

RESEARCH ARTICLE

Ecofriendly management approaches for sugar beet root-knot nematodes *Meloidogyne* ssp. by seed treatment with bio-origin compounds and Oxamyl

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Received: 23 April 2024\ Revised: 29 May 2024\ Accepted: 05 June 2024\ Published online: 10 May 2024

Abstract

Egypt's principal sugar crop, sugar beet, is severely affected by root-knot nematodes, *Meloidogyne* ssp., causing significant losses in yields and economic returns. Conventional pesticides are effective in the short term but pose growing threats to human and environmental health. Alternative management strategies, such as quick germination, can help plants escape soil-borne diseases and infestations. This can help prevent diseases from taking hold and spreading throughout the plant population.

The study investigated the effectiveness of phytochemical-based seed treatment on sugar beet seeds, using growth regulators Nano-NPK and Oxamyl %24.L at variable combinations. It also examined the reduction of root penetration and damage rates of root-knot nematode, *Meloidogyne javanica*, on sugar beet productivity and plant growth traits under non-infected or RKN-infected conditions. The use of Nano-NPK against sugar beet root-knot nematode significantly improved germination followed by Nano-NPK% + Oxamyl L 24%, and Nabta-Bio + Oxamyl. Seeds treated with a combination of Nano-NPK and growth regulators showed the best galling reduction percentage. The combined data showed significant variances in female numbers, egg masses, and juvenile root system⁻¹ between the two seasons. Nabta-Bio, Nano-NPK, and Oxamyl treatments significantly reduced female root system⁻¹, gall index, disease severity, and treatment efficacy. Nabta-Bio + Nano-NPK seed treatment, followed by Nabta-Bio and Nano-NPK seed treatment, achieved the highest records for actual field emergence, plant density, leaves weight, and roots yield. The qualitative reaction of sugar beet technological characteristics showed no significant difference among treatments, except for control treatment. Sugar yield differed significantly between seed treatments, suggesting a phytochemical-based approach could be an effective, environmentally friendly solution for managing RKN and developing bio pesticides to manage pests sustainably. Seeds treated with growth regulators, Nano-NPK, and Oxamyl 24% show enhanced growth and protection against soil-borne diseases. These treatments promote quick germination, strengthen root systems, and improve plant health, enhancing disease resistance.

The study suggests that a phytochemical-based approach could be a sustainable solution for managing RKN nematodes and accelerating the development of seed treatment. Seeds treated with growth regulators, Nano-NPK, and Oxamyl 24% show improved growth, protection against root-knot diseases, and enhanced plant health, thereby enhancing disease resistance.

Keyword: *Beta vulgaris*; Nematode; *Meloidogyne javanica*; Growth regulators; Nanofertilisers.

Introduction

Plant parasitic nematodes (PPNs) pose a significant threat to agricultural crops, causing annual yield loss of over \$100 billion worldwide (Thoden et al. 2011). Root-knot nematodes (RKNs) are the most yield-limiting group, easily multiplying within plant roots of over 3,000 plant species (Abad et al. 2003). These biotroph sedentary endoparasites are widespread worldwide and can increase in soil under suitable conditions.

Sugar beet (*Beta vulgaris* ssp. *vulgaris*), the major sugar crop in Egypt, contributes about 62% of sugar production, with an average production of 21.1 tons per feddan (Annual Report of Sugar Crops Council, December 2022).

The increasing industrial demand for sugar beet has led to a surge in beet planting, reaching 272,460 hectares (GAIN 2022). However, challenges such as root-knot nematodes, particularly *M. javanica* and *M. incognita*, pose significant threats to the crop's quantity and quality (Gohar and Maareg 2005).

Despite their economic importance, there is a growing need to improve ecological management strategies and handling for RKN control. Agricultural controls are limited due to the wide host range of *Meloidogyne* spp. The use of resistant varieties is an effective control tool, but not many are commercially available and resistance may be broken by emerging RKN species (Xiang et al. 2018).

Nematicides have been the primary short-term management strategy against RKN (Medina-Canales et al. 2019), but recent years have seen the discontinuation of chemicals like methyl bromide and aldicarb due to ecological and human health concerns, toxicity to non-target organisms, and residues in products (Xiang et al. 2018). Despite these changes, they have not achieved equivalent efficacy levels (Desaeger et al. 2017).

Researchers are exploring novel strategies and alternative agents for nematode management in sugar beet production, including biological control methods like live microbes, essential oils, plant extracts, organic and amino acids, natural bioactive substances, green manure, and industrial wastes. These environmentally benign treatments have been studied for their efficacy against RKN.

Biological agents with plant growth stimulating factors are used to replace chemical compounds in agriculture. These agents help plants by enabling resource gain, creating cytokinin and gibberellins, and indirectly producing antibiotics and lytic enzymes (Glick 2012). They help plants mitigate soil biotic stresses like pathogens like RKN, and the interaction between biological control agents and roots primes plants against RKN infection.

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Seed treatment is an eco-friendly method for managing sugar beet root-knot nematode pathogens. It requires less chemical input than large-scale field nematicide applications, reducing ecological impact and investment costs. Chemical seed treatment only affects the soil's rhizosphere, reducing undesired accumulation. Seed treatment is quicker to handle than liquid or granular formulations, especially in unskilled labor areas (Zaim et al. 2023).

This study hypothesizes that promoting rapid germination can be an effective strategy for avoiding soil-borne disease infestations. This is because quicker germination can help plants establish stronger root systems and grow faster, making them less susceptible to diseases.

This study aimed to evaluate the impact of treating sugar beet seeds with compounds and substances containing growth regulators, Nano-NPK, and Oxamyl %24.L on reducing early root penetration and damage rates caused by the root-knot nematode, *M. javanica*, and on sugar beet productivity.

Materials and methods

This study utilized two methods to evaluate the germination ability of sugar beet seeds. The first involved testing the effectiveness of seed treatments using various compounds for three soaking periods over 21 days. The second involved evaluating the performance of sugar beet seeds treated with these compounds under natural infestation with *Meloidogyne javanica* in a field trial over two seasons.

The planting material of tested seeds is sugar beet Nadir

The multigerm Nadir commercial variety, originating from Germany, is a diploid × diploid (CECD 2019). It's from the certified group of sugar beet varieties owned by Nubaryia Sugar and Refining Company (NSRC). It is susceptible to root-knot nematodes, as per Canto-Saenz host suitability designations modified by Gohar et al. (2013).

Table 1. Modified scheme for assigning host suitability designations for sugar beet by Gohar et al. (2013).

Resistance Level (RL)	R-factor (host efficacy)*	Plant Injury (Gall index) ^y
Resistant (R)	≤1	≤2
Moderately Resistant (MR)	≤1	≈2
Tolerant (T)	>1	≤2
Susceptible (S)	>1	>2
Hyper susceptible (HYS)	≤1	>2

* reproductive factor: $RF = Pf/Pi$ where Pi = initial population density and Pf = final population density, ^y Gall index: 0 = no gall formation; 5 = heavy gall formation source: Sasser et al. (1984) designations modified by (Gohar et al. 2013).

Pathogenicity test for the sugar beet variety Nadir as a host for root-knot nematodes

Nematode eggs were collected from the galled roots of *Solanum nigrum* L., a weed in the Solanaceae family, scattered in the soil with a history of repeated sugar beet plantations, and root-knot nematodes *Meloidogyne* ssp. Females of root-knot nematode were collected under a microscope to identify *Meloidogyne* species.

A minimum of 10 females per site was used to recognize the RKN species. Black Nightshade plants were up-rooted. Enumeration of eggs was placed in 1 mL⁻¹ suspension with the assistance of a counting cell slide, under a light microscope (10 x.); an average of three counts was represented for the number of eggs in mL⁻¹ suspension. Tests were initiated on 5 June 2020 at outdoor pots. Sandy loam soil collected from sugar beet fields of West Nubaryia province was air-dried, homogenized and steam sterilized using an autoclave for 3 h at 85°C. Pots (20 cm diameter) were filled with soil (3.5 kg pot⁻¹). Two Nadir var. sugar beet seeds were sown per pot, followed by Nematode inoculums of 4000 as an initial population (Pi) *M. javanica* eggs one h, after sowing. The inoculums were distributed into two holes, covered with soil, and watered immediately after inoculation.

The plants were then watered regularly and 15 g of compound fertilizer (15:15:15) was added to the 3-week-old plants. Sixty days after sowing, plants were uprooted by placing them in a water-filled pan, shaking gently to clean the soil and clean the roots.

The roots were examined and rated for galling responses using a scale of 1 - 2 galls, 3 - 10 galls, 11 - 20 galls, 4 - 31 - 100 galls, and 5 - 101 galls and above, as per Taylor and Sasser (1987) method. Before removing the plants, 250 cm³ of soil around each plant was gathered to a depth of 10 - 15 cm.

The extraction of second juvenile larvae (J2) from each soil sample was done using a 2 ml suspension, following a modified Bearman's tray technique outlined by Barker (1985). The J2 was subsequently enumerated under a dissecting microscope, and this procedure was reiterated 10 times (20 mL) to gauge the population in 250 cm³ of soil as the final population (Pf).

The host efficiency (reproduction factor 'RF') was calculated, where 'RF' = Pf/Pi , with Pf being the final population in 250 cm³ of soil and Pi being the initial inoculums. The final assessment of the various genotypes was based on modified Canto-Saenz's host resistance designations scheme (Gohar et al. 2013) as given in Table 1.

Characterization of compounds and substances used

Nabta-Bio®: is a local production growth regulator produced by Biotech Company for fertilizers and natural alternatives. It contains gibberellin acid, acetic acid, and cytokinin, and is recommended for sudden temperature fluctuations and uneven plant growth. It increases vegetative total, improves seed germination, speeds up growth and cell elongation, and increases plant tolerance to environmental stress. Nabta-Bio is suitable for all field crops, vegetables, ornamental, medicinal, and aromatic plants.

Nano®: Nano-NPK: The synthesis of Nano-NPK nanoparticles involved polymerizing methacrylic acid in chitosan solution and coating it in buffer solution for 18 h, at room temperature. The particles, ranging from 44.2 to 54.3 nm, had a crystal structure and 98.5% purity Figure 1 as described by Mahmoud and Swaefy (2020). The application of mixture of Nano nitrogen, phosphorus and potassium resulted in positive outcomes on both the plant and environment, represented in; high growth characteristics, chemical composition of the plant and reduced water stress.

Viva 24%®: Oxamyl 24. L: is a carbamate chemical group pesticide and nematicide that inhibits acetyl cholinesterase, causing paralysis and death in insects and nematodes. It is Systemic and contact nematicide in the form of concentrated liquid (L).



Figure 1. The Nano NPK' image after Mahmoud and Swaefy (2020).

Treatments of seeds

Sugar beet seeds were soaked in the tested compounds and substances used, namely Nabta-Bio®, Nano-NPK, and Oxamyl L 24%, as well as possible combinations of them, at the recommended application rate for 1 h, 2 h, and 3 hours, respectively, in an Erlenmeyer flask shaken by hand, then left to dry on paper in the open fresh air. Untreated sugar beet seeds were used as controls throughout the study.

Germination experiment

The treated and untreated sugar beet seeds were sowed at 0.5 cm depth in a seed raising trays locally purchased containing organic material mixture (peat moss) and sand 1:2. Each block as a substrate to preserve continuous humidity during the experimental period, propagating was carried out in a seedling tray with 25 cells per replicate and untreated sugar beet seeds were used as controls throughout the study of the initial growth assessments (21 DAS) following seed treatment and were placed at random in a greenhouse ($23 \pm 5^\circ\text{C}$ & 60 ± 5 RH), they were watered daily. Each treatment was replicated four times.

Standard germination test

The germination percentage (GP) was determined after 7, 14 and 21 days from sowing. Germination was assessed as the percentage of seeds producing normal seedlings as defined in the handbook of seedling classification (ISTA 1993). Also, the germination Index (GI) and Germination Rate Index (GRI) were calculated as follows.

$$GP = \frac{\text{Total.No.seedlings.that.emerged.in.the.final.count}}{\text{Total.No.of.seeds.planted}}$$

GI=

$$\frac{\sum(N_x)(DAS)}{\text{Total.No.of.seedlings.that.emerged.the.final.count}}$$

$$\frac{\sum(N_x)(DAS)}{\text{Total.No.of.seedlings.that.emerged.the.final.count}}$$

where, N_x is the number of seedlings that emerge on day x after sowing, and DAS is the number of days after planting.

$$GRI = \frac{GI}{GP.(0-1.scale)} \quad GRI = \frac{GI}{GP.(0-1.scale)}$$

Field experiments site description

At the Nubaryia Sugar and Refining Company's (NSRC) Research Farm, which is situated at (30°63' 88.93" N latitude and 30°22' 46.21 E longitude), El-Behaira Governorate, two field experiments were conducted over the two successive growing seasons in 2020/2021 and 2021/2022. This was naturally infested with *Meloidogyne javanica*. The soil type was sandy soil containing a distinctly low percentage of organic matter (0.38%), with a pH of 8.23. The average particle size distribution was 92.0% sand, 3.2% silt and 4.8% clay. The field had been planted for sugar beet for numerous years before launching this study. The experiment included three replicates each one had the same soil cultivation depths in strip tillage: strip tillage at a depth of 30 cm. Tillage was performed with a 4 × 4 drive wheel FIAT® 130-90

tractor, with 130 hp. engine powers. All plots received primary tillage by moldboard plowing in the last week of October. Plowing depth was controlled by the gauge wheel of the moldboard plow in all experiment areas. Secondary tillage operations consisted of disking and land leveling done for all plots at the same way before planting sugar beet at the 4th of November for both studied seasons. The preceding crop in both seasons was none (summer fallow). Conventional crop management was followed as recommended for sugar beet production in the region. All crop production practices were performed by the grower, and fertilization was based on soil nutrient analysis. Irrigation was sprinkler irrigation. Soil samples were taken at random from the experimental site at 0 to 30 cm from the soil surface and prepared for physical and chemical analysis according to Ankerman and Large (1974) as shown in Table 2.

Table 2. Particular physical and chemical properties of the experimental soil in the 2019/2020 and 2020/2021 seasons.

Soil properties	Season		The average of the two seasons
	2019/2020	2020/2021	
Mechanical analysis			
Sand %	92.00	92.00	92.00
Clay %	4.83	4.87	4.85
Silt %	3.17	3.13	3.15
Soil texture	Sandy	Sandy	Sandy
Chemical properties			
pH 1:1	8.16	8.22	8.19
E.C. (ds/m)	1.44	1.45	1.45
Soluble cations (1:2) (Cmol/kg soil)			
K ⁺	0.88	0.97	0.93
Ca ²⁺	2.83	2.80	2.82
Mg ²⁺	1.81	1.95	1.88
Na ²⁺	8.98	7.71	8.35
Soluble anions (1:2) (Cmol/kg soil)			
CO ₃ ⁻ + HCO ₃ ⁻	5.2	5.3	5.3
CL ⁻	7.19	7.21	7.20
SO ₄ ⁻	1.03	0.93	0.98
Calcium carbonate	6.22	6.17	6.20
Total nitrogen (mg/kg)	2.3	2.4	2.4
Available Phosphorus (mg/kg)	0.5	0.6	0.6
Organic matter %	0.37	0.39	0.38

Experimental design and setup

Treatments were arranged in a randomized complete block design with three replications. Statistical comparison was made at the P=0.05 level of significance. Each plot consisted of six rows (50 cm spacing) by 7.0 m in length (3 m×7.0 m = 21.0 m² superscript). i.e. 1/200 Fed. All treatments were planted at a rate of 5 seeds m⁻¹ per row. Then manual sowing of seeds of sugar beet variety was carried out

on one side of the ridges keeping hill to hill distance of about 15 cm according to the layout plan to obtain a rate of 53000 plants fed⁻¹. Soil samples were collected on 19th October for each studied season. The sowing date was 1st week of November; the experimental setup was repeated for the following fall season of 2022. Experiments were harvested in the 1st week of May in both studied seasons.

Table 3. Characterization of treatment groups and field application strategies.

Treatment symbols	Treatment groupings	Application strategies
T1	Nabta-Bio® 100%	Seed treatment + (1 ground applications through irrigation)
T2	Nano®: Nano-NPK 100%	Seed treatment + (1 foliar application)
T3	Nabta-Bio® 50%+ Nano®* 50%	Seed treatment + (1 foliar + 1 ground applications each).
T4	Viva 24 L%®: Oxamyl 24.L %	Seed treatment
T5	Nabta-Bio® 50%+ Oxamyl 24.L 50%	Seed treatment
T6	Nano-NPK% 50 + Oxamyl 24% L® 50%	Seed treatment
T7	Nabta-Bio® 33.3%+ Nano® 33.3% + Oxamyl 24% L® 33.3%®	Seed treatment
T8	Viva 24 L%®%: Oxamyl 24.L	2 foliar applications each
T9		Standard seed & free ground of chemicals

Treatments of seeds

The sugar beet seeds, soaked in the tested compounds and substances, i.e., Nabta-Bio®, Nano-NPK and Nano-NPK individually and/or in mixture as described in Table 3 at the recommended application rate for approximately 2 h, for each in an Erlenmeyer flask shaken by hand, then left to dry on a paper at open fresh air, Using four replicates per treatment and 25 seeds per replicate, the seeds were moistened with distilled water after being enfolded in filter paper.

Nematode soil population densities

Enumeration and identification

Nematode densities were estimated from composite soil samples taken from each plot just before applying the soil treatments (Pi) at a depth between 20 and 30 cm, before harvest directly for all treatments by taking composite soil samples dug with a spade around the roots of 10 to 12 plants distributed randomly at each site to determine (Pf) to calculate reproduction factor (RF). On each sampling time, twelve soil cores were taken per plot using a vertical soil core sampler and cores were mixed in a composite soil sample. Nematodes were extracted from sub-samples using a modified Bearman's tray method as described by Barker (1985) and identified and counted under a compound microscope. To identify the *Meloidogyne* Species, ten females were collected from infected roots under a stereo microscope to identify the RKN species following their perineal pattern.

Also, the rest of treatments were allotted at plots with the same average Pi equals to 250 (± 7) juveniles/ 200 g soil. Three replicates were maintained for each treatment. Observations on the number of galls/plants, number of egg masses/ plant, number of juveniles / 200 g soil by sieving and decanting methods (Barker 1985) and number of juveniles/root systems by incubation method, as well as nematode build-up rate which was deduced by a formula adopted after Maareg et al. (2018), whereas,

Build-up = Total count of nematodes in root and soil / initial population at sowing time.

To evaluate the experimented efficacy% of management strategies against root-knot nematode, disease severity was determined. For the disease severity detection, the gall indices we rerecorded at the termination of experiments on the scale rating chart, and roots were cleaned. The roots were examined and rated for galling responses on a scale from 0 to 5, where 0 indicated no galls and 5 indicated more than 100 galls per root system (Taylor and Sasser 1987). The efficacy of nine treatments including the control treatment was calculated as described by (Xue et al. 2009).

Disease severity (DS) and control treatment efficacy % were calculated as follows:

DS% =

$$\frac{\sum \text{Class frequency} \times \text{Score of rating class}}{\text{Total number of plants investigated} \times \text{Maximal disease index}} \times 100$$

Treatment efficacy % =

$$\frac{\text{disease severity in control treatment} - \text{disease severity in strategy treatment}}{\text{disease severity in control treatment}} \times 100\%$$

Also, the following characteristics of sugar beet yield and quality were determined at harvest (six months from planting). The number of survival plants/ plot was counted for each treatment in all replicates to estimate their number/ fed. A sample of 10 guarded plants representing each treatment in all replicates was collected to determine: root length and diameter (cm), roots were washed and cleaned, and then the length and diameter of each sole root were measured using a measuring tape and a caliper.

plant weight (g), root and foliage weight (g) /plant were done by the common method is to manually weighing each root using a digital scale and recording the weight., as well as, Total Soluble Solids percentage (TSS %) which was determined using Hand Refractometer, sucrose was determined, purity % was estimated as it is equal (Sucrose % / TSS %) × 100 and sugar content g /plant was assessed as root weight (g) × sucrose %. Sugar beet plants of each plot were up-rooted, topped, cleaned and weighed to determine root yield in tons/fed. Whereas, sugar yield per feddan was estimated as it is = root yield (ton/fed) × sucrose % × purity %.

Determination of yield components and quality traits

The actual field emergence (FE) and actual plant density (PD) were calculated as the total number of the plants that emerged ne per theoretical number of the plants (application rate) times a hundred (%).

$$FE = \frac{n_c}{n_k} \times 100\%$$

ne: number of plants after the field emergence

nK: number of theoretically applied seeds

Also, the actual plant density (PD) is defined as the number of plants per feddan at harvest was determined as follows:

$$PD = \frac{nP \times 100}{lR} \quad (\text{Plants}^{-1}) \quad (\text{Kromer et al. 2004})$$

nP: number of plants

l: row length (m)

Root and top yields were also calculated. Root samples (10 from each treatment) were collected randomly and sent to the (NSRC) Quality Assessment Lab to determine technological characteristics. i.e., Brix and sucrose contents (Pol %). Then the sugar recovery (%) in different sugar beet seed treatments was estimated with the assistance of the following formulas:

$$\text{Sugar Recovery (\%)} = [3P/2\{1-(F+5)/100\}-B/2\{1-(F+3)/100\}] \times 0.93 \quad (\text{Anonymous, 1970}), \text{ where}$$

P = Pol % of juice, B = T.S.S % of juice, F = Fiber % beet and 0.93 = Recover factor.

Statistical analysis

Data of the two experiments growing seasons were subjected to Bartlett's test (Snedecor and Cochran, 1989) to check their homogeneity of variances before combined for analysis of variance (ANOVA) using MSTAT version 4 (1987), followed by testing significant differences among means were compared with the LSD test ($P \leq 0.05$) described by Steel and Torrie (1980).

Results

Nematode identification

The RKN species were identified as *M. javanica* (Kofoid and White) Chit wood, with the assistance of perennial pattern according to Taylor and Netscher (1974) (Figure 2). A perineal pattern of ten female RKNs, *M. javanica*, was photographed at the same magnification. An image processing software was used to invert images to sketch and match them with a sketch for a perineal pattern of *M. javanica* (Eisenback and Hirschmann 1980).

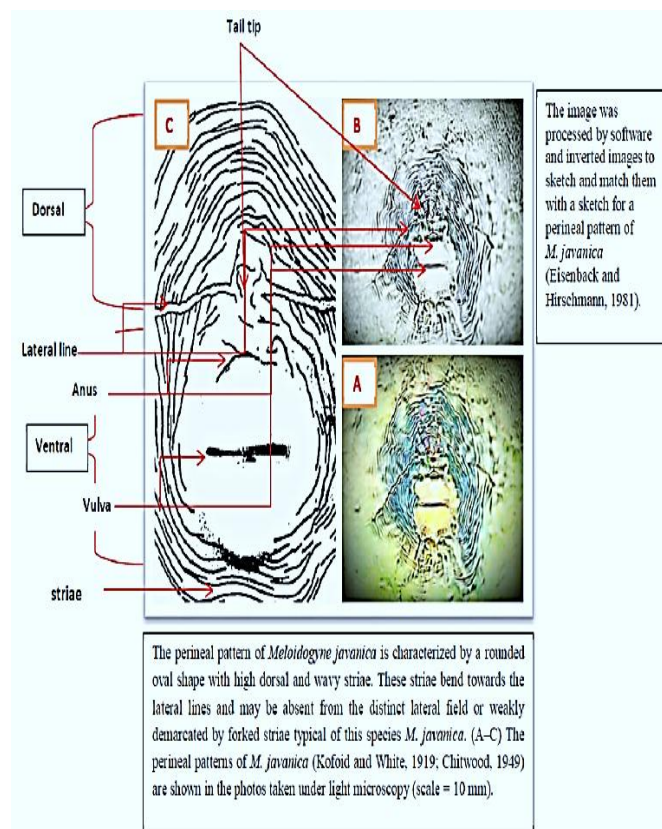


Figure 2. The perineal pattern of root-knot nematode *Meloidogyne javanica*, where (A) is taken under the light microscope, (B) is a sketch of A made by the Photo Sketch Maker App. Ver. 2.0.3 and (C) a drawing after Eisenback et al. 1981.

Pathogenicity test for the Nadir sugar beet variety as a host for *Meloidogyne javanica* For Canto-Saenz's assignment, sugar beet variety Nadir. Was infected by *M. javanica* to conduct a host suitability test.

The gall index measurement was used to assess plant damage and host efficiency, as well as to determine the reproductive factor (RF). By establishing baselines for the relationship between these factors, the host suitability was deduced. According to Canto-Saenz's assignment, the Nadir variety was found to be susceptible, maintaining RF value of 4.4 and a high plant damage (DI) of 3.7 (Table 4; Figure 2).

Table 4. The host suitability of sugar beet variety Nadir. for root-knot nematode, *Meloidogyne javanica*, by the adopted Canto-Saenz quantitative scheme.

Plant Injury (Gall index)*	J2/250 cm ³ of soil (Pi)	R-factor (host efficacy)**	Resistance Level (RL)
3.7	1750	4.4	Susceptible

*The gall index scale is as follows: 0 = 0 galls; 1 = 1-2 galls; 2 = 3-10 galls; 3 = 11-30 galls; 4 = 31-100 galls; 5 = 101 galls or above.

**The reproduction factor (RF) was calculated as the average final egg count divided by 400 eggs (the number of eggs with which ea.



Figure 3. The sugar beet variety Nadir reaction to *Meloidogyne javanica* sixty days after inoculation. A: infested plant, B: plant free of infestation.

Germination parameters as affected by compounds and substances used in seed treatments

The results in Table 5 showed that after 21 days of germination, the differences in seed germination parameters were influenced by the different compounds and substances used, such as nematicide, Nano-NPK, and growth regulators, as well as their combinations or application methods and soaking duration.

As the days passed after sowing, the germination percentage increased. The highest seed germination rate occurred after 21 days. Among all tested treatments, the results show that T2 had the highest germination percentage (84.5%), followed by T6 and then T5. However, the control treatment (T9) recorded the lowest germination percentage (52.6%).

All treatments of tested compounds and substances that were soaked for 2 h showed the best germination ability (Table 5), while the opposite was observed for seeds soaked for 1 h and/or 3 h. Sugar beet seeds soaked in T2 at the recommended rate for 2 h showed the highest germination rate (99.3%) after 21 days, followed by T3 and then T1.

The lowest germination rates were seen in T3-treated seeds soaked for 1 h, regardless of the control treatment (T9), with rates of 50.2%. T8 (Viva 24 L%® -2 foliar applications, where seeds were soaked in water as in the control treatment) had germination rates of 51.1% after 3 hours and 52.4% after 1 h. Table 5 indicates that T2 seed treatment yielded the highest GI value (40.4), followed by T6 (38.0) and then T5 treatments (35.2).

However, the control treatment had the lowest value (23.6). Furthermore, seed treatments soaked for 2 hrs showed higher values of GP and GI compared to other soaking periods (1 hr and 3 hr). T2 seeds soaked for 2 h, exhibited the highest GP and GI values (49.1 and 49.3), with T3 following closely at (43.1 and 47.6), respectively.

While GP and GI showed almost significant differences, overall values of GRI had negligible significance. Briefly, the optimal germination treatments involve consideration of the germination percentage (GP), index (GI), and/or germination rate index (GRI). This includes the use of Nano-NPK, Nabta-Bio® (a growth regulator), and Viva 24%® (Oxamyl 24% L).

Table 5. The effect of seed treatments containing multiple compounds on germination percentage (GP), germination index (GI), and germination rate index (GRI) after 21 days from sowing.

Seed treatments	Soaking period (hours)	Germination (%)			GI 21 DAS	GRI 21 DAS
		7	14	21		
		Days after sowing (DAS)				
T1	1	84.0	61.0	75.3 cd	33.3 c	44.2b
	2	51.0	73.5	90.7 ab	43.1 a	47.5 a
	3	33.0	44.4	54.9 f	23.3 d	42.5 c
	Mean	56.0	59.6	73.6	33.2	44.7
T2	1	43.0	65.2	80.4 c	38.3 b	47.6 a
	2	55.0	80.4	99.3 a	49.1 a	49.4 a
	3	39.3	59.7	73.7 cd	33.7 c	45.7 b
	Mean	45.8	68.4	84.5	40.4	47.6
T3	1	23.0	40.7	50.2 f	22.7	45.2 b
	2	50.0	73.7	91.0 ab	43.1 a	47.4 a
	3	40.7	61.0	75.3 cd	35.3 b	46.9 a
	Mean	35.7	56.4	69.6	32.8	47.1
T4	1	23.7	51.0	63.0 de	29.3 d	46.5 a
	2	44.0	60.2	74.3 cd	34.7 c	46.7 a
	3	39.3	58.0	71.6 cd	34.3 c	47.9 a
	Mean	35.7	56.4	69.6	32.8	47.1
T5	1	39.3	60.2	74.3 cd	33.7 c	45.4 b
	2	41.0	66.7	82.3 c	39.3 b	47.8 a
	3	39.0	62.4	77.0 cd	32.7 c	42.5 c
	Mean	39.8	63.1	77.9	35.2	45.2
T6	1	38.2	58.0	71.6 cd	32.3 c	45.1 b
	2	51.3	70.2	86.7 c	41.1 b	47.4 a
	3	35.0	61.3	75.7 cd	35.7 b	47.2 a
	Mean	41.5	63.2	78.0	38.0	48.9
T7	1	23.3	51.0	63.0 de	27.3 d	43.4 b
	2	43.3	61.3	75.7 cd	35.7 b	47.2 a
	3	22.7	58.0	71.6 cd	33.3 c	46.5 a
	Mean	29.8	56.8	70.1	32.1	45.7
T8	1	22.3	42.5	52.4 f	23.0	43.9 b
	2	30.0	52.4	64.7 de	31.7 d	49.0 a
	3	22.1	50.0	51.1 f	24.0	47.0 a
	Mean	24.8	48.3	56.1	26.2	46.6
T9	1	21.0	41.3	51.0 f	23.3 d	45.7 b
	2	29.0	52.9	53.0 f	23.7 d	44.7 b
	3	25.1	45.7	53.7 f	23.7 d	44.1 b
	Mean	25.0	46.6	52.6	23.6	44.8

GI; germination index; (GRI) ; germination rate index.

Means having different letters in the same column are significantly different ($P \leq 0.05$).

Effect on sugar beet root galling

The analysis of variance for the combined data of two seasons, illustrated in Table 6, and Figure 4, revealed that the maximum reduction in root galling (over 80%) occurred in plots treated with T1 (86.1%), T2 (86.9%), T3 (87.6%), and T4 (80.3%).

The remaining treatments, T5, T6, T7, and T8, achieved galling reductions of 78.5%, 76.1, 68.1, and 64.5%, respectively.

The reduction in sugar beet root galling compared to the control treatment is considered reasonable. It is also worth noting that seeds treated with a combination of Nano-NPK techniques and/or growth regulators achieved the best results in reducing galling percentage.

The lowest reduction (64.5%) was observed in treatment T8, which involved foliar application only with untreated seed (Table 6).

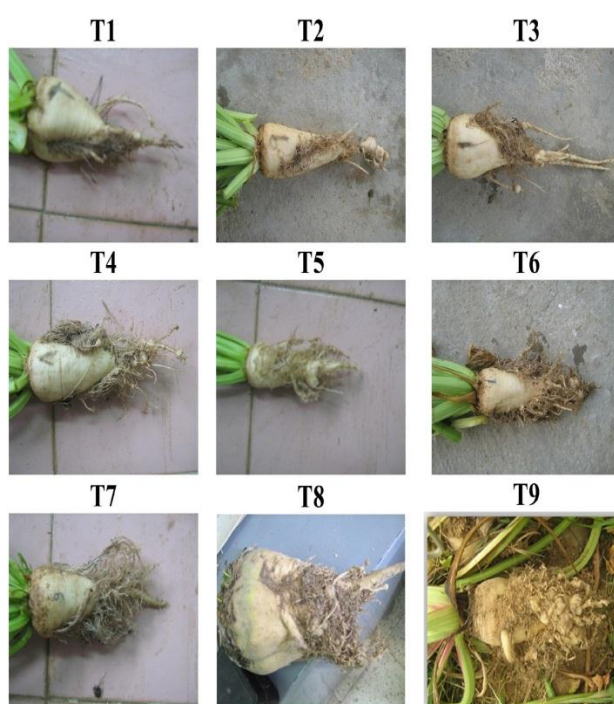


Figure 4. The impact of seed treatments with various compounds on sugar beet root gall formation.

Effect of seed treatment on the development of females, egg masses, and juveniles

Data in Table 6 indicated substantial variations in the combined data of both seasons for various aspects such as females, egg masses, juveniles per root system⁻¹, and juveniles per 200 g of the soil. The treatments involving the application of Nabta-Bio®, Nano-NPK, and Oxamyl 24%.L either alone or in combination showed a notable decrease in the number of females, with the most significant reduction ranging from 88.7% to 90.5% observed in T1, T2, and T3. Among these, T2 had the highest reduction at 90.5%, followed by the other treatments (T4, T5, T6, T7, and T8) with reductions ranging from 62.4 to 79.2%, where T4 showed the highest reduction at 79.2% (as a seed treatment). Subsequently, the same above-mentioned treatments achieved a similar tendency of reduction on the number of egg masses, juvenile's root system⁻¹, and juveniles 200 g of the soil, ranging from 56.0% for the treatment T8 as foliar application up to 89.0 % reduction assigned by T2 with same treatments that attained almost the same percentage reduction with females root system⁻¹, and in groups of similar significance. Juvenile's root system⁻¹ and juveniles 200 g of the soil were affected severely by the most promised treatments, i.e., T1, T2, and T3, whereas, the reduction percentage ranged between 83.5 to 90.2 %. The second-ranked effect on the previous parameters was exhibited by treatments T4 to T8 which attained a range of 32.9 (Juvenile's root system⁻¹) up to 68.6% reduction.

The superlative results were due to those treatments contained in one way or another Nano-NPK and/ or growth regulators (Nabta-Bio®) plus Oxamyl 24.L % as seed treatment. Due to the lack of direct seed treatment, oxyamyl-24% foliar application achieved the lowest reduction percentages in all studied nematode parameters.

Effects of seed treatment on nematodes build-up rate

The first three treatments in Table 6 (T1, T2, and T3) exhibited a significant reduction in the build-up rates of *M. incognita*, with no significant differences among them at $P \leq 0.05$ (87.2, 85.7, and 88.8%, respectively). These reductions were observed when T1 and T2 were applied individually, and when T3 was used as an aid, compared to the control treatment. The percentage of build-up reduction ranged from 61.4 to 79.2% for treatments T4 to T7, with T4 achieving 79.2%, T5 achieving 77.8%, and T6 achieving 75.9%. Treatment T8 recorded the lowest reduction percentage at 61.4%. Based on the findings presented in Table 6, it is evident that the key to effectively managing root-knot nematodes in these experiments lies in the combined impact of Nano-NPK, growth regulators (Nabta-Bio®), and the nematicide Oxamyl.

Data in Table 6 indicates that the most effective single application for reducing root galling and suppressing all nematode stages was associated with the use of Nano-NPK and growth regulators (Nabta-Bio®). They indirectly affect the nematodes in the soil, leading to improved early seedling development.

As shown in Table 5, there were positive effects on germination percentage (GP), germination index (GI), and germination rate index (GRI). This prevents early penetration of newly hatching larvae (J2) or limits the hatching of eggs and the movement of juveniles into roots when these treatments are combined with a nematicide. Contrary to the treatment of nematode alone, where the build-up rate reached the maximum (7.2 times), the treatment of T8 [Oxamyl 24. L % as foliar application] recorded the lowest reduction percentage in all studied nematode parameters and a build-up of 2.8 times.

All favorable conditions for the increase in nematode population were present, such as degree days, a susceptible host, a relatively long crop maturation period (six months), and the absence of any control measures. Furthermore, using Oxamyl 24. L% as a foliar treatment (T8) alone caused a delay in early nematode penetration. Even during an infestation, this could have primed plants to tolerate and harbor additional nematodes (supplementary tolerance).

Table 6. The effect of seed treatments containing multiple compounds on nematode population, egg masses, juveniles/root, and juvenile in soil, females, and build-up factor of *Meloidogyne incognita* infesting sugar beet plants (a combined analysis over the 2019/2020 and 2020/2021 seasons).

Treatments	No.of galls/ root	Red. (%)	No.of females / root	Red.(%)	No.of egg masses / root	Red.(%)	No.of juveniles			Build-up	Rate Red. (%)	
							Per root	Red. (%)	200 gm soil			
T1	35f	86.1	25f	88.7	26e	87.0	30.0f	88.2	150g	86.7	0.9d	87.2
T2	33f	86.9	21f	90.5	22e	89.0	28.0f	89.0	186f	83.5	1.0d	85.7
T3	31f	87.6	24f	89.1	23e	88.5	25.0f	90.2	130h	88.4	0.8d	88.8
T4	45d	82.1	46de	79.2	45d	77.5	74.0e	71.0	210de	81.3	1.5c	79.2
T5	54c	78.5	50d	77.4	49d	75.5	80.0d	68.6	220d	80.4	1.6b	77.8
T6	60c	76.1	58c	73.8	61c	69.5b	90.0d	64.7	225d	80.0	1.7c	75.9
T7	80b	68.1	79b	64.3	83b	58.5	151.2c	40.7	285c	74.7	2.4b	66.8
T8	89b	64.5	83b	62.4	88b	56.0	171.2b	32.9	353b	68.6	2.8b	61.4
T9	251a	0.0	221a	0.0	200a	0.0	255.0a	0.0	1125a	0.0	7.2a	0.0

Values are averages of four replicates.

Means having different letters in the same column are significantly different at 5% level of significance.

Effect of seed treatments containing multiple compounds on gall index, disease severity%, and treatment efficacy% in sugar beet

Data in Table 7 shows a significant reduction in gall index, disease severity, and treatment efficacy. These parameters measure the effectiveness of nematode control strategies used in the study. At harvest, T1, T2, and T3 were the most effective compounds, with a maximum reduction of 60.0% for gall index and 81.6% for disease severity. In return, the treatment efficacy was superior for

T1, T2, and T3, ranging between 77.0% and 81.2%, while T4 to T7 achieved lower efficacy, ranging between 11.4% and 46.6%. Data in Table 7 indicated that treating seeds with Nabta-Bio and/or Nano-NPK alone resulted in a significant decrease in disease severity percentage, while combinations boosted the management of root-knot nematode on sugar beet.

Table 7. The effect of seed treatments containing multiple compounds on gall index, disease severity%, and treatment efficacy % for *M. incognita* infestation of sugar beet plants with a combined analysis of the 2019/2020 and 2020/2021 seasons.

Treatments	Gall index Red. (%)	Disease severity Red. (%)	Treatment efficacy (%)
T1	2.0 (60)	10.9d (81.6)	81.2a
T2	2 (60)	11.6d (80.6)	80.0a
T3	2.0 (60)	13.3d (77.8)	77.0a
T4	2.5 (20)	31.3c (47.7)	11.4c
T5	3.0 (40)	33.3c (44.4)	42.5b
T6	3.0 (40)	30.9c (48.4)	46.6b
T7	4.0 (20)	50.6b (15.2)	12.6c
T8	4.0 (20)	49.6b (17.2)	14.3c
T9	5.0 (0.0)	57.9a	0.0

Different letters in the same column are significantly different at a 5% level of significance. A scale from 0 to 5, where 0 indicated no galls and 5 indicated more than 100 galls per root system (Taylor and Sasser 1987).

Results from field trials, as presented in Table 8, indicate that T3 seed treatment resulted in the highest actual field emergence at 91.0%, followed by T1 at 89.7% and T2 at 85.3%. These were not significantly different from each other. Following these treatments are T7 (77.3%), T5 (75.3%), T6 (74.6%), and T4 (74.3%) seed treatments, as well as sugar beet seed treatment at 68.5%, all showing similar effects. The lowest actual field emergence at 64.7% was observed for T5, which involved foliar application without seed treatment and significantly differed from the control

treatment. The data shows a consistent trend in actual plant density, leaf weight (tons per feddan), and root yield (tons per feddan). The T3 seed treatment had the highest yield (18.2 tons per feddan for leaves and 36.2 tons per feddan for roots), followed by the T1 seed treatment (17.9 tons per feddan for leaves and 35.7 tons per feddan for roots), and then T2 seed treatment (16.7 tons per feddan for leaves and 33.9 tons per feddan for roots). There were no significant differences between T1 and T2 treatments.

Table 8. The effect of seed treatments containing multiple compounds on actual field emergence%, actual plant density ($\times 10^3$), leaves weight and roots yield (tons fed⁻¹) for sugar beet plants infested by *M. incognita* with a combined analysis of the 2019/2020 and 2020/2021 seasons.

Treatments	Quantitative response as				
	Actual field emergence (%)	Actual plant density ($\times 10^3$)	Leaves weight (ton fed ⁻¹)	Root yield (ton fed ⁻¹)	Rank of beet (roots) yield
T1	89.7a	47.5a	17.9a	35.7a	2
T2	85.3a	45.2a	16.7a	33.9a	3
T3	91.0a	48.2a	18.2a	36.2a	1
T4	74.3b	39.4b	14.3b	29.5b	7
T5	75.3b	39.9ab	14.9b	29.9b	5
T6	74.6b	39.5b	14.7b	29.7b	6
T7	77.3b	41.0ab	15.0ab	30.7b	4
T8	64.7c	34.3	13.0b	25.7c	8
T9	53.0d	28.1c	11.6c	21.1d	9

Different letters in the same column are significantly different at a 5% level of significance by the Waller-Duncan K-ratio t-test. The layout plan was to obtain a rate of 53000 plants fed⁻¹.

Regarding the qualitative reaction of sugar beet technological characters (Table, 9), there was no significant difference found among almost all treatments except T8 and control treatment whereas they didn't receive seed treatment which had the lowermost values with significance for T.S.S %, Pol % and sugar recovery % (18.4, 16.8 and 10.7, respectively).

The sugar yield tons fed⁻¹ differed significantly between the two groups of the seed treatments based

on the level of productivity (Table 9; Figure 5) the first high yielded implied (T1, T2, and T3) and the second low to medium yield implied (T4, T5, T6, T7 and T8), their sugar yield tons fed⁻¹ oscillated between 3.1 – 3.7 tons fed⁻¹ but it was comparable to the highest sugar yield achieved under T3 seed treatment (5.0 tons fed⁻¹) followed by T1, and T2 seed treatment (4.5 and 4.3 tons fed⁻¹), respectively.

There were no significant differences within each group (Table 9).

Table 9. The effect of seed treatments containing multiple compounds on the qualitative reaction of sugar beet technological characters of plants infested by *Meloidogyne incognita* with a combined analysis of the 2019/2020 and 2020/2021 seasons.

Nematicide seed Treatments	T.S.S (%)	Pol (%)	Qualitative response as		
			Sugar Recovery (%)	Sugar Yield (ton fed ⁻¹)	Rank for sugar Yield
T1	22.2a	20.3a	12.8 a	4.5 a	2
T2	21.6a	20.0 a	12.7a	4.3a	3
T3	23.4a	21.3a	13.0a	5.0a	1
T4	20.2a	18.0a	11.9a	3.6b	5
T5	19.6ab	16.4b	10.9ab	3.3b	8
T6	20.6a	17.8b	11.3a	3.7b	4
T7	19.4ab	16.8b	10.8ab	3.3b	6
T8	18.4b	16.8b	10.7ab	3.2 c	7
T9	18.0b	15.4	10	2.8 c	9

Different letters in the same column are significantly different at a 5% level of significance by the Waller-Duncan K-ratio t-test.

Discussion

The great germination outcomes were linked to Nano-NPK and Nabta-bio, essential growth regulators containing auxin, cytokinin, and gibberellin. Nano fertilizers are seen as promising for enhancing nutrient retention in seed and crop growth.

The study indicated that treating sugar beet seeds with Nabta-Bio®, Nano-NPK 50%, and occasionally Oxamyl 24% produced positive results, improving early-stage development and leading to a higher number of viable plants post-thinning with reduced losses. Each treatment had specific effects on various parameters.

The research focused on the effects of different treatments alone or combined on root-knot nematode parameters such as nematode density (250 nematodes per gram of soil) and disease severity. The findings demonstrated that the management treatments were most successful in decreasing all studied root-knot nematode parameters.

The study found that compounds increased sugar beet seed germination at different rates, consistent with Orzeszko-Rywka and Podlaski's (2003) findings. Variations in germination parameters were mainly due to nematocidal seed treatments. The excellent germination parameters were attributed to Nano-NPK and Nabta-bio, essential growth regulators, which contain auxin, cytokinin, and gibberellin. Nano fertilizers are thought to be promising fertilizer candidates because they have the potential to improve nutrient retention in seed and crop growth (Tarafder et al. 2020). Nano fertilizers also have a positive effect on the microbial communities surrounding seeds, which indirectly contributes to seed germination (Nongbet et al. 2022).

It has been discovered that Nano fertilizers containing nitrogen, phosphorous, and potassium (NPK) improve plant development and crop production (Bhardwaj et al. 2022). Furthermore, Nano fertilizer increases seed tolerance to both biotic and abiotic stresses by activating a variety of molecular mechanisms (Seleiman et al. 2020). The study discovered that treatments T1 (Nabta-Bio® 100%), T2 (Nano-NPK 100%), and T3 (Nabta-Bio® 50% + Nano-NPK 50%) resulted in the highest germination percentage and speed. This was attributed to the components of Nano-NPK and Nabta-Bio®, which are essential growth regulators containing auxin, cytokinin, and gibberellin.

The study also highlighted that nano fertilizers show promise in improving nutrient retention in seeds and promoting crop growth (Tarafder et al. 2020). Several studies have shown that nano-fertilizers can improve seed germination in ways that traditional fertilizers cannot. These findings suggest that nano-fertilizers can penetrate seed coats, enhance water absorption by up-regulating aquaporin genes, and consequently enhance seed germination while reducing the negative effects of salinity, drought, and heavy metal stresses. PGR is a compound that enhances plant growth and development by combining auxin, gibberellin, and cytokinin .

Auxins promote seed germination by promoting cell division in the embryo, forming the radicle, and regulating the seed coat's opening for water and nutrients. Gibberellins break seed dormancy by stimulating hydrolytic enzymes, providing energy for germination and enhancing cell elongation. Cytokinins counteract auxins by promoting cell division in the shoot meristem, preventing premature aging, maintaining seedling vigor, and regulating nutrient uptake and transport (Chen et al. 2015). Enhancing seed germination involves various mechanisms, one of which includes the use of nanomaterials to create nanopores in seed coats.

Additionally, reactive oxygen species (ROS) are known to contribute to this process. Signaling molecules such as ROS and phytohormones also play a crucial role in regulating germination. Studies have shown that understanding and manipulating these mechanisms can significantly improve the germination success of seeds (Shelar et al, 2023). It promotes germination and regulates seed dormancy and lipid mobilization. Gibberellic acid boosts germination by reducing abscisic acid levels, while auxin promotes rhizogenesis and stem cell multiplication. Cytokinins, plant hormones, govern various plant functions, including seed germination, activities of meristemic cells in roots, and leaf senescence (Riefler et al. 2006).

The research revealed that using Nabta-Bio®, Nano-NPK 50%, and occasionally Oxamyl 24% as seed treatments led to positive outcomes for sugar beet plants. These positive outcomes were attributed to improved early-stage development, resulting in a higher number of viable plants after thinning and a reduced loss percentage. Each of these treatments had specific effects on different parameters.

The study focused on the impact of different treatments, either used alone or in combination, on root-knot nematode parameters such as nematode density (250 nematodes per gram of soil) and severity of knot disease. The results showed that the management treatments yielded the most effective outcomes in reducing all the studied root-knot nematode parameters. This underscores the growing significance of understanding and implementing cropping practices and seed technology as alternative approaches to chemical pesticides (Dewar 2017; Kathage et al. 2018). Seed treatments for plant parasitic nematodes involve applying nematicides or biological control agents to seeds before planting. These methods protect emerging seedlings from nematode damage and improve crop yields. The findings of this study are consistent with those found by Rady et al. (2021) who stated that using EPD (Early Plant Development or fast initial growth), accelerating the speed of sugar beet germination and seedling emergence offers the most important escape strategy from root-knot nematodes.

Conclusions

The use of Nano-NPK and growth regulators in seed treatment has shown promising results in reducing galling percentage, females, egg masses, juveniles per root system, and sugar recovery. When used alone, Nabta-Bio and/or Nano-NPK significantly decreased disease severity. However, when combined, they improved root-knot nematode management in sugar beet. Applying Nabta-Bio 100%, Nano-NPK 100%, Nabta-Bio 50% + Nano 50%, or a mixture with Oxamyl 24% resulted in positive outcomes for sugar beet, making this approach a valuable component of integrated root-knot disease management.

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