Journal of Medicinal Plants Research Vol. 5(20), pp. 5138-5141, 30 September, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

*Full Length Research Paper*

# Nematicidal potential of *Impatiens bicolor* Royle

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Accepted 4 July, 2011

**This study was designed to evaluate the nematicidal effects of *Impatiens bicolor* Royle extracted with various solvent systems to explore its potential use in agriculture as pesticide. Extracts of *I. bicolor* Royle obtained from n-hexane (A), dichloromethane (B), ethyl acetate (C), n-butanol (D), aqueous (E) fractions as well as crude (F) were tested *in vitro* for their nematicidal activities against *Cephalobus litoralis, Helicotylenchus indicus, Meloidogyne javanica* and *Meloidogyne incognita.* All fractions as well as crude extract showed toxicity in a dose and time dependent manner. Ethyl acetate fraction however showed more potent nematicidal effects.**

**Key words:** *Impatiens bicolor* Royle, nemacitidal, pesticide.

## INTRODUCTION

Plant parasite nematodes are an economically important group of soil borne pathogens which have inflicted serious damage on agricultural crops and plants. Plant-parasitic nematodes may be controlled by cultural practices, chemical nematicides and the use of resistant cultivars. However, nematicides do not provide long-term suppression of nematodes, and environmental and human health concerns are resulting in increased restrictions on their use. Some safe procedures for nematode control have been developed based on biological control agents and organic amendments; however, there is still a need for alternative, environmentally friendly measures or compounds for effective nematode control to be developed (Noling and Becker, 1994). Biocontrol has drawn great interest from researchers in the prevention of nematodes due to the environmental pollution problems induced by chemical insecticides (Li et al., 2005). *Impatiens bicolor* Royle is an important medicinal plant distributed in northern areas of Pakistan. Although some research work has been done on this plant, however its nematicidal screening was totally ignored. In this context as part of our continuous studies on exploring the hidden potential of indigenous flora of Pakistan (Ahmad et al., 2010; Nisar et al., 2010a, b) we have screened the extract of *I. bicolor* Royle for *in vitro* nematicidal activity to evaluate its potential use in agriculture as pesticide. The present investigation will provide a broad base for the possibility of further detailed biological studies on *I. bicolor* Royle.

## MATERIALS AND METHODS

### Plant material, preparation of crude extract and fractionation

Whole plant of *I. bicolor* Royle was collected from Khwazakhela, Swat, KPK, Pakistan, during September 2008. A taxonomist, Dr. Hassan Sher, Jahan Zeb Post Graduate College Saidu Sharif, Swat (Pakistan), identified the plant. A voucher, specimen No.18-NH-4-008 was deposited in the National Herbarium Islamabad. Shade-dried *I. bicolor* Royle (10 kg) was grounded and extracted with MeOH and water at room temperature. The combined methanolic extract was filtered and evaporated under vacuum to obtain a thick

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**Table 1.** Nematicidal activity against *Cephalobus litoralis.*

| Sample | % Mortality observed against different concentration | | | | | | Control |
|---|---|---|---|---|---|---|---|
| | 24 h | | | 48 h | | | |
| | 2 | 1 | 0.5 | 2 | 1 | 0.5 | |
| n-butanol | 40 | 28 | 10 | 65 | 37 | 18 | 2 |
| n-hexane | 12 | 2 | 1 | 15 | 8 | 5 | 1 |
| Dichloromethane | 70 | 50 | 30 | 85 | 72 | 42 | 2 |
| Ethyl acetate | 80 | 60 | 34 | 88 | 77 | 52 | 1 |
| Aqueous | 60 | 38 | 30 | 80 | 55 | 48 | 2 |
| Crude | 32 | 14 | 6 | 46 | 22 | 11 | 2 |

greenish black gummy mass. It was fractionated into n-hexane, dichloromethane, ethyl acetate, n-butanol, aqueous as well as crude fractions. All these fractions were tested for nematicidal activities.

**Nematicidal activity**

Culture of *Cephalobus litoralis* was prepared by using a single egg. Green peas (*Pisum sativum*) were mashed in small Petri dishes. A single female was picked and placed beside pea meal paste. Female layed eggs within 12 h and then nematode eggs hatched within 72 h and after 10 days, large number of nematodes in various stages of life cycle was obtained. These were used for screening crude extracts (Qamar et al., 1989). For isolation of *Helicotylenchus indicus* nematodes, 500 mg soil samples (Depth 15 to 25 cm) collected from paddy (*Oryza sativa* L.) fields. Soil samples were processed by Cobb sieving (Cobb, 1918) and modified Baermann funnel method (Baermann, 1917). Soil sample was put in a large bucket containing water and the mixture was vigorously stirred into a suspension, which was allowed to settle for about 2 min. The heavy soil particles sank to the bottom but nematodes remained suspended in the water. The remaining suspension was slowly poured over a coarse sieve (60 mesh aperture), which was continuously tapped by hand to avoid blocking. The deposit on the sieve was washed with a gentle jet of water into a beaker. This water suspension, containing eel shaped nematode, was passed through 200 and 300 mesh sieves. The nematodes thus recovered were mixed and water was decanted after allowing sufficient time for the nematodes to settle down. Then nematode suspension was poured over a piece of tissue paper attached to a perforated plastic sheet placed in a funnel fitted with a rubber tube and clamped at the lower end. The water contained in the funnel barely touched the bottom of the tissue paper. Care was taken not to allow the debris to float off the edges of the tissue paper. After 24 h the nematodes wriggled out into the clear water in the funnel and settled at the bottom, and then 100 ml of water containing the nematodes was drawn into a beaker. The nematode suspension was allowed to settle for 2 h or more, the excess supernatant water was poured off, and the remaining concentrated content was transferred into a cavity block for examination under the stereomicroscope and nematodes picked (Naqvi et al., 1992). Crude extracts were dissolved in water (passed through whatman filter paper No. 1) to make dilutions of 2, 1, and 0.5%. Experiments were performed under laboratory conditions at 28±2°C. Glass tubes 15 cm long and 8 cm were taken for bioassay. Three ml were taken from all dilutions in each tube. The required amount of nematode suspension (100 freshly hatched second stage juveniles/3 ml suspension) were poured in to tubes to each of which equal amount of plant extract had already been poured). Distilled water with nematode larvae was taken as control. The dead nematodes were observed under stereoscopic binocular microscope after 24, 48 and 72 h and percentage mortality was calculated. Nematodes were considered dead if they did not move when probed with a fine needle (Cayrol et al., 1989). For *Meloidogyne* spp., fresh egg masses of *Meloidogyne javanica* and *Meloidogyne incognita,* collected from stock culture maintained on tomato (*Lycopersicon esculentum*) root tissues were kept in water for egg hatching. The eggs suspension were poured on a cotton-wool filter paper and incubated at 28±2°C to obtain freshly hatched juveniles (J2). Juveniles collected within 48 h were used (Nazli et al., 2008). Crude extracts were dissolved in water (passed through whatman filter paper No. 1) to make dilutions of 2, 1, and 0.5%. Experiments were performed under laboratory conditions at 28±2°C. Glass tubes 15 cm long and 8 cm were taken for bioassay. 3 ml were taken from all dilutions in each tube. The required amount of nematode suspension (100 freshly hatched second stage juveniles/ 3 ml suspension) were poured in to tubes to each of which equal amount of plant extract had already been poured). Distilled water with nematode larvae was taken as control. The dead nematodes were observed under stereoscopic binocular microscope after 24 and 48 h and percentage mortality was calculated. Nematodes were considered dead if they did not move when probed with a fine needle (Cayrol et al., 1989).

## RESULTS AND DISCUSSION

Root-knot nematodes, *Meloidogyne* spp., are the major nematode pests of economic crops worldwide. The various species of *Meloidogyne* induce major morphological and physiological changes within roots, attack nearly every crop sown where not only yields are greatly affected but quality is also reduced (Sasser, 1980). The damage caused by root-knot nematodes, represents one of the major obstacles for the production of an adequate food supply (Carter and Sasser, 1982). Plant parasitic nematodes are controlled by different methods. Methyl bromide was the most effective and widely used fumigant for soil-borne diseases and pests including nematodes. In the 4th Meeting of Montreal Protocol in November 1992, it was proposed to ban the use of methyl bromide because it has been implicated in the destruction of the ozone layer. It is necessary to look for natural compounds with less toxicity and low environmental adverse impact (Duschatzky et al., 2004). Of the various fractions tested for nematicidal activity against larvae of *C. litoralis, H. indicus* and *Meloidogyne* spp., (Tables 1 to 4), ethyl acetate appeared to be the most active, as it caused

**Table 2.** Nematicidal activity against *Helicotylenchus indicus.*

| Sample | % Mortality observed against different concentration | | | | | | Control |
|---|---|---|---|---|---|---|---|
| | 24 h | | | 48 h | | | |
| | 2 | 1 | 0.5 | 2 | 1 | 0.5 | |
| n-butanol | 36 | 22 | 6 | 62 | 33 | 14 | 2 |
| n-hexane | 10 | 2 | 1 | 12 | 6 | 3 | 2 |
| Dichloromethane | 65 | 45 | 28 | 75 | 62 | 50 | 1 |
| Ethyl acetate | 78 | 60 | 35 | 83 | 67 | 49 | 2 |
| Aqueous | 54 | 27 | 28 | 68 | 42 | 21 | 2 |
| Crude | 38 | 19 | 11 | 50 | 28 | 17 | 2 |

**Table 3.** Nematicidal activity against *Melidogynae incognita.*

| Sample | % Mortality observed against different concentration | | | | | | Control |
|---|---|---|---|---|---|---|---|
| | 24 h | | | 48 h | | | |
| | 2 | 1 | 0.5 | 2 | 1 | 0.5 | |
| n-butanol | 30 | 20 | 10 | 45 | 31 | 19 | 2 |
| n-hexane | 8 | 2 | 1 | 17 | 10 | 8 | 1 |
| Dichloromethane | 66 | 53 | 25 | 77 | 68 | 45 | 2 |
| Ethyl acetate | 77 | 61 | 35 | 84 | 72 | 50 | 1 |
| Aqueous | 58 | 34 | 28 | 69 | 50 | 42 | 2 |
| Crude | 23 | 12 | 1 | 33 | 17 | 8 | 2 |

**Table 4.** Nematicidal activity against *Melidogynae javanica.*

| Sample | % Mortality observed against different concentration | | | | | | Control |
|---|---|---|---|---|---|---|---|
| | 24 h | | | 48 h | | | |
| | 2 | 1 | 0.5 | 2 | 1 | 0.5 | |
| n-butanol | 28 | 17 | 8 | 39 | 27 | 16 | 2 |
| n-hexane | 6 | 1 | o | 12 | 8 | 4 | 1 |
| Dichloromethane | 57 | 49 | 19 | 66 | 58 | 38 | 2 |
| Ethyl acetate | 70 | 53 | 28 | 79 | 63 | 44 | 1 |
| Aqueous | 50 | 28 | 21 | 61 | 43 | 36 | 2 |
| Crude | 29 | 16 | 4 | 39 | 22 | 10 | 2 |

more mortality of the nematode larvae after 48 h exposure to its extract. Aqueous and crude fractions were found to be least active in its nematicidal activity. Despite differences among investigated legumes, all fractions indicated time and concentration dependent activity. The activity was higher at high concentrations and increased with time.

## Conclusion

From this study we concluded that *I. bicolor* Royle provided a broad base for nematicidal activity and can be used as a potential source in agriculture as pesticide. However, incorporation of plant parts/extracts into soil alone or with biocontrol agents has been suggested as an alternative, safe and effective control method for management of plant parasitic nematodes. It is also suggested that before commercial use as biopesticides, *I. bicolor* Royle must be screened against other nematode species also.

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