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Evaluation of the Efficacy of Fluopyram and Cyclobutrifluram against *Meloidogyne incognita* on Cucumber under Greenhouse Conditions

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ABSTRACT

Background: Root-knot nematodes are considered the most prevalent pathogens worldwide due to their extensive host range, which includes approximately 200 hosts.

Methods: This study evaluated the impact of two nematicides, fluopyram and cyclobutrifluram, at a concentration of 400 ppm/liter of water against *Meloidogyne incognita* under greenhouse conditions during the spring season of 2025. Treatments were made every four weeks over two intervals. *M. incognita was* identified through morphological and molecular diagnosis.

Result: The adult female's perineal patterns and molecular diagnosis of RKN isolates identified as *Meloidogyne incognita*. The sequencing of the *M. incognita was* deposited at the GenBank under the accession number (PQ600915.1). The most effective application was fluopyram and cyclobutrifluram, which applied by hand injector, showed such a significant impact against gall index, the quantity of juveniles (J2) after application, the percent reduction of juveniles (J2), eggs, females, final population and reproduction factor with values of 0.33, 0.67, 31.67, 49.00, 98.48%, 97.58%, 108.333, 346.00, 0.667, 1.000, 140.667, 396.000 and 0.132, 0.381, respectively, as well as increased plant height (cm), fresh shoot weight (g), dry shoot weight (g), yield per plant (kg/m^2), root length (cm) and fresh root weight (g) with values of 364.33, 331.67, 416.67, 393.67, 106.33, 99.67, 13.45, 12.45, 33.667, 29.667 and 17.103, 16.287, respectively, compared to the control treatment.

Key words: Cucumber, Cyclobutrifluram, Flopyram, *Meloidogyne incognita,* Nematicides.

INTRODUCTION

The cucumber vegetable crop *Cucumis sativus* L. belongs to the family of Cucurbitaceae and can be cultivated in home gardens, open fields, or greenhouses in milder climes. Cucumber fruits contain sulfur, silicon, potassium, acid and sodium, producing substances that help keep human blood alkaline. It also contains manganese, fiber, magnesium and vitamins C, K and A (Mallick, 2022; Ettah *et al*., 2025). Furthermore, cucumbers are one of Iraq's most economically significant vegetables grown in greenhouses. Meanwhile, Iraq produced 195,924 tons in 2022 from an estimated 20,381 hectares of cropland (FAO, 2022).

Root-knot nematodes are the most prevalent pathogens globally, which has motivated researchers to focus on this pest due to its severe impact on plant production (El-Saadony *et al*., 2021). Among the many species of plant-parasitic nematodes (PPNs) is *Meloidogyne* spp., considered the most dangerous due to its large host range, with approximately 200 plant hosts (Khalil, 2013). *Meloidogyne* spp. are extremely difficult to control due to their enormous reproduction rate, rapid development time and sedentary and entophytic character. The RKNs feeding activities in the root system cause the formation of giant galls of various sizes on the root tissue (Shihab and Abood, 2019; Sumita and Vivekananda, 2024).

Several methods have been used to manage root-knot nematodes (RKN), including chemical nematicides, organic substances, botanical extracts, resistant varieties and biological agents (Yass *et al*., 2020; Ikram *et al*., 2025). Currently, the most common method for managing root-knot nematodes is the application of nematicides (Nnamdi *et al*., 2022). Over the past fifteen years, newly developed nematicides with the lowest toxicity to vertebrates have been introduced, including fluopyram, cyclobutrifluram, fluazaindolizine and fluensulfone. All these compounds contain a trifluoro (CF_3) group in their chemical structure (Umetsu and Shirai, 2020). Fluopyram has been demonstrated to exhibit acute toxicity against second-stage juveniles of *Meloidogyne incognita*, *M. javanica* and *Heterodera schachtii* (Kim *et al*., 2016; Oka and Saroya, 2019).

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Cyclobutrifluram is a newly developed nematicide and fungicide that can be applied to seeds and soil. Its action mechanisms were disclosed and classified in meetings by the Insecticide Resistance Committee. It functions against nematodes as a mitochondrial complex II electron transport inhibitor, specifically targeting succinate-coenzyme Q reductase (IRAC, 2023). However, it functions as a complex II succinate dehydrogenase inhibitor (SDHI), classified by the Fungicide Resistance Committee (FRAC, 2022). The purpose of this study is to investigate the effectiveness of two nematicides, fluopyram and cyclobutrifluram, in suppressing the root-knot nematode that infects cucumber plants cultivated in the greenhouse.

MATERIALS AND METHODS

Laboratory tests

Morphological diagnosis of *Meloidogyne incognita*

Adult females were used to identify the *Meloidogyne* species collected from greenhouse infested samples by applied the female perineal pattern which subjected to dissection of the back of females bodies by following these steps: A section of infected roots placed in 1% sodium hypochlorite solution (NaCl) and females dissected from roots under a dissecting microscope with the aid of needles and a scalpel, then a small drop of 45% lactic acid added to the females in a petri dish and a females bodies were pushed out of the drop in a small isthmus of lactic acid solution. Then the end of an eye scalpel was embedded into a petri dish and cut off the posterior of the female, then the body tissue was gently removed from the posterior section with a dissecting needle, the cuticle was trimmed into a square with the perineal pattern in the center. Finally, the perineal pattern was moved to a microscope slide and drop of glycerin was added then a cover slide was applied and sealed.

Molecular diagnosis of *Meloidogyne incognita*

The egg masses of *Meloidogyne incognita* were isolated from infested tomato roots collected from the greenhouse. Six homogenized groups were prepared for molecular identification, each consisting of ten randomly chosen infested root samples. Genomic DNA of *M. incognita* from six samples was extracted from 100 egg masses (10 from each root system) using the ABIOpure TM Total DNA extraction procedure (ABIOpure, USA). The extracted DNA is stored at -20°C until it is used for PCR reactions. Universal primers MF 28S rRNA and MR 28S rRNA were utilized for root-knot nematode identification, as detailed in Table 1. These primers were obtained from Macrogen, a South Korean company. The PCR mixture was prepared by combining the following components: Master Mix (12.5 µl), each forward and reverse primer (1 µl) at a concentration of 10 picomoles/µl, DNA extract (2 µl) and nuclease-free water (8.5 µl). The samples were then placed in a PCR device (Thermo Fisher Scientific, USA) and programmed according to the manufacturer's instructions. The PCR products of six samples were sent for Sanger sequencing and then analyzed using MEGA6 software. *Meloidogyne incognita* was identified and confirmed using sequencing techniques based on its 28S rRNA gene.

Greenhouse experiments

Determination of nematode population densities before planting

The greenhouse previously surveyed, where naturally infected tomato plants were identified in the summer of 2024 as being affected by *Meloidogyne incognita*, was selected for planting cucumbers. The greenhouse land was divided into three rows, each 60 cm wide and 30 meters long, with a 1-meter distance between each row. Each row was further divided into three sections, every 10 meters in length and each section contained three replications. soil samples were collected from each row to determine the population densities of *M. incognita*. Juvenile extraction was conducted by using the technique of Baermann sieve funnel, as described by Southey (1986). The suspension was collected and examined under a microscope and the density of juveniles was counted.

Table 1: The primers used for the diagnosis of *Meloidogyne* spp.

Species	Primers name	Sequence	Primer sequence	Size of product (bp)	Source
Meloidogyne spp.	MF 28S rRNA	F	GGGGATGTTTGAGGCAGATTTG	500	(Nunn 1992)
	MR 28S rRNA	R	AACCGCTTCGGACTTCCACCAG		

Table 2: List of nematicides used against root-knot nematode.

Trade names	Active ingredient	Mode of action	Manufacturer
Velum prime	Fluopyram 400 g/L SC	Inhibiting mitochondrial respiration in nematodes, specifically complex II of the respiratory chain	Bayer crop Science - Germany
Tymirium®	Cyclobutrifluram 450 g/L SC	Inhibiting mitochondrial complex II, specifically succinate dehydrogenase (SDH).	Syngenta - Switzerland

The impact of flopyram and cyclobutrifluram on root-knot nematode

The experiment was conducted in greenhouse conditions to evaluate the impact of two nematicides, fluopyram and cyclobutrifluram, against root-knot nematode during the growing season of February 1, 2025. The active ingredients and trade names of these nematicides are shown in Table 2, as well as the mode of action of fluopyram and cyclobutylfuran presented in Fig 1. Cucumber F1 hybrid plants (Cadiar) were transplanted four weeks after sowing in the raised nursery beds to the rows of greenhouse naturally infested with *Meloidogyne incognita* at an initial population density ranging from 1039 to 1096 J2/500 g soil. Each treatment contained 18 plants, six plants per replicate and the distance between each plant and the others was 50 cm. Conditions such as temperature, humidity and light were maintained within optimal ranges to support cucumber growth. Each nematicide was prepared at a concentration of 400 ppm/liter of water. Applications were made every four weeks over two intervals according to the designated treatment method for each experimental unit. Three application methods were tested for each nematicide as follows: Fertilizer tank: The prepared solution was transported *via* canals or pipelines into the irrigation system using a fertigation unit to deliver the solution from its source to the plants. Watering can: 30 ml of solution was applied manually as a drench to the base of the plant at transplanting using a watering can. Injector application: 30 ml of solution was injected directly into the soil near the root zone using a hand injector. The trial included seven treatments as illustrated in Fig 2.

Root gall index and reproductive factor parameters

Cucumber plants were carefully uprooted at the end of the growing season and the shoots were separated from the root system of three randomly chosen plants in each replication. The roots were washed to remove soil and other debris. The gall index was assessed using a scale by Taylor and Sasser (1978), as follows: 0 = no knots per root system; 1 = 1-2 knots; 2 = 3-10 knots; 3 = 11-30 knots; 4 = 31-100 knots and 5 = over 100 knots. The number of females within the root system was estimated by staining 1 g of root according to the method described by Bybd *et al*. (1983) (Fig 3).

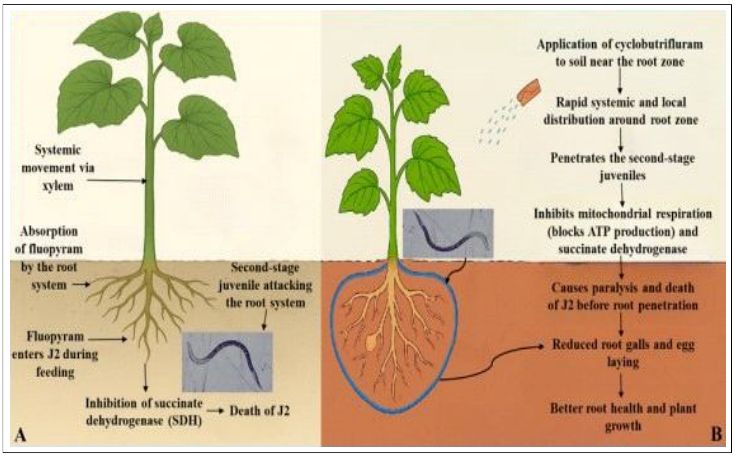


Fig 1: (A) Fluopyram mode of action and (B) cyclobutrifluram action mechanisms.

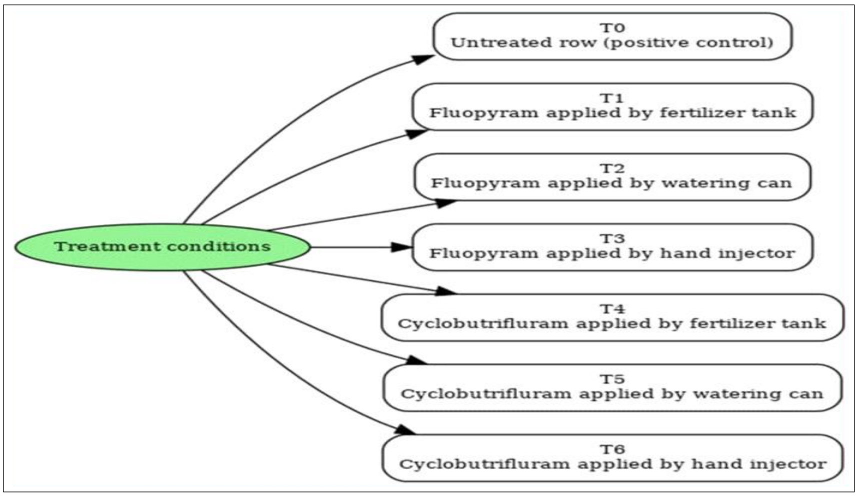


Fig 2: The trial treatments used in the current study.

The eggs were extracted using the approach described by Hussey and Barker (1973). The second-stage juveniles (J2) in the soil were extracted using the Baermann sieve funnel technique. The reduction of second-stage Juvenile (J2) in soil was calculated using the following equation (Handerson and Tilton, 1955). The final population of *Meloidogyne incognita* was calculated by adding the average number of eggs and females per root system to the average number of juveniles per 500 g of soil for each treatment. All stages of the root-knot nematode were observed, as illustrated in Fig 4. The reproduction factor (Rf) was estimated according to the following equation (Tedford and Fortnum, 1988).

Reduction% =

$$\left(1-\frac{\text{Density of J2 in treatment after application}}{\text{Density of J2 in treatment before application}}\times\frac{\text{Density of J2 in control before application}}{\text{Density of J2 in control after application}}\times 100\right)$$

$$\text{Reproduction factor}=\frac{\text{Final population density}}{\text{Initial population density}}$$

Plant growth and yield parameters

The data were collected from the same plants used to assess the gall formation index, the female count and the number of eggs within the root system for each replication. At the end of the growing season, observations on vegetative growth included measurements of plant height (cm), root length (cm), root fresh weight (g), shoot fresh weight (g) and shoot dry weight (g). Additionally, plant yield (Kg/m^2) was measured twice a week.

Statistical analysis

The data from the greenhouse experiment were analyzed using analysis of variance (ANOVA). The randomized complete block design (RCBD) was laid out. All trials included three replications, with three plants per replication. The data were analyzed using the statistical program GenStat 12 and the data were arranged using Excel. The significant differences between means were compared with the least significant difference (LSD) test at a significance level of 0.05.

RESULTS AND DISCUSSION

Morphological and molecular diagnosis of *Meloidogyne incognita*

The microscopic analysis of the morphology features of the adult female's perineal patterns revealed that all the samples had a distinct perineal pattern associated with *Meloidogyne incognita* (Fig 5). This pattern is characterized by presenting an elongated form accentuated by a prominent, square dorsal arch. This specific morphology closely corresponds with the descriptions documented in the literature regarding *Meloidogyne incognita* (Taylor and Sasser, 1983), highlighting its unique structural traits. The amplification of ribosomal DNA using universal primers MF and MR produced a specific fragment of 500 bp for the genus *Meloidogyne* in all samples derived from purified single egg mass cultures, as shown in Fig 6. All isolates of the root-knot nematodes were identified and documented using sequencing techniques based on their 28S rRNA genes, which revealed the presence of *Meloidogyne incognita*. The results of the sequencing of the *Meloidogyne incognita* isolate under the name Kh.M.11 was deposited at the National Center for Biotechnology Information (NCBI) under the accession numbers (PQ600915.1), where alignment with ten universal nucleotide sequences, obtained from the GenBank database, revealed 100% similarity to the *Meloidogyne incognita* isolate of South Africa, Ethiopia and Iran under the accession numbers (MF673762.1, KX752315.1 and KU380337.1) respectively, which belong to the same clade as illustrated in Fig 7. The morphological and molecular diagnostic results are consistent with those of Bastidas *et al*. (2019), who investigated morphological

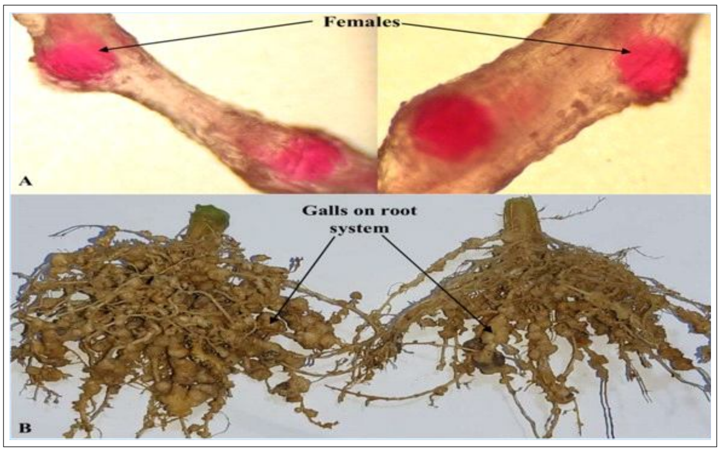
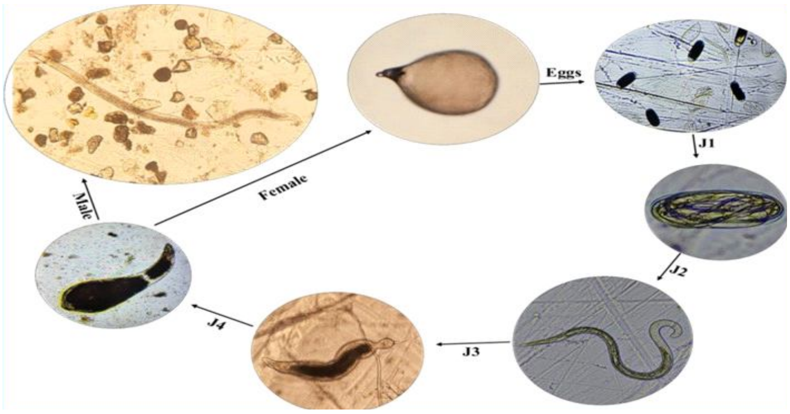


Fig 3: A: Root-knot nematode females within the root system stained by fuchsin acid dye observed under a compound light microscope 10X. B: Cucumber roots infected with root-knot nematodes.

investigated morphological characteristics and molecular methods to identify *Meloidogyne* species. The crop samples were identified through the morphological analysis of perineal patterns. However, DNA was extracted from these females and specific genes, as reported in the literature for different species, were amplified using universal primers MF/MR. These findings corroborate the morphological diagnosis of *Meloidogyne incognita*.

The effect on the root gall index and reproductive factor

The results presented in Table 3 reveal significant differences between nematicide treatments and the comparison treatment for gall index and second-stage juveniles (J2). The application of (T3) showed a substantial impact on the decrease of the gall index, the quantity of J2 in the soil after application and the percent reduction of J2 were in the ranges of 0.33, 31.67 and 98.48%, respectively,



The first-stage juvenile (J1) develops inside the egg and molts into the second-stage juvenile (J2) before hatching. J2 hatches from the egg and penetrates the plant root tissue, then develops through the third (J3) and fourth (J4) juvenile stages. Mature females have a pear-shaped body, while males may become vermiform and migrate toward the soil.

Fig 4: Life cycle stages of RKNs. The eggs are deposited in a gelatinous matrix on the plant root system.

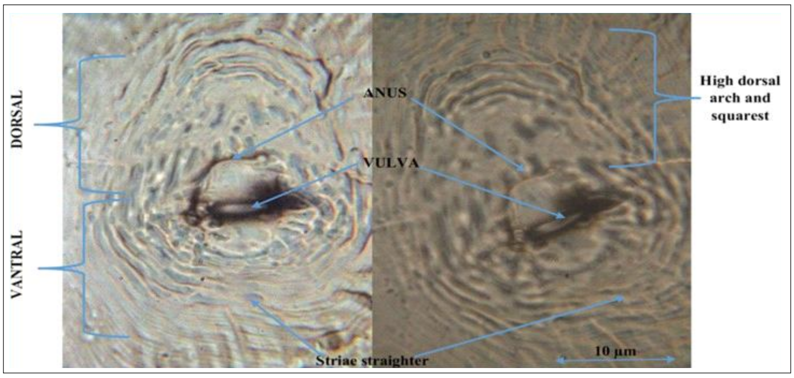


Fig 5: The perineal patterns microphotography obtained from root-knot nematode females of *Meloidogyne incognita*.

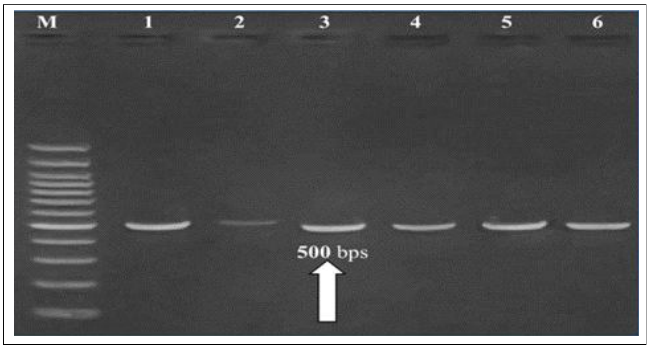


Fig 6: Agarose gel electrophoresis of PCR products for confirmatory molecular detection of *Meloidogyne* spp.

followed by (T6) and (T2) which reduced gall index and the number of J2 in the soil after application additionally to the percent reduction of J2 (0.67, 0.67, 49.00, 58.67 and 97.58%, 97.20% respectively) compared with control (T0). The least effect on reducing the gall index, the quantity of J2 in the soil after application and the percent reduction of J2 were observed in the (T4), with a mean value of 1.33, 124.67 and 94.11%, respectively. While the data in Table 4 showed significant differences between nematicide treatments and control. The two most successful applications (T3) and (T6) decreased eggs per root, females per root, final population and reproduction factor by 1.000, 108.333, 346.00, 0.667, 1.000, 140.667, 396.000 and 0.132, 0.381, respectively. However, (T4) and (T1) had the least activity, reducing the number of eggs per root, females per root, final population and reproduction factor to the ranges of 800.667, 666.667, 3.667, 2.000, 929.000, 750.667 and 0.856, 0.691, respectively.

A study by Schleker *et al*. (2022) demonstrated that fluopyram, a succinate dehydrogenase (SDH) inhibitor, is highly effective against plant-parasitic nematodes and has an excellent safety profile. By inhibiting SDH, fluopyram disrupts ATP generation, leading to paralysis in the plant-parasitic nematode and *Caenorhabditis elegans*. Continuous exposure to micromolar to nanomolar concentrations of fluopyram effectively prevents the infection and development of root-knot nematodes and *Heterodera schachtii* at the root level. Moreover, it significantly reduces the gall formation of RKN. Another study by Flemming *et al*. (2025) reported that Cyclobutri-fluram is a chiral molecule, particularly a phenyl-cyclobutyl-pyridine amide compound, belonging to the pyridine-3-carboxamide chemical class. has demonstrated broad effectiveness against all economically significant plant-parasitic nematodes and many soil-borne diseases, especially those caused by *Fusarium* spp. A study conducted by Mohammed and Abdel Reda (2021) revealed that Velum Prime at a concentration of 400 ppm reached a 100% decrease in the number of dead eggs and juveniles. Also, Velum Prime showed a substantial impact on the decrease in the number of root-knots per root system compared to the control treatment.

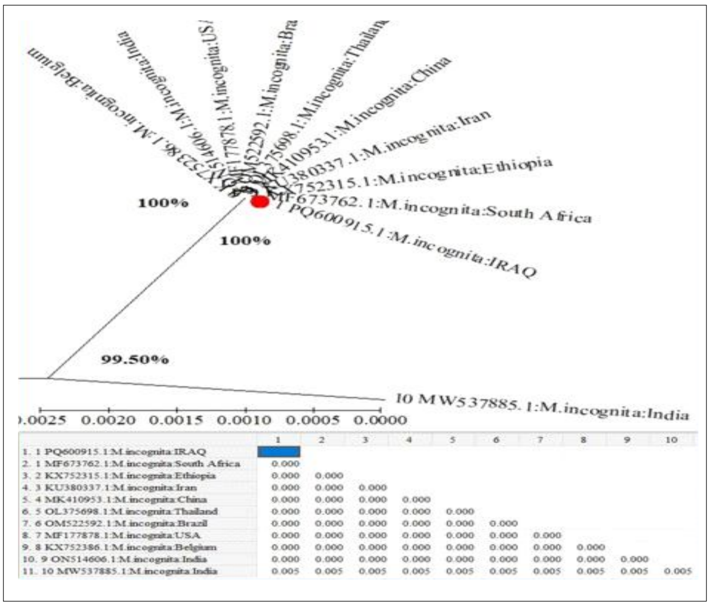


Fig 7: The evolutionary relationships of *Meloidogyne incognita* Kh.M.11 isolate and ten of their nearest phylogenetic relatives obtained from the Genbank database were determined for confirmatory analysis of *M. incognita*.

Table 3: The impact of fluopyram and cyclobutrifluram on gall index and second-stage juveniles in soil.

Treatment	Gall index	No. of Juveniles/500 g soil J2 before application	J2 after application	Reduction%
T0	5.00	1091.33	2130.00	0.00
T1	1.00	1086.33	82.00	96.13
T2	0.67	1072.00	58.67	97.20
T3	0.33	1066.00	31.67	98.48
T4	1.33	1084.67	124.67	94.11
T5	1.00	1096.67	79.00	96.31
T6	0.67	1039.00	49.00	97.58
LSD 5%	0.7924**	90.1**	225.4**	2.806**

**Means are averages of three replicates.

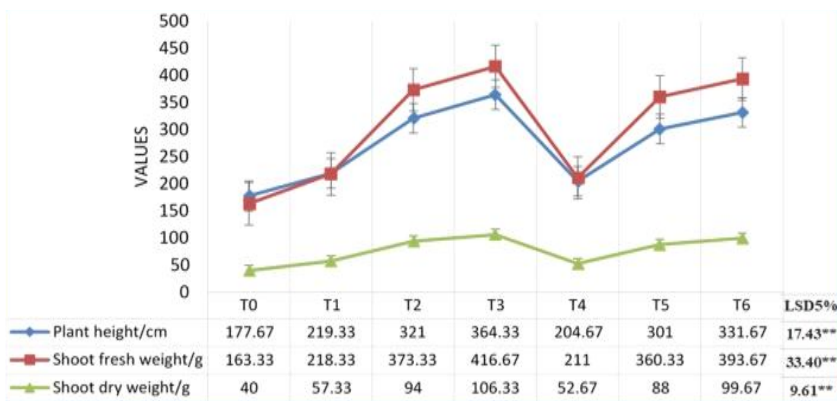
The effect on the plant growth and yield parameters

The data in Fig 8 indicated significant differences between nematicide treatments and the control. The most effective applications, (T3) and (T6), increased plant height (cm/plant), shoot fresh weight (g/plant) and shoot dry weight (g/plant) by 364.33 cm, 331.67 cm, 416.67 g, 393.67 g and 106.33 g, 99.67 g, compared with the control (T0). However, (T1) and (T4) with significant differences were observed compared to other treatments ($p \leq 0.05$) revealed the lowest plant height, shoot fresh weight and shoot dry weight with values of 219.33 cm, 204.67 cm, 218.33 g, 211.00 g and 57.33 g, 52.67 g, respectively, compared to other treatments. Whereas the data presented in Fig 9 showed that the application of two nematicides with different additions significantly increased the yield per plant, root length and root fresh weight compared to the control

Table 4: The impact of fluopyram and cyclobutrifluram on the reproductive factor of *Meloidogyne incognita.*

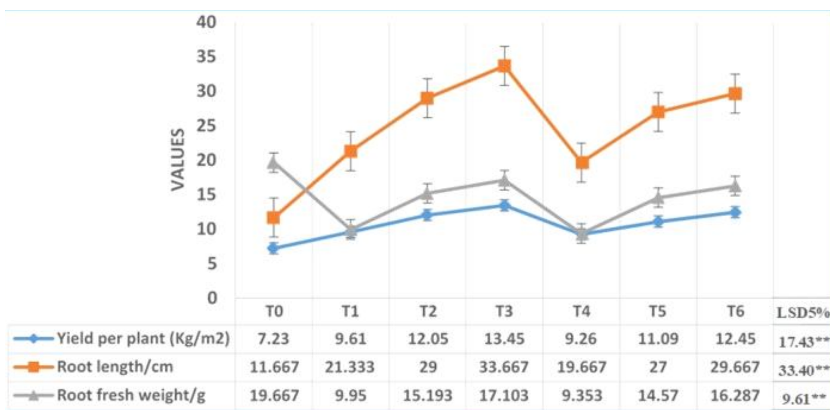
Treatment	Eggs /root	Females/root	Final population	Reproduction factor
T0	41598.0	173.000	43901.000	40.226
T1	666.667	2.000	750.667	0.691
T2	403.667	1.333	463.667	0.432
T3	108.333	0.667	140.667	0.132
T4	800.667	3.667	929.000	0.856
T5	447.667	1.667	528.333	0.482
T6	346.00	1.000	396.000	0.381
LSD 5%	3092.9**	17.07**	3094.8**	1.511**

******Means are averages of three replicates.



Means are averages of three replicates.

Fig 8: The impact of nematicides fluopyram and cyclobutrifluram on plant height, both fresh and dry shoot weight of cucumber plants under greenhouse conditions.



Means are averages of three replicates.

Fig 9: The impact of nematicides fluopyram and cyclobutrifluram on yield, root length and root fresh weight of cucumber plants under greenhouse conditions.

treatment, with significant differences. Both treatments of T3 and T6 led to the highest increase of the yield per plant, root length and root fresh weight with values of 13.45 kg, 12.45 kg, 33.667 cm, 29.667 cm and 17.103 g, 16.287 g, compared to the control (T0), except root fresh weight in the control treatment was the highest value (19.667 g), this increase is due to cell enlargement and the formation of galls that occur as a result of the interaction of root cells with chemicals secreted by nematodes. However, (T1 and T4) revealed the lowest value of yield per plant, root length and root fresh weight, which were in the range of 9.61 kg, 9.26 kg, 21.333 cm, 19.667 cm and 9.950 g, 9.353 g, respectively, compared to other nematicide treatments.

Nimgarri *et al*. (2024) used several nematicides for the management of RKNs in cucumber under a farmer's polyhouse. The most effective treatments were metham sodium, followed by fluopyram, which suppressed root-knot nematode parameters and increased plant yield. Hajihassani *et al*. (2019) conducted a study to evaluate the impact of four nematicides: oxamyl, fluopyram, fluensulfone and fluazaindolizine. All four nematicides effectively reduced the root gall index compared to the untreated control. Furthermore, regression analysis revealed no significant effect of nematode inoculation densities on the yield of cucumbers treated with these non-fumigant nematicides. Heydari *et al*. (2023) found that cyclobutrifluram significantly affects both the survival and fertility rates of the *Caenorhabditis elegans* by reducing the germ cells numbers. Additionally, they demonstrated through genetic analysis that cyclobutrifluram works by inhibited the mitochondrial succinate dehydrogenase (SDH) complex.

CONCLUSION

All treatments significantly decreased the gall index and final population as well as improved various growth parameters compared to the control treatment. The most successful applications, fluopyram and cyclobutrifluram applied by hand injector, showed such a significant impact on towered plant height (cm), shoot fresh weight (g), shoot dry weight (g), yield per plant (kg/m^2), root length (cm) and root fresh weight (g) compared to the control treatment. Additionally, the gall index and reproduction factor were decreased. The findings indicate that using fluopyram and cyclobutrifluram via hand injector could be a promising and sustainable method for reducing nematode damage and promoting cucumber growth. We recommend conducting additional studies to explore the mechanisms and modes of action of these nematicides.

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Conflict of interest

All authors declare that there is no conflict of interest.

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