

Impact Of Exposure To Phymar Csl Application On The Activity Of Pollinating Bee *Tetragonula Laeviceps* Smith (Hymenoptera; Apidae)

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ABSTRACT

*The stingless bee *Tetragonula laeviceps* (Hymenoptera; Apidae) is an important pollinator that supports agricultural productivity, yet its population may be affected by pesticide use, including botanical pesticides. This study aimed to evaluate the toxicity of the botanical pesticide Phymar CSL to *T. laeviceps*, assess the effects of its residues on bee survival, and determine whether Phymar CSL acts as an attractant or repellent. The study was conducted at the Plant Protection Laboratory, Faculty of Agriculture, Halu Oleo University, from November-December 2025. Toxicity tests were carried out using five concentrations of Phymar CSL (5, 10, 20, 40, and 80 mL/L) mixed with a 10% honey solution and analyzed using probit analysis, while residue and preference tests were analyzed descriptively. The results showed that the LC_{50} value of Phymar CSL for *T. laeviceps* was 16.194 mL/L, which was lower than the recommended field application dose (20 mL/L). Residue exposure resulted in relatively low bee mortality, even at LC_{50} and LC_{95} concentrations. Preference tests using a Y-tube olfactometer indicated that Phymar CSL was not attractive but exhibited repellent properties toward *T. laeviceps*. These findings suggest that although direct exposure to Phymar CSL may pose toxic risks to *T. laeviceps*, its residues are relatively safe and do not attract bees; therefore, application should be avoided during flowering stages and peak foraging periods to support pollinator conservation and sustainable agriculture.*

Keywords: *Botanical Pesticide, Repelen, Residuals, Toxicity.*

INTRODUCTION

The agricultural sector plays a vital role in supporting life, particularly in providing food, industrial raw materials, and as a source of foreign exchange for the country. The success of agricultural enterprises is greatly influenced by two main factors: the availability of natural resources such as land, water, and climate conditions, and the presence of organisms that play a role in supporting plant growth and reproduction. One important aspect that often receives less attention is the presence of pollinating insects. Pollinating insects function in the pollination process, namely the transfer of pollen from the stamen to the stigma, which then allows for the formation of fruit and seeds. (Wulandari et al., 2017).

The stingless bee (*Tetragonula laeviceps*) is a pollinator that plays an important role in the agricultural ecosystem. (Mubin et al., 2025), Pollination activity by *T. laeviceps* has been proven to increase the quality and quantity of production of various horticultural crops such as chilies, tomatoes, and oranges, as well as plantation crops such as cocoa and coffee. In addition to its role as a pollinator insect, *T. laeviceps* also produces honey which has high economic value and health benefits. (Wulandari et al. 2017). Therefore, efforts to preserve and protect the existence and activities of *T. laeviceps* need to be carried out to maintain the sustainability of the agricultural system and the balance of the ecosystem.

In modern agricultural practices, farmers often face serious problems due to pest and plant disease attacks. Plant pests can reduce productivity and even cause crop failure. To overcome this, synthetic chemical pesticides are still widely used because they are considered practical and effective. However, the continued use of chemical pesticides has a negative impact on the environment, leaving harmful residues on crops, and causing the death of non-target organisms (Wardana et al., 2023). Therefore, alternative pest control that is more environmentally friendly, effective, and safe for pollinators is needed.

A widely used alternative pest control method is the use of botanical pesticides. Botanical pesticides are considered safer because they contain active ingredients derived from plants, are readily available in nature, have relatively low residues, and work more selectively (Aziz et al., 2021). Phymar CSL, a highly potent botanical pesticide, contains the active compound anacardic acid extracted from cashew nut shells. Phymar CSL has only been approved for use on cocoa, but research conducted by (Srihidayati 2024), reported that the application of Phymar CSL was also able to reduce the intensity of fruit fly (*Bactrocera* sp.)

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attacks on chili plants.

The effectiveness of Phymar CSL in controlling pests and diseases has been widely reported, including the use of Phymar CSL in reducing attacks of fruit rot and stem cancer caused by *Phytophthora palmivora* in cocoa plants (Mariadi et al., 2018). However, studies on its effects on pollinating insects, especially *T. laeviceps* bees, are still very limited. Research related to this is important because, although botanical pesticides are known to be more environmentally friendly, the possible effects of residues, aromas, and active compounds on pollinator behavior still need to be considered.

This research is important because the results of previous research conducted showed that *T. laeviceps* actually showed an interest in the pesticides imidacloprid and deltamethrin, thus the results of this study are expected to not only provide academic contributions in the development of science, but also have practical benefits for farmers and the community in implementing the concept of sustainable agriculture. Therefore, the aim of this study was to determine the response of the botanical pesticide Phymar CSL to the pollinating insect *T. laeviceps*.

METHODS

This research was conducted in the Plant Protection Unit Laboratory, Faculty of Agriculture, Halu Oleo University, from November to January 2025. The materials used in this study were *T. laeviceps* bee colonies, Phymar CSL botanical pesticide, 10% honey, gauze, cotton, and label paper. The tools used in this study included a *T. laeviceps* maintenance box/hive, plastic jars, test tubes, olfactometer tubes, measuring pipettes, brushes, a cellphone camera, and writing utensils.

Preparation of *Tetragonula laeviceps* Bees

The study began with the preparation of *T. laeviceps* bee colonies obtained from a bee farm cultivated at the Halu Oleo University Botanical Garden (Awaluddin et al., 2018). Test bees were captured from the nest entrance using a 30 mm x 200 mm test tube. The colonies were then taken to the laboratory and prepared for research.

Phymar CSL Toxicity Test on *Tetragonula laeviceps*

The test began by preparing six series of Phymar CSL concentrations, namely 5 mL/L, 10 mL/L, 20 mL/L, 40 mL/L, 80 mL/L, each of which was mixed into a 10% honey solution and a control without Phymar CSL treatment referring to the modified Awaluddin et al., (2024)

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research. Each series of treatments and controls was repeated 4 times using 10 adult *T. laeviceps* bees. The bees were placed in a 300 ml test container with 3x3 vents, where at each vent was placed cotton that had been dipped in the treatment feed solution according to the specified concentration. Mortality observations were carried out for 3x24 hours to determine the percentage of LC₅₀ in each Phymar CSL concentration treatment.

Phymar CSL Residue Test on *Tetragonula laeviceps* Bees.

The residue test was carried out referring. The test procedure began with the preparation of the recommended Phymar CSL concentration solution of 20 mL/L, LC₅₀ concentration of 16.194 mL/L, LC₉₅ concentration of 75.179 mL/L, control solution and 10 bees. The prepared Phymar CSL concentration solution was then sprayed into a test tube, the inner surface of the test tube was attempted to get an even coating of the test material solution. After that, the tube was dried for 5 minutes until the test material solution evaporated, then the previously prepared bee colony was released into the test medium and allowed to interact with the test medium for 10 minutes, after the exposure period was complete, the bees that had been in contact with the medium were transferred into a 300 ml test container that had been fed 10% honey. Observations on the level of bee mortality were carried out for 3 x 24 hours to determine the effect of exposure to Phymar CSL residues on bee survival, with each test repeated 5 times.

Feed Preference Test (Attractant) of *Tetragonula laeviceps*.

The study began by preparing a test medium in the form of a Y-shaped tube (*Y-tube olfactometer*) referring to Li et al. (2014), and a bee colony that had been fasted for 2 hours, the test was carried out using a choice test, where each branch of the tube was given a different treatment, on the upper branch, Phymar CSL solution was applied with a concentration according to the recommended dose of 20 mL/L, an LC₅₀ concentration of 16.194 mL/L and an LC₉₅ concentration of 75.179. Meanwhile, the lower branch was given a 10% honey solution which functions as a natural food for *T. laeviceps* bees with a flowmeter set at a flow rate of 0.5 bar (Figure 3.4). After the media was ready, a bee was released into the tube and given three minutes to determine the direction of choice. During the observation period, the response or level of interest of the bees to each treatment was observed. Each test was repeated 10 times with each bee only used once.

Phymar CSL Toxicity Test

Observation of the Phymar CSL toxicity test was carried out by observing bee mortality

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or the percentage of *T. laeviceps* bee deaths.

$$\text{Mortality} = \frac{r}{n} \times 100\%$$

Information:

- r = Number of dead bees
- n = Number of bees tested

Phymar CSL Residue Test

Observation of Phymar CSL residue test was carried out by observing the number of bees that died after being exposed to Phymar CSL residue.

$$\text{Corrected mortality (\%)} = \frac{(M_t - M_k)}{(100 - M_k)} \times 100\%$$

Information:

- M_t = Percentage of deaths in treatment
- M_k = Percentage of deaths in controls

Feed Preference Test (Attractant) of *Tetragonula laeviceps*

Observations of food preference tests (attractants) were carried out by observing the bees' responses in determining the direction of choice towards Phymar CSL or 10% honey solution.

$$\text{Preference Index (IP)} = \frac{(N_t - N_k)}{(N_t + N_k)} \times 100\%$$

Information:

- N_t = Number of bees choosing the branch with the treatment solution (Phymar CSL)
- N_k = Number of bees that chose the control branch (10% honey)

RESULTS AND DISCUSSION

RESULTS

Phymar CSL Toxicity Test against *Tetragonula laeviceps*

Phymar CSL toxicity test was conducted by making 5 (five) series of concentrations with serial dilutions, namely (5, 10, 20, 40, and 80 mL/L) which were diluted with feed solution (10% honey) and as a control 10% honey (Table 1). Mortality observations were carried out for 3x24 hours (72 hours) after application, to find the relationship between concentration and mortality between 5-95%.

Table 1. Percentage of mortality observed at each concentration series

Phymar CSL Concentration (mL/L)	Number of Bees Tested (n)	Number of Dead Bees (r)	Observed Mortality (%)
Control (0)	40	0	0.00
5	40	8	20.00
10	40	15	37.50
20	40	23	57.50
40	40	32	80.00
80	40	40	100.00

The results of the Phymar CSL toxicity test on *T. laeviceps* showed differences in mortality rates at each treatment concentration as presented in Table 2, where increasing Phymar CSL concentrations tended to be followed by an increase in the percentage of test bee deaths. Based on the results of the probit analysis, the Phymar CSL LC₅₀ value was obtained, which represents the pesticide concentration capable of causing 50% death of the total test bee population. The LC₅₀ value is presented in Table 4.2.

Table 2. Toxicity Value (LC) at 95% level of Phymar CSL against *Tetragonula laeviceps*

Toxicity Index	Effective Concentration (LC) (mL/L)	Lower 95% Confidence Limit (mL/L)	Upper 95% Confidence Limit (mL/L)
LC ₅	2.122	1.985	3.274
LC ₁₀	4.124	2.983	6.117
LC ₂₀	7.383	4.696	9.852
LC ₃₀	9.926	6.916	12.713
LC ₄₀	12.784	9.518	15.989
LC₅₀	16.194	12.633	20.115
LC ₆₀	20.514	16.431	25.826
LC ₇₀	26.419	21.245	34.571
LC ₈₀	35.523	27.978	49.889
LC₉₅	75.179	52.862	134.185

Description: Probit Analysis.

Phymar CSL Residue Testing against *Tetragonula laeviceps*

Residue testing was conducted to determine the mortality rate of *T. laeviceps* bees after exposure to Phymar CSL at different concentrations. Mortality data was calculated using the Abbot formula based on observations over 3 days (72 hours) after application.

Table 3. Effect of Phymar CSL Residue on Mortality of Tetragnola laeviceps at various concentrations

Concentration (mL/L)	Total Insect Test	Total Deaths (72 Hours)	Mortality Percentage (%)
Control (0)	50	2	4%
(LC ₅₀) 16,194	50	3	6%
Product Recommended 20,00	50	9	18%
(LC ₉₅) 75,179	50	11	22%

The results of Phymar CSL residue tests on T. laeviceps showed that the resulting mortality rate was lower than that of direct toxicity tests. The results of these tests are presented in Table 3, which shows that even at concentrations equivalent to the LC₅₀ value, the mortality of bees exposed to residues was still relatively low, below 25%.

Feed Preference Test (Attractant) of Tetragnola laeviceps

Attraction testing was conducted using a Y-tube olfactometer to observe the bees' response to three concentrations of Phymar CSL compared to the control (10% honey). The results were analyzed using simple tabulated descriptive analysis and Preference Index (PI) calculations Table 4.

Table 4. Results of the Calculation of the Preference Index (IP) of T. laeviceps towards Phymar CSL

Phymar CSL Concentrate	Number of Test Bees	Bees Choose Phymar (Nt)	Bees Choose Control (Nk)	Preference Index (IP) (%)	Information
Product Recommended 20 mL/L	50	2	8	-60%	Repelen
LC ₅₀ (16,194 mL/L)	50	1	9	-80%	Repelen
LC ₉₅ (75,179 mL/L)	50	4	6	-20%	Repelen

Description: A negative IP value (-) indicates repellent properties.

Based on 4.4, it shows that T. laeviceps bees consistently prefer the tube branch containing 10% honey solution compared to the branch containing Phymar CSL concentration.

At the recommended dose of 20 mL/L, as many as 8 test bees chose the 10% honey treatment, *Journal Of Agriculture, Agribusiness, Welfare, Technology, Humanity, Environment, Social, And Economy* 216

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while only 2 chose the Phymar CSL treatment, at the LC₅₀ solution (16.194 mL/L) as many as 9 bees chose the 10% honey treatment, while only 1 chose the Phymar CSL treatment and at the LC₉₅ concentration (75.179 mL/L) as many as 6 test bees chose the 10% honey treatment, while only 4 chose the Phymar CSL treatment. This shows that Phymar CSL is not an attractant (attractant), but tends to be a repellent (repellent) for these pollinator insects.

DISCUSSION

The results of the toxicity test can be seen in Table 1, showing that the percentage of *T. laeviceps* bee mortality increased as the concentration of Phymar CSL given increased. (Sial et al. 2018) stated that the pattern of increasing mortality indicates a clear dose-response relationship, where the higher the concentration of botanical pesticide applied, the greater the toxic effect on the test insects. stated that the dose-response relationship is an important indicator in toxicology studies, because it reflects the interaction between the amount of active compound entering the organism's body and the level of physiological disturbance that occurs (Lengai et al., 2020). Thus, these results confirm that the bioactive compound in Phymar CSL has biological activity against insects, including bees as non-target organisms, so that evaluating its safety level is a very crucial aspect before its widespread application in the field.

Based on the results of the probit analysis in Table 2, the LC₅₀ value of Phymar CSL against *T. laeviceps* was 16.194 mL/L. This LC₅₀ value describes the pesticide concentration capable of causing death in 50% of the test bee population under laboratory conditions, when compared to the recommended field application dose of 20 mL/L, the LC₅₀ value is in a relatively close range, but still shows that Phymar CSL has lower toxicity compared to many synthetic chemical pesticides which generally have much smaller LC₅₀ values against pollinator insects. This is in line with (Aziz et al., 2025), that plant-based pesticides tend to have lower levels of toxicity against non-target organisms, making them more suitable for supporting sustainable agricultural systems.

The residue test results in Table 3 show that the mortality rate of *T. laeviceps* due to exposure to Phymar CSL residues is much lower compared to direct exposure through feed, even at concentrations equivalent to the LC₅₀ value, the percentage of bee mortality is still low, namely below 25%. This phenomenon indicates that the toxic effectiveness of the active compound Phymar CSL decreases after going through the evaporation and drying process so that it is not contact-based, which is likely caused by degradation of the active compound or

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reduced penetration ability into the insect's body. Physiologically, the wax layer on the bee's exoskeleton acts as a physical barrier that limits the entry of chemical compounds into the body, thereby reducing the lethal effects of pesticide residues attached to the surface (Balabanidou et al., 2018).

Based on the results of the feed preference test presented in Table 4, it can be seen that *T. laeviceps* consistently showed a tendency to choose 10% honey solution as a natural feed compared to solutions containing the botanical pesticide Phymar CSL at various test concentration levels. The Preference Index (IP) value which reached -60% at the recommended dose (20 mL/L), -80% at the LC_{50} concentration (16.194 mL/L), and -20% at the LC_{95} concentration (75.179 mL/L) indicated that most of the test bees avoided Phymar CSL and preferred 10% honey.

The high preference for 10% honey is closely related to the bees' physiological need for an easily digestible energy source. Honey contains simple sugars, primarily glucose and fructose, which play a vital role in supporting foraging activity, energy metabolism, and bee survival. Honey's natural aroma can also stimulate olfactory receptors on bees' antennae, increasing their attraction and frequency of visits to food sources (Branchiccela et al. 2019).

The low number of bees choosing the Phymar CSL solution indicates that this botanical pesticide is not an attractant, but rather tends to have repellent properties against *T. laeviceps*. Phymar CSL contains the active compound anacardic acid derived from cashew nut shell extract. This compound belongs to the phenolic group and has a distinctive and relatively sharp aroma. According to phenolic compounds with strong aromas can be detected by the insect sensory system as signals of incompatibility or potential danger, thus triggering an avoidance response (Arena et al., 2025).

Kang et al., (2017) stated that Phymar CSL's repellent properties against *T. laeviceps* can actually be seen as an advantage from an ecological and sustainable agriculture perspective. Bees that avoid areas or crops where pesticides have recently been applied tend to have a lower risk of direct exposure or ingestion of pesticide residues. Thus, the avoidance behavior exhibited by *T. laeviceps* can minimize the negative impact of botanical pesticides on the survival and pollination activity of bees in the field.

This finding is in line with the general characteristics of botanical pesticides which work not only through toxic mechanisms, but also through behavioral effects such as rejection.

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Pavela et al., (2025). In contrast to several synthetic chemical pesticides that are reported to be attractants to bees, such as imidacloprid, Phymar CSL actually shows a safer response for pollinator insects because it does not encourage bees to approach or consume Phymar CSL.

CONCLUSION AND IMPLICATIONS

Based on the research results and discussion, it can be concluded that the LC₅₀ of Phymar CSL is lower, namely 16.194 mL/L compared to the recommended product dose of 20 mL/L, exposure to Phymar CSL residue that has dried on the surface does not have a harmful impact on *T. laeviceps*. And Phymar CSL is not an attractant to *T. laeviceps*, but rather a repellent.

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