Research Article

# Evaluation of Some Medicinal Plants for the Management of Root-Knot Diseases of Banana

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**Abstract** | The present research study was undertaken to evaluate the nematicidal property of four indigenous medicinal plants viz., *Ferula oopoda*, *Zataria multiflora*, *Achillea santolina* and *Nepeta cateria* against *Meloidogyne incognita* at banana orchards of District Lasbela, Balochistan, Pakistan. Crude extracts, essential oils and fixed oil of these selected plants were tested separately. The crude extracts of *Z. multiflora* and *F. oopoda* showed higher mortality as compared to *A. santolina* and *N. cateria*. The results showed a maximum mortality of 76% by *Z. multiflora* and *F. oopoda* at highest dose of 3 g/ liter which was followed by 72% of *N. cateria* and 49% by *A. santolina* at the same dose. However, essential oils of *A. santolina* showed the highest mortality (79%) as compared to *Z. multiflora* (74%), *F. oopoda* (70%) and *N. cateria* (68%) at the same dose of 1.2 ml/ liter. While results of fixed oils showed maximum mortality of 74% by *F. oopoda* followed by 71%, 70% and 66% in *Z. multiflora*, *A. santolina* and *N. cateria*, respectively at a formulation of 3ml/ liter. A nematicide Furadan was kept as standard for efficacy comparison.

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## Introduction

Banana (*Musa* spp.) is the most ancient fruit and food crop cultivated by humans. It is an important member of family *Musaceae* having its order Scitamineae in monocotyledons. They are propagated by vegetative means and are parthenocarpic. It develops fruits without pollination. South East Asia is to be considered the origin of banana plant. The fruit is a good source of Vitamin B6, Vitamin C and Manganese (Arias *et al.*, 2003). It is one of most predominant fruit crops of tropical areas of Pakistan as well as of the world and is a valuable crop in many areas of the world (Oka, 2001). It is cultivated at the area of about 34,800 ha, with production of 154,800 tonnes annually. Sindh ranks first as production contributor in Pakistan. Balochistan and Punjab stand second in banana-cultivating provinces whereas Khyber Pakhtunkhwa (KP) province is third one in order (Anon, 2014, 2016). Production of banana crop in Pakistan is very low than other banana producing countries of the world due to the lack of awareness about latest technologies, poor management system, diseases and insect pests. Favorable climatic conditions of the country favor a variety of diseases and insect pests attack on banana plants causing severe economic losses. Plant parasitic nematodes are the top most destructing threats of banana crop. Quality and quantity of production as well as vigor of plant were also affected by plant parasitic

nematodes (Araya *et al*., 2002). The most widespread and important nematodes of banana are *Radopholus similis*, *Helicotylenchus multicinctus*, *Pratylenchus* spp., *Rotylenchulus reniformis* and *Meloidogyne* spp., (Gowen *et al*., 2005). The root-knot nematode caused severe losses at all life stages of banana round the year (Nasira *et al*., 1995; Shahina and Maqbool, 1992, 1993; Bridge, 1993).

Pakistan is a quite rich in medicinal herbs and hundreds of medicinal plants are wildly grown in varied environmental conditions. Most of these medicinal plants are used for the treatment of different diseases in traditional medicines (Taimoor *et al*., 2017; Mittal and Goswami, 2003). These medicinal plants have bio-active compounds possessing properties of pesticides (Bhattacharjee, 2004; Elbadri *et al*., 2008; Taimoor and Shahina, 2018). Several number of plant products are being formulated and have been used for the management of plant parasitic nematodes (Gupta *et al*., 2011).

Medicinal plants have remained unexploited for its commercial use as nematicide in Pakistan. These plant extracts can be used for development of eco-friendly and sustainable nematicides. Therefore, this study has been planned to evaluate the nematicidal property of four indigenous medicinal plants against *Meloidogyne incognita*.

## Materials and Methods

Current research study was undertaken during the month of June to August, 2020 at banana orchards of District Lasbela, Balochistan, Pakistan.

*Survey and site selection*

Five different banana orchards showing disease symptoms have been randomly selected for evaluation of different doses and fractions of four medicinal plants. A 500 g of 10 soil samples along with banana roots from diseased plants of each selected area has brought to the laboratory for isolation and identification of nematodes.

*Extraction and identification of root-knot nematodes*

Collected roots of banana plants were cut into 1-2 cm small pieces in a beaker containing water and observed them under a stereoscopic microscope in a Petri dish. With the help of dissecting needles galls were dissected to examine the root-knot females, juveniles and egg-masses. Recovered females were transferred to glass cavity blocks with the help of a dropper. Root-knot nematodes were identified based on perinneal pattern, morphological and morphometric characteristics according to Siddiqi (2000).

*Selection of four medicinal plants for nematicidal evaluation*

Four plants were screened for their nematicidal evaluation viz., *Achillea santolina*, *Nepeta cateria*, *Ferula oopoda* and *Zataria multiflora*; a standard nematicide Furadan was selected for comparison. These medicinal herbs were further processed for isolation of bioactive fractions.

*Extraction of plant bioactive compounds*

For this purpose, different techniques viz., extraction of crude compounds, extraction of essential oil and extraction of fixed oil have been used for extraction of all possible bioactive compounds of these medicinal plants as follow:

**Extraction of crude compounds with distilled water:** 100 g dehydrated powder for each four medicinal plants were soaked individually in sterilized conical flask containing 1000 ml distilled water. The caps of conical flasks were closed tightly. These were shaken at 100 rpm on an orbital shaker for 24 hours. After that, conical flasks containing plant extract were then removed and filtered through filter paper of 40 #. Filtered solutions of each plant were more concentrated individually into a hot water-bath. Finally, excess water of each concentrate was evaporated in a vacuumed oven at a temp of 60° C for two hours (Taimoor and Shahina, 2018).

Following dilutions were prepared from each concentration in sterilized distilled water:
g/ liter water; 1.5 g/ liter water; 2.0 g/ liter water; 2.5 g/ liter water and 3.0 g/ liter water.

**Extraction of essential oil:** Essential oils were extracted through steam distillation method by taking 500 g of fresh plant. It was steam distilled in a steam distillation unit for up to two hours. The condensed essential oils were collected in a capped test tube. These were stored in a refrigerator for further required studies (Sosamma and Jaysree, 2002).

Following doses were prepared from essential oils for

each plant: 0.2 ml/ lit solvent; 0.4 ml/ lit solvent; 0.6 ml/ lit solvent; 0.8 ml/ lit solvent; 1.0 ml/ lit solvent and 1.2 ml/ lit solvent.

*Extraction of fixed oil*

Fixed oils of four selected medicinal plants were extracted through solvent extraction method from 500 g of plant material. Plant materials were grinded in a grinder and soaked separately each in 1000 ml Cyclo-hexan. The extract was kept at room temperature (25°C ± 2°C) for about 3 days. The solvent containing fixed oil was filtered through 42# filter paper. This process was repeated three times for total extraction of all possible fixed oils. Five gram of activated carbon (Charcoal) was mixed with the extract for purification. The extract was again filtered to remove charcoal and impurities. Extracts were then concentrated on Rotary Evaporator at 50°C until the whole solvent evaporated from the oil. The concentrated oils were collected in a capped test tube for nematicidal studies. Finally, doses were prepared for field test as: 0.5 ml/ lit solvent; 1.0 ml / lit solvent

*Evaluation of plant extracted bioactive compounds as nematicide*

For this study, five-banana plants heavily infested with *Meloidogyne incognita* were randomly selected at five different farmer fields near Uthal for each assessment. Soil and root samples were collected for pre-treatment analysis of the nematode population. Prepared doses of all plants extracts were applied as soil drench. Furadan (Carbofuran) was used at the rate of 10 kg/acre as a comparable treatment. Untreated plants served as a check (Fazal *et al*., 2001; Fatoki, 2001). All treatments were arranged in a RCBD in three replications. Soil and root samples were collected after one week of soil drench for nematode mortality analysis by sieving and decanting methods (Hauser and Coyne, 2008).

*Statistical analysis*

Obtained data were statistically analyzed using multiple range Duncan's test and estimated mortality percentage was determined by Anderson and Tilton formula as:

$$\text{Reduction \%} = \frac{\text{Population of treated plants after application} \times \text{Population of check plants before Treatment}}{\text{Population of treated plants before application} \times \text{Population of check plants after Treatment}} \times 100$$

(Duncan, 1999; Puntener, 1981)

## Results and Discussion

*Comparison of crude extracts of medicinal plants with a conventional nematicide in field against Meloidogyne incognita*

The comparative effect of crude extract of *Achillea santolina*, *Ferula oopoda*, *Nepeta cateria* and *Zatoria multiflora* at six different doses with Furadan (carbofura) against *M. incognita* is depicted in Table 1.

The overall results exhibited the highest mortality at the highest doses of tested medicinal plants. *Z. multiflora* and *F. oopoda* showed higher mortality as compared to *A. santolina* and *N. cateria*. The results revealed maximum mortality of 76% by *F. oopoda* and *Z. multiflora* followed by *N. cateria* 72% and *A. santolina* 49% at dilution of 3 g/ liter water. These were followed by 60%, 40%, 31% and 21 % by extracts of plant of *Z. multiflora*, *N. cateria*, *F. oopoda* and *A. santolina*, respectively at dilution of 2.5 g/ liter water. *Z. multiflora* extract has shown (30%) mortality in followed by *Nepata cateria* (22%), *Ferula oopoda* (21%) and *A. santolina* (16%) after the application of 2 g/ liter water. Low mortality of 1% was revealed by *Z. multiflora* at a dose of 1.0g/ liter.

In the field test of highest rate of mortality was recorded 76% at a dose of 3 g/ liter water of *Z. multiflora*, followed by 60%, 30%, 4%, and 1% at doses of 2.5, 2.0, 1.5, and 1.0 g/ liter water. However, the dose of 0.5 g/ liter water showed no mortality at all.

Similarly, in the field test highest mortality rate of 72% was exhibited by the dose of 3 g/ liter water of *N. cateria*, this was followed by 40%, 22% and 3% at dilutions 2.5, 2.0 and 1.5 g/ liter water. However, dilution of 1.0 and 0.5 g/ liter water showed no efficacy. Moreover, after the application of *F. oopoda* highest population reduction was 76% at the dilution of 3 g/ liter water, which followed by 31%, 21%, 16% and 7% in serial dilutions of 2.5, 2.0, 1.5 and 1.0 g/ liter water. However, the dilution of 0.5 g/ liter water has shown no efficacy. A standard nematicide,

Furadan used at a dose of 3 g/ liter, for comparison which showed 100% mortality of the nematode *M. incognita.*

*Comparison of essential oils of medicinal plants with a conventional nematicide in field against Meloidogyne incognita*

The comparative effect of essential oils of *Z. multiflora*,

*A. santolina*, *N. cateria* and *F. oopoda* at six different doses along Furadan (carbofuran) against *M. incognita* is depicted in Table 2. The obtained data has shown highest mortality at the highest doses of essential oil of medicinal plants. *A. santolina* showed higher mortality as compared to *Z. multiflora*, *N. cateria* and *F. oopoda*. The results showed maximum mortality of 79% by *A. santolina* followed by *Z. multiflora* (74%), *F.oopoda* (70%) and *N. cateria* (68%) at the same dose of 1.2 ml/ liter.

**Table 1:** *Comparison of crude extracts of medicinal plants with a conventional nematicide in field against Meloidogyne incognita.*

| Treatment (Dose/Liter) | Mortality % | | | |
|---|---|---|---|---|
| | Zatoria multiflora | Achillea santolina | Nepeta cateria | Ferula oopoda |
| 0.5 g | $0_{f}$ | $0_{e}$ | $0_{f}$ | $0_{g}$ |
| 1.0 g | $1_{f}$ | $0_{e}$ | $0_{f}$ | $7_{f}$ |
| 1.5 g | $4_{e}$ | $0_{e}$ | $3_{e}$ | $16_{e}$ |
| 2.0 g | $30_{d}$ | $16_{d}$ | $22_{d}$ | $21_{d}$ |
| 2.5 g | $60_{c}$ | $21_{c}$ | $40_{c}$ | $31_{c}$ |
| 3.0 g | $76_{b}$ | $49_{b}$ | $72_{b}$ | $76_{b}$ |
| Furadan (Carbofuran) | $100_{a}$ | $100_{a}$ | $100_{a}$ | $100_{a}$ |
| LSD | 2.84 | 1.13 | 1.74 | 1.66 |

*Same alphabats represent non significant difference where as different alphabats represent significant difference among treatments.*

In the field test trials, essential oil of *Z. multiflora* has shown highest population percentage of 74% at the dilution rate of 1.2 ml/ liter water, it was followed by 34%, 11%, and 0.33% at serial dilutions of 1.0, 0.8 and 0.6 ml/ liter. However, the doses of 0.4 and 0.2 ml/ liter have shown no efficacy.

While field test of *A. santolina*, highest population reduction was found to be 79% at a dilution rate of 1.2 ml/ liter water, followed by the population reduction rate of 63%, 54%, 4% and 3% at serial dilutions of 1.0, 0.8, 0.6, 0.4 and 0.2 ml/ liter water.

Similarly, the field test of *N. cateria* showed the highest population reduction of 68% at a dilution of 1.2 ml/ liter water, which was followed by 43%, 27% and 6% at serial dilutions of 1.0, 0.8 and 0.6 ml/ liter water. However, the doses 0.4 and 0.2 ml/ liter water has shown no efficacy.

Moreover, in field test of *F. oopoda*, highest population reduction was observed (70%) at a dilution of 1.2 ml/ liter water, this was followed by 63%, 29%, 16%, 7% and 1% at serial dilutions of 1.0, 0.8, 0.6, 0.4 and 0.2 ml/ liter water.

In comparison to all applications of medicinal plants the positive control (Furadan) has given maximum mortality rate i.e., 100%.

**Table 2:** Comparison of essential oils of medicinal plants with a conventional nematicide in field against *Meloidogyne incognita.*

| Treatment (Dose/Liter) | Mortality % | | | |
|---|---|---|---|---|
| | Zatoria multiflora | Achillea santolina | Nepeta cateria | Ferula oopoda |
| 0.2 ml | $0_{e}$ | $3_{e}$ | $0_{f}$ | $1_{g}$ |
| 0.4 ml | $0_{e}$ | $3_{e}$ | $0_{f}$ | $7_{f}$ |
| 0.6 ml | $0.33_{e}$ | $4_{e}$ | $6_{f}$ | $16_{e}$ |
| 0.8 ml | $11_{d}$ | $54_{d}$ | $27_{d}$ | $29_{d}$ |
| 1.0 ml | $34_{c}$ | $63_{c}$ | $43_{c}$ | $63_{c}$ |
| 1.2 ml | $74_{b}$ | $79_{b}$ | $68_{b}$ | $70_{b}$ |
| Furadan (Carbofuran) | $100_{a}$ | $100_{a}$ | $100_{a}$ | $100_{a}$ |
| LSD | 1.15 | 2.94 | 1.54 | 1.71 |

Mortality values exhibited by different doses of medicinal plants have shown significant differences when compared separately, However, *Z. multiflora* and *N. cateria* have shown no mortality of *M. incognita* at 0.4 and 0.2 ml/ liter. In *A. santolina* mortality values at 0.6, 0.4 and 0.2 ml/ liter were very low and statistically non-significant from each other.

*Comparison of fixed oils of medicinal plants with a conventional nematicide in the field against Meloidogyne incognita*

The comparative effect of fixed oils of *Z. multiflora*, *A. santolina*, *N. cateria* and *F. oopoda* with Furadan (Carbofuran) against *M. incognita* is depicted in Table 3.

Overall results exhibited highest population reduction at highest doses of tested medicinal plants. *F. oopoda* showed highest mortality as compared to *A. santolina*, *Z. multiflora* and *N. cateria*. The results showed maximum mortality of 74% by *F. oopoda* followed by 71%, 70% and 66% in plants, *Z. multiflora*, *A. santolina*, and *N. cateria* at a dilution rate of 3ml/ liter water. Minimum mortality (1%) was shown by *Z. multiflora* at a dose of 1.5ml/ liter, *N. cateria* at 1.0 ml/ liter, *A. santolina* and *F. oopoda* at 0.5 ml/ liter.

During the field test of *Z. multiflora*, highest population reduction rate of 71% was shown at a dilution rate of 3 ml/ liter water, followed by 39%, 7% and 1% at dilution rate of 2.5, 2.0 and 1.5 ml/ liter water, respectively. However, dilution rate of 1.0 and 0.5 ml/ liter water has shown no population reduction effect.

While field test of *A. santolina* high population reduction rate of 70% was shown by the dilution of 3 ml/ liter water, followed by 60%, 51%, 7%, 2% and 1% at dilution rates of 2.5, 2.0, 1.5, 1.0 and 0.5 ml/ liter water, respectively.

Similarly, field test of *N. cateria*, high population reduction rate of (66%) obtained by the dilution of 3 ml/ liter water, followed by 52%, 27%, 6% and 1% at dilution rates of 2.5, 2.0, 1.5 and 1.0 ml/ liter water, respectively, however the dilution rate of 0.5 ml/ liter water has shown no efficacy. Moreover, in field test of *F. oopoda*, high population reduction rate was of 74% by the dilution of 3 ml/ liter water, which followed by 71%, 67%, 41%, 19% and 1% at the dilutions of 2.5, 2.0, 1.5, 1.0 and 0.5 ml/ liter water, respectively.

**Table 3:** *Comparison of fixed oils of medicinal plants with a conventional nematicide in field against Meloidogyne incognita.*

| Treatment (Dose/Liter) | Mortality % | | | |
|---|---|---|---|---|
| | Zatoria multiflora | Achillea santolina | Nepeta cateria | Ferula oopoda |
| 0.5 ml | $0_{e}$ | $1_{f}$ | $0_{f}$ | $1_{g}$ |
| 1.0 ml | $0_{e}$ | $2_{f}$ | $1_{f}$ | $19_{f}$ |
| 1.5 ml | $1_{e}$ | $7_{e}$ | $6_{e}$ | $41_{e}$ |
| 2.0 ml | $7_{d}$ | $51_{d}$ | $27_{d}$ | $67_{d}$ |
| 2.5 ml | $39_{c}$ | $60_{c}$ | $52_{c}$ | $71_{c}$ |
| 3.0 ml | $71_{b}$ | $70_{b}$ | $66_{b}$ | $74_{b}$ |
| Furadan (Cabo-furan) | $100_{a}$ | $100_{a}$ | $100_{a}$ | $100_{a}$ |
| LSD | 1.70 | 3.64 | 3.05 | 2.17 |

There is a significant difference among fixed oil of different medicinal plants and also in different applied doses of each tested plants as compared to positive control.

The results of this research revealed that essential oils of tested medicinal and aromatic plants were high in mortality against *Meloidogyne incognita* as compared to its crude extracts. Maximum mortality of 79 % has reported by the essential oil of *Achelea santolina* at the dose 1.2 ml/liter. Whereas maximum mortality of 76% showed by the crude extract of *Zataria multiflora* at 3g/ liter was second in order. High mortality of essential oils of these medicinal plant is due to presence of diversified and pure bioactive compounds as compared to crude extracts. On the other hand, lowest doses showed the lowest mortality and highest doses having the highest mortality against the *Meloidogyne incognita*. Similar results were reported earlier by the Alireza and Moosavi, (2016), El-Sherbiny and Manal (2012). They found that higher doses of *Ferula, Achelea, Zataria, Nigella* and *Peganum* were directly proportional to the mortality of *Meloidogyne incognita*. The highest mortality values of *Meloidogyne incognita* varied from one plant to another plant during different experiments, such as in crude extract test experiment *Zataria multiflora* was highest in mortality of 76% at the dose rate of 3g/ liter, where as in essential oil test experiment *Achelea santolina* showed highest mortality of 79%. This may be due to the presence of a novo-volatile compound of *Achelea santolina*. Ghazalbash and Abdollahi (2013) also obtained closely related results.

## Conclusions and Recommendations

Banana *(Musa* spp.) is one of the most important fruit crops of the area and the area cultivation of banana crop is increasing day by day. Optimal weather favors the attack of many insect pests and diseases on banana crops. Plant parasitic nematodes are the most important pathogens of bananas. Proportionally with the increase in banana cultivation, the population of nematodes is also increasing. Present study was therefore carried out to evaluate natural products for management of *Meloidogyne incognita* in the field. Four medicinal plants were selected for evaluation of bioactive fractions in the field against *Meliodogyne incognita*. The comparative efficacy of crude extract of *Achillea santolina*, *Nepeta cateria*, *Zatoria multiflora* and *Ferula oopoda* with Furadan (carbofuran) against *M. incognita* showed competent results but effect of essential oils of *Achillea santolina* were more or less comparable with the results of Furadan. This showed that bioactive compounds of this plant are more effective as compared to others.

Finally, it is concluded that the findings of this research work with encouraging results will help to formulate a non-hazardous, environmentally safe, sustainable and low cost nematicide. This product anticipated to

be inexpensive, cost effective and suitable for the end users.

## Novelty Statement

First time selected indigenous medicinal plants have evaluated in the field for management of root knot nematode disease of Banana.

## Author's Contribution

**Khan Tamoor:** Major contribution in the article such as research, design, wrote the article and supervised.
**Hafsa:** Did application of plant axtracts, recorded data, compilation and interpretation of data.
**Hanif Maryam:** Formulated and prepared doses from plant extracts, statistical analysis.

*Conflict of interest*
The authors have declared no conflict of interest.

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