Effect of (+)-usnic acid on the shot-hole borer (Xyleborus fornicatus Eichh.) of tea

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The shot-hole borer (SHB), Xyleborus fornicatus Eichh. is a small, wood boring ambrosia beetle belonging to the family Scolytidae. SHB is considered as the most serious pest of Tea (Camellia sinensis var. assamica) in Sri Lanka and is of greater economic importance than the other teainfesting insects and mite pests put together1. Certain tea cultivars show a higher degree of susceptibility to this pest. The use of utilization of persistent insecticides such as DDT, dieldrin, aldrin and telodrin to control SHB has caused serious secondary pest outbreaks2. This paper deals with the potent activity of (+)-usnic acid against X. fornicatus.

(+)-usnic acid was isolated from an Usnea sp. collected from the surface of a rotting Acacia decurrens tree in Ambewela, Sri Lanka3,4. The control medium was prepared using sucrose (1.409 g), casein (0.929 g), yeast (0.929 g) Wesson salt (0.118 g), agar (3.479 g), cellulose (17 g) and distilled water (75 ml)5,6,7. (+)-Usnic acid was dissolved in acetone (2 ml) and then distilled water was added to yield 50, 75 and100 ppm solutions. Solutions were incorporated into cellulose powder and mixed with the rest of the medium and ground thoroughly to a fine pulp.

An equal quantity of each of the diet mixtures and the control was dispensed into six separate sets of boiling tubes. A test tube was placed upright in the center of each boiling tube so that there was a thin layer of the medium between the walls of the inner test tube and the outer boiling tube, mimicking an artificial tea stem inside the tubes7. Six replicates (control and tests) were prepared and the boiling tubes were sealed with cotton plugs and aluminum foils. The tubes were autoclaved at 1.2 kg/cm² at 120°C for 15 min., allowed to cool to room temperature, and kept in a refrigerator for 3-4 days before use. TLC was used to confirm that no decomposition had taken place during autoclaving.

Healthy adult females of X. fornicatus were collected from beetle infested tea bushes (clone TRI 2025) at the Tea Research Institute sub-station in Hantane. The beetles were surface sterilized with 0.1% NaOCl (4-5 min)7. Two sterilized adult female beetles were introduced into each culture tube. The tubes were kept at 26 ± 2°C in darkness (24 h). Daily observations upto 60 days were made from 11.45 a.m. to 1.00 p.m. during which the beetles are most active.

(+)-Usnic acid has been found to show remarkable antibiotic, antitumour, antimutagenic, plant growth inhibitory and enzyme inhibitory activities8. In a previous study, we reported the antitermite and antifungal activity of (+)-usnic acid.9

Growth and development of SHB is adversely affected by the presence of (+)-usnic acid in the artificial dietary media (Table 1). At 75 and 100 ppm, only 10 and 4 mother beetles produced progeny, respectively. The galleries constructed in the test media containing (+)-usnic acid, were of an irregular shape compared to the control.

The late appearance of the fungus Monacrosporium ambrosium (which maintains a symbiotic relationship
with the beetle), correlated with the increase in the concentration of (+)-usnic acid. Interestingly, a separate recent study showed the antifungal activity of caffeine in tea on *M. ambrosium* and we had previously reported potent activity of (+)-usnic acid against several fungal species including *M. ambrosium*. Thus, it is likely that the antifungal activity of (+)-usnic acid against *M. ambrosium* is a key factor in the retardation of the growth and development of the beetle. The number of adults that emerged from the test media containing 75 and 100 ppm of (+)-usnic acid were 16 and 7 respectively which were relatively small compared to the control in which the number was 107. Prior to this study, there had been only one report of the insecticidal activity of usnic acid which was against the polyphagous herbivorous insect *Spodoptera littoralis*.

### Table 1: Average time (days) taken for development of galleries, *M. ambrosium* and the various stages in the life cycle of *X. fornicatus* in the test media

| Observation                  | (a) | (b) | (c) | (d) |
|------------------------------|-----|-----|-----|-----|
| Eggs                         | 6-9 | 7-10| 9-14| 13-15|
| Larvae                       | 11-14| 12-14| 15-17| 17-20|
| Immature beetles             | 20-24| 22-27| 18-23| 27-31|
| Mature beetles               | 24-27| 26-30| 26-31| 31-34|
| Number of emerging beetles   | 90 F | 65 F | 14 F | 6 F |
| (a) Control; (b), (c), (d): 50, 75, 100 ppm (+)-usnic acid, respectively. F (Female); M (Male). | 17 M | 14 M | 2 M | 1 M |

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