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Article

Potential weeds as a trap crop for *Meloidogyne enterolobii* and *Nacobbus aberrans*

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Abstract

The use of weeds as an agroecological management strategy for phytonematodes has gained importance due to their implementation as trap plants that interfere with their biological cycle; therefore, this research aimed to evaluate the percentage of reproduction of *Meloidogyne enterolobii* and *Nacobbus aberrans* in five weeds. This experiment was carried out at the College of Postgraduates, Montecillo *Campus*, State of Mexico, Mexico, in 2023. The chili genotype CM-334 (control) was used as a susceptibility reference and each experimental unit was inoculated with 1000 J2 of each nematode species. The response variables were galling, egg masses, eggs, number of females and juveniles per g of root at 35 days after inoculation (dai) for *Meloidogyne enterolobii* and 45 dai for Na. A completely randomized experimental design with factorial arrangement was used. The weeds *Tagetes erecta*, *Portulaca oleracea*, *Dysphania ambrosioides*, *Malva parviflora*, and *Oxalis corniculata* showed a 100% decrease in the number of galls, egg masses, and eggs per g of root for *Nacobbus aberrans*, compared to the control. These last two parameters were similar for *Meloidogyne enterolobii*. All weeds evaluated showed a differential reproduction percentage for both nematodes in the number of females and individuals per g of root (7.34-100%). The results obtained indicate that these weeds can be used as a potential trap crop for the management of the nematodes *Meloidogyne enterolobii* and *Nacobbus aberrans*.

Palabras clave:

Dysphania ambrosioides, Malva parviflora, Oxalis corniculata, Portulaca oleracea, Tagetes erecta.

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Introduction

The root-knot nematodes *Meloidogyne* spp., present in several agricultural areas of Mexico, attack vegetables, fruit trees, ornamentals, and staple crops, causing losses in their yield (Cid del Prado *et al*., 2001); studies have focused on both *M. incognita* and *Nacobbus aberrans* (Na) (Moens *et al*., 2009) because they are polyphagous species; however, in recent years, the incidence of *M. enterolobii* (Me) (Villar-Luna *et al*., 2016) has been reported in Sinaloa, which is one of the largest chili producers in Mexico (SIAP, 2019).

The losses in production and in the income of producers caused by these nematodes make it necessary to search for control alternatives. Among these, the implementation of weedy or cultivated plants has been chosen since they can act as susceptible or resistant hosts to phytoparasitic nematodes (Rich *et al*., 2009; Ntidi *et al*., 2016). This characteristic can be used in phytosanitary management through the use of trap plants, either as indicators or differentials between nematode species, considering local conditions such as predominant nematode species, availability of sowing material, and geographical distribution of the evaluated species.

Additionally, some weeds can act as antagonists to phytoparasitic nematodes, whose properties affect their biological cycle, and it is attributed to nematicide/nematostatic metabolites present in the tissues of these species (Ferraz and Valle, 1997); these compounds can be released into the external environment or act only within the plant (Moreira *et al*., 2015). These plants can also be used as green cover or organic matter, or to improve overall soil quality (Moreira *et al*., 2015).

The use of species with antagonistic characteristics, such as *Tagetes* spp., *Phyllanthus amarus*, *Trianthema portulacastrum*, *Solanum xanthocarpum*, *Coccinia grandis*, and *Leucas cephalotes*, can have a significant impact on the reduction of the galling index caused by root-knot nematodes mediated by their phytochemical and physiological composition (Khan *et al*., 2019). This potentially inhibitory action on the mechanisms of host-nematode interaction, either individually or interspersed with other antagonist species, is proposed as a strategy for the control of root-knot nematodes in agricultural systems.

In this sense, it is suggested that the use of weeds could have a positive impact on the reduction of reproductive parameters of phytopathogenic nematodes and consequently on the damage caused by these species in agricultural crops. The research aimed to evaluate the percentage of reproduction of *Nacobbus aberrans* and *Meloidogyne enterolobii* in five weeds.

Materials and methods

Experimental site and plant material

At the College of Postgraduates, Montecillo *Campus*, State of Mexico, Mexico, in 2023, the reproduction factor of the nematodes *N. aberrans* (Na) and *M. enterolobii* (Me) was evaluated on the following weeds: *Tagetes erecta* L., Asteraceae, *Portulaca oleracea* L., Portulacaceae, *Dysphania ambrosioides* L., Amaranthaceae, *Malva parviflora* L., Malvaceae, and *Oxalis corniculata* L., Oxalidaceae grown in pots with 236 cm^3 of substrate (peat most:black soil:sand; 1:0.5:1) and they were kept under greenhouse conditions (38 °C max and -7 °C min). The chili genotype Criollo de Morelos CM-334 (CM-334) was used as a reference of susceptibility to Me and Na (Villar-Luna *et al*., 2015).

Inoculum and inoculation

The inoculum of Me and Na was maintained in plants of chili (cv. California Wonder) and tomato (Río Grande) in a greenhouse at the College of Postgraduates, Montecillo *Campus*. Egg was extracted according to Vrain (1977) methodology; the juveniles of the second instar (J2) were obtained from eggs incubated at 27 ±1 °C in Petri dishes with sterilized distilled water. Each plant species was inoculated when they had 3-4 pairs of leaves with an inoculum level of 1000 J2 per plant (Filialuna *et al*., 2022).

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Experimental design and variables evaluated

The experimental unit consisted of an inoculated plant with five replications per plant species evaluated. It was established under a completely randomized design with factorial arrangement, where one factor was the species of nematode and another factor was the species of weed; five variables were evaluated: 1) galling; 2) egg masses; 3) eggs; 4) females and 5) juveniles per g of root at 35 days after inoculation (dai) for Me and 45 dai for Na. Subsequently, in the substrate containing the weeds, a cherry tomato seedling with 3-4 pairs of true leaves was planted in order to evaluate the presence of inoculum in said substrate. At 35 days after the extraction (dae) of the weeds inoculated with Me, the same parameters mentioned above were evaluated in the same way as occurred for Na at 45 dae.

Determination of weeds differential to *M. enterolobii* and *N. aberrans*

Egg masses were stained with phloxine B (Hussey and McGuire, 1987) and acid fuchsin was used for the number of nematodes present in the root system (Byrd *et al*., 1983). The number of egg masses per root was counted under a magnifying glass with a 5x magnification and the number of masses per root was recorded. The number of nematodes present in the root was obtained with the use of a stereoscopic microscope (Zeiss Stemi DV4).

Eggs were extracted using Vrain (1977) method and the number of eggs per gram of root of each species evaluated was determined. The evaluation of the categorization of host, good host or non-host, was carried out according to the criteria reported by Oostenbrink (1966) with modifications, who establishes the reproduction factor RF= 0-0.09 as non-host; 0.1-0.9, poor host; 1-2, moderate host and >2, adequate host. Where the RF was determined by dividing the final population (Pf) by the initial population (Pi).

The reproductive parameters of the nematode were estimated in each experimental unit according to Kanchan *et al*. (2023), who indicate that the percentage of reduction in reproductive parameters is equal to: [(number of p in the control - number of p in the treatment)/number of p in the control] x 100%. Where: p= galls/number of egg masses/eggs per g of root/number of females and juveniles inside the root.

Statistical analysis

The data of the evaluated variables were subjected to an analysis of variance (Anova) with a confidence interval of 95%; the statistical differences between the means were compared using Tukey's test ($p \geq 0.05$) with the statistical program of Sas version 9.0 (SAS Institute Inc, 2002).

Results and discussion

Significant differences ($p \geq 0.05$) were observed in all evaluated variables caused by *M. enterolobii* and *N. aberrans*. Me presented greater galling and number of juveniles per gram of root compared to Na (Tables 1 and 2). There was a differential galling among the weed species evaluated and the severity caused by the root-knot nematodes evaluated (Figures 1 and 2).

Table 1. Reproductive parameters evaluated 35 days after inoculation with *M. enterolobii* in different weeds.

RP	*Portulaca oleracea*	*Tagetes erecta*	*Dysphania ambrosioides*	*Malva parviflora*	*Oxalis corniculata*	CM-334
Galls	24.4 ±9^{B}	0 ±0^{C}	21.4 ±8.6^{B}	169.2 ±49.7^{A}	0 ±0^{B}	80 ±27.1^{A}
Egg masses	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	1.2 ±0.4^{B}	0 ±0^{B}	64.6 ±9.7^{A}
Juveniles	54.9 ±26.6^{B}	0 ±0^{C}	45.2 ±24.8^{B}	90.8 ±30.7^{A}	1.6 ±1.8^{C}	96.6 ±20.6^{A}
Mature females	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	39.4 ±13.7^{A}
Immature females	18.6 ±4.9^{A}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}

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RP	*Portulaca oleracea*	*Tagetes erecta*	*Dysphania ambrosioides*	*Malva parviflora*	*Oxalis corniculata*	CM-334
Eggs	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0.7 ±0.4^{B}	0 ±0^{B}	1386.4 ±4110.4^{A}
Galls 2^{o} cycle	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	86.8 ±34.8^{A}

Mean comparison test (Tukey) with an alpha= 0.05; RP= reproductive parameters. Means with a common letter are not significantly different ($p \leq 0.05$).

Table 2. Reproductive parameters evaluated at 45 days after inoculation with *N. aberrans* in different weeds.

RP	*Portulaca oleracea*	*Tagetes erecta*	*Dysphania ambrosioides*	*Malva parviflora*	*Oxalis corniculata*	CM-334
Galls	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	94.4 ±19.4^{A}
Egg masses	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	98 ±12.6^{A}
Juveniles	31 ±11.1^{B}	0 ±0^{C}	15 ±4.35^{B}	70.4 ±24.71^{A}	0 ±0^{C}	93.6 ±19.84^{B}
Mature females	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	124.2 ±20.3^{A}
Immature females	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	32.4 ±21.92^{A}
Eggs	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	32088.4 ±12897.2^{A}
Galls 2^{o} Cycle	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	0 ±0^{B}	57.2 ±16.45^{A}

RP= reproductive parameters. Test= mean comparison test (Tukey) with an alpha= 0.05. Means with a common letter are not significantly different ($p \leq 0.05$).

Figure 1. Presence of *M. enterolobii* (Me) and *M. incognita* (Mi) in weed roots stained with acid fuchsin at 35 days a fter inoculation (dai). A) adult female of Me in *Malva parviflora* root; B) juvenile J3-J4 of Me in *Portulaca oleracea* root; C) *Tagetes erecta* root without the presence of nematodes; D) juvenile J2 of Me in *Oxalis corniculata* root; E) juvenile J2 of Me in *Dysphania ambrosioides* root; F) adult female of Me in CM-334 root and G) adult female of Mi in *Oxalis corniculata* root.

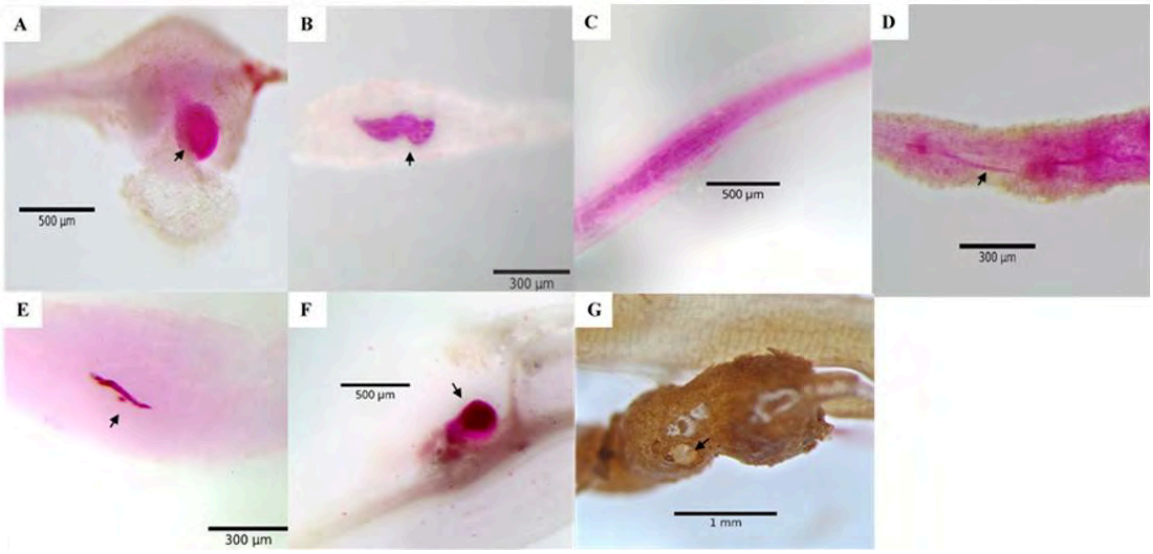

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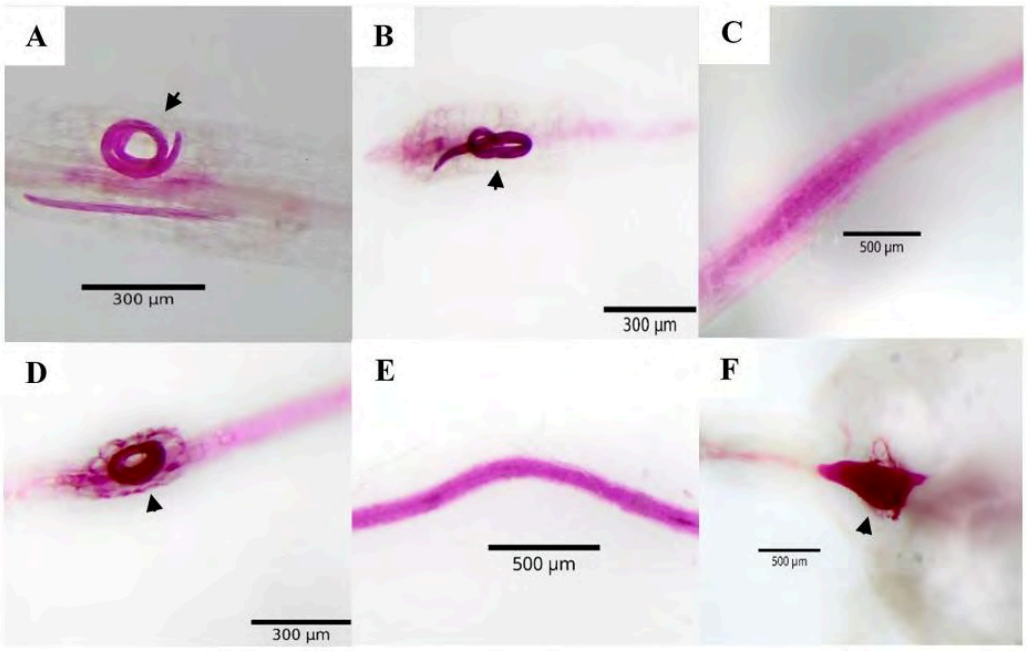
Figure 2. N. aberrans in weed roots stained with acid fuchsin at 45 days a er inocula on (dai). A) Juvenile J3-J4 in Malva parviflora root; B) juvenile J3-J4 in Portulaca oleracea root; C) Tagetes erecta root without the presence of nematodes; D) juvenile J3-J4 in *Dysphania ambrosioides* root; E) *Oxalis corniculata* root without the presence of nematodes and F) adult female in CM-334 root.



Malva parviflora, *Portulaca oleracea*, and *Dysphania ambrosioides* exhibited a greater susceptibility to Me infection; nevertheless, this behavior was different for Na, whose weeds did not present galling for this species (Table 2, Figure 2). On the other hand, *Oxalis corniculata* showed galling only for *M. incognita* (Figure 1), with an average of 270 galls per plant. In addition to a greater galling caused by Me (Table 1), a greater presence of J2-J3 juveniles was observed in all weeds evaluated compared to Na (Tables 1 and 2); the positive control CM-334 presented a greater number of juveniles in both species of root-knot nematodes (Tables 1 and 2).

Lower virulence was observed based on the number of Me juveniles in the weeds *Oxalis corniculata* (1.6) and *Tagetes erecta* (0), compared to the rest of the weeds evaluated (Table 1). In the case of Na, the weed *Oxalis corniculata* (0) and *Tagetes erecta* (0) were not susceptible to this nematode (Table 2). No mature females of Me or Na were observed in the weeds *Portulaca oleracea*, *Dysphania ambrosioides*, *Tagetes erecta*, and *Oxalis corniculata* (Figures 1 and 2, Tables 1 and 2).

All weeds evaluated showed no egg masses, except for *Malva parviflora* inoculated with Me, although this was not reflected in the galling of the second cycle, which corresponded to tomato plants (Table 1). On the other hand, *Oxalis corniculata* recorded an average of 77 egg masses per plant and 767 eggs per plant of *M. incognita*.

The virulence caused by Na had the same behavior in the production of eggs per gram of root (Table 2) and the reproduction factor that was equal to 0 in all weeds evaluated; that is, there was no presence of eggs, so all weeds evaluated according to Oostenbrink (1966) behaved as non-host species for both root-knot nematodes.

Although Me form galls in the weeds *Portulaca oleracea* and *Dysphania ambrosioides*, no adult stages were found; these findings are similar to those reported by Groover *et al*. (2019) as they found differences in galling in cotton (Fibermax 1944), corn (Mycogen 2R042) and soybean (Asgrow 5935) plants caused by species of the genus *Meloidogyne*, demonstrating that the differentiation of root-knot nematode species, through the implementation of hosts that show the variability of nematode populations in the same field, can contribute in a practical way to decision-making for the

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management of these species through the crop rotation thanks to the decrease in the reproductive factor, which would indicate that the formation of galls is not essential or indicative of the adequate development of the nematode (Cook and Starr, 2006).

The behavior of the weeds *Portulaca oleracea*, *Tagetes erecta*, *Dysphania ambrosioides*, and *Oxalis corniculata* may be related to what was pointed out by Villar-Luna *et al*. (2015) in the CM-334 chili, who reported a low penetration of J2 of *M. incognita* at 21 days after inoculation, which is highly resistant to this species, attributing it to a restriction in its establishment.

They also point out that this incompatible interaction (Mi-CM-334) may be involved in the overexpression of the EAS, HMG2, WRKY-a, PR-1 and POX genes associated with plant defense mechanisms, as well as with the accumulation of bioactive compounds, which they related them to the restriction of the establishment and reproduction of the nematode.

The inhibition of the development of adult females of Me and Na observed in plants of *Portulaca oleracea*, *Dysphania ambrosioides*, and *Oxalis corniculata* (Tables 1 and 2, Figures 1 and 2) could be based on a hypersensitivity response (HR), which plays a crucial role in immunity to plant pathogenic nematodes by influencing migration directly or indirectly through the release of nematostatic chemicals, nematicides or damage-associated molecular patterns that can activate other immune responses, such as creating a physical barrier between surrounding cells, inhibiting the supply of nutrients in the nematode's feeding cells, causing a reduction in fecundity in females and therefore in the reproduction factor (Sato *et al*., 2019), as observed in *Portulaca oleracea*, which presented only immature females for Me (Table 1).

This is consistent with Proita *et al*. (2008) results, who analyzed the post-infection development and histopathology of *Meloidogyne arenaria* race 1 on three species of *Arachis* spp. and observed a hypersensitive reaction with the formation of necrotic zones in the vascular cylinder and there were no giant cells or juvenile development until the second stage (J2); in the same way, in our results, there was only the presence of juveniles of different stages of Me and Na in *Dysphania ambrosioides* (Tables 1 and 2), of Na in *Portulaca oleracea* and *Malva parviflora* (Table 2), and of Me in *Oxalis corniculata* (Table 1).

In *Portulaca oleracea*, flavonoid levels have been reported to vary depending on the part of the plant, with the highest levels being present in the root followed by the stem and leaf (Zhou *et al*., 2015); likewise, seven different flavonoids have been found present in this plant, including kaempferol, myricetin, luteolin, apigenin, quercetin, genistein and genistin (Uddin *et al*., 2014), the same compounds that, in recent research, have been identified as chemicals that show high mortality and inhibition of hatching of eggs and juveniles of *Meloidogyne incognita* species (Wuyts *et al*., 2006; Khan *et al*., 2019).

Nonetheless, it has been reported as a host for *M. incognita*, as well as for Me and Na (Rich *et al*., 2009; Cid del Prado *et al*., 2018), which does not agree with what was observed in this work. On the contrary, we agree with what Manzanilla *et al*. (2002) report for *N. aberrans*, where they indicate that, although some species of Poaceae (= Gramineae) are reported as hosts, in these plants, there is usually only the presence of vermiform juveniles and females without the presence of obese females.

This behavior indicates that some species of weeds can serve as trap plants since they penetrate the infective stages but do not allow the biological cycle of the nematode to be completed as adult females are not found, which induce the formation of galls and interfere with the absorption of water and nutrients (Triviño, 2004).

Similarly, the action of secondary metabolites present in these species can affect the behavior of *M. incognita*, acting as attractants or repellents, inducing the inhibition of motility, reducing incubation, and causing its death (Yang *et al*., 2016). Wuyts (2006) observed that salicylic acid accumulates at the sites of nematode location, being able to migrate through tissues, acting as an elicitor that triggers the signal systems of plant cells, in such a way that endogenous salicylic acid induces gene expression, which results in the formation of proteins related to pathogenesis and the production of phytoalexins (Hussey and Janssen, 2004).

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On the other hand, it could be related to what was reported by Kirwa *et al*. (2018), who found that *M. incognita* can be attracted to five compounds (zeatin, quercetin, luteolin, solasodine, and tomatidine) isolated from tomato rhizosphere extract and that chemotaxis activity depended on concentration; some of these compounds, such as quercetin and luteolin, have been reported in the phytochemical profile of all weed species evaluated and their concentration depends on the host species.

Conclusions

Meloidogyne enterolobii (Me) induced galls in *Portulaca oleracea*, *Dysphania ambrosioides* and *Malva parviflora*, but the presence of galls by *Nacobbus aberrans* (Na) was not evident; for its part, in *Oxalis corniculata*, there was only the presence of galls by *Meloidogyne incognita* (Mi); this behavior suggests that these weeds can be used as differential species according to the presence of galls.

On the other hand, *Portulaca oleracea*, *Dysphania ambrosioides*, and *Oxalis corniculata* can be used as trap species given the presence of juveniles without adult females of the nematode's species Me and Na.

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