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IN VITRO EVALUATION OF SOME SEED-SPICES AGAINST ROOT KNOT NEMATODES, *MELOIDOGYNE JAVANICA* (TREUB) CHITWOOD

Anum Siddiqui, M. Javed Zaki and S. Zeeshan Abbas

Department of Botany, University of Karachi, Karachi-75270, Pakistan.
Department of Physics, University of Karachi, Karachi-75270, Pakistan.
Corresponding author:anumsiddiqui30@gmail.com

ABSTRACT

Root-knot nematode, *Meloidogyne javanica* (Treub) Chitwood is one of the major pest problem in countries with sweltering climate. Although utilization of nematicides and other synthetic chemicals to control nematode infestation in field crops are effective measures but these practices pose substantial threat to human health. Application of synthetic chemicals also results in environmental pollution. Recently, use of plant extracts has emerged as an effective alternative approach to control many plant pathogens including the nematodes. Some of the locally available seed spices including *Brassica juncea, Piper nigrum, Nigella sativa, Sinapis alba, Papaver somniferum* and *Trigonella foenum-graceaum* were used against root nematode, *M. javanica in vitro*. The nematode eggs and juveniles were exposed to the crude aqueous extract of plant seeds powder for 24, 48, 72 and 96 h *in vitro*. Significant results were obtained in egg hatching test in all treatments and time durations. *Papaver somniferum* was found to be the most effective in the control of egg hatching. In mortality test, all treatments showed significant mortality as compared to control. However, an absolute, larval mortality was observed when larvae were treated with aqueous extracts of *B. juncea, N. sativa, P. somniferum* and *T. foenum-graceaum* after 96 h interval. The present study shows *B. juncea, P. nigrum, N. sativa, S. alba, P. somniferum* and *T. foenum-graceaum* potentially natural source of nematicide against *M. javanica.*

Key-words: seed spices, nematicidal activity, root knot nematode

INTRODUCTION

Meloidogyne species were considered as a major threat towards global crop production and responsible for an annual estimated loss of approximately 100 billion US dollars worldwide (Brand *et al.*, 2010). The effect of *Meloidogyne* species is immense because they can attack over four to five thousand species of plants (Trudgill and Blok, 2001) affecting crop yields. *Meloidogyne* spp. attack plant roots, and produces galls or knots on roots of susceptible host plants. As a result of nematode's infection the nutrients and water uptake by the infected plants are reduced because of the damaged root systems that results in stunted plants and low yields (Abad *et al*, 2003). Therefore, root knot nematodes management is necessary so that we grow better quality crops of maximum yields. Nature and ecosystem friendly methods are being developed to control root knot nematodes such as application of pesticides made from plants (Olowe, 1992; Mangala and Mauria, 2006). There are substantially good number of plant species that have been reported to show nematicidal acitivities (Chitwood, 2002). There are many examples, where powder and extracts of different plant parts have been used for the management of root-knot nematodes For instance, plant parts from neem, basil, and drumstick tree have been reported to suppress nematode population and are being used against root knot nematodes (Jahn 1989 and Ajayi, 1990). In addition, seeds from different plant species, including *Acacia* sp., *Albizzia lebbak, Cassia* sp., *Sesbania* sp, *Medicago* sp., *Phaseolus* sp., *Pisum* sp., *Pongamia* sp., *Sesbania* sp., and *Trigonella* sp. have shown strong nematoicidal activities against root-knot and other nematodes (Khurma and Singh, 1997; Khurma and Chaudhry, 1999). Moreover, in few studies dried seed powder from Bishops weed, Castor, Mustard and Eucalyptus were tested and found to be very effective in the suppression of root-knot nematode population (Radwan *et al*., 2012). It is worth noting that low quantity of dried seeds, fruits, roots, bark or vegetative substances are commonly added in our food preparation. These substances not only add flavor to the foods but also serve as anti-bacterial agents (Burkill, 19865). Increasing evidence argue that plant parts including plant leaf extracts, seed powder, and root extracts have a major impact on plant disease control. This is an interesting but under-investigated area of research and obviously asking for more data. In addition, work on plant seeds powder to test their efficacy against root-knot nematodes has been limited. The nematicidal activity of some powder dried seed spices including *Brassica juncea, Piper nigrum, Nigella sativa, Sinapis alba, Papaver somniferum* and *Trigonella foenum-graceaum* that are locally available in Pakistan, against root-knot nematode, *Meloidogyne javanica* was investigated.

MATERIALS AND METHODS

Preparation of seed extract

We tested aqueous extracts from the seeds of six spices for their activity against *M. javanica in vitro*. All seeds were washed thoroughly under running tap water and air dried. Fully dried seeds were grinded into powder using an electric grinder. For aqueous extraction, 10g of freshly obtained seeds powder was soaked in 100mL of sterilized distilled water for 24 h. The solution thus obtained was strained through muslin cloth and filtered through Whatman filter paper no.1. This standard solution considered as 100% seed extract and used for further studies.

Effect of plant seeds extracts on nematodes egg hatch

To test the activity of seed extract on nematode egg hatching, root knots containing *Meloidogyne javanica* were obtained from heavily infested roots of eggplant (*Solanum melongena* L.). Roots were cut into small pieces and transferred in a capped bottle containing sodium hypochlorite solution (1 %). Bottle was shaken for 2 minutes. Sieves were stacked from top to bottom in order of 200 and 400-mesh (37 and 75 µm, respectively). Then roots were poured on 200 mesh sieve and eggs were collected on 400-mesh sieve (Hussey and Barker., 1973). The eggs collected in distilled water in a beaker and were concentrated as of 40-6 0 eggs / mL of suspension. Then 2 mL of egg suspensions and 2 mL of freshly obtained aqueous extracts of seeds powder were transferred into a 2.5cm diameter glass cavity block, and kept at room temperature 28 ± 2°C. Each treatment was replicated three times. A second glass cavity block containing 2 mL of water, in addition to egg suspension served as control. After 24, 48 and 72 hours, the number of hatched larvae were counted under low power stereoscopic microscope at a 60 X magnification.

Effect of plant seeds extracts on larval mortality

To study the effect of seed extract spices on the survival of nematode's larvae, nematode eggs or egg masses were incubated for 72 h at 28 ±2°C for egg hatching. Subsequently, second stage juveniles per mL were counted using a counting chamber and 30 to 40 juveniles per mL were maintained. Then, 2 mL of larval suspension was transferred to a 2.5 cm diameter glass cavity block containing 2 mL of aqueous seeds extracts. The experiment was conducted at room temperature. Each treatment was replicated three times. The glass cavity block containing 2 mL of water as well as 2 mL of larval suspension served as control. After 24, 48 and 72 h exposure, number of dead larvae were counted under a low power stereoscopic microscope at a 60 X magnification. The toxicity of seeds extracts was assessed as the mean percentage (%) of the dead nematodes. The nematodes viability was tested by a simple touch of eyebrow attached to a needle (Cayrol *et al*., 1986).

Statistical Analysis

Data was analyzed for descriptive statistics and analysis of variance (ANOVA) with SPSS ver. 16. The follow up included calculation of Least Significant Difference (LSD). Duncan's Multiple Range Test (DMRT) was employed to compare the treatment means (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

In the present study, aqueous extracts from seeds of *Brassica juncea***,** *Piper nigrum*, *Nigella sativa*, *Sinapis alba*, *Papaver somniferum* and *Trigonella foenum-graceaum* were tested to evaluate their effects on root knot nematodes. Significant results were obtained as all seed-spices extracts suppressed egg hatching and increased the larval mortality. Pandey *et al*. (2014) also reported the nematicidal activity of Essential oils from *Carum carvi, Foeniculum vulgare, Mentha rotundifolia,* and *Mentha spicata in vitro* and in pot experiments. Ground castor seeds significantly suppressed *M. incognita* infecting tomato (Rich and Rahi, 1995). Extracts obtained from *Papaver somniferum* seeds completely suppressed the hatching of eggs. Time required for nematodes eggs to hatch was also measured under various treatments. Major delay ($P < 0.001$) in egg hatching was observed when they were exposed to seed- spices aqueous extracts (Fig. 1). Similar results were found by Abbas and colleagues (2009). The author tested *Nigella sativa* and *Piper nigrum* seeds extracts against root knot nematode and found a significant reduction in egg hatching and increased mortality (Abbas *et al.,* 2009). Present experiment also suggested significant larval mortality in all treatments as compared to the larvae in the controlled environment (Fig. 2). However, larval mortality increased with increasing time interval; highest mortality was found after 96 hours exposure. We found 100% larval mortality when these larvae were exposed to the extracts of *Brassica juncea, Nigella sativa, Papaver somniferum* and *Trigonella foenum-graceaum* seeds extracts. Aqueous extracts of leaf and root of *Brassica juncea* are highly effective for immobilizing and killing root knot nematodes (Insunza *et al.,* 2001). Crops belonging to

genus *Brassica* are generally referred as trap crops for capturing nematodes. It is generally believed that *Brassica* species contain chemicals that suppress the mobility and reproduction of plant pathogenic nematodes (Lazzeri *et al.* 1993). In a screen house and field study, Seenivasan (2011) noted greater than 50% reduction in *Meloidogyne* infection when crops were drenched with *Brassica juncea* extract. So, these spices considered to possess compounds that inhibit nematodes**.** We have shown the efficacy of the aqueous extracts of the seed of the spices, *B. juncea, N. sativa, P. somniferum* and *T. foenum-graceaum* in reducing the population of *M. javanica in vitro.*

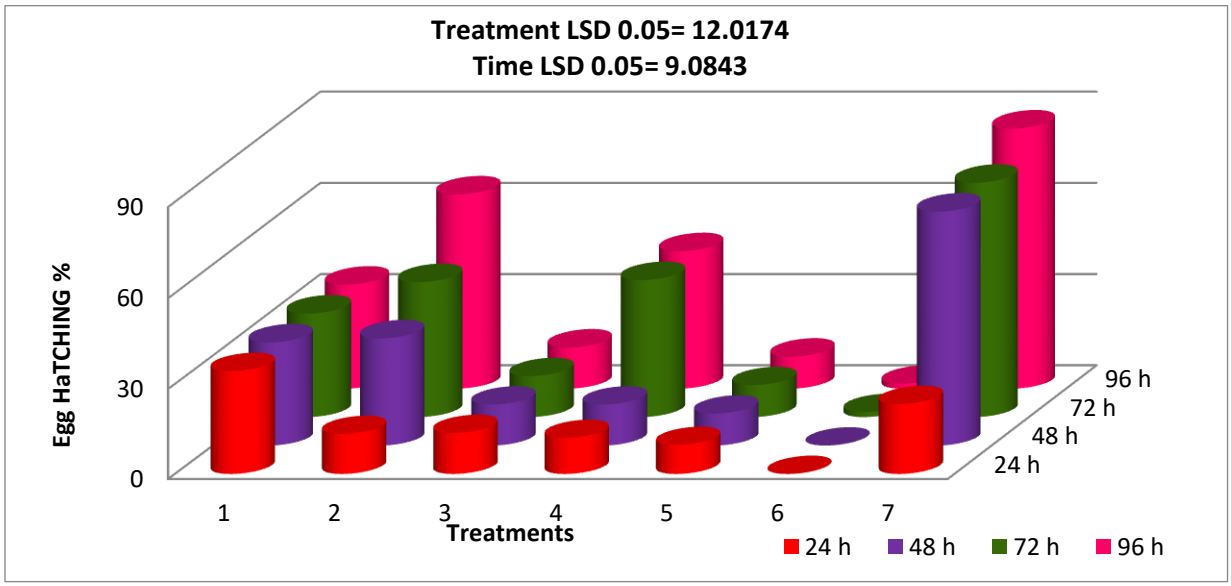

Fig.1. Effects of extracts of some seed spices on egg hatching of *Meloidogyne javanica.*
1- Control, 2- *Brassica juncea*, 3- *Piper nigrum*, 4- *Nigella sativa*, 5- *Sinapis alba*, 6- *Papaver somniferum*, 7- *Trigonella foenum-graecum*

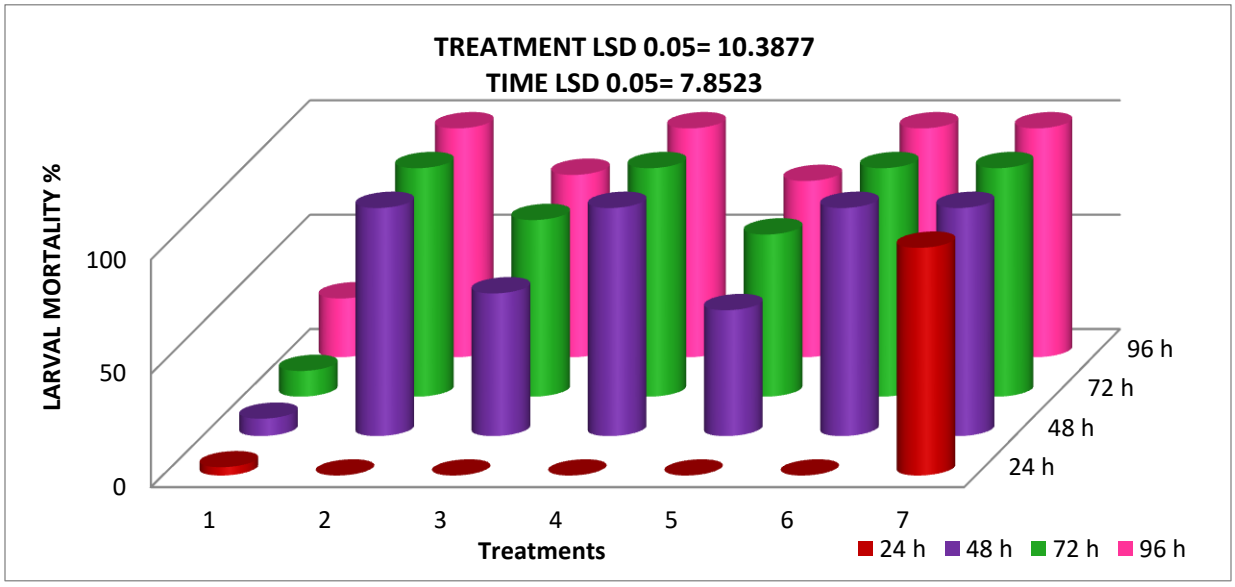

Fig 2. Effect of aqueous extract of some seed spices on Juvenile mortality of *Meloidogyne javanica.*
1= Control, 2- *Brassica juncea*, 3- *Piper nigrum*, 4- *Nigella sativa*, 5- *Sinapis alba*, 6- *Papaver somniferum*, 7- *Trigonella foenum-graecum*

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