

# First Report of Nematodes Associated with *Stevia rebaudiana* Bertoni ----- A Wonder Plant of Multifarious Medicinal Uses, from West Bengal, India



## Zoology

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### ABSTRACT

A survey was conducted on the medicinal plant *Stevia* [*Stevia rebaudiana* Bertoni cultivated at medicinal plant unit of Ramakrishna Mission Ashram West Bengal, India, in order to explore the nematode fauna associated with it.

A total of seven nematode species belonging to two orders and six families associated with the *Stevia* plant were recorded. Their absolute and relative frequencies and absolute and relative densities are also reported.

### INTRODUCTION

*Stevia* [*Stevia rebaudiana* Bertoni] a native plant of South America is one of the important medicinal plant with multiple medicinal values. It is probably best known as a source of natural sweetener. Centuries ago, Paraguay natives used the leaves of this small, herbaceous, semi-bushy, perennial shrub to sweeten their bitter drinks. Gaurani Indians extensively used this plant for more than 1500 years (Gupta et al., 2013). Likewise, it is also used as a sweetener in Japan, South Korea, Malaysia, Taiwan, Russia, Israel, Mexico, Paraguay, Uruguay, Venezuela, Colombia, Brazil, and Argentina. In the present days, stevia is cultivated and used in food elsewhere in East Asia, various parts of South America and Israel (Megeji et al., 2005). The *Stevia* leaves have sensory and functional properties superior to those of many other high-potency sweeteners and is likely to become a major source of natural sweetener for the growing food market (Goyal et al., 2010). It is commercially well known to exert beneficial effects on human health and has become an interesting area of research these days (Gupta et al., 2013). A large number of research work on the medicinal importance of these plants have been done in India and abroad like antimicrobial & anti-tumour (Satishkumar et al., 2008), antiviral (Kedik et al., 2009), antifungal (Silva et al., 2008), anti-hypertensive (Chan et al., 1998), anti-hyperglycaemic (Jeppesen et al., 2002), anti-inflammatory, anti-diarrhoeal, diuretic, anti-human rota-virus activities (Das et al., 1992; Takahashi et al., 2001), anti-HIV (Takahashi et al., 1998), hepatoprotective (Mohan and Robert, 2009) and immunomodulatory effects (Chatsudthipong and Muanprasat, 2009).

Like other medicinal plants, stevia plants are also attacked and affected by different pests which include insects, mites and nematodes. So far as nematode pests of stevia plants are concerned, only a single report of Sultan et al., (2010) on nematode fauna associated with stevia plantation from India is known, while Sen et al., (2012) studied the management of leaf spot disease of *Stevia rebaudiana* Bertoni with antagonistic bacteria. But, no further information is available about nematodes associated with stevia from West Bengal. Hence, the present study was conducted to explore the nematode fauna associated with stevia plant and to generate some data regarding their absolute and relative frequencies and absolute and relative densities.

### MATERIALS AND METHODS

During the present study, fifty soil samples (approx. 250 gms) associated with roots of stevia were collected from Medicinal Plant Garden of Ramakrishna Mission Ashrama, Narendrapur, Kolkata during July-October, 2014. Nematodes were extracted from soil samples using Cobb's (1918) sieving and decanting method and Bearman Funnel method (Christie & Perry, 1951). The extracted nematodes were fixed in hot FA (Formalin: Acetic acid mixture- 4:1) solution and then they were dehydrated slowly

following Seinherost method (1966) and thereafter were mounted in anhydrous glycerin (Seinherost, 1959) and sealed with paraffin wax. The nematodes were identified following classification of Jairajpuri & Ahmad (1992) in case of the Order Dorylaimida, Jairajpuri & Khan (1982) the order Tylenchida, Andrassy (1976, 1984) up to the species level and their population was recorded. The specimens were arranged in different trophic groups following Yeates et al., (1993). Moreover, the external morphological changes due to the occurrence of high nematode infection on the plants were also noted. The absolute frequency, relative frequency, absolute density, relative density and prominence value of the nematodes were calculated following Norton (1978), Srinivasan et al. (2011) using the formulae:

$$\text{Absolute frequency (AF)} = \frac{\text{Number of soil samples containing a species}}{\text{Number of samples collected}} \times 100$$

$$\text{Relative frequency (RF)} = \frac{\text{Frequencies of a species}}{\text{Sum of frequencies of all species}} \times 100$$

$$\text{Absolute density (AD)} = \frac{\text{Number of individuals of a species in all samples}}{\text{Number of soil sample collected}} \times 100$$

$$\text{Relative density (RD)} = \frac{\text{Number of individuals of a species in a sample}}{\text{Total number of all individuals in a sample}} \times 100$$

### RESULT AND DISCUSSION

The present study revealed seven nematode species belonging to two orders and six families associated with *Stevia* plant (Table 1). Those are *Ximphinema americanum* Cobb, 1913; *Hoplolaimus indicus* Sher, 1963; *Tylenchorynchus mashhoodi* Siddiqui & Basir, 1959; *Helicotylenchus dihystra* (Cobb, 1893) Sher, 1961; *Rotylenchulus* spp. Linford and Oliveira, 1940; *Aporcelaimellus chauhani* Baqri & Khera, 1975 and *Mesodorylaimus guarani* Andrassy, 1968. Among these nematode species the last two are omnivorous in feeding habit and the remaining are phytophagous (Yeates et al., 1993). All these nematodes were also reported earlier from different medicinal plants other than *Stevia* across India (Shukla, et al. 1986; Pandey & Akhtar, 1997; Rama & Dasgupta, 2000; Sultan et al. 2012), while, Sultan et al. (2010) reported *Meloidogyne* sp., *Pratylenchus* sp., *Macrorhynchus* sp., *Basioraimus* sp and *Helicotylenchus* sp. from *Stevia* plants from Punjab, India.

Moreover, in the present study it is observed that the nematode species, *Ximphinema americanum* showed a high absolute and relative frequencies (100% and 24.87%, respectively) while the same was lowest in *Rotylenchulus* spp. (10% and 2.48%, respectively). In the present study, *Ximphinema americanum*, *Hoplolaimus indicus*, *Tylenchorynchus mashhoodi* and *Aporcelaimellus chauhani* were found to be most prevalent (Table 1) with absolute densities 380%, 200%, 180%, 100%, respectively followed by *Mesodorylaimus guarani* (with AD of 70%), *Helicotylenchus*

*dihystera* (with AD of 56%) and *Rotylenchulus* spp. (with AD of 30%). The species *Helicotylenchus dihystera* showed high absolute frequency (70%) but low absolute density (56%). *Aporcelaimellus chauhani*, *Rotylenchulus* spp. and *Mesodorylaimus guarani* showed very less absolute frequency (30%,20%,10% respectively) but a high absolute density (100%,70%,30% respectively).

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**Table 1. A list of nematode species collected from Stevia Plants cultivated in Medicinal Plant Garden of Ramakrishna Mission Ashrama, Narendrapur along with their absolute frequency, relative frequency, absolute density and relative density.**

Sl.No.	Nematode species	Order	Family	Absolute frequency/AF(%)	Relative frequency/RF(%)	Absolute density/AD (%)	Relative density/RD (%)
1	<i>Ximphinema americanum</i> Cobb,1913	Dorylaimida	Ximphinematidae	100	24.87	380	37.40
2	<i>Hoplolaimus indicus</i> Sher, 1963	Tylenchida	Hoplolaimidae	92	22.87	200	19.60
3	<i>Tylenchorynchus mashhoodi</i> Siddiqui & Basir, 1959	Tylenchida	Telotylenchidae	80	19.90	180	35.43
4	<i>Helicotylenchus dihystera</i> (Cobb,1893) Sher,1961	Tylenchida	Hoplolaimidae	70	17.41	56	5.51

5	<i>Aporcelaimellus chauhani</i> Baqri & Khera,1975	Dorylaimida	Aporcelaimida	30	7.46	100	9.84
6	<i>Mesodorylaimus guarani</i> Andrassy,1968.	Dorylaimida	Dorylaimidae	20	4.97	70	6.88
7	<i>Rotylenchulus</i> spp. Linford and Oliveira, 1940	Tylenchida	Rotylenchulidae	10	2.48	30	2.95

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