



EXCESSIVE NECTAR INTAKE FROM *LANTANA CAMARA* L. PUNISH HONEY BEE, *APIS MELLIFERA* DURING THE DEARTH PERIOD

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ABSTRACT

A study was conducted to scientifically validate the beekeeper's perceptions about toxicity of *Lantana camara* to honey bee, *Apis mellifera*. Firstly, the floral visits to the source plant during honey flow and dearth period were recorded and correlated with the observed mortality in the bee colonies. A strong correlation was observed between floral visits and bee mortality. Lantadene was extracted and its effect was demonstrated through a randomized experiment to investigate the effect of lantadene on bee colonies. For this, colonies were fed with different concentrations of lantadene mixed sugar candy and the mortality was compared with those fed without it. It was found that excessive floral visits and nectar intake during dearth period is associated with bee mortality through lantadene.

Key words: *Lantana camara*, *Apis mellifera*, lantadene, correlation, nectar, dearth period, honey bee, honey flow period, nectar toxicity, honey bee mortality

Lantana camara, is one of the most widespread and troublesome exotic weeds of the old-world tropics. It invades pasture, crops and native ecosystems, causing substantial economic losses and environmental degradation (Negi et al., 2019). Ever since its arrival in India as an ornamental plant in the early 1800s, it has dispersed throughout the country. While more than 40% of Indian forests are invaded, the rest 50% holds the potential to conserve the native forms of our forest ecosystems (Gotemare and Kimmatkar, 2019). Nevertheless, this exotic weed is also a nectar and pollen source to many insect pollinators like honey bees. Being a melliferous plant it has an aesthetic feature evolved over time to fascinate the cross pollination by insects. For instance, it has a multicoloured inflorescence, which can display yellow, red, purple, pink and even white flowers. The lighter flowers are only in the centre of the inflorescence which contains pollen and aim to attract pollinators. However, the red flowers may distract a stingless native bee that may bite the bases of the stamens and takes the precious pollen of *L. camara*, but the plant doesn't get any pollination service in return. Therefore, honey bees comprise one of the most abundant insects visiting *L. camara*, accounting for 62.9% of all visits with effective pollination of this plant (Couvillon et al., 2015). A recent survey in the union territory of Jammu and Kashmir indicated that bees are dying through nectar poisoning especially from *L. camara* in outer plains and sub-tropical areas.

It was therefore imperative to scientifically validate the farmers' perception through systematic studies. Extensive search for the literature revealed little information on this aspect. However, many workers have reported the hepatotoxic effects of this weed on livestock and human health (Sharma et al., 2007; Kumar et al., 2016). The major constituent for toxicity has been attributed to lantadene which is a pentacyclic triterpenoid compound that is toxic to many organisms especially livestock and insects. Therefore, the effect of lantadene on honey bees health was investigated.

MATERIALS AND METHODS

The study was conducted at the research farm of ICAR-NRC near the apiary learning and experimental unit of SKUAST-Jammu, Chatha (Jammu and Kashmir) during 2020-21. The study was conducted on Italian honey bee, *Apis mellifera* colonies placed at the apiary, SKUAST-J, Chatha. The number of bees visiting the *L. camara* flowers were studied during the honey flow period (March-June) and dearth period (July-October). Ten plants were randomly selected in a randomized block design (RBD) and were observed randomly during morning to afternoon at one day interval. The mortality of bees was observed daily and the number of bees dying were recorded.

L. camara leaf powder (100 g) was extracted with 750 ml methanol with intermittent shaking and was

filtered through two layers of muslin cloth. The residue was extracted twice again with 750 ml methanol each time. The pooled methanolic extracts were decolorized with 20 g activated charcoal. The solvent was removed in vacuum at 60 °C and the residue was extracted twice with 100 ml chloroform. Chloroform extract was dried in vacuum at 60°C. The residue was dissolved in boiling methanol and was kept at 0-4°C for 2 hrs. Copious crystallization took place. The crystalized material was recovered by filtration using Whatman No. 1 filter paper. This provided partially purified lantadene (534 mg) and this fraction was subjected to chromatography fractional crystallization before subjecting the samples to HPLC (Sharma et al., 1999).

While devising the field evaluation experiments, it was found from the literature that Pereira (2005) performed bioassays using honey bee workers that received candy plus floral crushed of *L. camara* at variable concentrations (30%, 10%, 7.5%, 5% and 2.5%), that was compared with control groups that were fed only with candy. The survival analysis indicated that bees fed with candy plus the floral crushed at 30%, 10% and 7.5% have a shorter life time ($P < 0.0001$), in relation to the workers of the control groups. Taking above method in consideration, we found in our preliminary experiments that mean nectar load of 36.7 $\mu\text{l}/\text{bee}/\text{day}$ during dearth period while during the honey flow period, it was about 3.2 $\mu\text{l}/\text{bee}/\text{day}$ for lantana flower visits exclusively. Because strength of the colony also varies during the honey flow and dearth period, equivalent concentrations of lantadene extract viz., 0.2, 0.4, 0.6, 0.8 and 1.0 were finalized and incorporated in the sugar candy and fed to bees. For this, fifteen colonies with uniform (non-significant differences) parameters were selected and assigned to various treatment applications

and compared to the three replicates (colonies) of the control group during each season. During the initial period of treatments foragers were not allowed to escape from the colony by closing the entrance so that they must feed on the sugar candy. Once the sugar candy was consumed the foragers were set free to escape from the respective colony. The mortality of bees during the first seven days was inconsistent. Therefore, the observed mortality was checked following 1 week of treatment and the number of bees dying were recorded.

The data was subjected to one-way ANOVA using statistical software SPSS.

RESULTS AND DISCUSSION

A strong coefficient of determination (0.859) was observed between *A. mellifera* floral visits on *L. camara* and bee mortality during the dearth period (Fig. 1). However, in case of honey flow period such correlation (0.098) was very weak (Fig. 2). This is a kind of punishment to bees to avoid overexploitation of this plant during the dearth period only. In contrast, a nectar reward during honey flow period was observed with increased seed setting. The mortality was lower as there were abundant nectar supplies and bees are not forced to feed on *L. camara* nectar only but on many multifloral sources. In general, the mortality of bees increased with increasing concentration of lantadene. However, no significant difference was observed up to 0.6% of lantadene during both honey flow and dearth periods. A significantly higher number of bees died at higher concentration and the maximum level of mortality was 15.77% during honey flow period (Fig. 3) and 46.58% during dearth period (Fig. 4). This is because, during dearth period none other than this plant

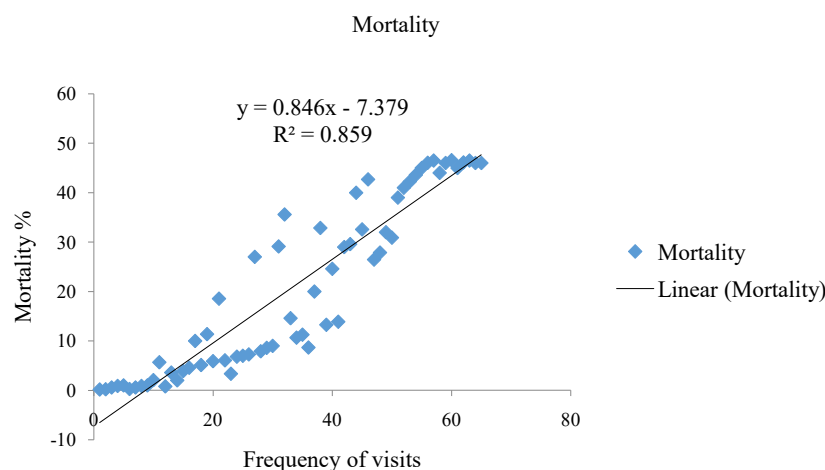


Fig. 1. Coefficient of determination between *A. mellifera* floral visits on *Lantana* during dearth period

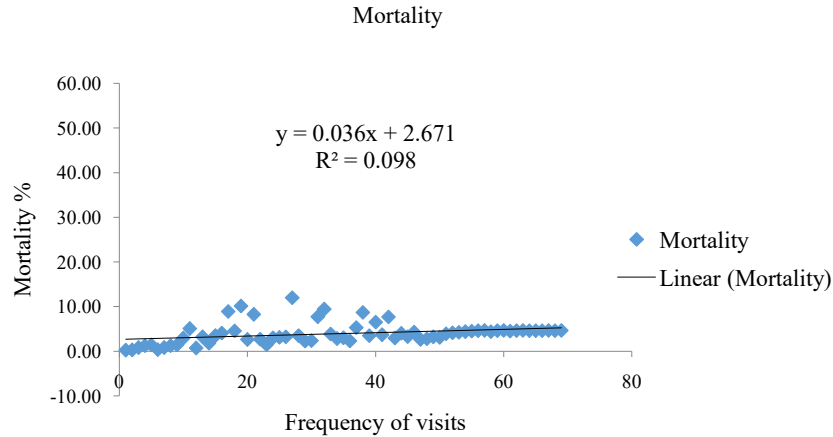


Fig. 2. Coefficient of determination between *A. mellifera* floral visits on *Lantana* during honey flow period

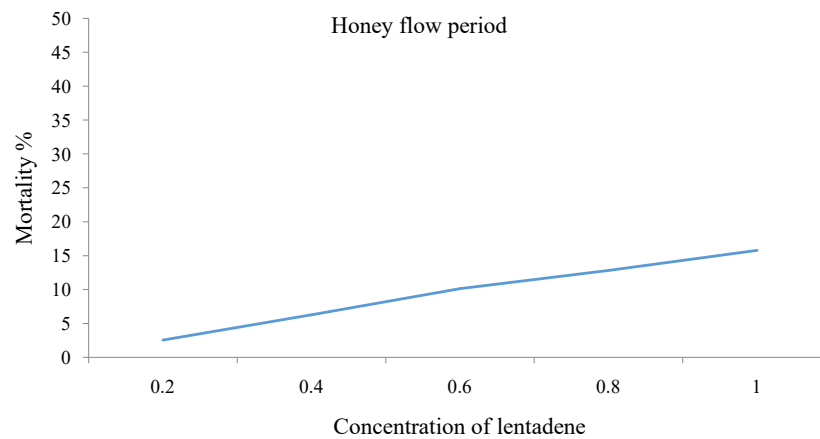


Fig. 3. Mortality (%) of bees during honey flow period due to lentadene

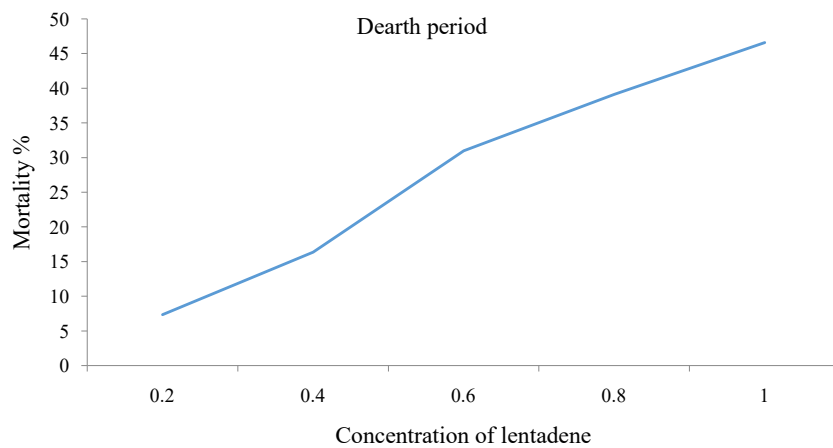


Fig. 4. Mortality (%) of bees during dearth period due to lentadene

was available at the experimental site as a nectar and pollen source for bees. During dearth period from July to October sugar feeding was given for survival of bees. Probably, a greater number of bees fed on the artificial sweetener during dearth period increasing the body intake of lentadene. Such mortality in animals is well

documented. However, effect on honey bees is rarely investigated. Ingestion of lantana foliage by grazing animals causes cholestasis and hepatotoxicity. Both ruminants and non-ruminant animals such as guinea pigs, rabbits, and female rats are susceptible to the hepatotoxic action of lantana toxins. The hepatotoxins

are pentacyclic triterpenoids called lantadenes (Sharma et al., 2007). Such effects on many harmful insects are evident when extracts of *L. camara* were used to protect the crops against pests. For instance, *L. camara* toxicity caused by lantadene was attributed to disruption of the nervous system in *Spodoptera litura* (Kasmara et al., 2018). The dead larva's body became brown to blackish and its stiffness indicated death due to toxins. Therefore, further studies to investigate the mode of action of lantadene on honey bees are urgently required.

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