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Management of Root-Knot Nematodes (*Meloidogyne* Spp.) in Cucumber Under Protected Cultivation System

Hekmatullah Nimgarri[1], Rahmatullah Nazir[2], Karamatullah Fazil[3] and Mirwais Yahyazai[4]

[1]Department of Plant Protection, Faculty of Plant Sciences, Afghanistan National Agricultural Sciences and Technology University (ANASTU), Kandahar, Afghanistan
[2,3]Department of Agronomy, Faculty of Plant Sciences, Afghanistan National Agricultural Sciences and Technology University (ANASTU), Kandahar, Afghanistan
[4]Center of Basic Sciences, Mathematics department, Afghanistan National Agricultural Sciences and Technology University (ANASTU), Kandahar, Afghanistan
✉Email: hekmatullah.n4@gmail.com (corresponding author)

ABSTRACT

Cucumber (*Cucumis sativus* L.) is a valued vegetable crop produced on a large scale throughout the year in a protected cultivation system. Its growth, development, and production are best in well-drained, fertile soil with 6.5 to 7.5 pH. Due to minor crop rotation and monoculture in protected structures, they are severely attacked by soil-borne pathogens. Between them, root-knot nematodes (*Meloidogyne* spp.) are the most intractable root endo-parasites that cause dramatic damage and severe crop yield losses. Environmental conditions of protected structures favor root-knot disease incidence and development. The non-availability of resistant crops, biological agents, and technology limitations are the key obstacles to their control. Therefore, agrochemicals are the only reliable method of root-knot disease management. In the present investigation, available nematicides (abamectin, metham sodium, fluopyram, and phorate 5G) were engaged in randomized block design with four replications at naturally infested farmer polyhouse (626.66 J2/ 200 cc soil) located at Mirwais Mina area of Kandahar city. Data on disease incidence, disease severity, soil nematode population, and fruit yield of cucumber were recorded 45 days after sowing the seed and at the termination of the field. All the data were statically analyzed using SPSS software (v.24.0.0). Among the treatments, metham sodium was found superior on plant and nematode parameters, followed by fluopyram. About 3.23 kg yield/plant loss was recorded caused by root-knot nematodes in protected cultivation systems. Based on findings, rotating fumigant and non-fumigant nematicides could be a reliable management method in a highly infested commercial protected structure.

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Introduction

Cucumber (*Cucumis sativus* L.) is a tropical vegetable crop cultivated worldwide in open fields and protected cultivation systems. Bearing in mind the valued vegetable crops of

Afghanistan, it is a key vegetable produced on a large scale in different parts of the country. Its production is limited to spring-summer in open fields, while it is made throughout the year in protected structures (Sadiq et al., 2019). The current production of cucumber is 171 t/ha and 30 t/ha in protected cultivation and open fields, respectively, in the country (Nimgarri et al., 2023). Its growth, development, and production are best in well-drained, fertile soil with 6.5 to 7.5 pH (Emede & Ehizogie, 2018). With minor crop rotation and monoculture in protected areas, they are severely attacked and badly affected by soil-borne pathogens and environmental stresses carefully managed in protected cultivation systems (Patil et al., 2017).

Root-knot nematodes (RKN) *Meloidogyne* spp. are the most intractable and dangerous root endo-parasites of various crops worldwide and occur mostly in Asian vegetable farms (Bui et al., 2022). The nature of their damage, the symptoms of plants, and few management options attract researchers to focus on these pests (Buttar et al., 2022). Considering the economically important nematodes, RKN is listed as the most vital one, while they are also viewed as among the most important biotic stresses to crop production, particularly to vegetable production in the tropics (Jones et al., 2013; Torto et al., 2018). The threat of RKN is based on their wide host range and the build-up of their species (Abad et al., 2003). Out of so far 101 described species, the riskiest are *M. incognita, M. javanica, M. arenaria,* and *M. hapla,* with 52%, 31%, 8%, and 7% incidence, respectively, in vegetable crops (Singh & Chahar, 2021; Tóth et al., 2019). Typical symptoms of infected plants are wilting, chlorosis, stunted growth, and root galls (Jones et al., 2013). The incidence of *Meloidogyne* species is 50-100% in tomato and up to 80% in cucumber under protected cultivation (Ami et al., 2018; Nimgarri et al., 2023). Polyphagous nature and wide host range are the key features of these species. Considering the overall nematode damage, 90% is caused only by root-knot nematodes (El-Marzoky et al., 2022). They cause up to 80% yield losses in different vegetable crops (Aydinli & Mennan, 2016). Cucumber yield losses caused by root-knot nematodes were reported ~35% in protected cultivation systems from Kandahar, Afghanistan (Nimgarri et al., 2023).

Managing RKNs in protected cultivation is vital; meanwhile, it could be riskiest for food security in a country due to crop yield losses. Laterally, with the availability of crop rotation, genomic upgrading, and bio-agents in developed countries, the use of pesticides is a method of selection worldwide. In countries where access to developed technology is restricted, and cost-benefit factors are of dominant importance, the use of pesticides is the first option for pest control according to its comfort of application and accessibility. (Desaeger & Csinos 2006). In addition, nematicides are the vital force of the integrated nematode management (NIM) program. Also, it is required in the commercial production of cucurbits in protected cultivation to prevent yield losses (Jiang et al., 2010). Furthermore, maximum population development and required improved farming production, the outdated scheme of nematode management is entirely dependent on nematicides (Chen et al., 2020; Eldeeb et al., 2022; Loubser & Deklerk, 2017; Maru & Patel, 2020). Therefore, the current study is made to clarify the best effect of fumigant and non-fumigant available nematicides against root-knot nematodes and the prevention of yield losses in naturally infested polyhouse in Kandahar province, Afghanistan.

Problem Statement

Recently, cucumber production in polyhouses has widely increased in Afghanistan as the farmers obtain maximum yield and returns. The country is self-sufficient in cucumber production and exports to neighboring countries. However, there are still challenges to lowering its yield potential and limiting the production area. Root-knot nematode is one of the most dangerous soil-borne pathogens. It is also known as the worst biotic stress in polyhouse conditions, limiting proper crop growth and maximum yield loss.

Research Questions

1. Is chemical nematicide effectively control the root-knot nematode?
2. What is the Efficacy variation among the selected nematicide?

Methods and Materials

A polyhouse experiment was led out at a farmer polyhouse located at Mirwais Mina area of Kandahar city situated on 31° 37′ 52″ N, 65° 38′ 45″ E. Total area of the polyhouse was 200 m^2 shielded with 200-micron thick stabilized low-density polyethylene and aluminate sheets. Entirely 5 treatments including (T_1) atomic (abamectin 2% SC) @ 1 cc/lit., (T_2) metham sodium Aria 32.7% SL @ 200 lit./ha, (T_3) velum prime (fluopyram 400 SC) @ 1cc/lit., (T_4) phorate 5G 10% GR @ 1.5 kg a.i./ha and (T_0) untreated control were engaged in a randomized block design (RBD) with four replications.

Soil samples were taken using an Oak-land tube 2.5 cm in diameter. Core samples were mixed thoroughly to prepare a composite sample, then picked in polythene bags, labeled, and carefully brought to the Afghanistan National Agricultural Sciences and Technology University (ANASTU) plant pathology laboratory. Samples were kept at 4 °C in a refrigerator before processing.

The extraction of nematodes from the soil was accompanied by Cobb's sieving and decanting method (Cobb's, 1918), followed by Baermann's assembly technique (Christie & Perry, 1951; Bezooijen, 2006). Estimation of J_2s was carried out for five ml suspension and then was calculated for 200 cc soil. Root-knot nematode species were identified based on cutting the perineal pattern of mature adult females using standard perineal pattern charts for comparison (Eisenback et al., 1991).

Plant samples were taken by gently uprooting plants from the field randomly. Uprooted plants were placed in polythene bags, labeled, and carefully carried to the lab.

The land of the polyhouse was plowed, and big clods were crushed. Rows were prepared 30 cm raised above ground level. The unite row net size was 5.5 m x 1.4 m. Standard cultivation procedures were followed to grow cucumbers in the polyhouse. The required amount of fumigant nematicide (Wapam) was applied 25 days before sowing the seeds, and the area was kept close to prevent the evaporation of chemicals. Cucumber Nagene 792 F1 hybrid seeds were planted in the experimental plots, maintaining row-to-row and plant-to-plant space of 140cm and 30cm, respectively. All the remaining treatments were applied after germination of seedlings. During crop season, required irrigation, weeding, and related intercultural operations were made based on the recommendation of the crop (Maitra et al., 2020).

Root-knot disease incidence and severity were recorded 45 days after sowing (DAS) and at the end of the season. Ten plants per row were gently uprooted and carefully cleaned with tap water. Calculation of disease incidence was done using the following formula (Elsharkawy et al., 2022):

$$\text{Incidence} = (\%) \quad \frac{\text{Number of samples containing juveniles}}{\text{Number of samples collected}} \times 100$$

Disease severity was calculated based on 0-5 scale, where 0= (no galls), 1= (0-1 gall/root system), 2= (2-10 galls/root system), 3= (11-30 galls/root system), 4= (31-40 galls/root system) and 5= (>100 gall/root system)(Nimgarri et al., 2023). Data on fruit yield were recorded from five randomly tagged plants per row, and the final fruit yield was expressed in kg/plant.

All the data were subjected to one-way analysis of variance (ANOVA), and multiple comparisons among the treatments were conducted using Duncan's multiple range test (DMRT) at $p < 0.05$. Two samples of the t-test were performed for chemical checks and untreated control treatments to realize the significant yield variations.

Results and Discussion

Significant variation was observed among all the treatments in increasing cucumber yield, suppressing soil nematode population, disease incidence, and disease severity at both periods of data records. The maximum fruit yield/plant was recorded by T_2 (4.45 kg/plant) next to (T_0) chemical check treatment (5.94 kg/plant), and the least fruit yield per plant was observed in the untreated control treatment (2.71 kg/plant). All the treatments were significantly higher than the untreated control. T_2 recorded a substantially higher yield than other treatments, followed by T_1 and T_3 (Table 1). The increase in cucumber yield could be attributed to the direct effect of blocking egg hatching and mortality of active juveniles (Huang et al. 2014; Qiao et al. 2012). Giannakou et al. (2002) reported that metam sodium can suppress root-knot nematode populations in cucumber plants' soil and roots. Regardless of protection against root-knot nematodes, the application of metam sodium recorded a 58% increase in fruit produce of cucumber (Jiang et al., 2010; Parmar et al., 2018; Thies et al., 2005). At the end of the season, highly infested roots of cucumber and weeds were cut off by small pieces and stained with acid-fuchsin stain. Semi-permanent slides were prepared using the perineal pattern of adult mature females. Confirmation of root-knot nematode species (*Meloidogyne incognita*) was done through a cut of a mature adult female perineal pattern by comparing with standard charts (Eisenback et al., 1991).

Soil nematode population was significantly suppressed with the application of T_1 (58.68%) compared to all other treatments, followed by a chemical check (55.35%) and T_2 (53.31%) at 45 DAS. The untreated control treatment detected maximum J2s (806.663 J2s/200 cc soil). At the end of the season, the highest percent reduction was recorded by T_2 (70.88%), followed by T_3 (55.09%) next to chemical check treatment (75.79%). The maximum soil nematode population was recorded by untreated control treatment (949.998 J2s/200 cc soil) (Table 2). Variability of effects of abamectin, metam sodium, and fluopyram was reported in different crops. Suppression of soil nematode population could be attributed to adequate product volatility and good chemical distribution in the soil (Huang et al., 2014; Thies et al., 2005).

Maximum disease incidence was recorded by untreated control treatment at both observation periods, 85% and 95%, respectively. No significant variations were observed between T_2 and T_3 at 45 DAS. At the end of the season, the maximum reduction in disease incidence was achieved by applying T_2 (55.26%) followed by T_3 (42.11%) over untreated control treatment. In general, the application of T_2 was superior to other treatments (Table 3). In the polyhouse, self-sown weeds belonging to Amaranthaceae were found 100% infested (Plate 1) by root-knot nematode (*M. incognita*).

Regarding the polyphagous pest, *M. incognita* can attack many weeds (Ahmad et al., 2015; Charabadiyan et al., 2022). The presence of host weeds in the field aggravates the harmful potential of the pest. Generally, all the treatments positively impacted the mortality of active J_2s of RKN in the soil. Therefore, the roots of plants could be saved from the attack and adverse effects of pathogens (Yucel et al., 2009).

T3 (72.86%) application recorded the highest disease severity reduction, followed by T_2 (68.57%) at 45 DAS. While at the end of the season, T_2 was found superior (25.75%) over other treatments. The lowest disease severity was 4.5% and 15.5% in chemical check treatment at 45 DAS and at the end of the season, respectively. However, the highest disease severity was 71%, recorded at the end of the season in the untreated control treatment (Table 4). A hundred percent severity of root-knot disease was recorded in self-sown weeds (*Chenopodium* spp.) in cucumber fields (plate 1). Infestation of root-knot nematodes in weeds hosts, including weeds from the Amaranthaceae family, was reported based on gall index of 1-5 from different regions of the world (Bakr & Mahdy, 2021; Charabadiyan et al. 2022; Ismael & Mahmood, 2020; S. K. Singh et al., 2010). However, in the case of low population density, disease incidence and severity could be lower compared to high population density in soil. Systemic and broad-spectrum nematicides can target the active juveniles in plants' soil and root systems (Huang et al., 2014; Thies et al., 2005; Zasada et al., 2010).

Table 1: efficacy of treatments on fruit yield of cucumber infested with *M. incognita*

Treatments	Fruit Yield of Cucumber	
	Yield (Kg/plant)	(%) increase over control
(T_1) Atomic (Abamectin 2% SC)	3.95[c]	45.78
(T_2) Metam Sodium 32/7% SL	4.45[b]	63.95
(T_3) Velum Prime (Fluopyram 400 SC)	3.38[d]	24.51
(T_4) Untreated control	2.71[e]	-
(T_0) Chemical Check (Phorate 10% GR)	5.94[a]	-

DAS= Day After Sowing, Hrt= Harvesting

Values in a column followed by the same letter are not significantly different at ($p < 0.05$).

Table 2: Efficacy of treatments on soil nematode (*M. incognita*) population

Treatments	Soil population of *M. incognita*			
	45 DAS	(%) reduction over control	At Hrt.	(%) reduction over control
(T_1) Atomic (Abamectin 2% SC)	473.33[b]	58.68	339.99[bc]	64.21
(T_2) Metam Sodium Aria 32/7% SL	376.66[b]	53.31	276.66[c]	70.88
(T_3) Velum Prime (Fluopyram 400 SC)	483.33[b]	4.97	426.66[b]	55.09
(T_4) Untreated control	806.66[a]	-	949.00[a]	-
(T_0) Chemical Check (Phorate 10% G)	446.50[b]	55.35	229.99[c]	75.79

DAS= Day After Sowing, Hrt= Harvesting *Pi* (Initial Nematode Population): 626.66 J2s/200 cc soil

Values in a column followed by the same letter are not significantly different at ($p < 0.05$).

Table 3: Efficacy of treatments on root-knot disease incidence in cucumber

Treatments	Root-Knot Disease Incidence (%)			
	45 DAS	(%) reduction over control	At Hrt.	(%) reduction over control
(T_1) Atomic (Abamectin 2% SC)	70.00[ab]	17.65	65.00[b]	31.58
(T_2) Metam Sodium Aria 32/7% SL	45.00[bc]	47.06	42.50[cd]	55.26
(T_3) Velum Prime (Fluopyram 400 SC)	45.00[bc]	47.06	55.00[bc]	42.11
(T_4) Untreated control	85.00[a]	-	95.00[a]	-
(T_0) Chemical Check (Phorate 10% G)	35.00[c]	58.82	32.50[d]	65.79

DAS= Day After Sowing, Hrt= Harvesting

Values in a column followed by the same letter are not significantly different at ($p < 0.05$).

Table 4: Efficacy of treatments on root-knot disease severity on cucumber

Treatments	Root-Knot Disease Severity (%)			
	45 DAS	(%) reduction over control	At Hrt.	(%) reduction over control
(T_1) Atomic (Abamectin 2% SC)	22.50^{b}	35.71	45.00^{b}	36.62
(T_2) Metam Sodium Aria 32/7% SL	11.00^{bc}	68.57	25.75^{cd}	63.73
(T_3) Velum Prime (Fluopyram 400 SC)	9.50^{c}	72.86	30.50^{c}	57.04
(T_4) Untreated control	35.00^{a}	-	71.00^{a}	-
(T_0) Chemical Check (Phorate 10% G)	4.50^{c}	87.14	15.50^{d}	78.17

DAS= Day After Sowing, Hrt= Harvesting

Values in a column followed by the same letter are not significantly different at ($p < 0.05$).

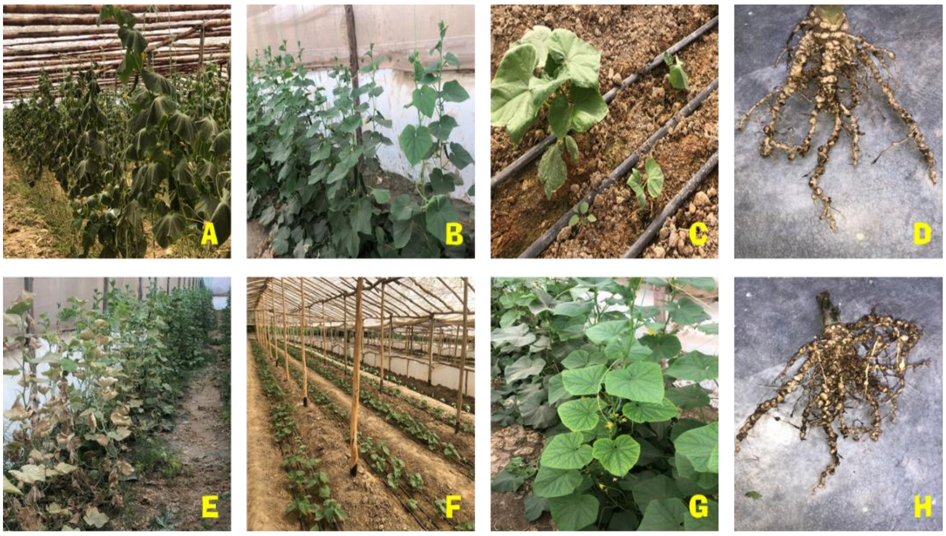

Plate 1: Symptoms of the experimental field.: A: Wilting of cucumber plants infected by *M. incognita.* B: fifty days old cucumber plants in polyhouse. C: primary infection of *M. incognita* and stunted growth of cucumber plants. D and H: Damage nature of *M. incognita* on roots of *Amaranthus* spp. E: Death of cucumber plants infected with *M. incognita*. F: 13-day-old cucumber plants in the field. G: Chlorosis of cucumber leaves infected with *M. incognita*.

Conclusion

The study suggests that under protected conditions, applying metam sodium and velum prime at random rotation and tested rates effectively controls root-knot nematodes in cucumber cultivation. It emphasizes the need for further research to enhance the application methods within protected structures, indicating a potential avenue for optimizing nematode management strategies in commercial settings.

Metam sodium emerges as a potent, broad-spectrum fumigant pesticide with significant potential in effectively managing root-knot nematodes. Caution is warranted due to its potential to induce abnormalities in soil texture and disrupt nutrient absorption upon repeated use. Justifying these concerns, a reasonable approach involves incorporating it into a rotation strategy with non-fumigant nematicides like velum prime and abamectin. This diversified approach enhances nematode control and minimizes the adverse effects on soil quality.

It's crucial to underscore that all pesticides, including metam sodium and velum prime, should be applied meticulously, following label directions, adhering to appropriate doses, and considering the optimal timing for specific crops. Failure to comply with these guidelines could compromise efficacy and incur significant costs, rendering it an expensive venture for farmers. Therefore, a balanced and well-informed application of these pesticides is essential to ensure effective pest management and economic viability for agricultural practitioners.

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