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# Efficacy of knockdown insecticide based on Permot (*Passiflora foetida* L.) leaf extract against mortality of German cockroach (*Blattella germanica* L.)

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**ABSTRACT**. Chemical insecticides have a detrimental effect on not only the target species, but also on non-target species and the environment. Transfluthrin is a pyrethroid class II insecticide that is considered to be safe for humans. However, repeated use over an extended period of time can result in cockroach resistance. For this reason, this study sought out alternative materials, specifically Permot leaf (*Passiflora foetida* L.). This study aims to determine the neurotoxic efficacy of insecticides derived from Permot leaf extract on German cockroach knockdown time and death. A total of 120 German cockroaches and was replicated three times. After 1 h of exposure to insecticides containing transfluthrin 3000 ppm and Permot leaf extract up to a dose of 4000 ppm, knockdown time and cockroach death were observed. The probit test was used to determine the cockroach knockdown time. The study's findings indicated that when cockroaches were exposed to insecticides derived from Permot leaves at a dose of 4000 ppm, the knockdown efficacy was $KT_{50}$ for 8 min and $KT_{90}$ for 30 min. The blocking test on German cockroach spiracles established that exposure via cockroach spiracles was more effective than exposure via other cockroach organs in killing.

**Keywords**: disease vector; knockdown time; neurotoxic efficacy; stinking passionflower; transfluthrin (TFT)

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## INTRODUCTION

Cockroaches are a type of disease vector found in damp places such as home kitchens, bathrooms, toilets, restaurant kitchens, waste fertilizers sites, and others, active at night or in dark spots as nocturnal animals. Cockroaches coexist with humans due to their habitat, which includes abandoned buildings with a lot of leftover food and other garbage, as well as bathrooms with clogged drains (El-Sherbini & El-Sherbini, 2011; Zhukovskaya *et al*., 2013; Dehghani *et al*., 2014).

The German cockroach (*Blattella germanica* L.) is a cosmopolitan pest as the primary vector of disease in urban areas. The use of synthetic insecticides become one method of their population control, which have the disadvantage of leaving residues or residual use of the chemicals content (Jensen *et al*., 2016; Jang *et al*., 2017). This pest has been regularly controlled with insecticides in Indonesia and other parts of the world (Rahayu *et al*., 2016; Zhu *et al*., 2016; Schapheer *et al*., 2018). However, the extensive and continuous use of insecticides to control these cockroaches can pose a threat to human health and has resulted in the development of cockroach resistance to several large classes of insecticides, complicating control programs and allowing cockroaches to continue attacking the urban environment (Shahraki *et al*., 2013; Wu & Appel, 2017; Pietri *et al*., 2018; Fardisi *et al*., 2019).

*Passiflora foetida* L., also known as Permot (Indonesia), is one of the plants suspected to contain active ingredients capable of using bioinsecticides. Permot is a type of vine that grows wild among other plants and is frequently found in yards, on streets, and open walls of buildings in Indonesia; as a result, this plant is considered a weed and is discarded or burned. Our previous study (Susilowati & Hartono, 2017) established the effectiveness of Permot leaf as a bioinsecticide, which was later confirmed by Junayed *et al.* (2020) and Nurcahya *et al.* (2020). Additionally, it has been demonstrated that Permot fruit acts as a bioinsecticide (Olla *et al*., 2020). The success of Permot leaf extract as an insecticide is inextricably linked to its chemical constituents, specifically flavonoids and phenolics (Asir *et al*., 2014; Sisin *et al*., 2017; Ajane & Patil, 2019). The flavonoid and phenolic content of aqueous extracts of Permot leaves and roots were significantly greater in aqueous and ethanolic extracts than in petroleum ether extracts (Asir *et al*., 2014). A total of 65 chemical compounds were isolated to be used as active ingredients in insecticides, including flavones, flavonols, and derivatives such as flavone glycosides and hexosides (Asir *et al*., 2014; Nguyen *et al*., 2015; Sisin *et al*., 2017; Song *et al*., 2018). The major chemical constituents of Permot leaf extract are flavonoid glycosides in O-glycosides or C-glycosides (Xiao *et al*., 2016). Isoquercitrin and isorhamnetin-3-O-glucoside are examples of flavonoid glycosides in the O-glycoside format, whereas vitexin, isovitexin, orientin, and isoorientin are examples of flavonoid glycosides in the C-glycoside format (Zucolotto *et al.*, 2012; Simirgiotis *et al*., 2013). Zucolotto *et al.* (2012) investigated the chemical structure of C-glycosyl flavonoid compounds extracted from Permot leaves and pericarp. Furthermore, a compound called isorhamnetin-O-hexoside was isolated from Permot leaves and roots methanol extract.

The research on botanical insecticides focuses exclusively on the deadly concentration and knockdown time of insects, particularly cockroaches. This is a continuation of our previous research (Susilowati & Sari, 2018; Susilowati & Sari, 2021). The study aims to assess the neurotoxic efficacy of insecticides generated from Permot leaf extract on the knockdown time and death of German cockroaches. This study puts a focus on the effectiveness of pesticides capable of killing the test insects, specifically spiracles. The study's findings will assist end users in making more informed decisions regarding the management of these pests and may also be used utilized in a resistance management capacity.

## MATERIALS AND METHODS

The Permot leaf (*Passiflora foetida* L.) and transfluthrin, a sort of synthetic pyrethroid, were utilized. The experimental animal was a German cockroach (*Blattella germanica* L.) purchased on Jl. Pramuka, Mataram District, East Jakarta**.**

**Research treatment.** Twenty adult female German cockroaches with three replications were placed in a glass tank measuring 30×30×30 $cm^3$ for each treatment group. The cockroaches were acclimated in the glass tank for four days, during which they were fed and hydrated ad libitum. To prevent cockroaches from emerging from the glass aquarium, the surface of the tank is firmly covered with wire, allowing air to penetrate. The methods used was following our previous studies (Susilowati & Sari, 2018; Susilowati & Sari, 2021).

**Direct spray test.** A direct sprayer was used to perform this test. After four days of acclimatization, the cockroaches were treated with an insecticidal spray made from transfluthrin 3000 ppm and Permot leaf extract in graded doses (500 ppm, 1000 ppm, 2000 ppm, 3000 ppm, and 4000 ppm). After 1 h of spraying, the cockroaches were transferred to a clean petridish without exposure to insecticides and observed for knockdown with an observation time of 1 min, 2 min, 3 min, 4 min, 5 min, 6 min, 7 min, 10 min, 20 min, 40 min, 60 min, 120 min, 180 min, 240 min, 300 min, 360 min, and 420 min (Susilowati & Sari, 2018; Susilowati & Sari, 2021).

**Blocks cockroach spiracles.** The wings of an adult female German cockroach were cut off. Adult female cockroaches were anaesthetized using diethyl ether. The cockroach wings were cut using stainless steel pins, and lacquer droplets were applied to the mesothoracic spiracles and observed using a substance microscope. To explain the effect of cockroach knockdown efficacy after exposure to insecticides made from transfluthrin 3000 ppm and Permot leaf extract up to a dose of 4000 ppm from blocking spiracles, two groups of cockroaches were used. One group of cockroaches occluded one side of the mesothorax spiracles, and the other group consisted of cockroaches with both sides of the mesothorax spiracles blocked or obstructed. In the two groups that had been divided, insecticides made from transfluthrin 3000 ppm and Permot leaf extract were applied to a dose of 4000 ppm in the cockroach's ventral mesothorax. The $KT_{50}$ and $KT_{90}$ values of the two groups of cockroaches that had their spiracles inhibited were calculated and observed the inhibitory effect of cockroach spiracles (Susilowati & Sari, 2018; Susilowati & Sari, 2021).

**Data analysis.** The $KT_{50}$ value (time needed to reach 50% knockdown) and the $KT_{90}$ value (time needed to reach 90% knockdown) were calculated using the probit method (Finney, 1971). This test was repeated three times. The 95% confidence limit was calculated by t-test.

## RESULTS AND DISCUSSION

According to observations on German cockroaches exposed to spray insecticides containing transfluthrin 3000 ppm and multidose Permot leaf extract after 1 h, the cockroach's behavior changed, the cockroach appeared restless and made up down movements in the glass aquarium, over time its body weakened (movement became slow), and it eventually became immobile and died. The dead cockroach's body color changes to a blackish-brown tint due to contact with the insecticide. Table 1 and Fig. 1 provide $KT_{50}$, $KT_{90}$, and German cockroach mortality.

**Table 1**. Mean knockdown time and mortality of *Blattella germanica* L. exposed to 3000 ppm transfluthrin and multi-dose *Passiflora foetida* L. leaf extract.

| Group | Knockdown time (minute to-) | | Mortality (%) |
|---|---|---|---|
| | $KT_{50}$ | $KT_{90}$ | |
| Control | 0.00 | 0.00 | 0 |
| TFL 3000 ppm | 8.738[a] | 22.258[a] | 100 |
| PL 500 ppm | 78.046[b] | 484.431[b] | 100 |
| PL 1000 ppm | 40.191[b] | 249.907[b] | 100 |
| PL 2000 ppm | 18.494[b] | 92.209[b] | 100 |
| PL 3000 ppm | 9.474[b] | 34.723[b] | 100 |
| PL 4000 ppm | 8.760[a] | 30.495[a] | 100 |

Notes: TFL= transfluthrin; PL= Permot leaf; a,b = are significantly different (P<0.05).

According to Table 1, there was a significant difference in the $KT_{50}$ and $KT_{90}$ knockdown time graphs of cockroaches subjected to pesticides containing transfluthrin 3000 ppm and Permot leaf extract up to 4000 ppm. In other words, the knockdown efficacy of cockroaches subjected to 3000 ppm transfluthrin and 4000 ppm Permot leaf extract was more or faster than that of cockroaches treated to less than 3000 ppm Permot leaf extract. This suggests that insecticides derived from Permot leaf extract are also neurotoxic, as are insecticides derived from transfluthrin 3000 ppm. Aerosol particles in spray insecticides block sodium channels, which are the primary pathways for entering hazardous chemicals, resulting in cockroach death.

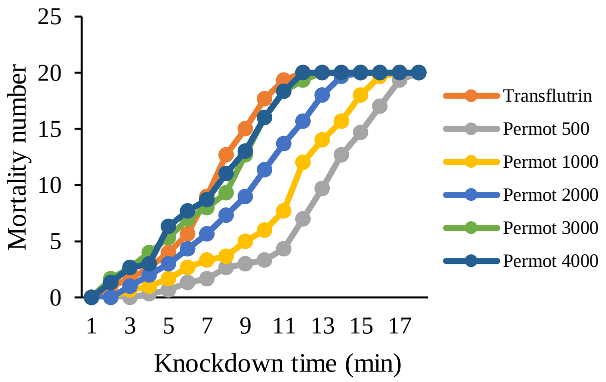


**Fig. 1.** The deaths of *Blattella germanica* L. exposed to transfluthrin 3000 ppm and graded dose of *Passiflora foetida* L. leaf extract.

The observations on German cockroaches after 1 h of insecticide exposure revealed that their movement became slower and slower until they eventually stopped moving altogether (knockdown) and died. The t-test revealed a significant difference between the insecticide exposure groups using transfluthrin 3000 ppm and 4000 ppm Permot leaf extract and the Permot leaf extract groups using less than 3000 ppm Permot leaf extract ($p<0.01$) (Fig. 1). Our earlier research (Susilowati & Sari, 2018; Susilowati & Sari, 2021) established that spray insecticides derived from Permot leaf extract at a concentration of 4000 ppm were efficient in killing German cockroaches in less than 30 min. Meanwhile, it takes around 10 min for German cockroaches to be knocked down when exposed to insecticides composed of a mixture of synthetic pyrethroids, transfluthrin 600 ppm, and 1500 ppm d-allethrin. A dose of less than 1000 ppm of Permot leaf extract was ineffective in knocking down and killing German cockroaches. The effective doses obtained were $LC_{50}$ of 1216 ppm and $LC_{90}$ of 3252 ppm based on the number of German cockroaches killed due to spray pesticide exposure.

Flavonoids are one of the largest natural phenolic groups and have a proclivity for binding proteins, interfering with metabolic activities in cell membranes (Mierziak *et al*., 2014; Arroyo-Maya *et al*., 2106; Brodowska, 2017). Permot leaf extract containing flavonoid chemicals, which can enter the insect's body via the respiratory system in spiracles, causing nervous system weakening and respiratory system damage, resulting in knockdown and even death during assaults due to inability to breathe (Fernando & Karunaratne, 2012; Hikal *et al*., 2017). Tannins are another component found in Permot leaf extract, classified as polyphenols. After entering the insect's body, tannin bond complexes chelate with metal ions, increasing their toxicity and ability to harm insect cell membranes (Asadujjaman *et al*., 2014; Belete *et al*., 2018). Additionally, Permot leaf extract includes saponin that, when ingested by an insect, can affect the metabolism of body cells and result in movement paralysis (Kamal *et al*., 2017; Chinnasamy *et al*., 2018). Saponin chemicals can reduce food intake, resulting in insect knockdown. Another major component of Permot leaf extract is alkaloids, which are poisonous substances that can interfere with the peptidoglycan and topoisomerase of insect body cells, resulting in an incomplete cell membrane layer and cell death (Thawabteh *et al*., 2019; Tandoro *et al*., 2020).

According to Balabanidou *et al.* (2018), pesticide susceptibility varied according to the thickness of the cuticle and the degree of sclerotization of the insects. Insects with thin sections such as sensory organs, spiracles, and intersegmental membranes allow for rapid penetration or entry of pesticides. In contrast, insects with thick sections such as body segments and appendages make insecticides less permeable to cell membranes or cuticles (Bass & Jones, 2016; Xiong *et al*., 2018; Balabanidou *et al*., 2019). After insecticide exposure, the ventral prothorax and other areas of the insect closest to the CNS will produce the quickest response. Additionally, the results of this study indicated that insecticide exposure to portions of the ventral mesothorax spiracles close to the CNS resulted in a relatively rapid knockdown of insects, as opposed to insecticide exposure to portions of the insect's body distant from the CNS, which resulted in a lengthy response. However, topical pesticide application to the abdominal spiracles can elicit a faster response than insecticide application to the labium or dorsal membrane of the head or thorax of insects, although these sites are closer to the CNS than the stomach (Sumita *et al*., 2016). Moreover, pesticide application on the mesothoracic spiracles nearest to the CNS elicited the quickest response. In line with our previous studies (Susilowati & Sari, 2021) that pyrethroid pesticides are neurotoxic with the intent of impairing the CNS's function. The findings of this study indicate that insecticide penetration via the spiracles, particularly the mesothoracic spiracles, is critical for inducing the knockdown effect and quick insect death.

When a transfluthrin pyrethroid aerosol was sprayed onto German cockroaches, adequate pesticide adherence was seen at areas near the CNS, resulting in low knockdown of insects (Chandelia & Dubey, 2014; Prasad *et al*., 2015). Furthermore, the pesticide that can penetrate the tissue was not excessive. However, based on the results of this investigation, the response of German cockroaches to pesticides containing transfluthrin 3000 ppm and Permot leaf extract up to a level of 4000 ppm was extremely rapid. The $KT_{50}$ and $KT_{90}$ values for German cockroaches subjected to insecticides containing transfluthrin at a concentration of 3000 ppm were 8 and 22 min, respectively. In comparison, those exposed to Permot leaf extract at a concentration of 4000 ppm were 8 and 30 min. According to thereby impeding the spiracles' function, it appears that the dorsal mesothorax spiracles are more susceptible than the ventral mesothorax spiracles. These findings revealed that the quick knockdown of insects using the direct sprayed pyrethroid approach was mostly attributable to the pesticide flowing into the mesothoracic spiracles and the sequential entry of insecticides through the mesothoracic spiracles' inner wall. The results suggested that spraying pesticides on one side of cockroaches with blocked mesothorax spiracles resulted in a less efficient knockdown, indicating that insecticide entry into the mesothorax spiracles was a significant cause of knockdown in cockroaches. In the group of cockroaches with blocked spiracles, the amount of insecticide that can penetrate to the cockroach's body surface will flow into the spiracles and is deemed equal to the amount of insecticide that can penetrate the cockroach's body surface in unblocked or blocked spiracles. Additionally, it was hypothesized that the volume or concentration of pesticide flowing into mesothoracic spiracles was smaller in insects with blocked spiracles than in insects with unblocked spiracles, implying that knockdown occurred more slowly and without insect death. However, a more in-depth research is required to fully understand how pesticides operate until they reach insect organs such as spiracles, the inner wall of the tracheal system, or the CNS directly.

## CONCLUSION

The neurotoxic efficacy of insecticides derived from *Passiflora foetida* L. leaf extract concentrations up to 4000 ppm correlates with *Blattella germanica* L. rapid knockdown time and death. The quickest $KT_{50}$ was approximately 8 min at 4000 ppm, and the fastest $KT_{90}$ was approximately 30 min at 4000 ppm, with 100% German cockroach mortality.

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