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**Potential role of acetone extract of Lichen species, *Usnea baileyi* (Stirt.) Zalbr. for controlling Root knot diseases of vegetables**

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**Abstract**

Recently research on bioactive compounds is gaining worldwide attention. Lichens which are symbiotic association between algae and fungi are reported to contain more than a thousand active secondary metabolites. In this direction antinematocidal property of *Usnea baileyi*, a fruticose lichen collected from hilly tracts of Manipur was tested. Acetone extract of thallus of *Usnea baileyi* when applied to *Meloidogyne* spp., root parasites isolated from infected roots of common vegetables like, Tomato, French bean, etc. was found to be highly effective. The parasites became inactive within 5-10 minutes of treatment and then stop their movement completely within 3-4 hours and get killed. When these killed specimens were observed under high power microscope (40X), the lichen extract was found accumulated in their guts. Acetone extract of this *Usnea* species contains Norstictic acid, which is reported from several other lichen species too. Lichens thus proved to be a promising species for extraction of bioactive compounds for maintaining Root knot diseases of vegetables.

**Key words:** Root knot nematodes, lichen extracts, permanent endoparasite, biological control.**INTRODUCTION**

Lichens contain more than 1050 active secondary metabolites<sup>1</sup> due to which many of possess antibacterial, antifungal and antioxidant properties. In the present

investigation a fruticose lichen species, *Usnea baileyi* collected from hilly tracts of Manipur state is selected for testing its Antinematocidal property. Such type of work on lichens is in a very beginning stage in India and so also in the world.



Fig. 1. Thallus of *Usnea baileyi*

**METHODOLOGY****Collection and identification of the material**

*Usnea baileyi* is found commonly on barks of trees in the forests of hilly regions in Manipur in comparatively undisturbed environment. The species could be easily identified with the help of its morphological (Fig. 1) and anatomical characters (macerated axis)

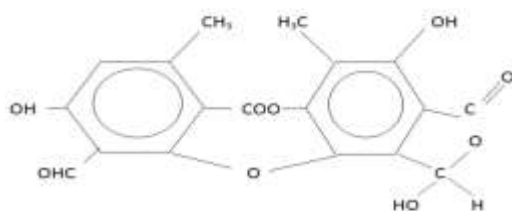
assisted by its chemistry. Some of the thallus was kept fresh while some were dried and transferred to a suitable herbarium packet. It was identified with the help of well-established lichenological methods. Morphological characters were studied under binocular microscope. Thin vertical sections of thallus were prepared with the help of a sharp blade, stained in cotton blue-lacto-

glycerol solution for anatomical studies under compound microscope. For determining lichen chemicals, spot colour tests were performed with the chemicals such as 10% K (Potassium hydroxide solution), C (Calcium hypochloride solution), KC and P (*p*-phenylenediamine solution), by putting directly on the thallus and medulla of the specimen and TLC (thin-layer chromatography) was done following the method described by White & James<sup>2</sup>. The specimen was identified with the help of authentic literature<sup>3,4,5,6</sup> and consulting ASSAM herbarium at Botanical Survey of India, Eastern circle, Shillong. The specimen was deposited in Dhanamanjuria Herbarium (DM Herb.) for future reference.

#### **Preparation of Lichen Extract and identification of bioactive compound**

Lichen thalli of *Usnea baileyi* were collected, cleaned and air-dried. About 200 mg of such thalli of *Usnea baileyi* was dissolved in 5 ml of absolute acetone and the filtrate was used for testing its antinematocidal property.

Using this extract the chromatograms were developed in solvent system A (toluene: dioxane: acetic acid; 180:60:8ml). The extract contained Norstictic acid (C<sub>18</sub>H<sub>12</sub>O<sub>9</sub>)<sup>7</sup>, a yellow acid, UV+ yellow, Pd+ve –orange, K+ve red, substance class: depsidones.



**NORSTICTIC ACID**

#### **Collection and isolation of *Meloidogyne* spp.**

For this, *Meloidogyne* spp. which are noticed to attack over 2500 host, mostly dicot vegetables, pulses, fibers, fruit crops like papaya, grapes, tobacco, peanut, tea, coffee, flowers, betel vine, mulberry and monocots like sugarcane, sorghum, rice, banana as well as large numbers of weeds were isolated from the infected roots of locally grown vegetables in Manipur. Collected infested root knots were washed with running tap water and chop into a of length of about 1 cm including the infected lesions, to make up to about 5 gm, then place them in 100 ml of water at room temperature (20°C-22°C). A piece of infested roots was taken, observed under a stereoscopic dissecting microscope (10X) and dissected with needles and forceps to extract nematodes which were then transferred into the cavity blocks.

#### **Treatment**

The eggs and 2nd stage juveniles were treated with lichen extract drop by drop to observe the metabolic activity.

#### **OBSERVATIONS**

After few minutes of treatment, the egg hatching was slowed down, then it was completely controlled after three hours. When the treatment was carried out in the 2nd stage juveniles (infective phase), they became inactive within 5-10 minutes and then stop their movement completely within 3-4 hours. When these killed specimens were observed under high power microscope (40X), the lichen extract were found accumulated in their guts. Acetone extract of this *Usnea* sp. contains Norstictic acid.

#### **CONCLUSION**

The chemical management of nematodes reduces nematode population for a limited of time and the use of chemical pesticides, however, is environmentally hazardous. Nematodes that escape treatment can resume feeding when the chemical is gone. So, such tested antihelminthic property of lichens could be utilized to control highly dangerous nematodes as an alternate means against the use of chemicals. From this study, we have found that the metabolic activities of this pathogenic nematodes are drastically reduced by using *Usnea* sp. extract. We have a rich report of 261 species of lichens reported from the state of Manipur<sup>6</sup> which if tested several of them will show positive result. We feel that management of this disease using plant extracts would be quite handy and successful. Meena, *et al.*<sup>8</sup> also used acetone extracts of *Tagetes* sp. to test the efficacy of acetone extracts of *Tagetes species* one egg hatching and larval mortality of *Meloidogyne incognita*.

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