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Assessment of Behaviourial Characteristics of *Poophilus nebulosus* Leth Spittlebug on *Alhagi persarum* Boiss and Buhse Camel Thorn Plant in Torbate Jam Region, Khorasan Razavi Province

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Abstract: This study was mainly conducted due to importance of plant manna and their production process regarding pharmaceutical use. Therefore, the behavioural characteristics of *Poophilus nebulosus* Leth spittlebug on *Alhagi persarum* Boiss and Buhse camel thron plant in Torbate Jam region, Khorasan Razavi province were assessed during 2006-07. Hedysarum or Taranjabin manna is a sweet liquid with pharmaceutical effects and it is exhausted by the spittlebugs after they nourish on the camel thorn plant. In European references, this manna is referred to as the 'persian manna'. In this study we found out that the spittlebug has 5 nymphal instars and 3 generation per annum. The first generation's activity starts from early April and the instars make a great deal of spittle at the bottom section of the plant while they reside inside the foam. The adult spittlebug appears in late April and moves out of the foam, creates a non-sweet and non-crystallized fluid which dries out as a white powder on the plant. The second generation which appears in mid June is behaviorally different from the first generation as they have an independent life, are resilient and enormously active, stay at the browse of the plant and do not create any spittle. The first hedysarum manna crystals are produced by this generation in late June. The activity of the third generation is similar to the second. Hedysarum manna production continues as mentioned until the end of autumn. By the end of autumn the female spittlebug lays the eggs underneath the skin of the stem. The spittlebugs in Torbate-Jam region prefer the plants which are less damaged due to exploitation of hedysarum. In this study we realized that this spittlebug is also able to hibernate as an adult insect. There is evidence that the bug was not capable of spittle production on the cut browse of the camel thorn plants and the process was ceased after a short period of time. We also found out about the rapid re-production of foam by the nymph instars after long-term spring rain when the plants were completely rinsed off.

Key words: *Poophilus nebulosus* (spittlebug), *Alhagi persarum* (camel thorn), Taranjabin-manna, behavioural characteristics

INTRODUCTION

One of the major disadvantages of modern medical science, despite of its conspicuous benefits in comparison with traditional medicine, is higher consumption of chemicals which unfortunately is becoming worse over time. Many scientists in this field believe that herbal medicine is a good substitute for the chemicals used in modern medicine. Plants have been used for medicinal purposes for as long as history has been recorded. With certain scientific knowledge on how to use such medicine, frequent consumption becomes a possibility. Herbal manna is a series of compounds (Aieenechy, 1987) with pharmaceutical and nutritional virtue and plays a specific role in traditional medicine. In fact it is the irregular, externally yellowish-white color, sweet tasted exudation of insects produced after nourishing on the host plant and can be found crystallized on different parts of the plant. Different types of manna are produced by different insects on their specific host plants. Hedysarum is an important pharmaceutical factor in traditional Persian medicine. It is referred to as Persian-Manna, Mereniabin-Manna, Alhaghi-Manna and finally Taranjabin-Manna in scientific references (Henriette, 2007).

Taranjabin is a gentle laxative that usually operates mildly. It is also used as sweetener in traditional medicines (Esmaeel, 2001).

Previously it was believed that hedysarum was produced and spread out spontaneously as a result of insect damage to the *Alhagi* sp. camel-thorn plant stem. Later on the role of one kind of a coleoptera was discovered. In recent years, it has been proven that hedysarum is secreted by spittlebugs on *Alhagi persaram*

camel thorn plant (Asgarzadeh, 1999). In this study they have also raised the presence of melezitose only in hedysarum manna and not in the camel thorn plant (Asgarzadeh, 1999).

In 1965, this species has been reported for the first time from Fars province of Iran (Shiraz and Fasa regions. Also in 1970 has been collected from *Alhagi*, camel thorn plant from Ghazvin and Shiraz regions in Iran (Jiri, 1981).

Lethierry *et al*. (1876) has classified this spittlebug in Aphrophoridae family in the super family Cercopoidea.

According to the latest classification, the spittlebug which produces hedysarum manna belongs to Cercopidae family of Homoptera order (Asgarzadeh, 1999; Borror *et al*., 2005; Jiri, 1981).

Insects belong to this family are small and more similar to froghoppers due to their leaping ability. They feed from the sap of the trees and plants. The body of instars is covered by foam-like exudates produced by the instars themselves; therefore it gives rise to the name spittle or frothy bugs (Borror *et al*., 2005; Daly *et al*., 1988).

There is no clear evidence among international references regarding the function of *Poophilus nebulosus* spittlebug and its manna secreting on camel thorn plant. In one study, the species has been reported as a terrestrial insect that lives in Kharazm region on the banks of the Aral Sea (Khamraev, 2003). Other studies have also reported a few of the insect's species to be agricultural pests (Ajayi and Oboite, 2000; Shih *et al*., 2005). In a study conducted in Afghanistan, hedysarum manna was reported as being triggered from *Alhagi camelorum* camel thorn plant but they have not mentioned anything about the spittlebug. In the same study, they have also high lightened the presence of hedysarum in Khaf region in Iran (Aitchison *et al*., 1988).

The object of the current study was to investigate the behavioral characteristics of this spittlebug and its part in Persian manna production.

MATERIALS AND METHODS

Behavioral characteristics of spittlebug on A. *persarum* camel thorn plant in Torbate Jam region was studied and samples were taken once a week for a period of one year during 2006-2007. A tea-spoon, a knife or in some cases a small paint brush was used for sampling. About 10 camel thorn plants were taken out of the natural growth area, planted in pots and transferred to a greenhouse for further investigation in spring 2007. Afterwards, the function of insects and stages of growth of the camel thorn plant underwent assessment by creating net shelves. Meanwhile these stages were frequently compared between our marked plants in their natural growth regions while sampling from insect's housing spot on the plant was continuously repeated every week and assessed using a stereomicroscope. Stages of growth and behavior of the insect in different environmental situations was also investigated. Finally the samples were sent to the National Institute of Plant Protection for recognition.

RESULTS AND DISCUSSION

Camel thorn plants located in the greenhouse and the region under study both grew at the same time in mid March. Once the plants grew about 10 cm in length during 21-25 April, the eggs underneath the skin of the plant belong to *Poophilus nebulosus* Leth. spittlebug hatched and the first instars came out, settled on the new stems of the plants and derived its nourishment from phloem sap of the plant by piercing their them with their stylets and sucking the sap. Exhaustion almost starts right after the instars start feeding and by using this fluid with the help of abdomen and the continuous movement of the abdominal segments, theinsect create a foam-like environment (Fig. 1 and 2).

Mechanism of foam making is explained in details further on. After creating froth, the insect uses the foam as a moist and secure habitat and starts moving. It is important to mention that in our studies on the foam, we found a type of parasitoid of hymenoptera which was dead after being trapped in the foam. It was then concluded that the foam creates a secure habitat for the delicate body of instars from predators at this age.

The body length of first nymph instars is approximately 1mm. On the 5th-7th day of nourishing, when the size of the body reaches about 2 mm in length, the insect starts molting. The second instars (Fig. 3), also produce spittle on the very lowest part of all parts of the plants above soil level.

Fig. 1: First generation activity on camel thorn plant

Fig. 2: Spittle made on the camel thorn plant

Fig. 3: Second instar of the first generation under ×40 magnification

After 5 days when the body length of the second nymph instars reached about 3mm, the second molting occurs and the instars enter the third nymph instar in which they also continue their spittle making function. The foam produced at this age will be located at about 10-15 cm height of the stem. This age of the instars is along with the width growth of the insects' body accompanied with the length growth so that by the end of their third instar the width of the body will be twice more than before at about 2 mm and the length will be about 5 mm. At this time the 4th molting happens. The fourth instars are more active compared with the 3rd and they drop spittle at the height of 20-30 cm of the stem. It should be mentioned that all the stem will eventually be covered with the sloping foam as well as the first nymph instars spitting function at the lower parts of the stem. At the end of this age which takes about one week to complete, the insect's body length will be about 7 mm and that's when the 5th instars are borne and the last molting happens. We should mention that the instars will have a soft and slippery body due to the moist habitat and the wing pads

Fig. 4: *Poophilus nebulosus* Leth adult bug under ×16 magnification

are not developed yet. The three-segmented stylet is quite long in the first instars however gradually becomes shorter. The fifth nymph instars produce a great deal of spittle in compare with the previous age and they settled on the host plants approximately at about 50 cm height. The abdomen grows a lot more than the rest of the body at this age. This stage takes about 7 days and at this time the body length will reach about 8.5 mm. Foam making function actually reduces and eventually stops by the end of this period as the insect gets closer to the time of molting which is explained in details below: The adult insect stay in the spit mass for the first few hours of it's appearance until the cuticle is hard and fully pigmented. The skin color first appears white but as it's exposed to the air it turns into a grayish color. The first adult insect appears in late May (Fig. 4). The body length is 9.5 and 9 mm in females and males, respectively and they have the capability.

In order to reduce the amount of energy consumption and passing the extreme heat of summer when out of spittle, the insect changes it's location during day and night so that it stays on the browse of the plants early in the morning and by the time it gets warmer it moves to the plants located in the shadow and shelters itself from the sun by hiding underneath the thorns of the plant.

In cases were population of the insects is too high on the plants, the adult insects move to other plants after mating. This is mainly important in providing enough sources of nutrition for instars belong to the next generation which will probably have a higher population than the generation before.

The spittle from the first generation insects on the *A persarum* camel thorn plant is non-sweet, non-crystallized and dries out as a white powder on the dry plants. Meanwhile, the discharge becomes sweeter during June but still does not solidate. It seems that hedysarum production is the result of changes that occur in the resin of the plant due to its mating phase.

Fig. 5: Seconnd and 3rd generation activity as Taranjabin manna

Fig. 6: Taranjabin manna separated from the plant

The adult female spittlebug lays eggs on the dry camel thorn plants after mating. When the eggs are hatched, first nymphs of the second generation appear which are behaviorally and apparently different from the first nymph instars of the previous generation; they live in a free habitat and don't need to make spittle to survive. The wings are slightly larger and cover the whole back and abdomen. The second generation emerge in early July and disappear when the adult insects turn up by latter August. Due to different timing of laying eggs it is possible to find 2-3 ages of nymph instars on the plants concurrently.

Instars are more active on the tip of the browses, they feeding from the sap of the plants and are the first age of nymphs of this spittlebug that can produce hedysarum. Therefore the first crystals of hedysarum are the result of the second generation insects' function. Although crystals produced by the first, second and even third nymph instars are very small but they grow larger in the coming ages by the adult bugs (Fig. 5 and 6).

The adult bugs in the second generation lay eggs to create the third generation and this activity starts from late August until early September.

The growth stages for the third generation are similar to the second. However, due to higher population density of the insect per unit surface and their simultaneous activity, hedysarum production raises extremely. This extremity reaches its maximum when some instars of the 3rd generation attain their last nymphal age, some adult bugs from the same generation appear and the autumn cool climate starts.

Although, due to the cool climate there is a possibility for not all the third generation to gain maturity or to lay eggs but the insects which had an earlier adulthood will move to the areas in which the camel thorn plants are less damaged due to commercial exploitation of hedysarum and lay their eggs underneath the skin of the plant. These eggs will hatch in spring when the weather becomes warmer and the first generation of instars starts their activity again. However, this study showed that not only eggs, some adult bugs also hibernate.

Foam making process: In order to understand the mechanism of the first generation nymph' spittle making, a few of them were removed out of the fluid and transferred to the non-spittle plants in the greenhouse and their behavior was closely observed. Behavioral characteristics of the insects, regarding foam making are as follows:

- The nymph instar finds its suitable location by moving along the stem. The proper distance from the crown of the plant is different regarding insect's age in a way that the older instars are settled in higher locations. In fact along with the plants growth and thickening of lower tissues, instars will also enter a new age and start functioning in the higher portions of the stems. Therefore we can say that only one part of the stem is most beneficial to each instar at a certain age. We also need to mention that if other instars are available on the stem the spittle bug prefers to stay and produce spittle along with the other available instars.
- After finding the suitable location for feeding, the spittlebug nymph usually rests head downward on the plant.
- After few minutes of continuous feeding the insect will frequently expand and contract the abdomen for a while.
- The first drop of fluid comes out the nymph's anus. This drop is trapped between the bug's legs because of its downward situation. The second drop comes out after a few seconds and the nymph tries to trap the other drops between the legs and create air bubbles by moving its abdominal caudal segments.

The rate of bubble making is one per second and after a short amount of time the insect's body will be covered with the spittle. Other instars also cooperate in this process and develop the spittle volume so that all the stem surface will be covered with foam and the nymph can easily move in it.

In the current study we realized that the spittlebug was unable to produce spittle on the cut stems of the camel thorn plant. This shows a close relationship between the foam making procedure happens in the bug's body and sap flow in the plant. It was also observed that spittlebugs restart producing spittle after a long rain. In complementary studies, death of spittlebugs after their detachment from the plant and exposing to artificial humidity was reported.

It should be pointed out that the sampled insects were sent to the National Institute of Plant Protection in Iran and the mentioned species was confirmed to be *Poophilus nebulosus* Leth. in Cercopidae family. There are 5 nymphal instars and 3 generations per annum. The first generation instars live thoroughly in the foam and create the second generation after spending their nymphal period and mating. The first crystals of hedysarum are created as the result of the 2nd generation activity on *Alhagi persarumi* camel thorn plant in late July. However, exploitation is normally postponed due to the warm climate, young age and low population of the instars. A number of factors are involved in hedysarum production but the certain role of only a few of them has been determined. The most important issue is the insect's relation with the plant and regional conditions which requires additional investigation. Further studies on the insect and discovering its complicated relations with the habitat can be a great assistant to technicians to protect and spread Taranjabin as a valuable product.

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