



Management of root-knot nematodes, *Meloidogyne incognita* in Okra using wheat flour as bionematocides



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Abstract: The current study aims to determine how well root-knot nematodes (*Meloidogyne* sp.) are reduced when wheat flour is used as bio-nematicides to increase agricultural productivity. Root-knot nematodes inflict a significant amount of annual loss by parasitizing plant species, many of which are vegetable crops. They harm plants to the extent that they contribute between 10% to 40% of India's annual agricultural losses. A multi-cropping system and the advent of high-yielding crop types have increased the demand for efficient crop protection. Different strategies, including biological, physical, and cultural usage of resistant varieties, and pesticides, have been developed to control the phytophagous nematodes to deal with the situation. Organic raw materials used on the okra plants and the root-knot nematode are evaluated together with prospective bionematocides that aim to manage nematodes over the long run for a sustainable ecological system and profitable crop values. Using a greater dose of the liquid bioagent formulation significantly reduces the nematode population and increases the plant growth parameter. Plant materials provide effective nematicides that are easily biodegradable. The present study intends to further establish the effectiveness of bio-nematicides, such as wheat flour, in treating root-knot disease.

Introduction

Nematodes that parasitise plants are among the most destructive pests in global agriculture. According to thorough surveys (Sasser and Freckman, 1987), the average yield loss across all crops is around 10%, with certain crops seeing losses of as much as 20%. Global losses unquestionably exceed \$100 billion USD annually in monetary terms. In general, a relatively small number of the many nematode genera are responsible for most of the damage (Nickle, 1991; Patil et al., 2021), especially the secondary endoparasitic genera *Meloidogyne*, *Globodera*, and *Heterodera*, which cause significant crop losses (Sasser et al., 1987). These nematodes develop and preserve a close bond with their host (Sijmons et al., 1994). Root-knot nematodes infect thousands of plant species and significantly reduce the output of several crops all over the world (Kayani et al., 2017). They are so named because of the distinctive root galls or root knots that they

produce on many hosts. The application of bio-control agents is one potential supportive alternative for nematicides (Dutta and Thakur, 2017). The proliferation of bio-agents was mainly a result of the favourable conditions (micro climate) kept in safe structures. Additionally, several scientists found that handling complex diseases (*Meloidogyne* sp. and *F. oxysporum*) under controlled circumstances has been enhanced by bio-agent efficacy (Singh, 2019; Singh et al., 2021).

Because many other infections are also linked to nematode infection, it is challenging to estimate the crop losses caused by worms alone. The quantity of harm done by nematodes is mainly determined by their population and other elements like soil type, temperature, moisture, nutrients, and the host plants' vulnerability to the parasite. There has been a lot of interest in recent advancements in the field of proteinase and α -amylase inhibitors as natural plant defence mechanisms (Gatehouse et al., 1986;



Farmer and Ryan, 1990). These inhibitors are thought to provide resistance by rendering plants less appetising, even fatal, to insects. The enzyme inhibitors prevent the digestion of plant proteins and starch by interfering with the activity of digestive enzymes called proteinases and amylases found in insect guts. Pest management strategies could use six different groups of α -amylase inhibitors: lectin-like, knottin-like, cereal-type, Kunitz-like, γ -purothoinin-like, and thaumatin-like. These types of inhibitors exhibit striking structural diversity, resulting in various inhibitory mechanisms and specificity profiles against various α -amylases. Since the injected inhibitor must not negatively impact the plant's (α -amylases or the crop's nutritional value, specificity of inhibition is a crucial concern. An inhibitor family of α -amylase is present in wheat flour (Da Silva et al., 2000).

Recent research has revealed that there are at least two allelic variations of bean α -amylase inhibitor (α -AI). The isoform present in cultivated beans is now known as α -AI-1, while a second variation, α -AI-2, is discovered in some wild forms of the common bean (Moreno and Chrispeels, 1989 ; Suzuki et al., 1993). These two inhibitors, which have 78% identical amino acids, differ in the α -amylases they are specific to when used (Da Silva et al., 2000).

Certain mammalian and insect amylases are inhibited by the α -AI, while plant enzymes are not affected (Marshall and Lauda, 1975; Powers and Whitaker, 1977 a,b). Sweet potatoes have also been found to have α -amylase inhibitors (Rekha et al., 1999). The α -amylase inhibitors in wheat have a very apparent regulating function, according to Pace et al. (1978). According to Mulimani et al. (1994), newly formed sprouts exhibited α -amylase inhibitor activity, possibly serving as a defence mechanism for the developing shoot. The root-knot disease alters the host plants' morphology and biochemistry. The primary objectives of this work were to investigate the potential of α -amylase inhibitors generated from wheat flour to limit root-knot nematode infection in tomato and okra, two different host plants.

Materials and Methods

Nematode and plant material

The root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood was used in the study as the parasite. The egg masses collected from a field-maintained culture of tomatoes and okra were used to create the second-stage juveniles, which were then allowed to hatch.

The host plants selected for my study were:

1. Tomato (*Lycopersicon esculentum*), var. Pusa Ruby
2. Okra (*Abelmoschus esculentus*) L Purbani Kranti

In vitro test

Egg masses from a culture kept on tomato and okra were allowed to hatch into second-stage juveniles (J2) of *M. incognita*. Active J2 extracted from egg masses was stored in 100±25 cavity larvae blocks. The sterile tap water in the cavity blocks where active *M. incognita* J2 were kept was pipetted out and immediately replaced by 1ml of 5% wheat flour suspension, 1ml of 0.02% α -amylase, and 1ml of 0.003% α -amylase inhibitor. This was done to determine the effect of wheat flour, α -amylase, and α -amylase inhibitors on J2 of *M. incognita*. Two cavity blocks containing only sterile tap water were used as negative controls. Nematode mortality at room temperature (26±20C) was observed every hour for six hours.

Pot tests

One tomato seed per pot was aseptically planted in pots 32 cm in diameter filled with an autoclaved mixture of clay soil and compost (2:1 v/v). There were eight groups of nine pots altogether (Fig. 1). These are the groups: Group-A: Non-inoculated and untreated (NU); Group-B: Inoculated and untreated (IU); Group-C: inoculated and treated with 5% flour (20 g/plant) (IF); Group-D: Inoculated and treated with 5% flour (20 g/plant) and α -amylase (2 mg/plant) (IFA); Group-E: inoculated and treated with 5% flour (20 g/plant) and α -amylase inhibitor (300 μ g/plant) (IFI); Group-F: Inoculated and treated with α -amylase (2 mg/plant) (IA); Group-G: Inoculated and treated with α -amylase (2 mg/plant) and α -amylase inhibitor (300 μ g/plant) (IAI); Group-H: Inoculated and treated with α -amylase inhibitor (300 μ g/plant)

When the plants had six leaves, the IU, IF, IFA, IFI, IA, IAI and II groups were inoculated with 1500 ± 175 freshly hatched *M. incognita* J2 in 5 ml of water. The J2 was injected into the soil at a depth of 2 cm, halfway between the plant and the pot's side. Wheat flour was applied as a rhizospheric soil drench. Each plant from the groups IF, IFA and IFI, received a soil drench of 200 ml of flour suspension. α -amylase and α -amylase inhibitor was applied as a foliar spray by an atomizer. Each plant from the groups IA, IFA, IAI and II, IFI, IAI received a foliar spray of 2 ml of α -amylase and α -amylase inhibitor, respectively. Following an inoculation period of 24 hours, treatments were administered. The soil surface underneath each plant was covered with a polyethene sheet during spraying. A drenching and spraying with distilled water were applied to the plants that were either

not inoculated or inoculated but not treated in any way. After a period of four (4) days, treatment was resumed. The experiment was carried out outside at an ambient air temperature ($27\pm 3^{\circ}\text{C}$) and humidity ($81\pm 3\%$), and the plants received routine irrigation.

The same process as described above was used to plant okra in 90 pots, with 10 plants in each of the nine treatments. The groups were: Group-A1: Non-inoculated and untreated (NU); Group-B1: Inoculated and untreated



Figure 1. Growing plants in the pots.

(IU); Group-C1: Inoculated and treated with 5 % flour (20 g/plant) (IF); Group-D1: Inoculated and treated with 5% flour (20 g/plant) and α -amylase (2 mg/plant) (IFA); Group-E1: inoculated and treated with 5% flour (20 g/plant) and α -amylase inhibitor (300 μg /plant) (IFI); Group-F1: Inoculated and treated with α -amylase (2 mg/plant)(IA); Group-G1: Inoculated and treated with α -amylase (2 mg/plant) and α -amylase inhibitor (300 μg /plant) (IAI); Group-H1: Inoculated and treated with α -amylase inhibitor (300 μg /plant). The ambient air temperature ($26.6\pm 30^{\circ}\text{C}$) and humidity ($76.6\pm 6\%$) were comparable to those found in the cowpea experiment

Evaluations of plant development and nematodes

After 40 days of inoculation, measurements of the shoot length, shoot weight, longest root length, root weight, number of root galls (Fig. 2), and eggs per gram (g) of root were noted. Additionally, the quantity of juveniles and root nodules per gram (g) of root were counted (Fig. 3). The roots were treated with sodium hypochlorite to remove nematode eggs. Each plant had three root samples randomly selected, and the Folin-phenol method was used to calculate the amount of total protein in each sample (Lowry et al., 1951). Two times the experiment was conducted. The data from both experiments were pooled because there was no statistically significant difference in the outcomes between the two replications. ANOVA was used to evaluate the data ($P=0.05$), and the means of the

six replicates are shown together with the standard error of the mean difference.

α -Amylase extraction

The tomato and okra roots were used to extract α -amylase three days after the second treatment. Each group's three plants were uprooted, and the fresh roots were taken out in separate batches, mixed together, and sliced into little pieces. Each group utilised one gram (g) of these young roots, and the enzyme was extracted at 40°C in 10ml of calcium chloride at a concentration of 10mM. After centrifugation at 54,000g for 20 minutes at 40°C , the supernatant was employed as an enzyme source. The Bernfield method measured the activity of α -amylase. In a clean test tube with a glass stopper, 1ml of the enzyme solution and 1ml of the starch solution were combined. The test tube was then incubated at 27°C for 15min at pH 4.7. After 15 minutes, the hydrolysis of starch was stopped by adding two ml of the dinitrosalicylic acid (DNSA) reagent to the mixture. After that, the test tube spent five minutes in a bath of boiling water. After adding 1ml of 40% Rochelle salt, the tube was cooled to 27°C . The test tube was filled with five millilitres of distilled water. In a spectrophotometer, the solution's optical density (O.D.) was measured at 560 nm (Shimadzu, Japan). Each preparation received ten replicates. The standard error of the difference between the means of 10 replicates is provided alongside the mean values after an ANOVA analysis ($P=0.05$).



Figure 2. Morphological changes in the roots of Okra.



Figure 3. Morphological changes in the roots of Tomato.

Table 1. Plant growth, protein content and nematode infestation of tomato treated with 5% flour, 0.02% α -amylase and 0.003% α -amylase inhibitor at 24 and 110 h after inoculation with 1500 \pm 175 second stage juveniles of *M. incognita* (40 days after inoculation).

Treatment*	Tomato plant growth				<i>Meloidogyne incognita</i> infection		
	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Root galls	Eggs per g root	Root protein conc. (mg/g)
NU	54.3	71.2	24.5	24.5	–	–	4.3
IU	51.6	56.2	23.0	34.5	690.0	5233.3	9.0
IF	56.6	71.6	24.5	17.3	215.7	2468.3	4.2
IFA	60.8	75.8	25.3	30.3	347.5	3266.6	4.9
IFI	59.2	65.3	24.5	23.3	316.7	3466.7	4.4
IA	52.0	74.6	23.0	32.0	754.8	4400.0	3.9
IAI	52.5	68.3	27.3	31.0	788.3	4200.0	4.8
II	52.6	64.2	23.6	18.3	363.3	3833.3	5.5
SED	2.7	5.5	1.3	4.3	84.2	327.0	0.2
P	0.0008	0.02	0.01	0.0006	<0.001	0.0004	<0.001

*Dashes (-) indicate no root galls or eggs in this group; *NU*, non-inoculated, untreated; *IU*, inoculated, untreated; *IF*, inoculated and treated with flour; *IFA*, inoculated and treated with flour and α -amylase; *IFI*, inoculated and treated with flour and α -amylase inhibitor; *IA*, inoculated and treated with α -amylase; *IAI*, inoculated and treated with α -amylase and α -amylase inhibitor; *II*, inoculated and treated with α -amylase inhibitor. Means of six replicates.

Results

With just 3.8, 3.2, 3.6, and 3.0% mortality after 6 hours, nematodes survived in 5% wheat flour, 0.02% α -amylase, and 0.003% α -amylase inhibitor in vitro as well as in control. In comparison to the infected untreated plants, wheat flour dramatically enhanced shoot length, shoot weight, and root length in pots. Fewer galls and eggs were present in the roots of plants treated with

wheat flour than in untreated plants. Compared to non-inoculated plants, inoculated plants that were not treated had more root mass. Compared to plants treated with flour, infected plants with no treatment had higher root protein content (Tables 1 & 3). In terms of its impact on the bio-mass and quantity of eggs in roots, flour did not differ from flour and α -amylase, flour and α -amylase inhibitor, or flour and flour and α -amylase. In plants that

Table 2. α -Amylase activity expressed as the release of maltose (μ g/min) at 8 days after inoculation of tomato seedlings with 1500 \pm 175 J2 of *M. incognita* and treated tomato plants at 24 and 110h after inoculation with 5% flour (means of ten replicates)

Treatment	Amount of maltose released (μ g/min)
Non inoculated untreated	18.1
Inoculated untreated	38.4
Inoculated and treated with flour	18.2
Inoculated and treated with flour and α -amylase	21.0
Inoculated and treated with flour and α -amylase inhibitor	27.2
Inoculated and treated with α -amylase	21.7
Inoculated and treated with α -amylase and α -amylase inhibitor	37.8
Inoculated and treated with α -amylase inhibitor	39.4
SED	0.3
P	<0.001

Table 3. Plant growth, protein content and nematode infestation of okra treated with 5% flour, 0.02% α -amylase and 0.003% α -amylase inhibitor at 24 and 110h after inoculation with 2000 \pm 120 second stage juveniles of *M. incognita* (40 days after inoculation).

Treatment*	Okra plant growth				Meloidogyne incognita infection		
	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Root galls	Eggs per g root	Root protein conc. (mg/g)
NU	81.2	95.6	35.1	18.5	-	-	3.4
IU	70.3	59.1	25.2	35.6	342.5	4000	7.3
IF	65.9	52.5	31.2	34.2	55.6	920.1	3.3
IFA	72.9	62.9	25	28	60.7	1100	3.7
IFI	70.1	55.4	27	32.1	58.1	1050.3	3.5
IA	65.7	50.5	20.5	21	438	4200.3	2.9
IAI	65	70	28.1	21.5	440.3	4310.2	3.9
II	56	70.5	28.4	21.7	198.3	2200.3	4.1
SED	0.1	0.02	0.1	3.3	2.1	40.1	0.01
P	<0.001	<0.001	<0.001	0.02	<0.001	<0.001	<0.001

*Dashes (-) indicate no root galls or eggs in this group. *NU*, non-inoculated, untreated; *IU*, inoculated, untreated; *IF*, inoculated and treated with flour; *IFA*, inoculated and treated with flour and α -amylase; *IFI*, inoculated and treated with flour and α -amylase inhibitor; *IA*, inoculated and treated with α -amylase; *IAI*, inoculated and treated with α -amylase and α -amylase inhibitor; *II*, inoculated and treated with α -amylase inhibitor. Means of six replicates.

had been treated and inoculated, α -amylase increased susceptibility. In plants that had been infected and treated, the number of root galls and eggs in the roots significantly decreased (Table 1 & 3). It was superior to using flour in terms of root length, shoot length, root protein content, and the number of eggs in the roots (Table 1 & 3).

Tables 2 and 4 show the activity of α -amylase as measured by the amount of maltose released. Compared to untreated plants that were not inoculated, those that demonstrated higher enzyme activity. Comparing wheat flour-treated plants to untreated inoculation plants, the enzyme activity was drastically reduced. Compared to untreated, non-inoculated plants, enzyme activity was

Table 4. α -Amylase activity expressed as the release of maltose (mg/min) at 8 days after inoculation of okra seedlings with 2000 \pm 120 J2 of *M. incognita* and treated okra plants at 24 and 110h after inoculation with 5% flour (means of ten replicates).

Treatment	Amount of maltose released (μ g/min)
Non inoculated untreated	13.2
Inoculated untreated	28.4
Inoculated and treated with flour	12.9
Inoculated and treated with flour and α -amylase	15.2
Inoculated and treated with flour and α -amylase inhibitor	20.4
Inoculated and treated with α -amylase	16.7
Inoculated and treated with α -amylase and α -amylase inhibitor	26.9
Inoculated and treated with α -amylase inhibitor	30.1
SED	0.8
P	<0.001

dramatically increased by both α -amylase and α -amylase inhibitors (Table 2 & 4).

Discussion

For the purpose of determining the α -amylase activity in the wheat flour-treated roots, we used tomato and okra. The activity of α -amylase was reduced when rhizospheric soil was soaked in wheat flour at 24 and 110 hours after nematodes were introduced to the roots of the plant. Forty days after inoculation, it was determined that treating the host with flour at the root penetration decreased nematode proliferation. Compared to the inoculation control group that was not treated, all treatments greatly reduced the nematode populations (eggs and all stages of nematodes).

In vitro testing revealed that it did not kill infectious juveniles. As a result, the reduced activity of α -amylase in flour-treated roots suggests that α -amylase inhibitors are involved in preventing α -amylase activity. Additionally, it was found that rhizospheric soil drenching with amylase and amylase inhibitors had no appreciable impact on the tomato and okra plants. It was further observed in the present study that the rhizosphere soil drench of flour suspension containing Grisovin, a standard antifungal compound, did not differ from flour alone in its effect on the biomass and root galling of tomato plant. Nematode feeding is a promising target for nematode control, and it might be accomplished by preventing the formation of feeding cells or interfering with the digestive system (Gheysen et al., 1996). An active area of research for both cyst and root-knot nematodes is identifying oesophageal gland secretory products (Hussey, 1989; Hussey et al., 1994). Proteins and carbohydrates were discovered in stylet secretion composition analysis (Hussey, 1989). Because of the infection with root-knot nematodes, it is known that the amount of carbohydrates-metabolizing enzymes in roots increases (Veech and Endo, 1970). Enzyme activities, including cellulase α -amylase and proteinase, have been detected in exudates of nematodes (Bird, 1974; Koritsas and Atkinson, 1994). Feeding tests using fake seeds made from cowpea or azuki bean flour with variable levels of pure bean α -AI-1 added have shown that α -AI can act as a plant defence protein (Ishimoto and Kitamura, 1989). The growth of the azuki bean weevil and cowpea weevil larvae was hindered by the presence of inhibitors at concentrations greater than 0.3%. The combined effects of all the studied bio-nematicides may have greatly increased plant growth and decreased disease severity. Future research is required to determine how flour and an inhibitor of α -amylase reduce *M. incognita* in tomato and cowpea.

Conflict of interest

None.

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