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Research Article

Application of chicken manure and summer plowing to control root-knot nematode *Meloidogyne javanica* in muskmelon, *Cucumis melo* var. *inodorus,* farms

Hadi Karimipour Fard* **and Nazanin Doryanizadeh**

Plant Protection Research Department, Kohgiluyeh and Boyerahmad Agricultural and Natural Resources Research and Education Center, AREEO, Yasouj, Iran.

Abstract: Root-knot nematode *Meloidogyne javanica* is an economically significant plant parasitic nematode in muskmelon farms in Ardestan, Isfahan province, Iran. A two-year experiment was conducted in two muskmelon farms in this region. We assessed the application of 3, 6, and 9 t/ha fresh (unrotten) chicken manure separately or in combination with summer plowing. To evaluate the efficacy of each treatment, the number of galls, egg masses, reproduction factor, plant growth factors, and yield were recorded at harvesting time. The results showed that combining double summer plowing with nine t/ha chicken manure was the most effective treatment for controlling *M. javanica*. Nematode population indices including: the egg mass number, gall number, total population in root and soil, and reproduction factor, were significantly lower than the control treatment. The application of double plowing with nine t/ha chicken manure treatment caused 83.5%, 79.1%, 80.2%, and 78.3% decreases in egg mass number, gall number, total population per root, and soil, and reproduction factor, respectively. Moreover, total fruit weight and root dry weight in each plot (24 m^2) increased by 81.8% and 50.4% compared to the control.

Keywords: chicken manure, plowing, muskmelon, *Meloidogyne javanica*

Introduction

Among plant pathogens, plant parasitic nematodes are one of the most devastating agents causing crop damage, and their annual damage is estimated at 100 billion dollars in various crops (Oka *et al*., 2000). The root-knot nematode *Meloidogyne javanica* (Treub 1885) Chitwood, 1949 parasitizes a wide range of plants in tropical and temperate regions by penetrating plant roots and producing galls (Karssen *et al*., 2013). The root-knot nematode reduces photosynthesis and growth and causes nutrient deficiency, yellowing and wilting. It also increases the susceptibility of the root system to invasive fungal/bacterial plant pathogens. The damage to young plants can result in death and severe infection, often preventing the plants from growing to maturity (Jaffee and Muldoon, 1995).

Meloidogyne spp. is an important pathogen in vegetable and summer crops (Moens *et al.*, 2009; Collange *et al*., 2011). In general chemical pesticides, including nematicides, are

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* Corresponding author: karimipourfard@yahoo.com
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widely used as a control method (Javed *et al*., 2007). However, due to environmental considerations, chemical control of plant parasitic nematodes has become less desirable (Castillo *et al*., 2010) and very much restricted in developed coutries. Therefore, relying on safe methods such as resistant cultivars, organic materials, and cultivation practices is more emphasized (Widmer *et al*., 2002). Twice summer plowing and postharvest summer plowing in beet fields resulted in a greater reduction of root-knot nematode (Akhyani *et al*., 1984; Savage and Bramwell, 2002). Organic amendments such as chicken manure affect soil properties and nematode population (Akhtar and Malik, 2000; Litterick *et al*., 2004; Nahar *et al*., 2006; Hu and Qi, 2010; Oka, 2010; McSorley, 2011). They act by stimulating many potential predators or parasites of plant-parasitic nematodes in the soil (Akhtar and Malik, 2000; Oka, 2010). Also, they can enhance nematode-trapping fungi and egg parasitism (Mukerji and Garg, 1988; Singh and Sitaramaiah, 1973). Using chicken manure also improves plant growth and increases crop yield (El-Sherief *et al*., 2010; Abolusoro *et al*., 2012). The impact of organic manure depends on many factors, such as organic material type, composting of material, application rate, crop rotation, agronomic practices, soil type, climate, and other environmental factors (McSorley, 2011). Chicken manure is a rich source of organic matter, which, in addition to improving soil conditions, contains nutrients that are effective in plant nutrition. It is especially rich in nitrogen content compared to other animal manures (Hirzell and Walter, 2008). The efficacy of chicken and poultry manure on the reduction of root-knot nematodes has been reported in many types of research (Nasr-Esfahani, 2002; Antonio *et al*., 2005; Karimipour Fard *et al*., 2019). Osei *et al*. (2011) reported that chicken manure reduces the root-knot nematode population density by inhibiting egg hatching.

This study evaluated the efficacy of chicken manure and summer plowing for managing *M. javanica* in muskmelon farms. The aims of the study were: *i:* investigating the effect of different levels of chicken manure and summer plowing on root-knot nematode, *ii:* combining the summer plowing and chicken manure in reducing the nematode indices, *iii:* improving the muskmelon yield and growth indices in out-of-season muskmelon cultivation.

Materials and Methods

The experiment was conducted on two muskmelon fields with a history of root-knot nematode infestation in different regions of Ardestan in Isfahan province, Iran.

Soil samples

Five randomized core soil samples were taken from each plot (6 × 4 m) to assay the initial nematode population (Pi). The soil samples were collected from experimental fields at the beginning of the first week of July 2009 in a systematic zigzag method, from 0-30 cm depth. Nematodes were extracted by a modified centrifugal floatation method (Jenkins, 1964).

Application of chicken manure: The selected plots were tilled twice in July and August 2009. Unrotten chicken manure was applied two months before planting in plots based on the calculated amount. The trials were conducted in a randomized complete block design with eight treatments viz: double plowing, three tonnes chicken manure/ha, double plowing + three tonnes chicken manure/ha, six tonnes chicken manure /ha, double plowing + six tonnes chicken manure/ha, nine tonnes chicken manure/ha, and double plowing + nine tonnes chicken manur/ha, and control without applying plowing and chicken manure with four replications.

Planting muskmelon: Muskmelon seeds were sown at the end of the first week of February 2010 in two rows at 3-4 cm depth and 50 cm space between the rows and 70 cm apart along the row. In this winter cropping (out-of-season cultivation), the ridges were covered by plastic tunnel.

Qualitative and quantitative parameters determination: Plant height, fresh and dry weight (75 °C in the oven for 48 h) of the aerial shoot (whole aerial shoot), and root (whole root system) were measured. During the harvest season (last week of May 2010 to Mid-June 2010), fruit weight was recorded. From the beginning of the harvest time, the yield of each plot (24 m^2) was weighed separately. The total weight of the fruit was calculated through the sum of the total yield in consecutive harvests. After 100 days of planting (last week of May 2010), two plants from each plot were unrooted, and the number of egg masses and galls per root system was determined (Taylor and Sasser, 1978). The nematode developmental stages/cm root was determined by staining roots using acid fuchsin solution and Lacto glycerol (Bridge *et al*., 1982). The number of second-stage juveniles (J2s) was estimated by extracting nematodes from 200 cm^3 of soil samples of each plot using a modified centrifugal floatation method (Jenkins, 1964). The sum of eggs, juveniles, and adult populations of nematodes in the roots and the population of J2s in 200 cm^3 of soil per plot were considered the final population of nematodes in each plot. The Nematode reproductive factor (Rf) was also calculated based on initial and final population density.

Soil analysis: Before and after applying the chicken manure, some soil properties, including pH (by pH meter), organic carbon (by Walkley-Black method, Nelson and Sommers, 1996), available P (Olsen's method), and available K (by flame photometer) (Knudsen *et al*., 1982) were measured.

Results

The identification of *M. javanica* was confirmed by morphological and morphometric studies. The results showed that double plowing plus nine tonnes of chicken manure/ha was the best treatment for control of the root-knot nematode population in muskmelon fields. This treatment significantly reduced root-knot nematode population and increased the plant growth characteristics. The total population per root, soil and reproduction factor (RF) was reduced compared to other treatments (Table 1). It caused 83.5%, 79.1%, 80.2%, and 78.3% decreases in egg mass number, gall number, total population per root, and soil and reproduction factor, respectively. This treatment also resulted in significantly higher aerial shoot dry weight, plant and root dry weight by 81.8% and 50.4% increase in the factors of total fruit weight and root dry weight per plot (24 m^2), respectively. The total fruit weight was also significantly higher than the control (Table 1).

Analysis of soil samples before and after applying chicken manure for two years showed that there was a significant increase in soil salinity (electrical conductivity), organic matter, and the absorbable content of phosphorus and potassium and a significant decrease in pH ($p \leq 0.05$; Figure 1). This study also showed that using chicken manure at the rate of 9t/ha reduced soil pH by 0.31 (Fig. 1), which impacted the improvement of soil conditions and absorption of micronutrients.

Discussion

In our investigation, the lowest population density of root-knot nematode and the most quantitative characters in fruit yield and plant growth parameters belonged to the treatment of double plowing plus nine tonnes of chicken manure. The application of this treatment led to an increase in plant growth indices and a decrease of 78.3% and 81.8% in the overall reproduction factor and total population of nematodes in soil and roots, respectively.

Our results were in agreement with the results of the works done on this subject, which confirm the effectiveness of chicken manure for root-knot nematode control of different crops (Nasr-Esfahani, 2002; Lopez-Pérez *et al*., 2005; Oka *et al*., 2000; El-Sherief *et al*. 2010; McSorley, 2011; Abolusoro *et al*., 2012; Karimipour Fard *et al*., 2019).

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Table 1 The effectiveness of chicken manure, plowing, and combined treatments on nematode indices and muskmelon growth parameters in muskmelon fields.

Treatments	No. of Egg /whole root	No. of gall /whole root	Reproduction factor	Total population (in root & soil)	Aerial shoot fresh weight (g)	Aerial shoot dry weight (g)	Plant height (cm)	Root dry weight (g)	Root length (cm)	Total fruit weight (Kg)
Double ploughing+ 3 tonnes of chicken manure	18.62 c	7.87 d	6.14 bc	11516.1 e	1221.25 d	11.60e	112.87 a	3.06 ab	23.92 b	28.50 a
3 tonnes of chicken manure	35.87 b	13.12 bc	7.22 b	15150.6 c	1516.25 c	16.86 d	114.25 a	2.42 ab	22.90 b	19.75 b
Double ploughing + 9 tonnes of chicken manure	17.87 c	6.87 e	4.91 c	8858.4 f	1723.75 b	22.05 a	117.25 a	3.52 a	31.83 a	31.25 a
9 tonnes of chicken manure	30.75 b	12.12 bc	6.17 bc	13641.5 d	2246.25 a	19.91 b	121.62 a	2.83 ab	25.45 ab	27.87 a
Double plowing	18.50 c	11.37 bc	6.77 bc	14337.3 cd	1285.00 d	19.37 bc	113.50 a	3.05 ab	27.39 ab	27.75 a
6 tonnes of chicken manure	29.50 bc	14.12 b	7.83 b	17252.8 b	2173.75 a	18.60 c	110.25 a	3.32 ab	23.97 b	30.06 ab
Double ploughing + 6 tonnes of chicken manure	20.00 cd	10.37 cd	5.28 b	12213.8 e	1586.25 bc	19.57 bc	109.62 a	2.38 ab	22.44 b	30.56 a
Control (no plowing and no chicken manure)	108.50 a	33.00 a	22.67 a	42260.0 a	846.25 e	17.52 d	89.12 b	2.16 b	22.60 b	17.18 b

Note: Values in the same column followed by the same letter(s) are not significantly different ($p \leq 0.05$) using Duncan's Multiple Range test. From each plot of 24 m^2, two plants with roots were used to measure nematode and plant indices. Each treatment included four replicates; therefore, the values are the average of eight plants.

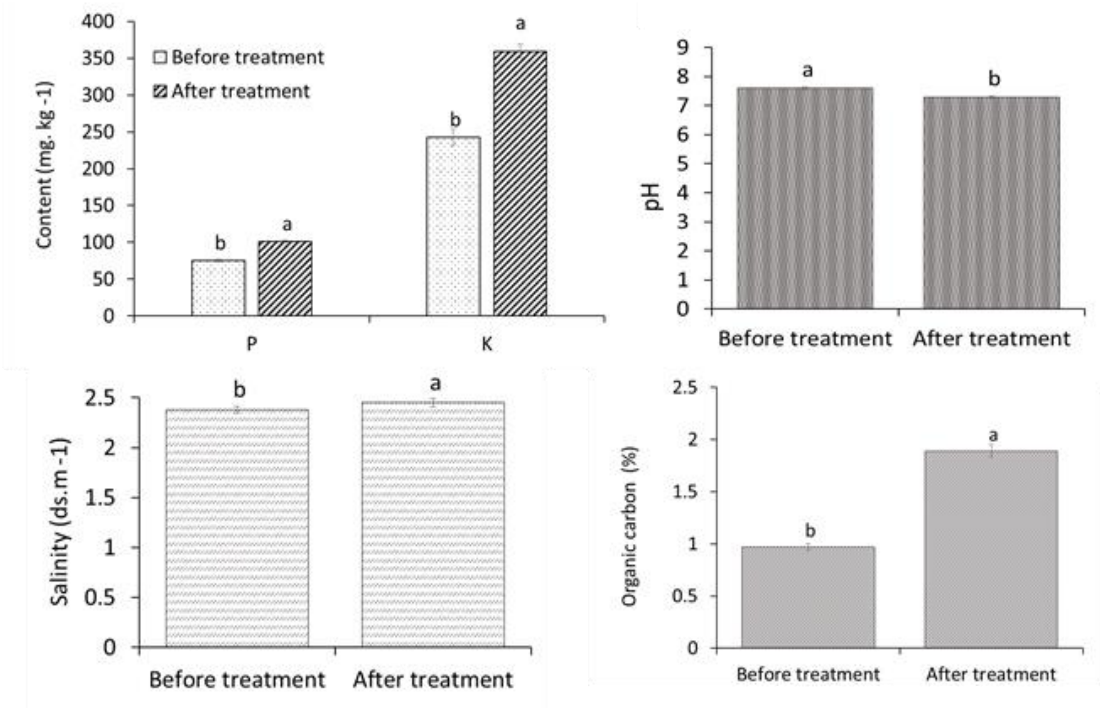

Figure 1 Comparing the soil properties before and after nine tonnes of chicken manure application.

In this study, unlike most previous studies, an unrotten and pure form of chicken manure from laying hens was used in a much lower rate than the amounts used in previous studies. Some studies suggest that chicken manure stimulates the decomposition of nematode cuticular layers (Rodriguez-Kabana, 1986; Stirling, 1991). Chicken manure releases toxic levels of ammonium and probably changes the soil structure during decomposition. It also causes stimulation of antagonistic organisms, increases plant tolerance, and promotes nematode control (Oka *et al*., 2000; Lopez-Pérez *et al*., 2005).

Our findings are similar to the results of Everts *et al.* (2006) who indicated that summer plowing can be considered an effective method to control *M. incognita* in potato fields.

Besides the efficacy of chicken manure for managing root-knot nematodes in muskmelon farms, it showed improved soil fertility through increased organic carbon, P, and K contents in the treated soils.

Cultural practices like amending soil with chicken manure and plowing are environment-friendly management tactics (Oka, 2010). Applying these methods prevents the hazards of chemical nematicides on the environment and on human health (Oka, 2010). Using chicken manure combined with summer plowing can be considered a practical management strategy that could be an alternative to chemical control for the root-knot nematode. Meanwhile, the costs of nematicide application and its effects on non-target species should be considered (Udo and Ugwuoke, 2010).

Use of chicken manure reduced soil pH and raised soil salinity, P, and K of the soil in the treatment of double plowing plus nine tonnes of chicken manure; however, applying pure chicken manure in this study reduced the amount of manure and also prevented high raising of soil salinity as a consequence of using high amounts of fresh (unrotten) chicken manure. Comparison of the salinity (EC) of the soil before and after application of 9 t/ha of chicken manure showed a slight rise of about 0.07 ds.m^{-1} in the soil. Although the tolerable salinity levels vary for different crops, this amount for muskmelon is up to 2.5 ds.$m^{-1,}$ while two ds.m^{-1} is ideal. However, the soil salinity of 2 ds.m^{-1} does not affect yield reduction, but 5% and 20% yield loss occurs at 3 and 4 ds.m^{-1}, respectively (Mass, 1986). Using 9 t/ha chicken manure increased the soil organic carbon by 0.92% and significantly increased absorbable potassium and phosphorus. This process, in addition to control of root-knot nematode, will improve the soil conditions and plant growth.

Adding any decomposable organic matter to the soil leads to changes in the physicochemical and biological properties of the soil. It also provides suitable conditions for the activity of antagonists and the establishment of biological control of soil-borne pathogens (Westphal *et al*., 2016). The use of organic compounds, such as chicken manure that is rich in nitrogen content due to the release and dispersion of ammonia after microbial degradation, causes a rapid reduction in the population of plant parasitic nematodes on the nematode population (Lazarovits *et al*., 2001). We used fresh (unrotten) chicken manure in this study because of its high fumigant activity (Zhang *et al*., 2021). Also, it is readily available and is cheaper than chemical fumigants with fewer environmental hazards.

Conclusion

This research showed that using chicken manure helped to control root-knot nematodes and increased muskmelon yield. In the tropical regions of Iran, summer crops cultivated out-of-season is done. Usually, after harvesting these crops in the summer, the farm is left uncultivated due to high temperatures and water shortages, which can be subjected to summer plowing. Using chicken manure in the recommended amount in these farms would provide a safe and environmentally friendly method for controlling root-knot nematodes. Therefore, according to the conditions and facilities, growers can manage muskmelon root-knot nematode by using chicken manure alone or in combination with summer plowing to enhance its effects.

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کاربرد کود مرغی و شخم تابستانه به‌منظور کنترل نماتد ریشه گرهی *Meloidogyne javanica* در مزارع خربزه

هادی کریمی‌پور فرد* و نازنین دریانی‌زاده

بخش تحقیقات گیاه‌پزشکی، مرکز تحقیقات و آموزش کشاورزی و منابع طبیعی استان کهگیلویه و بویراحمد، سازمان تحقیقات، آموزش و ترویج کشاورزی، یاسوج، ایران.

پست الکترونیکي نویسنده مسئول مکاتبه: karimipourfard@yahoo.com

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چکیده: نماتد ریشه‌گرهی *Meloidogyne javanica* یکی از نماتدهای انگل گیاهی مهم از لحاظ اقتصادی در مزارع خربزه اردستان در استان اصفهان است. آزمایشی دو ساله در دو مزرعه خربزه آلوده به این نماتد در منطقه مذکور، با کاربرد کود مرغی در مقادیر سه، شش و نه تن در هکتار به‌طور جداگانه یا همراه با شخم تابستانی جهت کنترل نماتد ریشه گرهی، انجام شد. برای ارزیابی تأثیر هر یک از تیمارها روی *M. javanica*، تعداد گال، توده تخم، فاکتور تولیدمثل، شاخص‌های مربوط به رشد گیاه و همچنین عملکرد محصول در زمان برداشت ثبت شد. نتایج نشان داد که ترکیب دو بار شخم تابستانه با نه تن کود مرغی نپوسیده، مؤثرترین تیمار برای کنترل *M. javanica* است. شاخص‌های جمعیتی نماتد شامل تعداد توده تخم، تعداد گال، جمعیت کل در ریشه و خاک و فاکتور تولیدمثل در این تیمار به‌طور معنی‌داری کم‌تر از تیمار شاهد (بدون کود مرغی و شخم تابستانه) بود. اعمال تیمار مذکور به‌ترتیب باعث کاهش ۸۳/۵، ۷۹/۱، ۸۰/۲ و ۷۸/۳ درصد در تعداد توده تخم، تعداد گال، کل جمعیت نماتد در ریشه و خاک و فاکتور تولیدمثل شد. هم‌چنین وزن کل میوه و وزن خشک ریشه در هر کرت (به ابعاد ۲۴ مترمربع) نسبت به شاهد ۸۱/۸ درصد و ۵۰/۴ درصد افزایش یافت.

واژگان کلیدی: کود مرغی، شخم زدن، نماتد ریشه‌گرهی، خربزه، *Meloidogyne javanica*

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