trichoderma viride* with 91.70 % control followed by chemical Fosthiozate with 86.33 % control. On the contrary, the lowest percentage of nematode control was obtained with *Allium sativum* extract (61.86 %) followed by *Lantana camara* extract (66.66 %).

#### Interaction Between Reproductive Factor (Rf) and Root Gall Index (GI)

A significantly positive relationship between root gall index and Rf was observed as shown in the figure 4. Linear regression equation revealed that if there was a unit increase in Rf, root gall index would have been increased by 1.4038 times. According to the coefficient of determination, contribution of Rf for increment in the root gall index was found 77.06 %.

#### Interaction between Reproductive Factor (Rf) and Shoot Weight, and Root Biomass

A significantly negative relationship between Rf and growth parameters like shoot weight and root biomass was observed as shown in the figure 5 and figure 6 respectively. According to the linear regression equation, if there was a unit increase in Rf, shoot weight and root biomass would have been decreased by 0.2892 and 0.1965 times respectively. Moreover, according to the coefficient of determination, contribution of Rf for reduction in shoot weight and root biomass in Kiwifruit seedlings was 19.74 % and 26.61 % respectively.

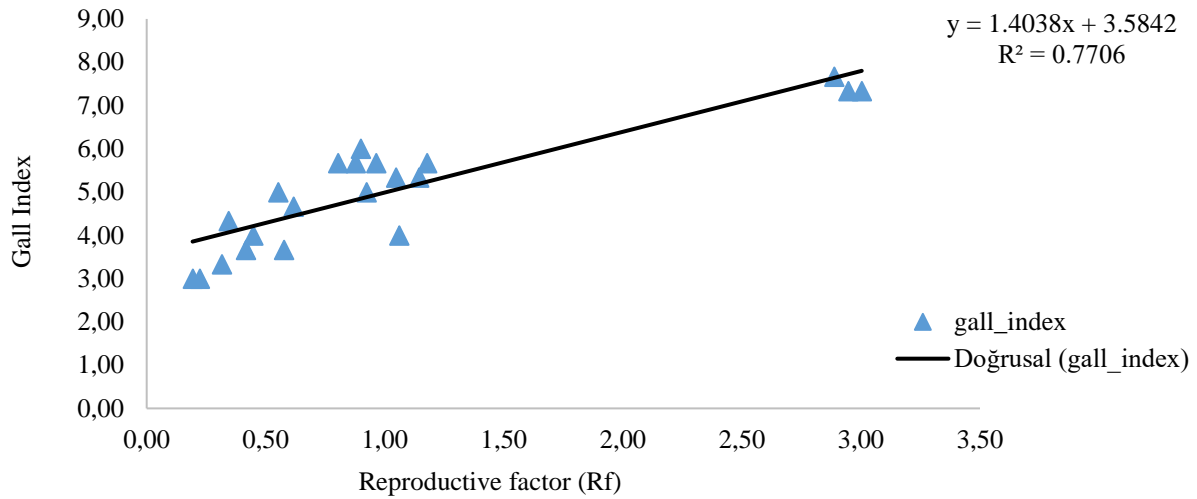


Figure 4. Regression between root gall index and Rf in Kiwifruit at WTHC, Kirtipur, Kathmandu

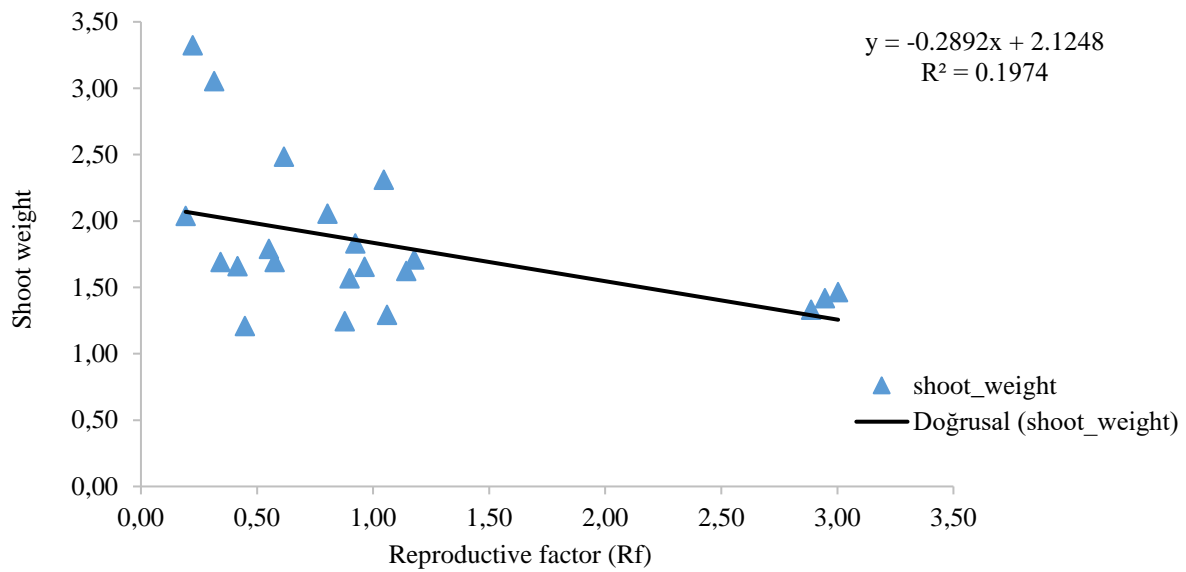


Figure 5. Regression between Rf and shoot weight on Kiwifruit at WTHC, Kirtipur, Kathmandu

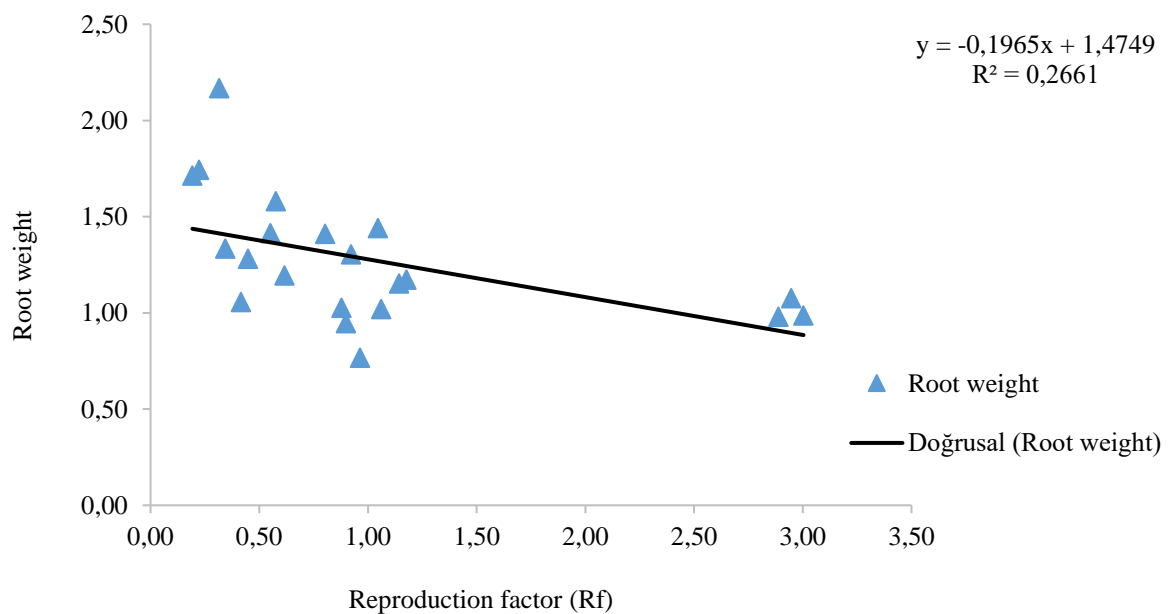


Figure 6. Regression between Rf and root weight on Kiwifruit at WTHC, Kirtipur, Kathmandu

## Discussions

### **Root Gall Index and Number of Root Galls per Plant**

Our investigation revealed a noteworthy distinction among various treatments tested against *Meloidogyne* spp. on Kiwifruit seedlings, particularly concerning the root gall index and the quantity of root galls per plant. Notably, the lowest values for both root gall index and the number of root galls per plant were observed in the case of *Trichoderma viride*. This finding aligns with the results of a similar study conducted by Subedi (2022) on tomatoes, where *Trichoderma viride* demonstrated a significant reduction in the gall index (4.00) compared to the control (8.33). Baños et al. (2017) also discovered that *Trichoderma viride* led to a 53.5% reduction in gall index caused by *Meloidogyne* spp. Furthermore, Baidya et al. (2023) found that *Trichoderma viride* significantly decreased both the gall index (3.43) and the number of root galls per plant (88.86) in tomato, lending further support to our own findings.

In addition, Shamalie et al. (2012) noted that the application of *T. viride* in soil led to only 13.32% of root gall formation in *Centella asiatica*. The formation of galls on the roots and the invasion of root tissue by these nematodes deprive plants of vital nutrients (Terefe, 2015), resulting in stunted growth and heightened vulnerability to mild stressors. *Pseudomonas fluorescens* also demonstrated a significant decrease in both the gall index and the quantity of gall formation. Abd-El-Khair et al. (2019) reported a 57% reduction in gall formation on cowpeas due to *Pseudomonas fluorescens*.

### **Egg Count, Juvenile Count, and Reproductive Factor (Rf)**

Amid the various treatments, *Trichoderma viride* emerged as the most effective in controlling the final nematode population, aligning with Subedi (2022) study on RKN in tomato plants, where *Trichoderma viride* displayed the lowest egg count (6720) and juvenile count (255). Baidya et al. (2023) also noted a significant reduction in the nematode population due to *Trichoderma* spp., with a reproductive factor of 0.45, slightly exceeding the findings of the current study. A reproductive factor less than one indicates that nematodes neither grow nor develop a population in the soil that could harm the crops of interest (Ferris & Noling, 1987). Elad et al. (1982) emphasized the production of antibiotics and extracellular lytic enzymes by *Trichoderma* spp. as effective tools against RKN. Additionally, Santos et al. (1992) reported that *Trichoderma* spp. acted as effective egg parasites of *Meloidogyne* spp. by growing on the surface of RKN egg masses and penetrating the eggshell. Destruction of the egg masses subsequently led to a significant reduction in the number of infective juveniles, ultimately decreasing the final nematode population and reproductive factor. Haran et al. (1996) highlighted the role played by enzymes like chitinase, glucanases, and proteases produced by *Trichoderma* spp. in parasitism.

Chemicals such as Fosthiazate and Cartap hydrochloride, along with the biocontrol agent *Pseudomonas fluorescens*, were also found effective against RKN in Kiwifruit seedlings. Hashem & Abo-Elyours (2011) reported that *P. fluorescens* caused approximately 45% mortality in J2 of RKN in tomato.

Moreover, Abd-El-Khair et al. (2019) found that *Pseudomonas fluorescens* significantly reduced the number of egg masses per plant by 60% and J2 in the soil by 76%.

Furthermore, the leaf extract of *Lantana camara* exhibited potential in reducing the reproductive factor, whereas *Allium sativum* was deemed ineffective, as the RF was determined to be 1.12, indicating the potential for RKN multiplication within the agro-ecosystem. The diverse chemical composition present in *L. camara*, including compounds such as 11-oxo triterpenic acid, lantanolic acid, lantoic acid, pomolic acid, and cimarín, may contribute to the reduction of the nematode population (Chitwood, 2002; Ntalli & Caboni, 2012). Similar findings were also reported in a study conducted by Abrar et al. (2020), where the leaf extract of *Lantana camara* significantly reduced the RF to 0.5306. Additionally, Feyisa et al. (2015) reported highly effective results from the use of *Lantana camara* extracts, with an RF as low as 0.26, differing from the current study's results.

### **Growth Parameters (Plant Height, Shoot Weight, Root Length, and Root Biomass)**

A noticeable distinction at the 0.1% level of significance was observed among the various treatments for plant height, shoot weight, and root biomass, while treatments demonstrated significant variations at the 1% level of significance for root length. Notably, the uninoculated control exhibited the highest values for all growth parameters, including plant height (6.00 cm), shoot weight (3.69 gm), root length (9.63 cm), and root biomass (2.56 gm). Conversely, the inoculated control displayed the lowest values for all growth parameters, indicating that the roots of Kiwifruit seedlings may have been deprived of water and nutrients uptake from the soil due to a severe infestation of *Meloidogyne* spp., consequently hampering the plants' growth performance.

Among the applied control measures, *Trichoderma viride* proved to be an effective treatment, consistent with the findings of Shamalie et al. (2012), which revealed that the application of *Trichoderma viride* in the soil led to an increase in stalk length to 14.07 cm, root length to 2.97 cm, and top fresh weight to 0.9 gm compared to the untreated control. Similarly, Subedi (2022) discovered that the application of *Trichoderma viride* in the soil resulted in a dry root weight of 40.75 gm, surpassing the results of the untreated control. Baidya et al. (2023) also reported similar findings regarding the impact of *Trichoderma viride* on tomato plants, where the fresh root weight was significantly higher (5.51 gm) compared to the control (3.65 gm). According to Hermosa et al. (2012), *Trichoderma* spp. can indirectly interact with roots, thereby enhancing the potential for plant growth. Alongside *T. viride*, *Pseudomonas fluorescens* also exhibited effectiveness against RKN. Abd-El-Khair et al. (2019) observed that the introduction of *Pseudomonas fluorescens* increased the fresh shoot weight and root weight by 35% and 19% respectively compared to the inoculated control in cowpeas, supporting the findings of our study.



### Percentage Nematode Control

It was noted that the application of different treatments significantly aided in controlling the nematode population in comparison to inoculated control. Soil drenching with *Trichoderma viride* emerged as an effective measure, leading to a 91.70% control of RKN on Kiwifruit seedlings. Given that chitin constitutes a major component of the nematode eggshell, the nematophagous egg-parasitic fungus *Trichoderma viride* can infiltrate the eggs, thereby managing the nematode population (Druzhinina et al., 2018; Kubicek et al., 2019). The biocontrol agent *Pseudomonas fluorescens* was also observed to be effective, controlling the nematode population by 80.25%, surpassing the results obtained by Abd-El-Khair et al. (2019) in cowpeas, where a 69.8% nematode control was achieved through the application of *Pseudomonas fluorescens*. The rhizobacteria under study might employ various mechanisms such as the production of antibiotics, enzymes, and toxins against plant-parasitic nematodes, leading to the reduction in nematode populations (Siddiqui et al., 2006).

*Allium sativum*, when compared with other treatments, was found to be less effective, resulting in a 61.86% nematode control rate. The release of various sulfur compounds from garlic clove extract could be implicated in nematode control. Leaf extracts of *Lantana camara* were also found to be effective in controlling the nematode population (66.66%). Similar findings were observed in a study conducted by Taye et al. (2012) in Ethiopia, where a 59% control rate on the final nematode population was observed in tomato roots with the use of *Lantana camara* leaf extracts. Similarly, in a study by Srivastava et al. (2006), *L. camara* was found to be effective against *M. incognita*, resulting in a mortality rate of 85-90%. Ntalli & Caboni (2012) reported that the presence of chemicals like 11-oxo triterpenic acid, lantanolic acid, lantoic acid, pomolic acid, camarin, lanatacin, and ursolic acid accounts for the antagonistic effect of *L. camara* against RKN.

### Conclusion

The findings of the present study indicated that *Trichoderma viride* effectively combated RKN, leading to a notable reduction in the formation of root galls on Kiwifruit seedlings. The treatment with *Trichoderma viride* resulted in the lowest counts of eggs and juveniles, along with a minimized reproductive factor, thereby achieving the highest percentage of nematode control. The growth parameters of Kiwifruit seedlings also displayed improved performance under the *Trichoderma viride* treatment, coming in second only to the uninoculated control group.

Moreover, a significant positive correlation was identified between the Rf and the root gall index, while a negative correlation was observed between the Rf and the growth parameters of Kiwifruit seedlings. Consequently, the application of *T. viride* is recommended to mitigate the infestation of RKN on Kiwifruit seedlings.

### Limitations of the Study

This research is limited to a single season only. In addition, this research was carried out in greenhouse

condition; hence, further research is advised to implement the same treatments in a Kiwifruit orchard, aiming to ascertain their effectiveness under field conditions.

### Acknowledgement

The authors would like to express their sincere gratitude to the Agriculture and Forestry University (AFU); Prime Minister Agriculture Modernization Project (PMAMP), and Warm Temperate Horticulture Center (WTHC) for providing the internship opportunity along with valuable support and guidance during the research. We would like to thank Mr. Lekh Raj Dhakal, Agriculture Extension Officer; Asst. prof. Sujan Mishra, AFU, and Mr. Rajendra Koirala, Chief, National Center for Fruit Development, Nepal for their moral support and constructive feedback throughout the research project.

### Declarations

#### Author contribution statement

*Kapil Simkhada*: Conceived and designed the research, performed the research, analyzed, and interpreted data, wrote the paper.

*Srijana Bhandari*: Design the research, contributed to the materials and methods.

*Chiranjivi Sharma*: Analysis and interpretation of data, contributed to paper writing.

#### Data availability statement

Data will be made easily available on request.

#### Declaration of interest statement

The authors declare no conflict of interest in publishing this scientific paper.

### References

- Abd-El-Khair, H., El-Nagdi, W. M. A., Youssef, M. M. A., Abd-Elgawad, M. M. M., & Dawood, M. G. (2019). Protective effect of *Bacillus subtilis*, *B. pumilus*, and *Pseudomonas fluorescens* isolates against root knot nematode *Meloidogyne incognita* on cowpea. *Bulletin of the National Research Centre*, 43(1), 64. <https://doi.org/10.1186/s42269-019-0108-8>
- Abrar, S., Seid, A., & Dejene, M. (2020). Integrated management of *Meloidogyne incognita* in tomato (*Solanum lycopersicum*) through botanical and intercropping. *African Journal of Agricultural Research*, 15(4), 492–501. <https://doi.org/10.5897/AJAR2019.14040>
- Annual Progress Report. (2022). Warm Temperate Horticulture Center, Kirtipur, Kathmandu.
- Atreya, P. N., Shrestha, C. M., Subedi, B. D., & Pandey, S. P. (2020). Emerging Fruits of Nepal: Pomegranate, Kiwifruit, Avocado, Dragon Fruit, and Grape; Opportunities, Challenges and Ways Forward. *Nepal Horticulture Society*, 11.
- Baidya, S., Manandhar, S., & Manandhar, C. (2023). Study on some species of *Trichoderma* for the management of root knot nematode (*Meloidogyne* spp.) in tomato. *Journal of the Plant Protection Society*, 8, 49–59.
- Baños, Y. S., Concepción, A. D. B., Torres, I. P., González, I. A., Sanchez, M. R., Izquierdo, E. M., Sánchez, A. C., & Guanche, L. H. (2017). Biostimulant and Nematicidal Effect of *Trichoderma harzianum* Rifai and Aqueous Extract of *Azadirachta indica* A. Juss. In *Solanum lycopersicum* L. *Universal Journal of Agricultural Research*, 5(5), 251–256. <https://doi.org/10.13189/ujar.2017.050501>

- Bridge, J., & Page, S. L. J. (1980). Estimation of Root-knot Nematode Infestation Levels on Roots Using a Rating Chart. *Tropical Pest Management*, 26(3), 296–298. <https://doi.org/10.1080/09670878009414416>
- Chhetri, B. (2019). Nematode Fauna Associated with Kiwi (*Actinidia deliciosa*, Chev.) Plants in Machchhegaun, Kathmandu, Nepal. *Nepal Journal of Biotechnology*, 7(1), Article 1. <https://doi.org/10.3126/njb.v7i1.26951>
- Chitwood, D. J. (2002). Phytochemical Based Strategies for Nematode Control. *Annual Review of Phytopathology*, 40(1), 221–249. <https://doi.org/10.1146/annurev.phyto.40.032602.130045>
- Davis, R., Bertrand, P., Gay, J., Baird, R., Padgett, G., Brown, E., Hendrix, F., & Balsdon, J. (2009). *Guide for interpreting nematode assay results*.
- Druzhinina, I. S., Chenthamara, K., Zhang, J., Atanasova, L., Yang, D., Miao, Y., Rahimi, M. J., Grujic, M., Cai, F., Pourmehdi, S., Salim, K. A., Pretzer, C., Kopchinskiy, A. G., Henrissat, B., Kuo, A., Hundley, H., Wang, M., Aerts, A., Salamov, A., ... Kubicek, C. P. (2018). Massive lateral transfer of genes encoding plant cell wall-degrading enzymes to the mycoparasitic fungus *Trichoderma* from its plant-associated hosts. *PLOS Genetics*, 14(4), e1007322. <https://doi.org/10.1371/journal.pgen.1007322>
- Elad, Y., Chet, I., & Henis, Y. (1982). Degradation of plant pathogenic fungi by *Trichoderma harzianum*. *Canadian Journal of Microbiology*, 28(7), 719–725. <https://doi.org/10.1139/m82-110>
- Ferris, H., & Noling, J. W. (1987). Analysis and prediction as a basis for management decisions. *Principles and Practice of Nematode Control in Crops / Edited by R.H. Brown and B.R. Kerry*. [https://scholar.google.com/scholar\\_lookup?title=Analysis+and+prediction+as+a+basis+for+management+decisions&author=Ferris%2C+H.&publication\\_year=1987](https://scholar.google.com/scholar_lookup?title=Analysis+and+prediction+as+a+basis+for+management+decisions&author=Ferris%2C+H.&publication_year=1987)
- Feyisa, B., Lencho, A., Selvaraj, T., & Getaneh, G. (2015). Evaluation of Some Botanicals and *Trichoderma harzianum* for the Management of Tomato Root-knot Nematode (*Meloidogyne incognita* (Kofoid and White) Chit Wood). *Advances in Crop Science and Technology*, 04(01). <https://doi.org/10.4172/2329-8863.1000201>
- Hafiza, A. A. R., Khandaker, M. M., & Mat, N. (2016). Occurrence and control of root knot nematode in crops: A review. *Australian Journal of Crop Science*, 10(12), 1649–1654.
- Haran, S., Schickler, H., & Chet, I. (1996). Molecular mechanisms of lytic enzymes involved in the biocontrol activity of *Trichoderma harzianum*. *Microbiology*, 142(9), 2321–2331. <https://doi.org/10.1099/00221287-142-9-2321>
- Hashem, M., & Abo-Elyousr, K. A. (2011). Management of the root-knot nematode *Meloidogyne incognita* on tomato with combinations of different biocontrol organisms. *Crop Protection*, 30(3), 285–292. <https://doi.org/10.1016/j.cropro.2010.12.009>
- Hermosa, R., Viterbo, A., Chet, I., & Monte, E. (2012). Plant-beneficial effects of *Trichoderma* and of its genes. *Microbiology*, 158(1), 17–25. <https://doi.org/10.1099/mic.0.052274-0>
- Hooper, D. J., Hallmann, J., & Subbotin, S. A. (2005). Methods for extraction, processing and detection of plant and soil nematodes. *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*, 53–86. <https://doi.org/10.1079/9780851997278.0053>
- Hussey, R. S., & Barker, K. R. (1973). Comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. *Plant Disease Reporter*. [https://scholar.google.com/scholar\\_lookup?title=comparison+of+methods+of+collecting+inocula+of+Meloidogyne+spp.%2C+including+a+new+technique&author=Hussey%2C+R.S.&publication\\_year=1973](https://scholar.google.com/scholar_lookup?title=comparison+of+methods+of+collecting+inocula+of+Meloidogyne+spp.%2C+including+a+new+technique&author=Hussey%2C+R.S.&publication_year=1973)
- Kepenekci, İ., Dura, O., & Dura, S. (2017). Determination of Nematicidal Effects of Some Biopesticides against Root-Knot Nematode (*Meloidogyne incognita*) on Kiwifruit. *Journal of Agricultural Science and Technology A*, 7(8). <https://doi.org/10.17265/2161-6256/2017.08.004>
- Kubicek, C. P., Steindorff, A. S., Chenthamara, K., Manganiello, G., Henrissat, B., Zhang, J., Cai, F., Kopchinskiy, A. G., Kubicek, E. M., Kuo, A., Baroncelli, R., Sarrocco, S., Noronha, E. F., Vannacci, G., Shen, Q., Grigoriev, I. V., & Druzhinina, I. S. (2019). Evolution and comparative genomics of the most common *Trichoderma* species. *BMC Genomics*, 20(1), 485. <https://doi.org/10.1186/s12864-019-5680-7>
- Lawes, G. S., & Anderson, D. R. (1980). Influence of temperature and gibberellic acid on kiwifruit (*Actinidia chinensis*) seed germination. *New Zealand Journal of Experimental Agriculture*, 8(3–4), 277–280. <https://doi.org/10.1080/03015521.1980.10426272>
- McClure, M. A., Kruk, T. H., & Misaghi, I. (1973). A Method for Obtaining Quantities of Clean *Meloidogyne* Eggs. *Journal of Nematology*, 5(3), 230.
- Muthulakshmi, M., Devrajan, K., & Jonathan, E. I. (2010). Biocontrol of root knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood in mulberry (*Morus alba* L.). *Journal of Biopesticides*, 3(2), 479.
- Ntalli, N. G., & Caboni, P. (2012). Botanical Nematicides: A Review. *Journal of Agricultural and Food Chemistry*, 60(40), 9929–9940. <https://doi.org/10.1021/jf303107j>
- Santos, M. A. dos, Ferraz, S., & Muchovej, J. J. (1992). Evaluation of 20 Species of Fungi from Brazil for Biocontrol of *Meloidogyne incognita* Race 3. *Nematropica*, 183–192.
- Shamalie, B. V. T., Fonseka, R. M., & Rajapaksha, R. (2012). *Effect of Trichoderma viride and Carbofuran (Curator®) on Management of Root Knot Nematodes and Growth Parameters of Gotukola (Centella asiatica L.)* (1). 23(1), Article 1. <https://doi.org/10.4038/tar.v23i1.4632>
- Sharma, A., Thapa, S., & Khatiwada, M. P. (2020). Production, Marketing And Future Prospects of Kiwifruit in Nepal. *International Journal of Applied Sciences and Biotechnology*, 8(2), Article 2. <https://doi.org/10.3126/ijasbt.v8i2.29083>
- Siddiqui, I. A., Shaukat, S. S., Sheikh, I. H., & Khan, A. (2006). Role of cyanide production by *Pseudomonas fluorescens* CHA0 in the suppression of root-knot nematode, *Meloidogyne javanica* in tomato. *World Journal of Microbiology and Biotechnology*, 22(6), 641–650. <https://doi.org/10.1007/s11274-005-9084-2>
- Srivastava, M., Sharma, S., Siddique, N., & Aslam, M. (2006). Microbial active triterpene from *Lantana camara*. *Biosci. Biotechnol. Res. Asia*, 3, 505–507.
- Subedi, S. (2022). *Evaluation of host resistance and management practices of root knot disease (Meloidogyne spp.) of tomato under green house conditions at Hemja, Kaski, Nepal*. Agriculture and Forestry University, Bharatpur, Nepal.
- Subedi, S., Thapa, B., & Shrestha, J. (2020). Root-knot nematode (*Meloidogyne incognita*) and its management: A review. *Journal of Agriculture and Natural Resources*, 3(2), Article 2. <https://doi.org/10.3126/janr.v3i2.32298>
- Taye, W., Sakhujia, P. K., & Tefera, T. (2012). Evaluation of plant extracts on infestation of root-knot nematode on tomato (*Lycopersicon esculentum* Mill). *Journal of Agricultural Research and Development*, 2(3), 086–091.
- Terefe, M. (2015). Evaluation of nematicidal action of some bio-agents and botanicals for eco-friendly management of root-knot nematodes, *Meloidogyne incognita* on tomato. *Science, Technology and Arts Research Journal*, 4(3), Article 3. <https://doi.org/10.4314/star.v4i3.11>