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2. Response of Honeybees Against Cyanogenic Glycoside Present in Pollen and Nectar of (Clove) Syzygium Aromaticum (L.) Merr. & L.M. Perry & Pliny the Elder

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Abstract:

Some plants are poisonous in nature. Because they contain cyanogenic glycosides in their different parts. Cyanogenic glycosides are natural plant toxins. Due to the presence of this toxic compounds, plants may be harmful for herbivorous, insects and honeybees. The poisonous parts of plants are mainly seeds, leaves, flowers, pollen, and nectar...Etc. plant pollen is a male gametophytes and nectar is a sweet viscous secretion from the nectaries. plants which containing cyanoglycoside like S. aromaticum may be toxic for honeybees, Amygdalin also known as Laetrile, is a cyanogenic glycoside found in the pits of many fruits, in raw nuts, and in other plants such as lima beans, cloves, and sorghum. This study aimed to understand the response of honeybees against cyanogenic glycoside present in s. aromaticum. Our result suggests that clove nectar is not very much rich in amygdalin to pose a hazard, but that clove nectar could be toxic if exclusively consumed by honeybees for much more time. Further tests are needed to determine if honeybee's cloves herbs are at risk, or if they somehow cope with toxin.

Keywords: *S. aromaticum (Cloves), Amygdalin, nectar, pollen, and pollination.*

2.1 Introduction:

Pollination is a natural process found in angiospermic plants. The process of pollination is done by wind, various insects, butterflies and honeybees. Bees and flowering plants having a mutualistic relationship: the honeybees pollinate the plants and the plants provide nutrition to the bees in the form of nectar. Floral nectar, stored as honey. Honey is the main energy source for honeybees. Protein-rich pollen provides most of the nutrients like essential amino acids, lipids and vitamins required for honeybees in their physiological development.

Cyanogenic glycosides (CNglcs) are secondary metabolites widespread in plants.

CNglcs act as a plant defense: when a stabilizing glucose molecule is removed from the compounds by a catalyzing glucosidase enzyme into toxic hydrogen cyanide (HCN) is released and inhibits cellular respiration. Herbivores can overcome from this plant defensive compounds by detoxification, excretion or sequestration and can even use these compounds in their own defense against natural enemies. If CNglcs found in other organs of plants rather than pollen, there is no issue for honeybee's health. The nectar and pollen of S. aromaticum are known to have the bitter testing due to the presence of a little amount of compound amygdalin which is a cyanogenic glycosidic compound. Since chemical compound Amygdalin is a toxic cyanoglycoside compound so it can influence negatively on honeybees foraging at the flowers.

These problems were faced by some beekeepers and reported by them because they lost colonies because colonies using clove nectar for their feed. Due to all these problems, it is necessary to make initial studies on the toxicity on amygdalin on honeybees. A study on honeybees found that honeybees in 88% cases goes to take clove nectar and increases pollination and seed yielding 30-fold due to their activity. Several clove species produce in large quantities of nectar suitable for honey production. The aim of this study was to test the toxicity of amygdalin fed in sugar syrup on honeybees to determine if it poses a possible risk to bees keeping.

2.2 Materials and Methods:

Honeybees (*Apis mellifera ligustica*) were obtained from hives at RPCAU (Bihar) on 15 March 2020. Hording cages were taken (similar to those used by (Kulencevic and Rothenbuhler, 1973). hording cages were of wood, plexiglass, and screen with glass vial feeders in the top measuring 9 x 12 x 8cm, width, hight and depth. approximately 40 -70 bees were introduced into hording cages. Saturated sugar taken as per concentration. Cages were kept in laboratory in open conditions at temperatures that ranged from about 17.5 to 25C, and RH that ranged from about 18 to 37%.

Mortality of died bees was measured by counting on days 1,2,3,4,5,6,7and 8 days following initial dosing on day 0. *ad libitum* was an experimental solution which was used for bees feeding at the duration of experiments. *Ad libitum* having the property to stimulate feeding within the hive or at the flowers of blooming clove. Water was not provided.

The data were analyzed in SAS by proc PROBIT to determine the duration of exposure, in days, at each experimental dose of amygdalin that would be required to kill 50% of the bees (LT50). The data also were analyzed to provide estimates of the dose of amygdalin (ppm) that would kill 50% of the treated bees after different amounts of time (LD50). Analysis of controls to determine an LT50 provided a significant model but had 95% fiducial limits of 1 to infinity.

To refine this answer, we added control bees from 2 tests done in 2020. Although those tests were associated with other plants products, the controls (i.e., unspiked sugar syrup) were treated in exactly the same fashion as described for testing amygdalin.

Both of those earlier tests had 8 control cages that were observed for 14 days. The numbers in Table 1are the results from the analysis of 214 bees in 4 control cages from the amygdalin test combined with 702 bees from the tests in 2020.

2.3 Results:

Table 2.1: The estimated duration of exposure, in days, at each experimental dose of amygdalin required to kill 50% of the bees (LT50).

Dose of amygdalin (ppm)	Estimated time (days) for 50 % mortality	Lower (95) % fiducial limit	Upper (95)% fiducial limit
0[a]	16.1	14.2	19.4
2,250	4.6	3.0	6.3
4,500	2.7	1.7	3.6
9000	2.3	2.1	2.4
18000	1.9	1.7	2.0
36000	1.8	1.5	2.0

a. Estimates based on controls from several tests. See methods for details.

Table 2.2: Estimates of the dose of amygdalin (ppm) that would kill 50% of the treated bees after different amounts of time (LD50).

Treated bees after different amount of time (LT50).

Times Days			
1	Not estimated		
2	15,300	10,400	25,100
3	5,900	4,200	7,900
4	2,300	2,000	2,500
5	2,100	1,900	2,300
6	1,800	1,500	2,000
7	1,600	1,300	1,800

2.4 Discussion and Conclusion:

Our study shows that the rate of toxicity of amygdalin on honeybees (Apis mellifera ligustica). According to the results, it can be said that doses which are similar to those found in pollen nectar of S. aromaticum is not having much toxic effects to kill honeybees, but the pollen of S. aromaticum having sufficient amount of amygdalin and can be harmful for those bees consuming a pure diet over about a week.

It is considered that the Amygdalin - a toxic compound found in the nectar of clove maybe come through the pollen of clove due to the natural events.

It may be also possible that pollen grains fell into nectar of cloves due to natural events or by winds and due to this reasons amygdalin transferred into nectar of cloves. some organisms and insects are resistant to amygdalin because they have B-glucosidase enzymes that break it down. Pollen of cloves may be harmful for honeybees at that time when it gathered on flowers at the time of consumption of the floral resources of S. aromaticum, but when honeybees stored it in the hive the effect of toxin gradually decreased. In both conditions honeybee's colony consumed a large amount over a brief period of time. After few days the stored pollen detoxified gradually due to the effects of acidification in bee breads or by the dilutions with other pollens. And then the stores become non-toxic relatively. Water consumption in their diet may also influence the toxicity of secondary plants compounds including amygdalin. However, there is no documented indication that honeybees used for S. aromaticum pollination are harmful because of amygdalin which is found in their nectar. It is recommended to the commercial bee keeping industries to assess the impact and risk of amygdalin (found in pollen of S. aromatica) which give a toxic and harmful effects on the health of honey bees.

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