

## STUDIES ON NEMATODE, *SPHAERULARIABOMBI* DUFOUR IN BUMBLE BEE, *BOMBUSHAEMORRHODALIS* SMITH IN INDIA

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**Abstract:** Bumble bees are some of the most important insect pollinators. However, knowledge on parasites associated to bumble bees in India is very limited. This study reports the first isolation of a sphaerularid nematode parasitizing queens of the native bumble bee, *Bombushaemorrhoidalis* Smith in Himachal Pradesh. Measurements and morphological characters of eggs, juveniles, and adults strongly suggests that the species is *Sphaerulariabombi*, a parasite that affects the reproduction and foraging behavior of the host. Nematodes are endoparasites of bumble bees which lay eggs inside the hind gut and juveniles develops inside the body thus cause death of the infected queens. Present studies were conducted during 2011-2014 to know the role of nematode, *Sphaerulariabombi* in bumble bee rearing. Seasonal incidences revealed higher infestation/infection during February-March with 10-12% infestation in bumble bee queens. Infested queens mostly do not brood but died after 10-16 days of captivity. Thus, *S. bombi* infestation reduces the successful establishment rate of bumble bee colonies. It is for the first time that the nematode *Sphaerulariabombi* was reported in bumble bees from Himachal Pradesh in India.

**Keywords:** Bumble bees, *Sphaerulariabombi*, Morphology, Seasonal incidence

### INTRODUCTION

Bumble bees belongs to order hymenoptera which is commercially reared for the pollination of different crops under protected conditions globally. Rearing methods have been developed in India [1-2, 3] but its rearing success rate is affected by different pests and diseases. Very little efforts have been made earlier in our country to study these pests and diseases [4]. Among different pests/ diseases of bumble bees, *Sphaerulariabombi* is one of the oldest known insect parasitic nematode as noticed in 1742 [5]. It was observed by various workers [6, 7] that during hibernation, the queens were infected by the nematode *S. bombi*.

Vander and Laan, observed queens of *Bombusterrestris*, *B. hypnorum*, *B. lucorum*, *B. hortorum*, *B. lapidarius* and *Psithyrus* sp., were infected with the parasitic nematode, *Sphaerulariabombi*. These were collected and studied in the Netherlands from April to September, 1971. In June, the infected queens gathered in a special wooded area which partially overlapped with a normal hibernation site. These queens were observed digging in

the soil and depositing third-stage juvenile nematodes through their anus. The nematodes entered the soil and molted to the adult stage in approximately two months. Mating occurred in the nematodes and the infective females were ready to penetrate a new host. Two molts occurred in the egg of *S. bombi* and the nematodes matured to third-stage juveniles in the host [8].

In New Zealand, *S. bombi* was presumably introduced accidentally with the first bumblebee release in 1885. By the early 1970s the parasite had spread by only about 40 km, about 0.5 km per year, while the bumble bees had colonized the whole of New Zealand within a few years of their release [9]. In Canterbury, New Zealand, the nematode (*S. bombi*) was isolated from bumble bees. It has been found infecting members of bombinae, including *B. terrestris*, *B. hortorum* and *B. subterraneus*. On dissection of the infected queens, the abdomens were having nematode juveniles and some eggs. Those queens that have *S. bombi* infection died after 9.0 days in captivity [10]. Another nematode species from Allantonematidae was also observed to be an internal parasite of *Mega bombussylvarumcitrinofasciatus* [11].

In Japan, a new species of genus *Sphaerularia* was reported by Kanzaki et al. [12]. On identification, it was found that this newly discovered nematode was *Sphaerulariavespae* sp., which infects the common Japanese hornet, *Vespa simillima*. By studying its characteristics such as parasitic form and uterus (sausage shape with many verrucae on its surface), it was found close to *S. bombi* [12].

Plischuk and Lange isolated sphaerularid nematode parasitizing queens of the native bumble bee, *B. atratus* in Argentina. Measurements and morphological characters of eggs, juveniles and adults indicated that this species was *S. bombi* [13]. It affects the reproduction and foraging behavior of the host. Fourteen overwintered queens of *B. terrestris* were captured from forest and observed for the incidence of pests by Macfarlane [10]. He found 50% of them infected with *S. bombi*. Out of which 43% queens have both nematode juveniles and eggs in their abdomen. The infected queens were found dead after 9.0 days of their captivity while the non-infected queens reared the colonies successfully. Parasitized queens on emergence from hibernation do not start the nest and died within few days of emergence from their hibernaculum.

In India, little efforts have been made earlier to study the pests and diseases of bumble bees due to less available literature on rearing of *Bombus haemorrhoidalis* [4]. Keeping in view the importance of different pests and diseases, present studies were undertaken to know the role of nematode, *Sphaerularia bombi* Dufour which is an important natural enemy of

bumble bee queens in Himachal Pradesh, India and act as a preliminary limiting factor in the rearing under captivity.

## **MATERIALS AND METHODS**

The studies were carried out during 2011-2014 in the laboratory and field area of the department of Entomology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India at an altitude (1250m amsl), longitude of 77° 11' 30" E and latitude 30° 52' 30" N subtropical areas.

### **Infestation of *S. bombi* in bumble bees**

The bumble bee queens get infested by *S. bombi* during hibernation in the soil under natural conditions. It enters in the body of the queen through the ovipositor and then started multiplying in the haemocoel. In the spring when the queen emerges from hibernation, the nematode begins to grow and multiply inside the body of queen. The number of juveniles ranged from 300-900 inside the infected abdomen of queen. With the increase of infection/multiplication, nematode juveniles spread in whole system and the queen dies in 10-16 days.

### **Identification**

The dead bumble bee queens were collected from the reared colonies as well as from the field and then dissected. The dissected bees were observed under the microscope for the presence of nematodes.

The different parameters used for the identification of nematode were:

- a. Biology and symptoms: Effect of infestation or infection due to the incidence of nematode was recorded on the basis of induced symptoms in the developing colonies with regard to death of queens/workers. The biology of *S. bombi* were studied.
- b. Seasonal incidence: The incidence of nematode, *S. bombi* was studied by collecting the foraging bumble bee queens and workers from the field during different months of the year January 2012-July 2014 and dissected them under the stereomicroscope. Out of the total dissected bees, the per cent infestation in bumble bee foragers was calculated as:

Per cent infestation = (Total number of infested bees/Total number of bees collected) x 100

- c. Morphology: After the isolation of the nematodes, permanent slides were made and the morphological characters were studied under the microscope with regard to size, shape and orientation.
- d. Identification: The identification of the nematode was got done from the concerned department, IARI, New Delhi.

## RESULTS

### Biology and Symptoms of *Sphaerularia bombi*

The nematode infects hibernating bumble bees while these were seeking out a hibernation site for the winter season during fall and autumn in the soil. It causes the infected bumble bee to take unsuccessful attempts to burrow into the ground. Hundreds of 3<sup>rd</sup> stage juveniles are discharged from the infested bee's anal opening into soil. Inside the bee, these developed into the 4<sup>th</sup> juvenile stage and at this stage, the infected bee dies. When a healthy bee finds its hibernation site where the 3<sup>rd</sup> juveniles nematode are present, infect the bee by penetrating it. The bee emerges to the surface during the spring season and by that time the nematodes has developed into a mature egg producing female within the bumble bee. The eggs then hatch and molt into the first and second stage juveniles which then rapidly molt into the 3<sup>rd</sup> stage. The third juvenile stage is the infective stage for *S. bombi*. These juveniles can be found in the bee's haemocoel, midgut and hindgut. Infected queens never lay any eggs. The number of juveniles ranged from 300-900 inside the infected abdomen of queen. With the increase of infection/ multiplication, nematode juveniles (Fig 1a) spread in whole system and the queen dies.

### Seasonal incidence

Seasonal incidence of *S. bombi* has been presented in Table 1 Infection of nematode, *S. bombi* was observed while dissecting the dead bumble bees. During 2012, the infection found in 1.88% bumble bees. In 2013 and 2014, the nematode infection was less (1.51% and 0.93%) as compared to 2012 (Fig 2). The infestation was recorded in dead queens which were captured during onset of spring till their availability in nature. The highest infestation was recorded in the month of February irrespective of years (2012 and 2013). While in 2014, the queens of bumble bees were not collected from field for rearing as the bumble bee culture was already present. In the months of April and May less infestation of nematode was recorded in 2012, 2013 and 2014 (Table 1).

### Morphology

It is vermiform in shape (Fig. 1 a and 1b) having a spear, an oesophageal tract, mid-intestine and anus. It is about 100 x 20  $\mu$  in size. Oesophageal tract is 180  $\mu$  long with 45  $\mu$  obtuse tail. Males are smaller than the females (880  $\mu$ ). Mating takes place in the soil. A fascinating feature of this nematode is the ability to completely evert the reproductive system. The size of uterus increased to 15000 x 20000 fold during eversion of uterus.

It belongs to order Rhabditida, Infraorder Tylenchomorphae, Superfamily Sphaerulariidae, Family Sphaerulariidae and genus *Sphaerularia* as per De lay and Blaxter [14].

### Identification

On the basis of different parameters studied, the samples were identified as *Sphaerularia bombi*. Similarly, the samples sent to IARI New Delhi were also confirmed as *Sphaerularia bombi*.

### DISCUSSIONS

Earlier, different researchers have also found *S. bombi* infection in the haemocoel and midgut of *Bombus* spp. [9-15-16-17-18, 19]. It was observed that infected *B. terrestris* queens never laid any eggs and is therefore, virtually castrated [20]. The nematode *Sphaerulariabombi* infests bumble bee queens during hibernation in the soil under natural conditions. It enters in the body of the queen through the ovipositor and then started multiplying in the haemocoel. Increased infestation led to death of queens [6]. *Sphaerularia bombi* affects the reproduction and foraging behavior of the host bumble bees [8]. Taylor et al, 2011, while rearing the bumble bee, *B. voskenenski* Radoszkowski, found *Sphaerularia bombi* infection in Oregon, west coast of USA for the first time. They collected total 360 bumble bee queens and observed that 147 queens did not laid the eggs to start the colony and died after few days of collection from the field [21].

The present studies further indicate that its infestation was very low (0.93 to 1.88%) during the period of experimentation i.e. 2012-14. Earlier, researchers have also recorded its varied infestation in different countries i.e. 3-10% in New Zealand, Europe and North America [22], 8.0 and 20.0% in Turkey [13] and 12.0% in Holland [23, 5]. Morooka described the infection of *S. bombi* for the first time from the overwintering gynes of *Parapolybia* sp. (polistine wasps) [24]. The low infestation in *B. haemorrhoidalis* queens in present studies may possibly be due to its short hibernation period as compared to other bumble bee species and different locations. This study will help in development of bumble bee management practices for the control of this important pest of *B. haemorrhoidalis* Smith.

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**Table 1. Seasonal incidence (%) of *Sphaerulariabombi* Dufour in *B. haemorrhoidalis* during February 2012 – July 2014**


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Number of bumble bees (queen/worker) infested with <i>Sphaerulariabombi</i> in the indicated year						
Month	2012		2013		2014	
	Total number of bees collected	Per cent Infestation	Total number of bees collected	Per cent Infestation	Total number of bees collected	Per cent Infestation
January	Nil	Nil	Nil	Nil	3	nil
February	19	10.52	25	12.00	19	nil
March	21	4.76	32	3.12	26	3.84
April	32	6.25	54	3.70	48	2.08
May	36	2.77	66	3.03	39	nil
June	39	Nil	74	Nil	42	nil
July	32	Nil	72	Nil	38	nil
August	39	Nil	54	Nil		
September	48	Nil	52	Nil		
October	44	2.27	39	Nil		
November	29	Nil	32	Nil		
December	32	Nil	29	Nil		
<b>Total</b>	371	1.88	529	1.51	215	0.93



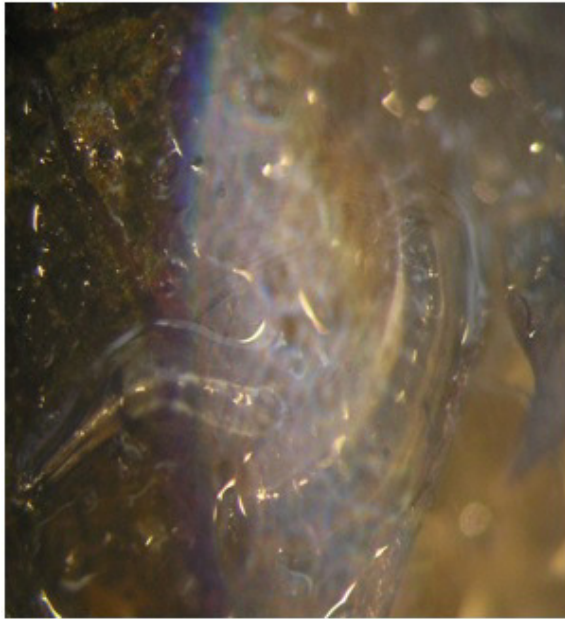


Fig 1. a.



Fig 1. b.

Fig 1. a. *Sphaerulariabombi* juveniles in abdomen of *B. haemorrhoidalis*  
 Fig 1. b. *Sphaerulariabombi* juvenile

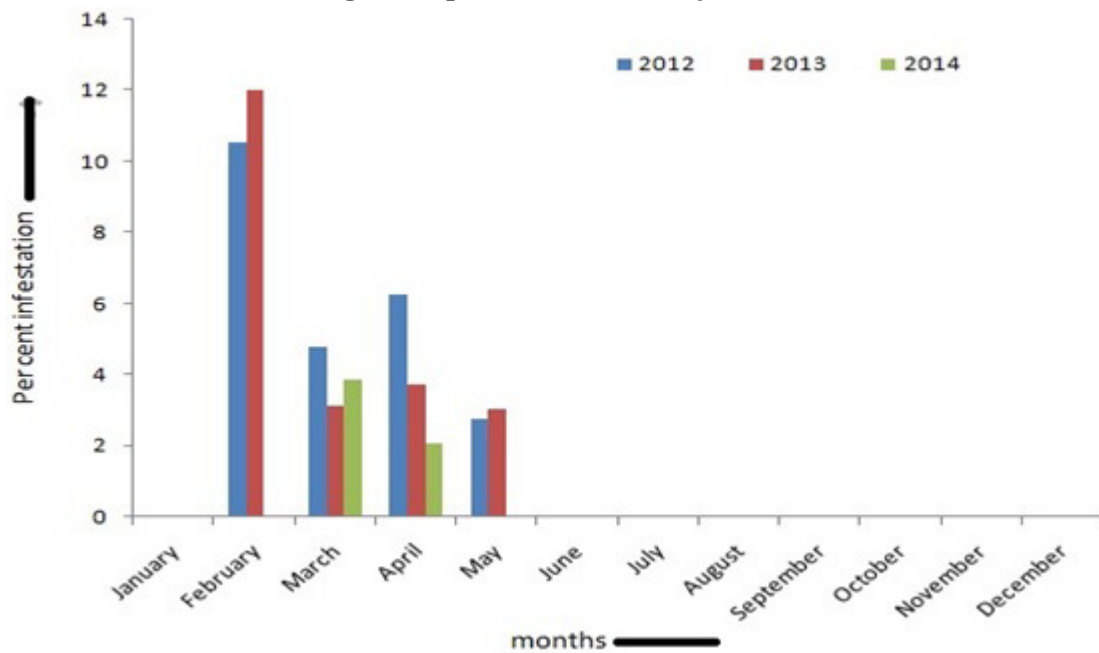


Fig 2. Incidence (%) of nematode, *Sphaerularia bombi* in bumble bees during different months in 2012 to 2014