



**International Journal of Biology, Pharmacy  
and Allied Sciences (IJBPAS)**

*'A Bridge Between Laboratory and Reader'*

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## NEMATICIDAL ACTIVITY OF *EUDISTOMA VIRIDE* TOKIOKA, 1955

C. PRATHEEPA AND M. PARIPOORANASELVI\*

P.G and Research Department of Zoology, St. Mary's College (Autonomous), Thoothukudi,  
Tamil Nadu, India

(Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tami Nadu, India)

\*Corresponding Author: Dr. M. Paripooranaselvi: E Mail: [mparipooranaselvi@gmail.com](mailto:mparipooranaselvi@gmail.com)

Received 15<sup>th</sup> May 2023; Revised 18<sup>th</sup> Aug. 2023; Accepted 4<sup>th</sup> Nov. 2023; Available online 1<sup>st</sup> Aug. 2024

<https://doi.org/10.31032/IJBPAS/2024/13.8.8255>

### ABSTRACT

Nematicidal activity refers to the lethal action of a nematicide on specific and vital life processes within tissues of the nematode. Now-a-days there is great urgency for discovering new ecofriendly tools for concerning about soil degradation and toxicity of agricultural chemicals to non-target organisms. The aim of study work was to investigate the nematicidal activity of the ethanolic extract of *Eudistoma viride* against root-knot nematode *Meloidogyne incognita*. Samples of *Eudistoma viride* Tokioka, 1955 were collected during the low tide from the intertidal rocky area of Hare Island. Egg masses of *Meloidogyne incognita* was isolated from the root of the Banana tree. The death rate of nematodes was noted on 2 days interval during the experimental study. Result indicated that the highest concentration of ethanolic extract of *Eudistoma viride* treated group has a maximum juvenile mortality (70%) and also showed nematicidal activity in a dose dependent manner. It was concluded that the ethanolic extract of *Eudistoma viride* can be utilized for biocontrol of root knot nematode and this method of management is cheap, eco-friendly and free from any hazards.

**Keywords:** Ascidian, *Eudistoma viride*, *Meloidogyne incognita*, nematicidal activity, mortality

### INTRODUCTION

Nematodes live at both high and low elevations, in polar and tropical climates, in fresh water, seawater and on land. They

have adapted to nearly every ecosystem on the world. Nematodes are thread-like worms, mostly about 1 mm in length and the

most ubiquitous organisms on Earth. The root-knot nematode (*Meloidogyne spp.*) is common and affects a wide range of crops [1, 2]. More than 3000 host species cause serious damage to most agricultural crops around the world. *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne arenaria*, and *Meloidogyne hapla* are the most damaging nematodes for crops, infecting over 3000 host species [3, 4]. Root-knot nematodes often occur on roots of banana. The damage caused by nematode species is more visible (root necrosis) and more destructive (toppling of plants). Root-knot nematode management in agriculture includes soil fumigation, soil pasteurization, soil solarization and crop rotation. The nematicides ethylene dibromide and 1,2-Dibromo-3-Chloropropane and carbamate are widely used. Chemical pesticides are commonly used to control plant parasitic nematodes. Notably, excessive use of such pesticides, known as "nematicides," has had a negative impact on the environment and human health [5]. Initially, applying chemical nematicides to contaminated soil can significantly reduce the damage caused by plant parasitic nematodes, but many of the regularly used nematicides are expensive. Therefore, it is essential to create novel, low-risk nematicides to manage root knot nematodes.

Among the marine invertebrates, ascidians constitute the biggest and most

varied class of the sub-phylum Tunicata, comprising about 3000 described species [6]. Ascidians are second on the list of potential drug sources [7]. Many different secondary metabolites have been produced by ascidians, some of which have physiological purposes, mostly for protection against their natural predators [8]. Ascidians harbour a great microbial community (including bacteria, actinobacteria, cyanobacteria, and fungi), which represents an additional source of natural products, many of which are extremely potent and mainly cytotoxic and antimicrobial, but also antioxidant, anti-inflammatory properties etc. [9, 10]. In India, studies on nematicidal property of ascidians especially in *Eudistoma viride* are lacking. As ascidians are available along the Tuticorin coast an attempt has been made to assess the nematicidal property of ascidians.

#### MATERIALS AND METHODS

The specimen - *Eudistoma viride* were collected during the low tide from the intertidal rocky area of Hare Island, Tuticorin harbour area. Identification up to the species level was carried out based on the key to identification of Indian ascidians [11]. Samples of animals were cleaned several times with sea water, dried at 45°C and homogenized to get a coarse powder which was stored in an air tight container. The extract was prepared from 100 g powder with ethanol using soxhlet apparatus, cooled

to room temperature and evaporated in a rotary evaporator to get a residue which was used for further investigations.

#### Isolation of *Meloidogyne incognita*

*Meloidogyne incognita* was isolated from the root of the Banana. Using sterile forceps, egg masses of *M. incognita* were removed from the severely affected roots. The egg masses were placed in Petri dishes with sterile water to keep the eggs moist after being cleaned three times with sterile distilled water. The hatched juveniles were removed from the Petri dishes every 24

hours and utilized for inoculation. To keep the eggs from drying out, fresh water was added to the dishes.

#### Nematicidal activity of *Eudistoma viride* against *Meloidogyne incognita*

The nematicidal activity of *Eudistoma viride* against *M. incognita* was carried out by maintaining the nematode numbers as constant, but with various concentrations of bio pesticide. Experimental animals were divided into six groups of five animals (n=5) each.

- |                  |   |  |
|------------------|---|--|
| <b>Group I</b>   | - | <b>Normal control without any ascidian extract</b>               |
| <b>Group II</b>  | - | <b>10 µg/ml of ethanolic extract of <i>Eudistoma viride</i></b>  |
| <b>Group III</b> | - | <b>50 µg/ml of ethanolic extract of <i>Eudistoma viride</i></b>  |
| <b>Group IV</b>  | - | <b>100 µg/ml of ethanolic extract of <i>Eudistoma viride</i></b> |
| <b>Group V</b>   | - | <b>250 µg/ml of ethanolic extract of <i>Eudistoma viride</i></b> |
| <b>Group VI</b>  | - | <b>500 µg/ml of ethanolic extract of <i>Eudistoma viride</i></b> |

One set of six petri plates were arranged on a table. Using a filler, nematodes of *Meloidogyne incognita* were transferred to petri plates. The sets of plates were labelled as a, b, c, d, e, and f. The concentrations 500 µg/ml, 250 µg/ml, 100 µg/ml, 50 µg/ml and 10 µg/ml of ascidian extract were taken in each petri plates respectively. The plate labelled 'f' acted as the control without any ascidian extract. The plates with each concentration were maintained at 28° C for 10 days. The death rate of nematodes was noted on 2 days interval during the

experimental study. Statistical data were analysed using mean values.

#### RESULTS

In the present investigation, ethanolic extract of *Eudistoma viride* were tested against root-knot nematode *Meloidogyne incognita* and the results were given in **Table 1 and 2; Figure 1 and Plate 1**. The groups which received 100, 250 and 500 µg/ml ethanolic extract showed an increasing mortality rate of 30, 50 and 70. The maximum juvenile mortality was recorded as 70% at the highest

concentration. The maximum juvenile mortality was recorded as 70% at the highest concentration. The minimum juvenile mortality was noted as 30% in 100  $\mu\text{g/ml}$

concentration. No juvenile mortality was noticed in control, 50  $\mu\text{g/ml}$  and 10  $\mu\text{g/ml}$  of the extract. The juvenile mortality was increased in a dose dependent manner.

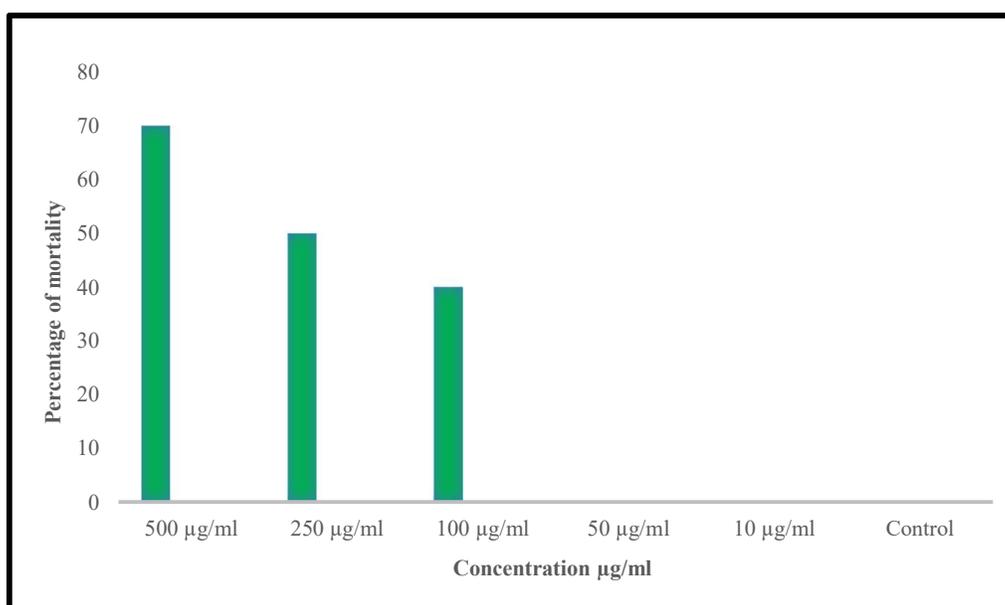
**Table 1: Nematicidal activity of ethanolic extract of *Eudistoma viride* against *Meloidogyne incognita***

Name of the sample	Groups											
	I – Control		II - 10 $\mu\text{g/ml}$		III - 50 $\mu\text{g/ml}$		IV - 100 $\mu\text{g/ml}$		V - 250 $\mu\text{g/ml}$		VI - 500 $\mu\text{g/ml}$	
<i>Eudistoma viride</i>	D	L	D	L	D	L	D	L	D	L	D	L
	0	5	0	5	1	4	2	3	3	2	4	1
	0	5	0	5	1	4	1	4	2	3	3	2

D – Dead nematode; L – Live nematode

**Table 2: Mortality percentage of ethanolic extract of *Eudistoma viride* against *Meloidogyne incognita***

Name of the sample	Groups					
	I – Control	II - 10 $\mu\text{g/ml}$	III - 50 $\mu\text{g/ml}$	IV - 100 $\mu\text{g/ml}$	V - 250 $\mu\text{g/ml}$	VI - 500 $\mu\text{g/ml}$
<i>Eudistoma viride</i>	0	0	0	40	60	80
	0	0	0	20	40	60
Mean value	0	0	0	30	50	70



**Figure: 1 Mortality percentage of ethanolic extract of *Eudistoma viride* against *Meloidogyne incognita***



Control

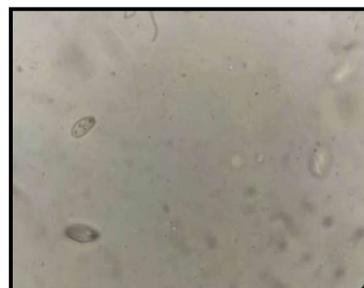
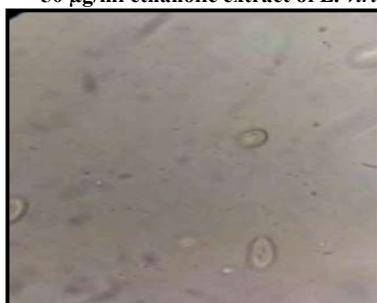
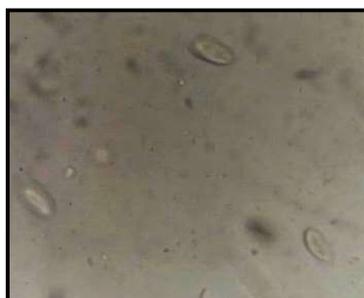
10 µg/ml ethanolic extract of *E. viride*50 µg/ml ethanolic extract of *E. viride*100 µg/ml ethanolic extract of *E. viride*250 µg/ml ethanolic extract of *E. viride*500 µg/ml ethanolic extract of *E. viride*

Plate: 1 Nematicidal activity of ethanolic extract of *Eudistoma viride* against *Meloidogyne incognita*

## DISCUSSION

The purpose of this research is to evaluate ascidian as a viable substitute for dangerous and harmful chemical nematicides. In the present study, ethanolic extract of *Eudistoma viride* were tested against root-knot nematode *Meloidogyne incognita*. The maximum juvenile mortality was recorded at the highest concentration (500 µg/ml). In earlier studies, phenolic substances such caffeic acid, benzoic acid and p-cumaric acid caused the death of

juvenile *Meloidogyne javanica* [12]. Nimin is regarded as a neem-derived chemical that can considerably lower the population of soil and root knot nematode *Meloidogyne incognita* and enhance plant growth [13]. Ferulic acid a phenolic compound also exhibited a similar effect on plant growth when tested against *Meloidogyne incognita* [14]. The nematicidal activity of *Serratia marcescens* was found effective against juvenile stages and it may be due to the presence of prodigiosin [15]. The

nematicidal activity of aqueous leaf extract of *Brugmansia suaveolens* possessed maximum mortality on second stage juveniles of *Meloidogyne incognita* and it may be due to the presence of phytocompounds in leaf [16]. It was reported earlier that *Eudistoma viride* contains phenols and flavonoids [17] which may have a role in controlling the nematodes. Further investigations are needed to confirm the nematicidal activity of *Eudistoma viride* against *Meloidogyne incognita*.

## CONCLUSION

The animal which are considered as the nuisance were used for this study. The present study indicated that the ethanolic extract of *Eudistoma viride* can be utilized for biocontrol of root knot nematode and this method of management is cheap, environmentally friendly and free from any hazards. It is a promising source for isolation of compounds which can be applied for nematode control.

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