In vitro nematicidal activity of Juglone against Meloidogyne incognita race 2 infesting pomegranate

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ABSTRACT

Introduction. Juglans regia L. (walnut) is also known as the medicinal plant and native to the mountain ranges of central Asia. Recently, a severe infestation of root-knot nematodes (RKN, identified as Meloidogyne incognita race 2), was observed on Punica granatum L. (pomegranate). Aim. The present study aimed to investigate the effect of in-vitro nematicidal activity of the purified bioactive compounds (Juglone: 5-Hydroxy 1, 4-Napthoquinone) isolated from medicinal plant like walnut on Meloidogyne species associated with pomegranate under field conditions. Methods. Plants are cultivated at the agricultural farm in Solapur, (M.S.), India. Results. Roots of infected plants were heavily galled and soil samples collected from the affected plants second stage juveniles (J2). The ability of treatment schedule of Juglone was tested using an in-vitro method against RKN - Meloidogyne incognita race 2 infesting pomegranate. Juveniles in the control (distilled water; carbofuran) were compared with treated groups. The Juglone showed a 100% mortality in 5 µl/10 ml of distilled water/ 100 nematodes.

INTRODUCTION

Pomegranate (Punica granatum L.) is an important fruit crop of India. Pomegranate is delicious, popular fruit in India and several other countries of the world. It is the natural food for man. It is excellent source of vitamins, minerals, and enzymes. All parts of the fruit, seed, rind, and membrane have been shown to have potential health benefits. One of the most studied constituent of pomegranates is a polyphenol calls ellagitannins and ellagic acid [1].

Nematodes associated with pomegranate trees causes' severe damage to whole thickness of the root and stunting. Lesions were formed at the site of entry with necrosis, enlargement of cells into giant cells, containing egg masses and sections of larvae [2].

There are different pest attacks on the pomegranate plants by bacteria, virus fungal, but plant parasitic nematode is one of the major yield limiting factors. Now the productivity of pomegranate in Maharashtra is highest in the country [3, 4]. Further, Meloidogyne incognita L. has been observed as a serious problem on pomegranate in Maharashtra and attributed 32% yield losses in the identified hotspots. In the present study, in vitro nematicidal activity of Juglone as bioactive compound was evaluated on root-knot nematodes collected from pomegranate. Juglone, also called 5-hydroxy-1,4-napthalenedione (IUPAC) or 5-hydroxynaphthoquinone, is an organic compound with the molecular formula C_{10}H_{8}O_{5}. In the food industry, Juglone is also known as C.I. Natural Brown 7 and C.I. 75500; with systematic name: 5-Hydroxy-1,4-napthoquinone.

MATERIAL AND METHODS

Preparation of plant extracts

Juglone, extracted by using polar and non-polar chemicals by cold extraction method; extract will be separated by using Column chromatography and tested by spectroscopy as follows: IR = Perkin-Elmer 599-B in CHCl_{3}; NMR = 200 MHz Bruker Ac 200 MHz in CDCl_{3}; Mass Spectra, Finnigan Mat 1020 usind direct inlet system at 70 V; For purity and structure determination of the pure molecules Juglone = 5-Hydroxy 1, 4-Napthoquinone.
Collection of root samples
Pomegranate (P. granatum L.) root samples with soil were collected from the field located in Solapur district, Maharashtra state, India. Two hundred grams of soil and roots from each sample were processed for the isolation of nematodes.

Cobb’s decanting and sieving method for collection of nematodes
Nematodes were extracted by Cobb’s decanting and sieving methods [4]. Direct contact toxicity of compound at different dose was analyzed by exposing freshly hatched J2 and J3 of Meloidogyne species [5].

In vitro phyto-nematicidal activity
For in vitro nematocidal activity, the method described by Dama [6] and Dama et al. [7], used for present study. The test animals are divided in 5 groups in which one distilled water and one for slandered carbofuran chemical, each group contains 100 phyto-nematodes, with test compound concentration of 1 µl to 5µl.

The observations were taken at 24 hrs after exposure of test compounds under compound and stereomicroscope, on Juvenile mortality was recorded and expressed as percentage of efficacy against Meloidogyne species in each group were estimated by statistical analysis on the basis of the arithmetic mean number of J2 recovered. Juveniles in the control (distilled water; carbofuran), and also treated groups were compared statistically.

Statistical analysis
For taxonomic identification, the generated data was compared with taxonomic aids. Rate of immobility (i) was calculated and the obtained data analyzed by using Standard statistical methods (like SAS method). Standard deviation or confidence intervals of the means of LC50 values are calculated and recorded. Formula for percentage of mortality is as follows:

\[
\text{Percentage of mortality} = \frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100
\]

RESULTS AND DISCUSSION

The Results are shown in table 1 and figures 1-6. The action of Juglone on Meloidogyne species associated with Pomegranate roots, demonstrated maximal activity. The spectral data of Juglone where shown in figure 1.

The spectral data analysis of Juglone are as follows: IR= CHCl3 : 3300, 1730, 1430 cm⁻¹; NMR= 1H mr (200 MHz); CDCl3 : 6: 92 s(2H); 7.30m (1H) and 7.72m (2H). 11.9 (s, 3H, OH, D2O exchange); Mass Spectra 174.

The results of spectral data namely IR, NMR and Mass Spectra showed the isolated pure bioactive compound as Juglone. Figure 2 shows the Juglans regia tree with green walnuts. Punica granatum L., the healthy plant, infected plant and infected roots were shown in figure 3. Figure 4 showed the extraction of plant paparasitic nematode by using Cobb’s decanting and sieving method for collection of Nematode.

Phyto-nematicidal activity of Juglone on the mortality (immobilized nematodes) of root-knot nematode Meloidogyne sp. in 24 hrs were shown in table 1 and figure 6. The results compared with the available observations pointed out by Shelke and Darekar [8] and Pawar et al. [3]. Although, observations suggested that Juglone was active compound which require future pharmacological and toxicological studies prior to their use in the integrated diseases management (IDM) of plant parasitic nematodes. The result indicated that Juglone was very effective to controlling Meloidogyne species 100% mortality in 5 µl.

The present work confirms that the effect of Juglone shows the phyto-nematicidal activity on M. incognita race II associated with Pomegranate at very low concentration. The uses of bioactive compounds are an interesting option to control the root-knot nematodes instead of the chemical control and have no effect on human health. There was no any reported information on the effect of Juglone on plant-parasitic nematode M. incognita race 2 infecting pomegranate. The seasonal incidence and biological management of root-knot nematode, Meloidogyne incognita [9], the infesting pomegranate, Punica granatum observed by Walunj [10].
Figure 1. Spectra of Juglone. a) IR; b) NMR; c) Mass Spectra

Figure 2. Juglans regia tree shows green walnuts
Figure 3. Punica granatum L.; a) healthy plant, b) infected plant, c) infected roots (root-knots of Meloidogyne spp.)

Figure 4. Cobb’s decanting and sieving method for collection of nematodes.

Figure 5. Meloidogyne sp. isolated from the soil and roots from Punica granatum L., (Root-knot nematodes- M. incognita race 2, magnification 10x - 45x); a) Juveniles, b) Female, c) Nematode entering in the pomegranate root.
**Table 1.** Phytonematicidal activity of Juglone on the mortality (Immobilized Nematodes) of root-knot nematode Meloidogyne sp. in 24 hrs

<table>
<thead>
<tr>
<th>Concentration of Juglone / 10 mal of D.W.</th>
<th>1 µl</th>
<th>2 µl</th>
<th>3 µl</th>
<th>4 µl</th>
<th>5 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of root-knot Nematodes</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Juglone</td>
<td>60</td>
<td>74</td>
<td>84</td>
<td>92</td>
<td>100</td>
</tr>
<tr>
<td>Distilled water</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>80</td>
<td>85</td>
<td>96</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**Figure 6.** Phyto-nematicidal activity of Juglone on the mortality (immobilized nematodes) of RKN nematode Meloidogyne spp. in 24 hrs.

**CONCLUSION**

Juglone is highly active phyto-nematicide as compared to control and other phyto-nematicide. Improved data management and decision-support systems should facilitate the integration of new and traditional IPM strategies and tactics. In conclusion the naphthoquinones are highly active against in controlling plant parasitic nematodes namely Meloidogyne Sp. To reduce the cost for collecting the naphthoquinones from various plant other raw materials like, Tendu leaves, Plumbago zylanica and leaves of Lawsonia inermis need to be further carried out. This research work findings are useful for plant pathologists, teachers, agriculturist, students, farmers and research workers in this field of study.

**DECLARATIONS**

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**Authors’ Contributions**

All authors contributed equally to this work.

**Competing interests**

The authors declare that they have no competing interests.
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