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## IN VITRO THE EFFECT OF GALANGIN ON ROOT-KNOT NEMATODE *MELOIDOGYNE INCOGNITA* RACE II ASSOCIATED WITH *PUNICA GRANATUM* L.

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**Abstract:** *The Root-knot nematode Meloidogyne Species causes severe damage and yield loss to fruit crops in India. Punica granatum L. (Pomegranate), it is an important fruit crop of India. The root-knot nematode Meloidogyne incognita race II causes the roots of pomegranate. The heavy infection of about nematode causes the yield loss in every year. In the present study to isolate and purify the bioactive compound Galangin in the Rhizome of Alpinia officinarum L. it shows the phytonematicidal activity on Meloidogyne incognita race II.*

**Key words:** *Alpinia officinarum L., Meloidogyne incognita race II, Phytonematicidal activity, Punica granatum L., Pomegranate*

### I INTRODUCTION

The pomegranate is an important fruit crop in India. The pomegranate is a vital crop [1], and medicinally important fruit crop. The seeds of Pomegranate consist of polyphenols and the juice consists of antioxidants. It is a good source of minerals, carbohydrates, iron, calcium, sulphur, citric acid, vitamin-C, Glucose fructose etc. The *Meloidogyne incognita* race II causes to the Pomegranate plant are the gall formation on the roots, drying of leaf tips, yellowing leaves loss of vigor, and other symptoms similar to a lack of water or nutrients distortion of stem and ear heads etc. [2], studied on the root-knot nematode, *M. incognita* infesting to Pomegranate.

Chemical nematicides are highly toxic to animals, humans. It is also harmful to beneficial organisms and problems in environment. For present study the bioactive compound Galangin is used for in vitro Phyto-nematicidal activity on *M. incognita* race II associated with Pomegranate. The galangin is isolated from *Alpinia officinarum* L., Family: Zingiberaceae occur mostly in tropical Asia. It is used to treat muscle spasms, fever, intestinal gas, inflammation, used as a stimulant and to kill bacteria [3]. Maharashtra State, is the leading producer of *P. granatum* L. followed by Gujarat, Tamil Nadu, Karnataka, Andhra Pradesh. Ganesh, Arakta, Mridula, Bhagwa and Ruby, are the different pomegranates varieties produced in Maharashtra State, India.

### II MATERIALS AND METHODS

#### Materials

Chemicals: Chloroform, Petroleum ether, Methanol, Acetone, Alcohol, TLC plates, Silica gel G, Glass column, Silica gel (60 - 120 mesh).

#### Methods

The plants to be screened were identified from a Botanist from the Department of Botany, D.B.F. Dayanand college of Arts and Science, Solapur, (M.S.), India. The above plant materials were shade dried and grounded into powder and extracted with methanol.

The isolation and purification of bioactive compounds from the Rhizome of *Alpinia officinarum* L. by sequential extraction method with increasing polarity, subjecting the appropriate fractions to column chromatography and preparative TLC described. The spectral data analyzed by IR, <sup>1</sup>H NMR, C-13 NMR and LCMS. Dama *et al.* [4], [5], described method is used for In vitro phytonematicidal activity on Root-Knot nematode *M. incognita* race II. The isolated bioactive compound Galanginin were used for further in vitro phyto-nematicidal activity on the second stage juvenile larvae of *M. incognita* race II. The bioactive compound is dissolved in 2% Dimethyl Sulphoxide (DMSO), to prepare concentration of (1mg/1ml). Observations were made for death time or immobility of worm. The Time of mortality was recorded at 24 hours' time intervals with 1 µl to 5 µl bioactive compounds.

**Pure culture *M. incognita* race-II**

Infected Pomegranate soil collected from various parts of Maharashtra state and maintained the pure culture *M. incognita* race-II. The culture thus maintained and multiplied the nematode; it is used for in vitro testing of Galangin in laboratory conditions. The Taxonomic identity of *M. incognita*: on the basis of perennial pattern reported by [6].

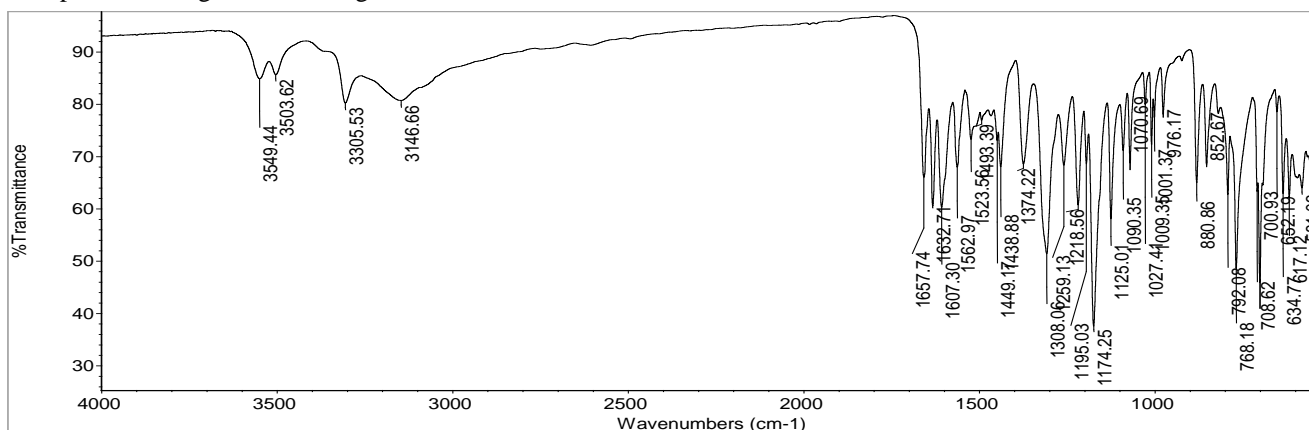
**Experimental**

Cobb's decanting method [6], were used for isolation of root-knot nematodes associated with Pomegranate root. Each petridish contains about 10 second stage juvenile larvae one group control with distilled water,

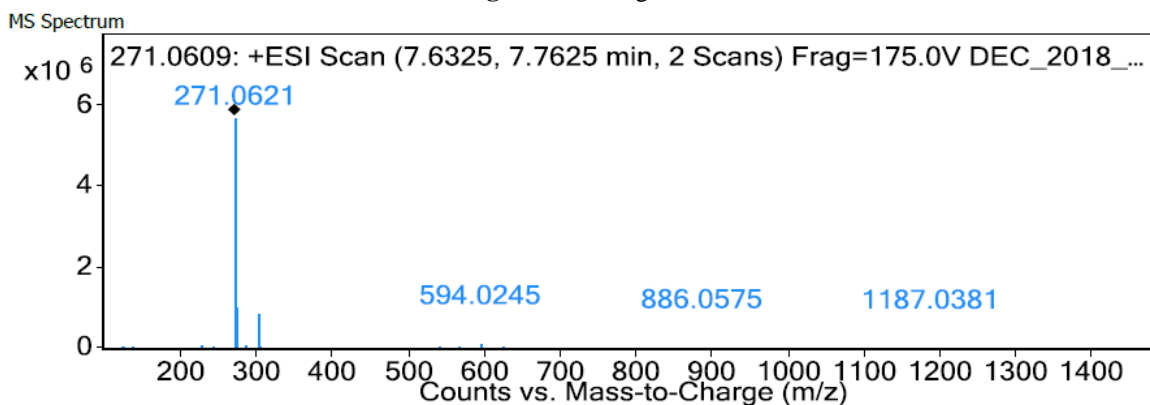
second group with standard Carbofuran chemical nematicides, Third group control standard bio-pesticide Neem cake and fourth group with different extracts of medicinal plants. The method used for counting of nematodes in the suspension is described by Bezooijen [7]. The experimental design will be randomized and all the concentrations along with the control were recorded and determined. For control 2% DMSO with DW, Neemcake and Carbofuran on *M. incognita* race II at 24 h exposure. The data were statistically analyzed by using Standard Statistical Methods [8].

**III RESULTS AND DISCUSSION**

The present observations are made to study the effect of purified bioactive compounds isolated from Plants on in vitro nematicidal activity against *M. incognita* race II associated with Pomegranate. Spectral data of isolated and purified bioactive compound Galangin shows in figure 1 to 4.



**Figure 1. Galangin : IR**



**Figure 2. Galangin: LCMS**

**Spectral data of isolated compounds**

Compound (a) : 3,5,7-Trihydroxy flavone (Galangin) mp : 213-214°C. FT-IR (KBr)  $\text{cm}^{-1}$  : 3549 , 1657, 1449 and 1027 ;  $^1\text{H-NMR}$  (400 MHz), in  $\text{CD}_3\text{OD}$  :  $\delta$  (ppm) 8.25 (2H, dd, H-2', 6'), 7.52 (2H, dd, H-3', 5'), 7.49 (1H, m, H-4'), 6.43 (1H, d, H-8), 6.20 (1H, d, H-6).  $^{13}\text{C-NMR}$  (100 MHz), in  $\text{CD}_3\text{OD}$  :  $\delta$  (ppm) 146.66 (C-2), 145.57 (C-3), 176.36 (C-4), 164.49 (C-5), 103.27(C-6), 16117 (C-7), 97.99 (C-8), 157.03 (C-9), 114.60 (C-10), 137.10 (C-1'), 129.51(C-2', 6'), 131.20 (C-3', 5'), 128.41 (C-4'). MS m/z :  $[\text{M}+1]^+$  peak = 271.0621.

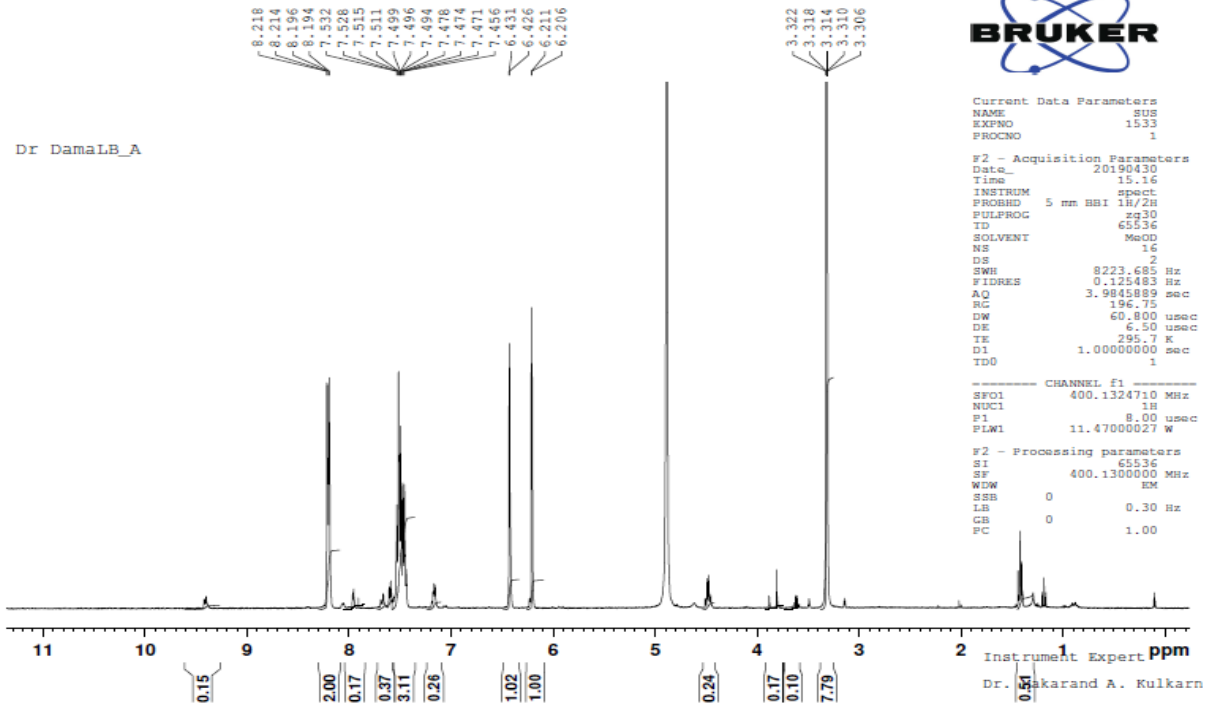


Figure 3. Galangin: 1H NMR

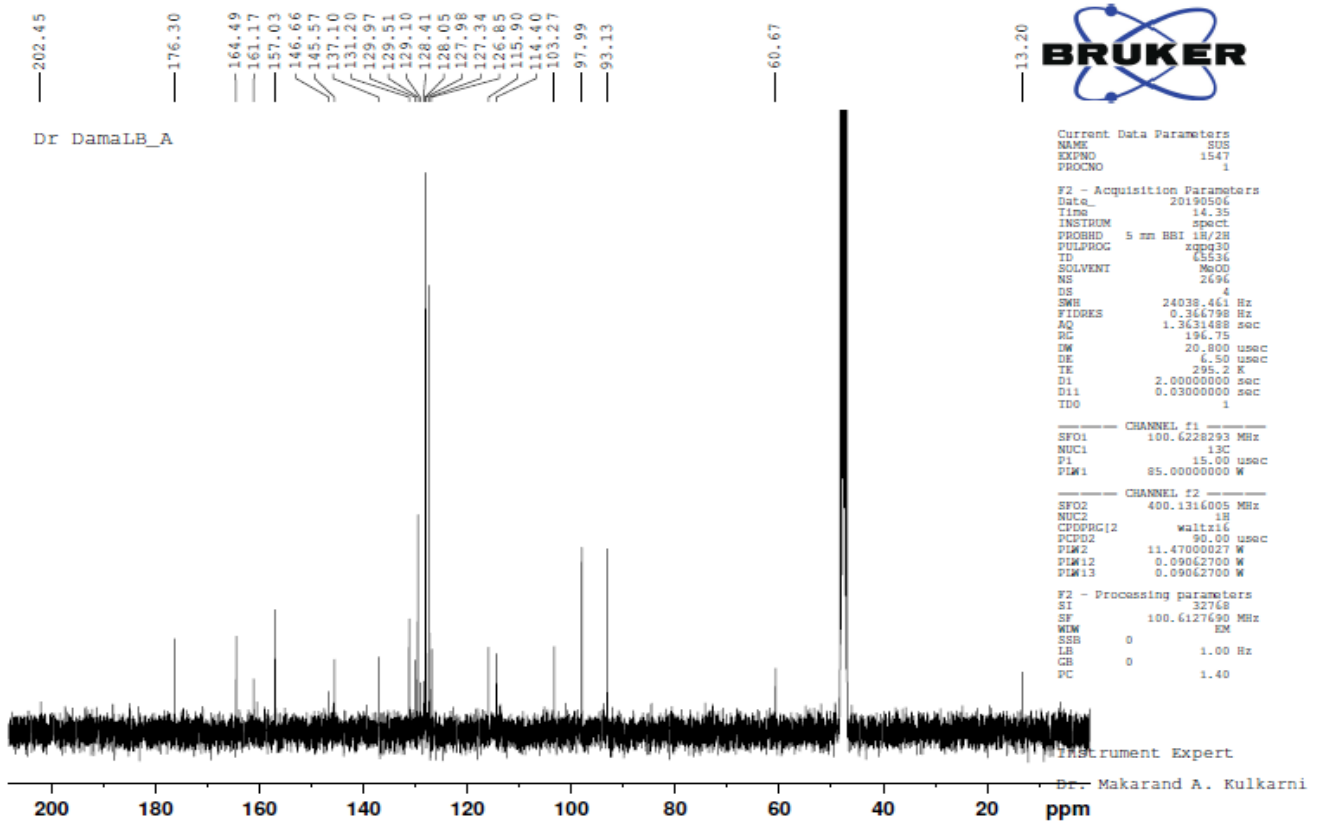
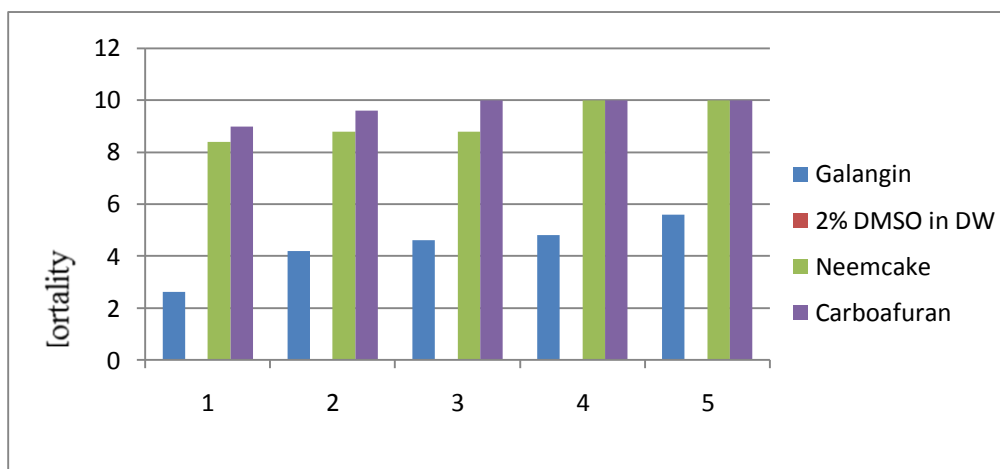


Figure 4. Galangin: C-13 NMR

The spectral data of Galangin shows in figure 1 to 4 and it is proved that the isolated purified bioactive compound is Galangin isolated from Rhizome of *Alpinia officinarum* L. The purified bioactive compound is galagin isolated from *Alpinia officinarum* L compared with the previous observation [9], [10].

**Table 1.** The effect of Bioactive compounds and with control, 2% DMSO in DW, Neemcake and Carboafuran on mortality of *M. incognita* race II associated with *P. granatum* L. at 24 hrs.

Galangin Conc. µg/ ml of 2% DMSO in DW		Galangin	2% DMSO in DW	Neemcake	Carboafuran
1	Mean	2.60	0.00	8.40	9.00
	SD	0.55	0.00	0.55	0.00
2	Mean	4.20	0.00	8.80	9.60
	SD	0.84	0.00	0.45	0.55
3	Mean	4.60	0.00	8.80	10.00
	SD	0.55	0.00	0.45	0.00
4	Mean	4.80	0.00	10.00	10.00
	SD	0.45	0.00	0.00	0.00
5	Mean	5.60	0.00	10.00	10.00
	SD	0.89	0.00	0.00	0.00



**Figure 5.** Effect of Galangin on average mortality of *M. incognita* race II associated with *P. granatum* L. at 24 hrs.

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The table 1 and figure 5 shows that the statistically average mortality of *M. incognita* race II associated with *P. granatum* L. by Bioactive compound Galangin was found to be 48% and 56 % mortality at concentration 4 and 5 µg/ ml of 2% DMSO in DW at 24h exposure. The obtained results are shows that Galangin used as natural phyto-nematicide instead of chemical hazardous nematicide. There is no work done on effect of Galangin as a phyto-nematicide on *M. incognita* race II associated with Pomegranate. However this work is novel and it is useful for researchers, agriculturists, scientists to control the root-knot nematode *M. incognita* race II Chitwood [11], studied to use of phytochemicals for nematode control. Darekar and Mhase [12], observed the yield losses due to parasitic root-knot nematode *M. incognita* in brinjal, tomato, and bitter gourd.

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