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## Original Article

# Effect of Initial Density of *Meloidogyne javanica* on *Salvia officinalis*

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## Abstract

The influence of different initial inoculum densities of the root-knot nematode *Meloidogyne javanica* on the growth of sage (*Salvia officinalis*), root-knot development and nematode multiplication was investigated 90 days after inoculation under greenhouse conditions. With the increase in the nematode initial inoculum densities, shoot fresh/dry weight, shoot/root length and root fresh/dry weight were decreased. The greatest reduction in growth occurred in plants inoculated with the highest density of nematodes (10,000 eggs and juvenile of stage 2.2 kg soil). The highest reproduction rate was observed in plants inoculated with the lowest level of nematode population tested (1,000 eggs and juvenile of stage 2.2 kg soil). The results on *S. officinalis* gave conclusive evidence of pathogenic potential of *M. javanica*.

**Key words:** Root-knot nematode, *Meloidogyne javanica*, Sage, Medicinal plant, Reproduction, *Salvia officinalis*

## Introduction

*Salvia officinalis* L. (common sage) is the most representative species within the genus *Salvia* in Labiateae family. This plant is mostly distributed in the Mediterranean Basin, in South East Africa and in central and South America, where it is largely cultivated for culinary and medicinal purposes. The curative properties of sage are particularly recognized since earliest times and its use as a tonic, stimulant, carminative, antiseptic and antihydrotic is reported [1]. Root-knot nematodes (*Meloidogyne* spp.) are among the most damaging nematodes in agriculture, causing an estimated US $100 billion loss/year worldwide [2]. *Meloidogyne javanica* (Treub) chitwood is one of the most common and important root-knot nematode species. It has a wide host range and is considered as a major agricultural pest [3]. Information on host status of *Salvia officinalis* for *M. javanica* is important not only for continued sustainable production but also determination of appropriate management practices. The objective of this study was to evaluate the effect of initial inoculum levels and reproduction factor of *M. javanica* on the growth of *S. officinalis*.

## Materials and Methods

### Nematode Isolates

Seven nematode populations, each obtained from a single egg mass, were maintained on tomato (*Lycopersicon esculentum* Mill), cv. Rutgers (nematode-susceptible) in greenhouse at 20-25 ºC. Species identification was performed using the morphology of female perineal patterns. Moreover, for molecular analysis, DNA was extracted from eggs and single juvenile nematodes according to the method of Silva et al. [4] and Orui [5] respectively. PCR amplification reactions were carried out using Mjavf /Mjavr specific primer pairs [6].

### Initial Population Densities of *M. javanica* on Sage

Seeds of sage, *S. officinalis*, were sown in plastic pots containing 2 kg of steamed-sterilized soil (15% sand, 42% silt, 43% clay). To obtain inocula for

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experiment, *M. javanica* eggs were extracted from infected tomato roots by the NaOCL procedure [7]. After 4 weeks of germination, plants were thinned to one/pot and individually inoculated with 1000, 2000, 3000, 4000, 5000 and 10000 of eggs and freshly hatched, second stage juveniles (J2) of *M. javanica* obtained from infected roots of tomato. For inoculation, five holes (4 cm deep) were made within a radius of 2 cm around the plants and a suspension was transferred to the holes using a sterilized pipette. The holes were subsequently plugged by pressing the soil gently. The control pots were left uninoculated. Each treatment was replicated four times. Pots were arranged in a complete randomized block design on benches in a greenhouse maintained at 25±2ºC. The experiment was terminated 90 days after inoculation. Plant growth was recorded by measuring length and fresh and dry weights of roots and shoots. Severity of root galling in infected plants was assessed on a 0-5 scale (0= no galls, 1=1-2, 2=3-10, 3=11-30, 4=31-100, 5= >100 galls per root system) [8]. Nematodes from 200 g soil samples of infested potting soil and eggs from roots of each plant were extracted by centrifugation [9]. Extracted nematodes and eggs were counted to estimate final nematode population densities in each pot. The reproductive factor, Rf = Pf/Pi, was calculated (Pf is the average final population count and Pi, the original inoculum of egg and J2).

Analyses of variance and regression analyses were carried out using the STATISTICAL ANALYSIS SYSTEM (SAS) 9.1. The Duncan′s multiple range test was employed to test for significant difference between treatments at $P<0.05$.

## Results and Discussion

### Nematode Diagnosis

With the existence of lateral line in perineal position (Fig. 1) and 1600 bp fragment amplification utilizing Mjavf/Mjavr specific primer pairs (Fig. 2) the species were recognized as *M. Javanica*.

### Initial Population Densities of *M. javanica* on *S. officinalis*

The data obtained at 90-day post-inoculation, showed that root-knot nematode, *M. javanica* decreased plant length, fresh and dry weight of sage directly proportional to the number of nematodes per pot, affecting all variables as compared with uninoculated controls (Table 1). The final population of *M. javanica* was highest in and around plants inoculated with 10000 J2/plants, while the lowest population was recorded in and around plants inoculated with only 1000 J2/plants. Similarly, root gall severity increased with the increase in Pi (Table 2). At the highest Pi, root gall severity was 5.0.

The results on *S. officinalis* gave conclusive evidence of pathogenic potential of *M. javanica* in terms of both quality and quantity of roots.

Han [10] has also reported that 20 and 30% decrease in root-growth of a medicinal plant, *Agelica gigas*, when inoculated with 1000 and 5000 J2s of *M. hapla* respectively [10]. The progressive decrease in plant growth with the increasing inoculums has also been reported by Rakesh et al.[11], Patel et al. [12], Pathak et al. [13] and Khan et al. [14] in different plants.

Wallace [15] reported that the increase in nematode population and subsequent reduction in the yield of crops or other manifestation of pathogenic effects are directly influenced by the initial density of nematodes in soil [15]. The highest reproduction rate observed for *M. javanica* was in pots with the lowest inoculation rate (Table 2).

Initial densities of plant parasitic nematodes affect multiplication rate, with lower initial densities resulting in higher multiplication [16,17]. The Rf of *M. javanica* on *S. officinalis* roots decreased with increase in Pi levels (Fig. 3).

Rf value of 21.82 was recorded at the lowest Pi, whereas the minimum Rf value was 8.52 at highest Pi on *S. officinalis*. The decrease in nematode multiplication at the highest population level was perhaps due to the destruction of the root system and competition for food and nutrition among developing nematodes within the root system and also due to the inability of the larvae to find out the new infection sites of subsequent generation [18].

Similar observations have been made by Pandey and Haseeb [19], Pathak et al. [13], Khan [20], Khan et al. [14] and Park et al. [21].

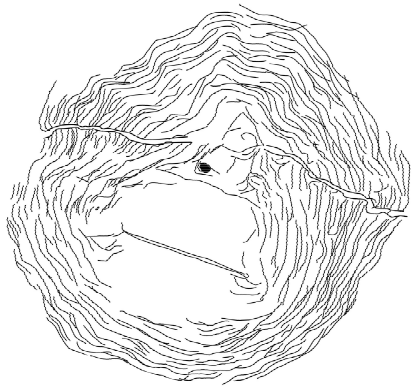

**Fig. 1** Perineal patterns of *M. javanica*

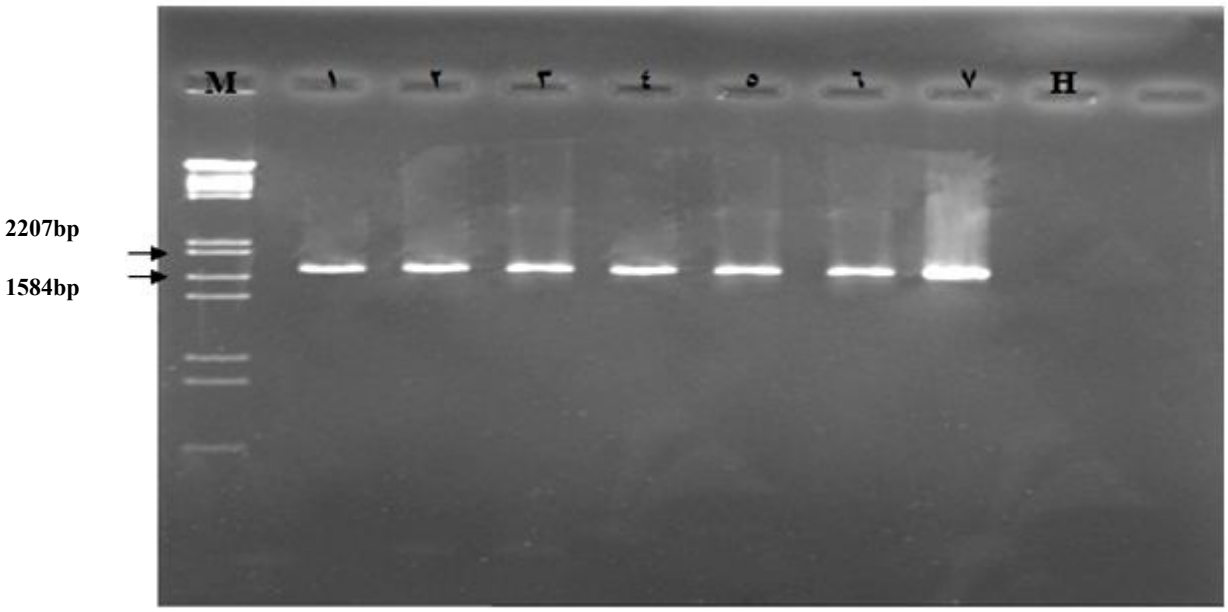


**Fig. 2** The amplified 1600 bp fragments with species specific primer, M: Marker III H: Negative control (water), 1-5: Different replications of DNA extraction of egg, 6-7: Different replications of DNA extraction of single larva

**Table 1** Effect of initial population densities of *M. javanica* on growth of *S. officinalis*

| Inoculum density($P_i$) | Shoot | | | Root | | |
|---|---|---|---|---|---|---|
| | Length (cm) | Fresh weight (g) | Dry weight (g) | Length (cm) | Fresh weight (g) | Dry weight (g) |
| 0 | 25.41 a | 19.47 a | 4.60 a | 17.08 a | 10.96 a | 2.65 a |
| 1000 | 24.43 a | 17.86 ab | 4.03 ab | 16.29 ab | 10.37 a | 2.22 ab |
| 2000 | 23.27 a | 17.38 ab | 3.86 ab | 14.52 bc | 10.09 a | 2.07 ab |
| 3000 | 21.07 ab | 15.06 bc | 3.53 ab | 13.02 cd | 9.25 ab | 1.87 abc |
| 4000 | 17.45 bc | 14.36 bcd | 3.49 ab | 12.35 de | 8.09 bc | 1.76 bc |
| 5000 | 15.88 bc | 13.25 cd | 3.26 ab | 10.92 ef | 6.70 cd | 1.46 bc |
| 10000 | 12.90 c | 10.54 d | 2.61 b | 9.90 f | 5.05 d | 1.19 c |

Mean values followed by the same letter do not differ significantly at P<0.05 according to Duncan's Multiple Range test.

**Table 2** Inoculum densities on development of root-knot nematodes at 90-day post inoculation

| Inoculum density($P_i$) | Nematode population | | | Reproduction factor ($P_f/P_i$) | Gall index |
|---|---|---|---|---|---|
| | Total roots | Soil | Total | | |
| 0 | 0 f | 0 f | 0 f | 0 d | 0 d |
| 1000 | 20270 e | 800 ef | 21070 e | 21.82 a | 4 c |
| 2000 | 35637 d | 1600 de | 37237 d | 18.61 a | 4 c |
| 3000 | 40335 cd | 2150 cd | 42485 cd | 14.15 b | 4.5 b |
| 4000 | 47988 bc | 2800 c | 50788 bc | 12.69 b | 5 a |
| 5000 | 55635 b | 3900 b | 59535 b | 11.89 bc | 5 a |
| 10000 | 78325 a | 7157 a | 85482 a | 8.52 c | 5 a |

Mean values followed by the same letter do not differ significantly at P<0.05 according to Duncan's Multiple Range test.

These results confirm the extremely high virulence and damage causing potential of the root-knot nematodes on *S. officinalis*. Therefore, it can be safely suggested that the cultivation of sage should be avoided in root-knot nematode infested fields or highly effective control measures should be applied before sowing.

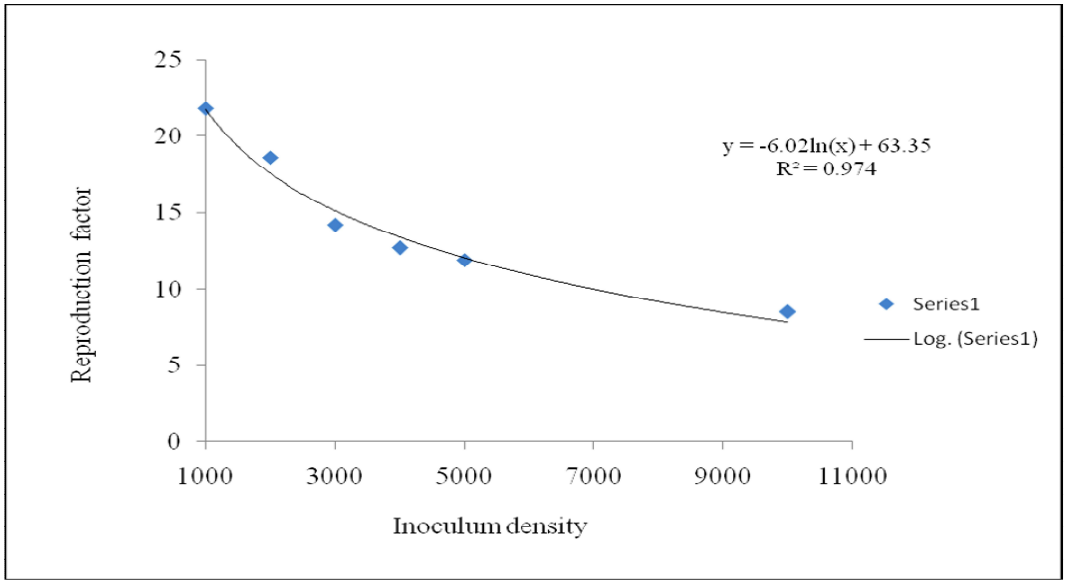


**Fig. 3** Relationship between inoculum densities of *M. javanica* and its reproduction on *S. officinalis* at 90-day post inoculation. The lines represent the predicted function calculated by fitting the expanded negative exponential model to data.

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