

## Research Article

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# Evaluation of 11 potential trap crops for root-knot nematode (RKN) control under glasshouse conditions

<https://doi.org/10.1515/opag-2022-0074>

received October 30, 2021; accepted February 22, 2022

**Abstract:** A preliminary study of the development and growth of lettuce crops trap cropped with 11 trap crops for root-knot nematode (RKN) control was studied under glasshouse conditions in Kadoorie Agriculture Research Centre in Palestine Technical University. Main crop growth parameters were evaluated by measuring the shoot height, lateral root length, wet and dry weight, ash percentage, and chlorophyll content. The total RKNs recovered from both main and trap crops from root systems were counted 6 weeks post RKN artificial inoculation. The first screening of the potential trap crops against RKNs showed a significant reduction in nematode numbers in lettuce and the average number of galls per gram fresh root when trap cropped with canola, B.G. pumpkin, mustard, and vetch cv. 976. No eggs were found by the end of the experiment. These trap crops attracted more nematodes and kept them from infecting the main crop. The effect of these trap crops on the growth and development of the main crop was variable. Trap cropping lettuce with canola, mustard, Armenian cucumber, and bottle gourd pumpkin caused a significant reduction of the total wet weight and dry weight compared with the control. Still, trap cropping did not significantly affect the lateral root length and wet weight. Removing the trap crops from the field after 6–8 weeks could be applied to reduce the competition between the main crop and the trap crops.

**Keywords:** trap cropping, artificial inoculation, *Meloidogyne* spp.

## 1 Introduction

Root-knot nematodes (RKNs) belong to the genus *Meloidogyne* and are the most economically important obligate polyphagous plant parasites. They cause significant damage worldwide to almost every crop and result in billions of dollars of losses annually [1]. They are also vectors for many fungal, bacterial, or viral diseases [2]. They increase root perfusion, malformations, and galls, affecting microbial communities and activity in the rhizosphere [3]. RKNs are one of the significant problems faced by Palestinian growers and farmers. They cause severe economic losses in most crops, especially tomatoes, cucumbers, beans, and other vegetables. RKN management is challenging; farmers use several control methods such as nematicides, fumigants, crop rotation, solar sterilization, organic amendments, resistant crop cultivars, and biological control. But still, none was proven to be an efficient method to achieve adequate control results [4]. Therefore, integrated pest management, using a combination of nematicides with other control approaches, is highly recommended to reduce potential pesticide residual toxicity to humans and the environment [5].

Trap or cover cropping strategy focuses on using an alternative host crop to either attract, interrupt, or capture targeted pests to reduce their damage to the main crop. The potential use of trap crops grown during the same periods between the main crop plants to suppress RKNs may be a better alternative to chemical control. These crops tend to attract nematodes more than the crop cultivated for production. Lettuce *Lactuca sativa* L. is one of the most important vegetable crops grown in all temperate regions worldwide, and it is one of the essential salad crops. RKN *Meloidogyne hapla* Chitwood attacks lettuce roots and causes substantial economic losses in production, reaching up to 60%, reducing plant growth, and producing smaller and unmarketable lettuce heads [6]. This research aimed to monitor the effects of using different trap crops on the growth and development of the main crop and nematode infection.

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## 2 Materials and methods

### 2.1 Crop and trap crop maintenance

11 experiments were carried out using various test plants and isolates of *Meloidogyne* spp. The plants examined were obtained from Palestine National Research Center (NARC): canola (*Brassica napus* L. cv. Hyoola 201); bitter vetch (*Vicia ervilia* L. cv. 1816); mustard (*Brassica juncea* L.), sesame (*Sesamum indicum* L. cv. 202); Armenian cucumber (*Cucumis melo* var. *flexuosus* L.); bottle gourd pumpkin (*Lagenaria siceraria* L.); sorghum (*Sorghum bicolor* L.); vetches (*Vicia* spp. cv. 976); local vetches (*Vicia* spp.); local coriander (*Coriandrum sativum* L.) and sesame (*Sesamum indicum* L. cv. 157). In all experiments, lettuce (*Lactuca sativa* L. cv. Noga) was used as a nematode-susceptible control. 10 days post sown day, the 11 trap crop seedlings listed above were transferred in 3 kg soil pots. The growing soil was a mixture of peat moss, coconut and compost (1:1:2). The pots were maintained under glasshouse conditions at  $25 \pm 5^\circ\text{C}$ , 75% R.H. and 18:6 L:D. Seedlings were irrigated every other day and fertilized every other week for 6 weeks after the planting. Then, lettuce seedlings were transplanted to each pot incorporated with a trap crop. Each treatment was replicated four times. When the lettuce was at the four-leaf stage, the seedling was inoculated with eggs and juveniles of *Meloidogyne* spp.

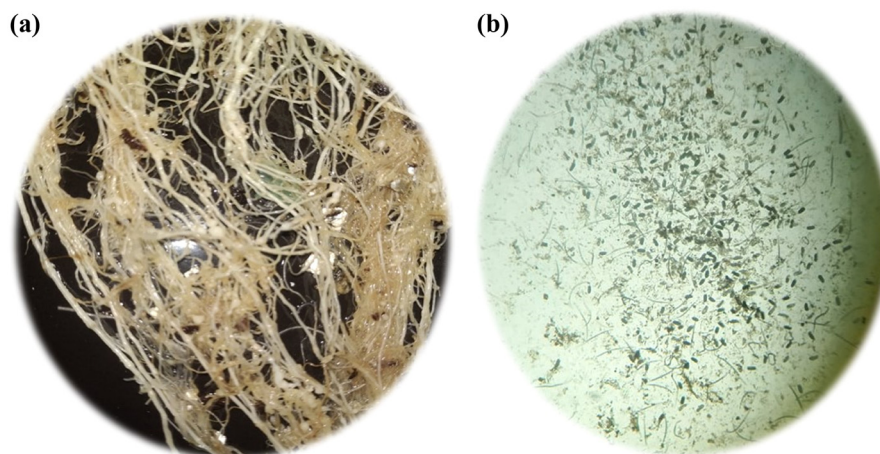
### 2.2 Isolation and inoculation of RKNs

Infected tomato plants with RKNs (Figure 1a) were maintained in the Kadoorie Agriculture Research Center's

glasshouse in Palestine Technical University – Kadoorie. The nematode suspension was extracted from infected tomato roots in 0.525% NaOCl following the procedure described by Hussey and Barker [7]. Each replicate received 20 mL of suspension containing  $\approx 6,000$  eggs and 400 viable nematodes (Figure 1b). The artificial inoculation was carried out in the trap crops and lettuce after 14 days from the sowing date. After 6 weeks, the number of galls, eggs, and juveniles per seedling was counted as mentioned above. Plant growth parameters were evaluated 6 weeks post artificial infection. When lettuce and trap crops were harvested, the lettuce shoot wet weight, dry weight, and root weight were recorded (Figure 2). Lettuce ash percentage was determined on a sample of 3 g each by igniting in a muffle furnace at  $550^\circ\text{C}$  for 8 h (Megatherm high-temperature chamber furnaces with  $\text{MoSi}_2$  heating elements [ $1,500\text{--}1,750^\circ\text{C}$ ]). The number of leaves and root length were recorded by the end of the experiment, while the level of chlorophyll content was monitored weekly for 3 weeks. The chlorophyll content of lettuce leaves was measured using soil plant analysis development (SPAD chlorophyll meter) (SPAD-502 plus, Konica Minolta Co., Ltd, Japan), which measures the radiation transmission within the leaf at the wavelengths between 650 and 940 nm. During this experiment, an average of three readings per leaf was recorded weekly.

### 2.3 Data collection and analysis

Collected data spreadsheets were analyzed using the analysis of variance test using the general linear model's procedure. Levels of significance were determined by



**Figure 1:** Infected tomato root plants with RKNs (a) maintained in the Kadoorie Agriculture Research Center's glasshouse in Palestine Technical University – Kadoorie. Extracted viable juveniles and eggs (b).

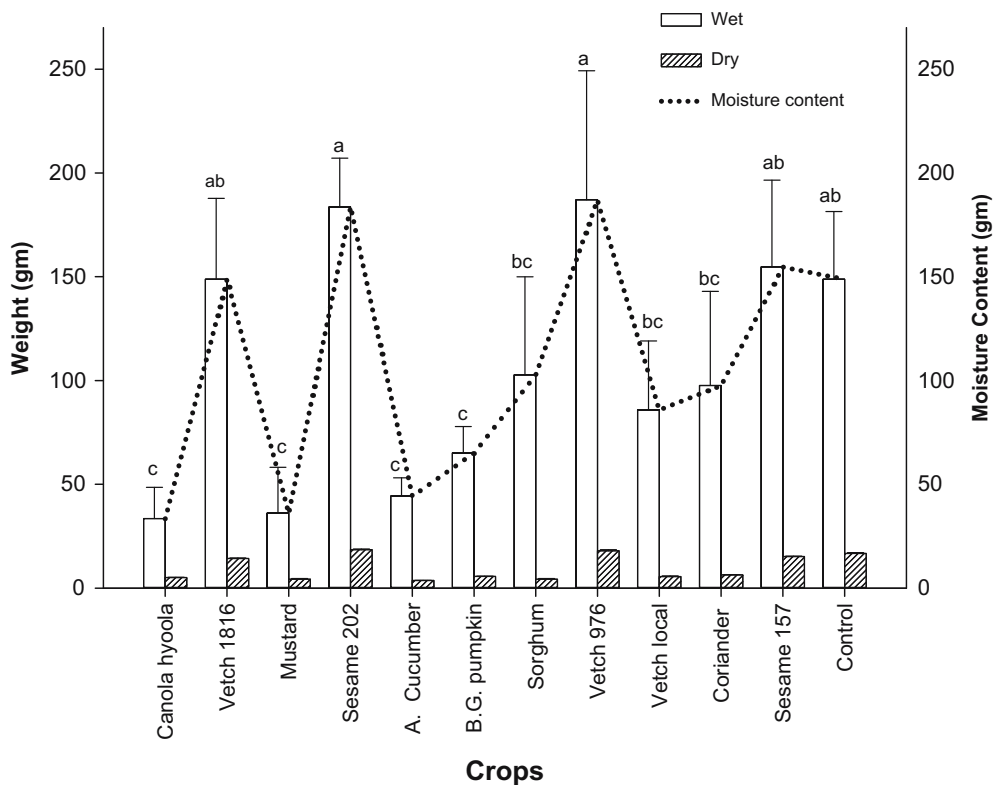
applying the Student–Newman–Keuls (SNK) method, all statistical analyses were performed using the Statistical Analysis System (SAS) (SAS Institute 2009). Figures and diagrams were carried out using the SigmaPlot 14 system.

### 3 Results and discussion

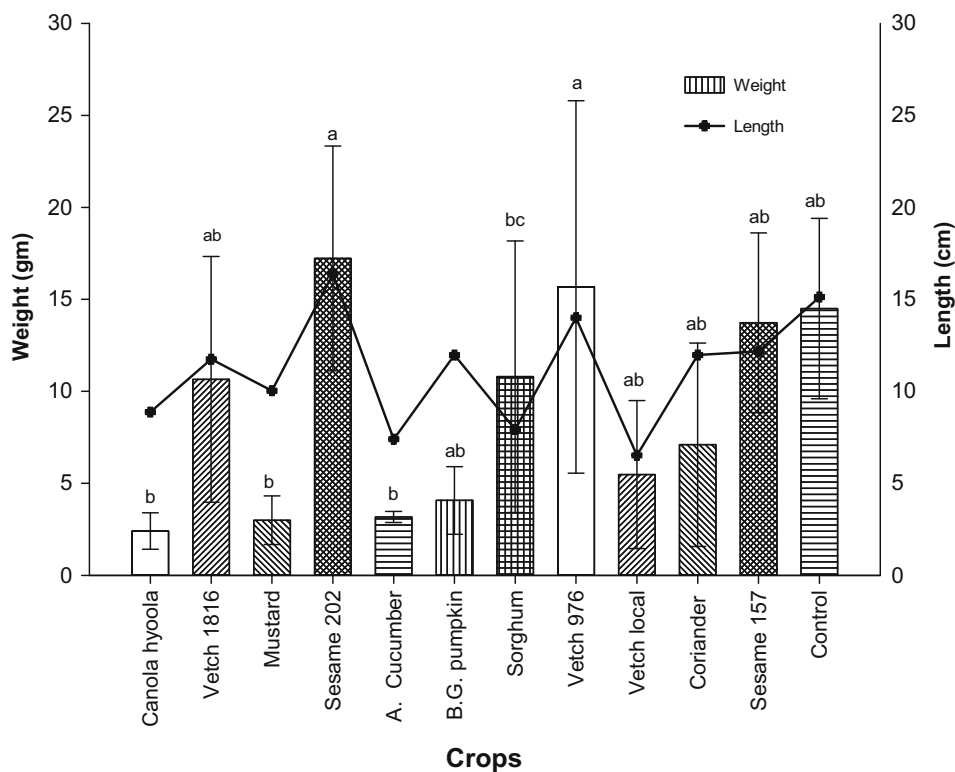
Trap cropping lettuce with 11 trap crops pre-artificially inoculated with RKN significantly impacted the lettuce wet weight and dry weight (Figure 3). Trap cropping



**Figure 2:** Comparison of lettuce plants’ vegetative and root growth post-artificially infested with *Meloidogyne* spp. Lettuce trapped with (a) sorghum (*S. bicolor*), (b) mustard (*B. juncea*), and (c) control.



**Figure 3:** Effects of artificial inoculation with *Meloidogyne* spp. and 11 trap crops on shoot fresh and shoot dry weight (g/plant) of lettuce plants (left-axis) and moisture content (g/plant) (right-axis). The standard deviation of the means is designated by the error bar for each mean. Means followed by the same letter are not significantly different (SNK,  $P > 0.05$ ).



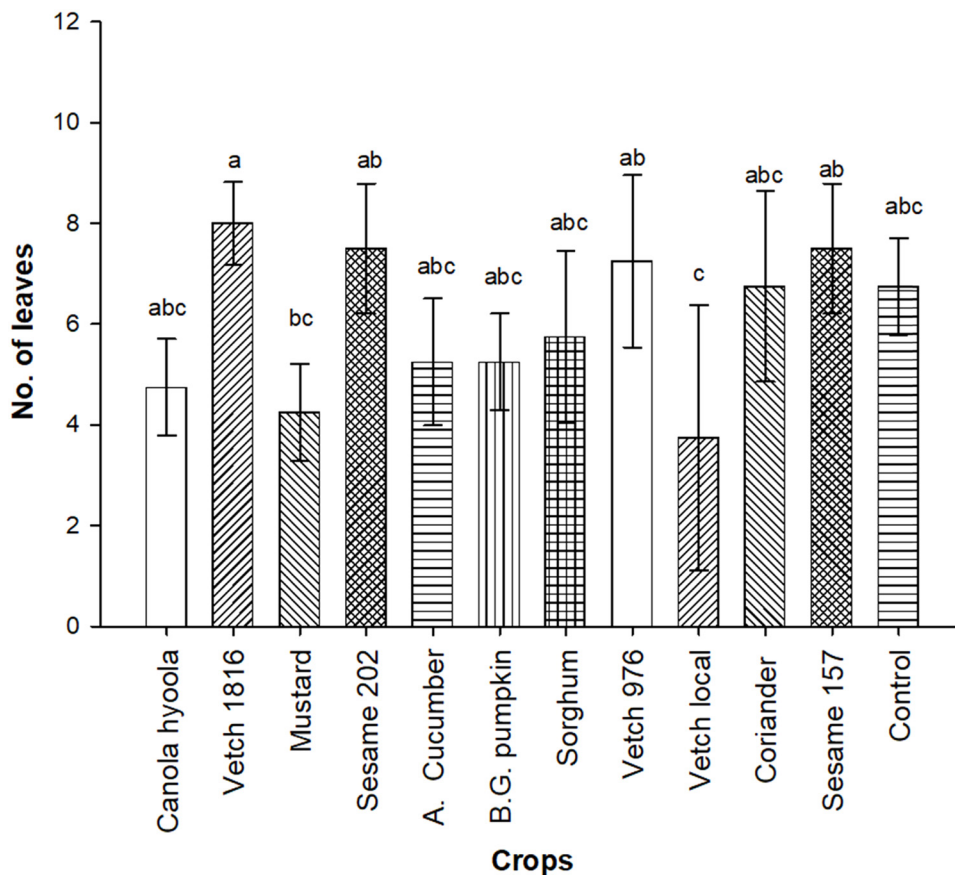
**Figure 4:** Effects of artificial inoculation with *Meloidogyne* spp. and 11 trap crops on fresh root weight (g/plant) (left-axis) and root length (cm/plant) (right-axis) of lettuce plants. The standard deviation of the means is designated by the error bar for each mean. Means followed by the same letter are not significantly different (SNK,  $P > 0.05$ ).

canola, mustard, Armenian cucumber and bottle gourd pumpkin caused a significant reduction of the wet lettuce weight (34, 36, 44, and 65 g, respectively) at ( $F = 10.5$ ;  $P < 0.0001$ ) and the dry weight (5, 4, 4, and 6 g, respectively) at ( $F = 197.6$ ;  $P < 0.0001$ ) compared with the control (148 and 17 g). The root wet weight (4, 3, 3, and 4 g, respectively) at ( $F = 3.7$ ;  $P = 0.0015$ ) and the root length were not significantly affected with trap cropping ( $F = 4.0$ ;  $P = 0.0008$ ) and the artificial inoculation of nematodes (Figure 4). Lettuce growth parameters were measured in an average number of leaves (Figure 5) and ash percentage (Figure 6). The ash percentage measured the total amount of minerals in the main crop after removing water and organic substances. There is no significant effect on viability in the average number of lettuce leaves ( $F = 3.7$ ;  $P = 0.0013$ ) trap cropped with the different trap plants. On the other hand, a significant change in the ash percentage ( $P < 0.0001$ ) for lettuce was recorded. Weekly monitoring of the chlorophyll content of lettuce crops showed no significant differences between treatments (Figure 7).

The trap crop influenced the number of juveniles (Figure 8), and the number of galls per grams of lettuce

root (Figure 9) is shown. A significant reduction of juvenile nematode numbers and gall numbers were recorded when lettuce ( $F = 8.5$ ;  $P < 0.0001$ ) was trap cropped with canola, B.G. pumpkin, mustard and vetch cv. 976. These trap crops attracted more nematodes and retained them from infecting the main crop. While a significant increase in nematode numbers was recorded in lettuce when trap cropped with sesame cvs. 202 and 157, A. cucumber and sorghum. These results showed that canola, B.G. pumpkin and mustard are good crops associated with lettuce in soils infested with root gall nematodes. The main effect of trap crops showed that canola, vetch cvs. 1816 and 976, mustard and B.G. pumpkin significantly affected the root and shoot dry weight of the main crop compared with the control in a negative direction. At the same time, they attracted significantly more nematodes and restrained them from the main crop. Thus, growing these trap crops could significantly reduce the number of nematode eggs and juveniles. But this does not compensate for the negative effects of the competition of the trap crop with the cash crop lettuce. Removing these trap crops at an earlier plant growing stage might reduce the competition between them and the main crop.





**Figure 5:** Effects of artificial inoculation with *Meloidogyne* spp. and 11 trap crops on the number of leaves in lettuce plants. The standard deviation of the means is designated by the error bar for each mean. Means followed by the same letter are not significantly different (SNK,  $P > 0.05$ ).

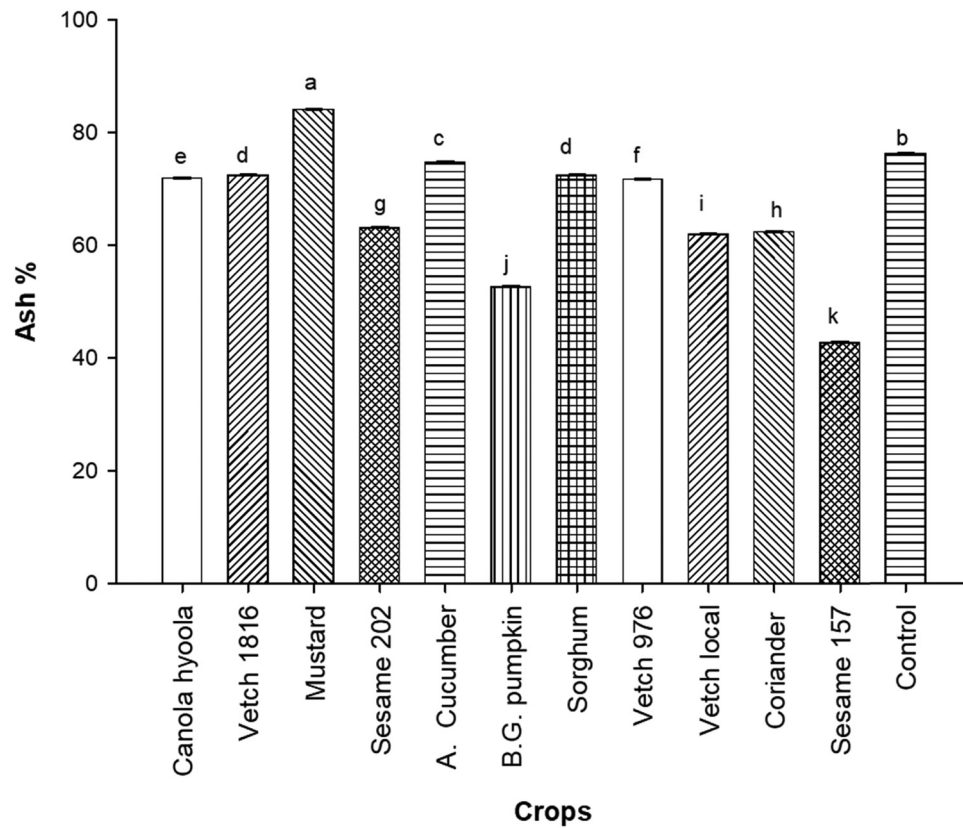
Excluding vetch 976 and sesame 202, most parameters in the other crops were lower than those of the control. This might be due to vetch being a nitrogen fixer.

The mechanism of the trap crop used to manage nematodes was reported by many researchers. Trap crops attract parasitic nematodes away from the main crop, thus, by removing these crops from the field, nematodes would be removed before they complete their life cycle [8]. Meanwhile some trap crops would perform as non- or poor-host [9], while others would release a toxic or allelopathic chemical that prevents nematode development or kill them [10]. Other may produce secondary metabolites that have nematocidal activities [11], or are considered as “dead-end” trap crop, where nematode eggs are triggered to hatch, then the juveniles would starve due to lack of a suitable host to parasitize on [12], and a few would create the non-favorable environmental condition by breaking down organic matters and increasing soil acidity [13].

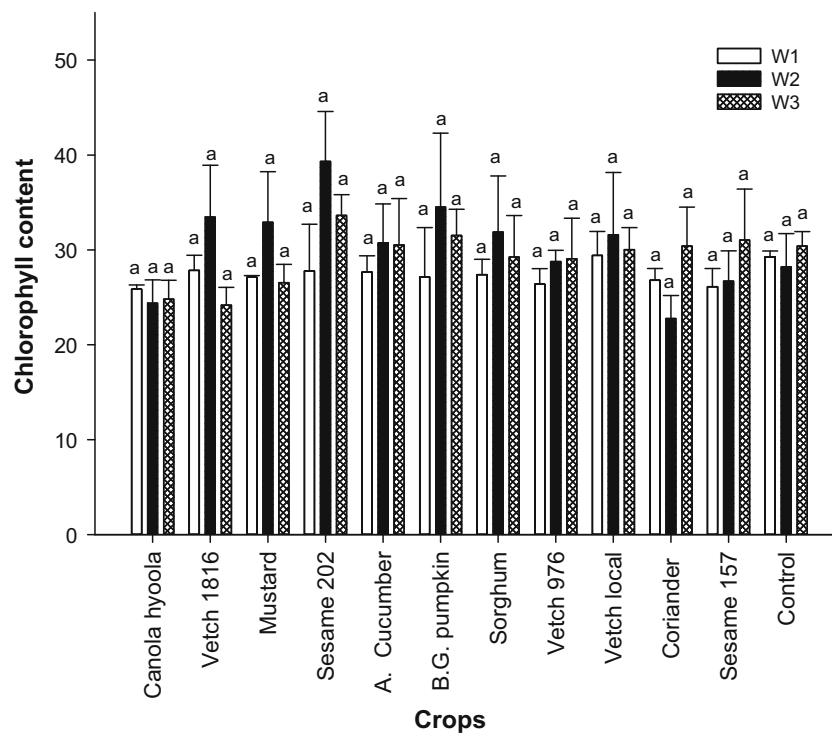
Many researchers investigated the effectiveness of trap crops belonging to the family Brassicaceae, Fabaceae,

Linaceae, and Poaceae on different root nematodes. Crimson clover, hairy vetch, white lupine, red clover, sweet clover, field pea and camelina cultivars were highly effective trap crops [14]. Mojtahed et al. [15] found that trap cropping in soil heavily infested with RKNs with rapeseed plants reduced the nematode population. Similar results were found when cropping canola and vetch with squash [16], *Solanum sisymbriifolium* and *S. nigrum* for potato cyst nematodes [17]. This could be attributed to the expanded root system of the trap crops, which could stimulate and promote more hatching of nematodes due to deeper extension of the root in the soil layers [18].

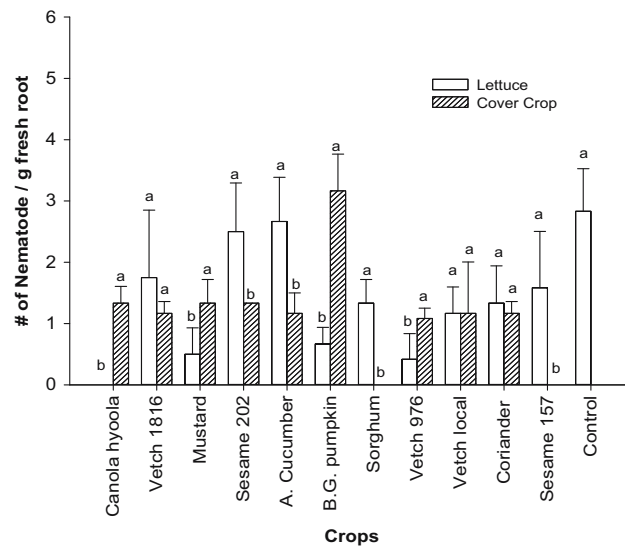
On the other hand, canola and other Brassica crops were reported to have phytotoxic effects on crops such as sunflowers, soybean, barley, and many soil-borne diseases and nematodes [19]. Cruciferous and cucurbit crops produce secondary metabolites such as triterpenoids and alkaloids with nematocidal activities [11,20]. Cruciferous secondary metabolites are glucosinolates [11], while cucurbit produces tetracyclic triterpenoids, cucurbitacin B,



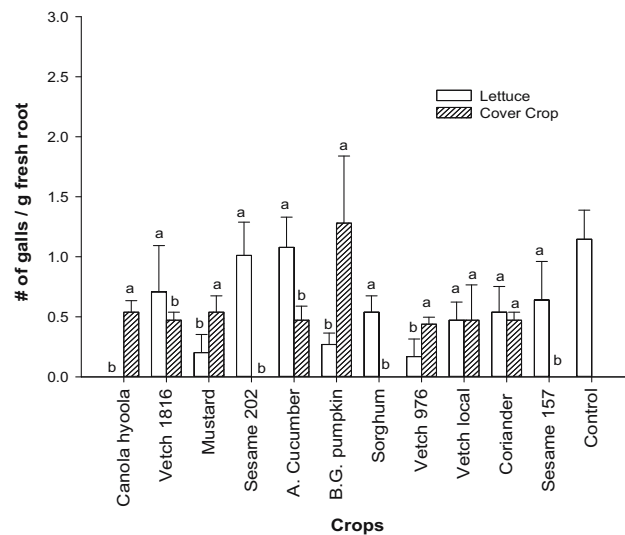
**Figure 6:** Effects of artificial inoculation with *Meloidogyne* spp. and 11 trap crops on lettuce ash content by igniting in a muffle furnace at 550°C for 8 h. The standard deviation of the means is designated by the error bar for each mean. Means followed by the same letter are not significantly different (SNK,  $P > 0.05$ ).



**Figure 7:** Lettuce chlorophyll content monitored weekly during this study. The standard deviation of the means is designated by the error bar for each mean (SNK,  $P > 0.05$ ).



**Figure 8:** The total number of RKN juveniles per gram fresh root in the root systems of the host plant lettuce (*Lactuca sativa* L.) planted with 11 local non-host crops as trap plants. The standard deviation of the means is designated by the error bar for each mean. Means followed by the same letter are not significantly different (SNK,  $P > 0.05$ ).



**Figure 9:** The total number of RKN galls per gram fresh root in the root systems of the host plant lettuce (*Lactuca sativa* L.) planted with 11 local non-host crops as trap plants. The standard deviation of the means is designated by the error bar for each mean. Means followed by the same letter are not significantly different (SNK,  $P > 0.05$ ).

saponins sterols and a non-proteic amino acid [20]. Moreover, it was reported that grafting cucumber on B.G. pumpkin as rootstocks reduced the damage caused by *Meloidogyne incognita* [21].

## 4 Conclusion

Trap cropping lettuce crops with 11 trap crops pre-artificially inoculated with RKNs significantly affected the

main crop size and weight, and this might be resolved by removing the trap crops from the field after 6–8 weeks, which will reduce the competition between the crops and at the same time it will decrease the number of viable RKNs in the soil. Canola, bottle gourd pumpkin, mustard, and vetch lured nematodes away from the main crop. At the same time, trap cropping with sesame, Armenian cucumber and sorghum resulted in more nematodes in the main crop. These results found in this research presented essential information on suitable trap crops such

as non-hosts and or poor hosts for RKNs. Further studies on these crops for reducing the RKN population under field conditions could provide more insight into the best alternatives to control RKNs with trap crops and their cost-benefit analysis or alternatives for bio-solarization or anaerobic soil disinfestation or chemical control.

**Acknowledgments:** The author is grateful to the National Research Center (NARC) for providing the plant seeds. The author thanks the capable students, Mr Alyat, Mr Nawahda and laboratory technicians for the technical assistance, support and culture maintenances.

**Funding information:** The scientific work was funded by Palestine Technical University-Kadoorie (PTUK).

**Conflict of interest:** The author states no conflict of interest.

**Data availability statement:** All data generated or analyzed during this study are included in this published article.

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