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DEVELOPMENT OF ANT AND COCKROACH CONTROL METHODS IN URBAN AREAS BASED ON BAITING SYSTEM

Dr. Idham S. Harahap
Trijanti A. Widinni A. SP., M.Si
Herni Widhiastuti S.Si

MINISTRY OF EDUCATION, CULTURE, RESEARCH AND TECHNOLOGY OF THE REPUBLIC OF INDONESIA
SECRETARIAT GENERAL
SEAMEO SEAMOLEC
SOUTHEAST ASIAN REGIONAL CENTRE FOR TROPICAL BIOLOGY
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APPROVAL SHEET

1.	Research title	:	Development of Ant and Cockroach Control Methods in Urban Areas Based on Baiting System
2.	Research Coordinator		
	a. Name	:	Dr. Idham Sakti Harahap
	b. Gender	:	Male
	c. Occupation	:	Researcher in SEAMEO BIOTROP
3.	Institution		
	a. Name of Institution	:	SEAMEO BIOTROP
	b. Address	:	Jl. Raya Tajur Km 6, Bogor
	c. Telephone/Fax.	:	+62-251-8323848/+62-251-8326851
	d. Email	:	idham@biotrop.org
4.	Research duration	:	9 months
5.	Research Budget	:	

Endorsed by Acting Manager of Research Hub Innovation Department SEAMEO BIOTROP	Bogor, 30 November 2021 Research Coordinator
Ir. Sri Widayanti, M.Si NIP. 19670822 200701 2 001	Dr. Idham Sakti Harahap NIP. 19591022 198503 1 002

Approved by,

Dr. Zulhamsyah Imran, S.Pi, M.Si
Director of SEAMEO BIOTROP
NIP. 19700731 199702 1 001

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ABSTRACT

Urban pests such as ants and cockroaches are two types of insects that are well adapted to human life and often cause disturbances to human comfort and health. The method of controlling ants and cockroaches using the safest insecticide (against building occupants) is baiting. This study aims to inventory the types of ants commonly found in Bogor and evaluate the effectiveness of bait combinations with botanical insecticide extract against cockroaches and ants. The results of the inventory and identification obtained 7532 individual ants from 4 subfamilies, namely Dolichoderinae, Formicinae, Myrmicinae, and Ponerinae, as well as 28 species. The diversity of ant species at each location shows that the ant species in the bus terminal environment are not significantly different from the mall area. However, the diversity of ant species in three locations, namely bus terminals, malls, and housing estates, showed significant differences with the diversity of ant species in office habitats. The study of seasonal differences did not show any effect on the diversity of ant species in each location. In the bait preference test, the cockroach *Blattella germanica* and the ant *Anoplolepis gracilipes* showed different preferences for the type of bait tested. The *B. germanica* cockroach showed a high preference for the combination of ground chicken meat + 25% sugar solution (1:1). In addition, the *A. gracilipes* ants preferred carbohydrate (sugar) and lipid-based baits, where the highest preference was found in 25% sugar solution baits followed by a combination of sugar + chicken fat solution (1:1). The LC_{50} and LC_{95} values of the *Tephrosia vogelii* extract against the third instar German cockroach were 0.023% and 0.058%, respectively. Meanwhile, the toxicity of *Cerbera manghas* extract was lower than that of *T. vogelii* against the third instar of German cockroach. The LC_{50} and LC_{95} values of *C. manghas* extract against the third instar of German cockroach were 0.027% and 0.073%, respectively. The addition of extracts of *T. vogelii* and *C. manghas* to the bait indicated a potential repellent effect by both types of insects.

Keywords: *Anoplolepis gracilipes*, *Blattella germanica*, botanical insecticide, baiting, identification

1. INTRODUCTION

1.1 Background

Ants and cockroaches are insects whose presence and life activities are alarming to humans. These two types of insects are a kind of pest that is very common in residential areas in Southeast Asia. Apart from the disturbance of comfort, these two groups of insects also can cause health problems to humans. Ants can bite or sting and can contaminate human food. In this latter condition, cockroaches have the potential to transmit disease-causing pathogens to humans. The habits of cockroaches mean that they have the potential to be a vector of the organism that causes human disease, as well as causing allergies and asthma (Miller & Peters 2004).

Ants can nest outside or inside a building, and the ones that become pests in a residential area are ants foraging in a building inhabited by humans, especially if the ants make their nests inside the building. While cockroaches generally nest inside a building because they need shelter with a slightly warm temperature and relatively high humidity, cockroach nests are generally found around kitchens and bathrooms. One species of cockroach, *Blattella germanica*, known as German cockroach has become a pest in urban areas. German cockroaches are nocturnal and forage for food and water at night. In the daytime, they hide in cracks and crevices in cupboards and kitchen appliances. Controlling the German cockroach is challenging to be done (Miller & Peters 2004).

Ant control techniques can be implemented in many ways, both chemically and non-chemically. However, what people generally do is chemical means, primarily if a pest control company assists the person. Various chemical methods are commonly practiced to control ants by spraying the perimeter of a building (known as perimeter treatment), local spraying (spot treatment) on nests in a building, and baiting them with food bait containing insecticides. This last method is also commonly used for cockroach control.

The safest method of controlling ants and cockroaches using insecticides is a baiting system. The baiting system is a reliable method to control many urban pest species, and a food bait base is usually formulated with slow-acting insecticide. Via trophallaxis, baits with toxicants that foraging workers consumed are transferred to other colony members, which will suppress the population or even cause the elimination of the population (Lee 2008). An insecticide is relatively safe to use in urban areas because it is not applied in open places but inside the bait stations, so it is more localized.

The problem in controlling ants and cockroaches with a baiting system is that the attractiveness of these two groups of insects to food bait can decrease if we apply this technique for a long time. This phenomenon has been seen in the baiting of one species of cockroach, *Blattella germanica*, which is known as the German cockroach. Commercial bait products have reportedly avoided this cockroach, a phenomenon is known as "bait aversion". Bait aversion phenomenon is thought to be caused by the formulation ingredients. Silverman and Bieman (1993) reported the food aversion of cockroaches, especially glucose, which led to cockroach bait products' failure in the 1990s. In addition, the use of bait in a high frequency can also trigger cockroach resistance to the bait. Resistance can develop as behavioral and physiological resistance (Wang *et al.* 2004). Therefore, it is necessary to continue to look for the types of bait whose attractiveness can last longer.

Food bait for ants and cockroaches should be based on the nutritional requirements of the insects. Ants generally need sugar, protein, and fat. In 4 season countries, during spring to summer, the ants generally need more sugar, while in the autumn, the ants need more protein and fat as reserves to do "overwintering" in winter. However, the need for the three types of nutrients is relatively balanced in tropical areas, although certain ants prefer protein or fat, especially those with predominantly predatory characteristics. Meanwhile, cockroaches are more omnivorous and tend to consume a combination of nutrients between sugar and protein.

The common toxicants used in commercial ant and cockroach bait formulations are synthetic insecticides. Not many people have used natural insecticides extracted from plants as a toxicant in food bait for ants and cockroaches. Several types of botanical insecticides that have the potential to control urban insect pests include *Schimus molle*, *Argemone mexicana*, *Nerium oleander*, and *Parthenium hysterophorus*, which are known to have a repellent effect and can cause mortality of American cockroaches (*Periplaneta americana*) (Ferrero et al. 2007; Khan & Qamar 2015). In this study, the use of extracts from two types of plants, bintaro or sea mango seed extract (*Cerbera manghas*) and fish poison bean leaf extract (*Tephrosia vogelii*) were explored.

1.2 Objectives

This study aims to (1) investigate the types of ants commonly found in the city of Bogor, (2) test the preferences of ants and cockroaches to food baits containing sugar, protein, lipid, and their combination, (3) test the effectiveness of two types of botanical insecticides (bintaro or sea mango seed extract and fish poison bean leaf extract) against ants and cockroaches, (4) making ready-to-use bait formulations and design traps for ant and cockroach bait applications in residential areas.

1.3 Expected Output

The expected results from this study are (1) a list of ant and cockroach species commonly found in residential areas in the Bogor area, (2) obtaining ready-to-use bait formulations containing botanical insecticides which set in a trap for ant and cockroach control in residential areas.

2. BENEFIT AND IMPORTANCE OF THIS RESEARCH

The more developed a nation is the higher its people's standard of living and health parameters, including hygiene and sanitation issues. Ants and cockroaches present in neighborhoods will be greatly lower these standards because of the sanitation problems they cause. In developed countries, people generally hire pest control services to overcome this problem. However, in Indonesia and perhaps some countries in Southeast Asia, the role of pest control services like this has not yet become an immediate need, especially in the residential environment.

The existence of simple technology that the community can use to solve the problem of residential insect pests will significantly assist them in controlling urban pests. Ants and cockroaches are the most common residential pests found in residential areas in Indonesia and other Southeast Asian countries. Avoidance of bait (bait aversion) shown by cockroaches against commercial bait can reduce the effectiveness of the bait. In addition, resistance issues of ants and cockroaches to synthetic insecticides will complicate the problems. Therefore, research aimed at developing natural bait formulations containing natural toxicants is essential.

3. METHODOLOGY

3.1 Inventory of Diversity of Ant Species in Different Environments

An inventory of the diversity of ant species in several different environments or habitats was carried out to determine the index of diversity and similarity of ants at each location, as well as knowing the differences in the abundance of ants in each different season (seasonal appearance study).

3.1.1 Time and Place

Inventory activities were carried out in four different locations: office area, mall, housing estate, and bus terminal. Each selected location represents a different type of habitat or environmental condition. Inventory activities were carried out for five months, namely in May - October 2021.

3.1.2 Material and Equipments

The materials used in the inventory activities include a solution of sugar and ground beef to attract ants and 70% alcohol to preserve ants obtained from the field before being identified. Meanwhile, the tools used consisted of plastic tubes, bottle caps, vaseline, Eppendorf bottles, thermohygrometer, brushes, stereo microscope, GPS, camera, and stationery.

3.1.3 Ant Sampling/ Collection Method

Ant collection was carried out from 09.00 to 15.00 every week at the exact location using the trapping method (Stringer *et al.* 2009, Rizali *et al.* 2008). Environmental conditions such as temperature and humidity at each collection location are always recorded when the collection is carried out. The trapping method was carried out using bottle caps, each containing a 25% sugar solution and ground beef as bait or attractant. The bottle caps containing the bait are then placed on the floor or the ground for ten traps each at each location (Figure 1). The sampling point at each location is replication for that location. There are ten replications with the same sampling points at each sampling location, namely office area, Mall, Housing, and Bus Terminal sampling points each month. The specifications for sampling points at each location can be seen in Table 1. Insect collection using traps was carried out for 30 minutes. The ants that come and are trapped in the bait are then put into an Eppendorf bottle containing 70% alcohol to be brought to the laboratory, and the number is counted and identified.

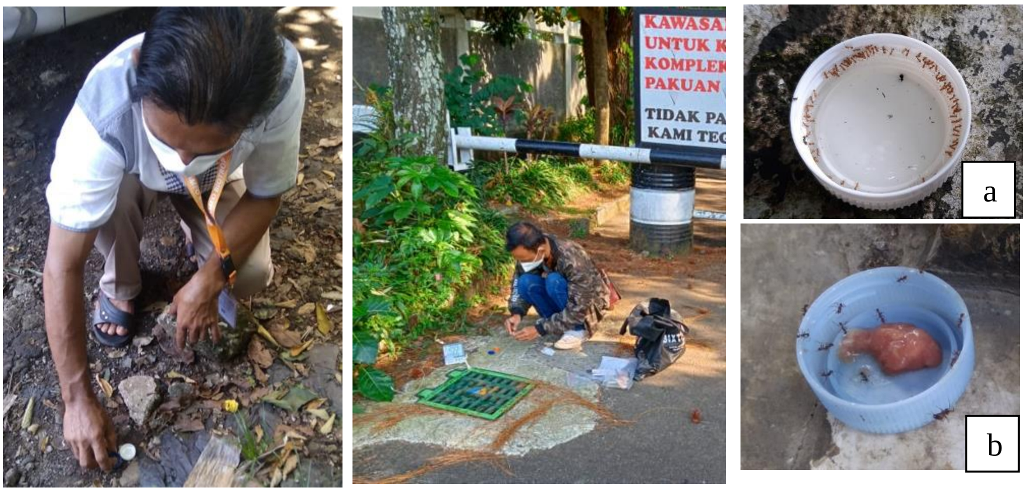

Figure 1. Sampling of ants with 25% sugar solution (a) and ground beef (b) on bottle caps.

Table 1. Specifications for ant sampling points in the office area, Mall, Housing, and Bus Terminal.

Sampling Point	Location of Sampling Area			
	Office (BIO)	Mall (ELS)	Housing (PK)	Bus Terminal (BS)
01	Laboratory corridor (office)	Front entrance 1	Security post	Back area 1
02	Parking area	Front entrance 2	Inner gate	Back area 2
03	Orange garden	Pavement garden 1	Central Post	Middle area 1
04	Teak garden	Pavement garden 2	Trash area	Middle area 2
05	The border between the teak garden and the parking area	Right middle entrance	In front of people's houses	Police Post
06	The outside area of the convention hall	Left middle entrance	Back post	Pavement
07	Park	Exit garden 1	Park	Front pavement 1
08	Guest house	Exit garden 2	Outside Park	Front pavement 2
09	Building yard	Exit 1	Front Post	Front pavement 3
10	Canteen	Exit 2	Gate	Front pavement 4

3.1.4 Identification of Ant Specimen

The ants collected from each location were then taken to the Entomology Laboratory, SEAMEO BIOTROP, to identify the species. Identification refers to several kinds of literature, such as *Key to the workers of ant genera and 12 ant subfamilies of Borneo in English and Malay* (Tom M. Fayle) and *Generic synopsis of the Formicidae of Vietnam. Part 1 – Myrmicinae and Pseudomyrmecinae* (Eguchi *et al.* 2011 in [antwiki.org]) to determine the genus and morphospecies (Figure 2).

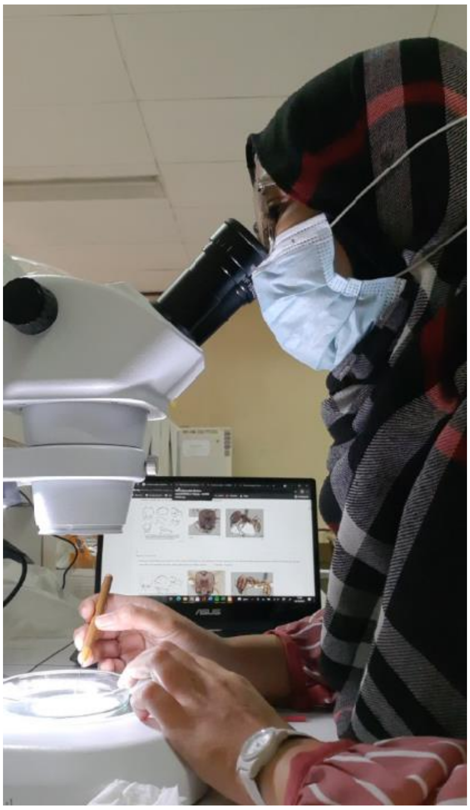

Figure 2. Identification the sample of ants

3.1.5 Data Analysis

The data obtained from the collection were then analyzed using a species accumulation curve (Colwell & Coddington 1994) to determine the differences in ant diversity between habitats/sample locations. Differences in ant species composition between habitats were analyzed using Ordihull analysis based on the Bray-Curtis dissimilarity index. The test of significant differences in the abundance of ant species in each different season, namely April - June and July - September, was carried out through the Tukey test. All data analysis activities were carried out using R statistical software and SPSS.

3.2 Preferences of Ants and Cockroaches to Food Bait Containing Sugar, Protein, and Lipid

3.2.1 Time and Places

Preference ttesting of ants and cockroaches against food bait were conducted at Entomology Laboratory SEAMEO BIOTROP for three months.

3.2.2 Material and Equipments

The bait material used consisted of chicken meat (protein), chicken fat (lipid), and a sugar solution with a concentration of 25%. The ant used in this test was the ant

Anoplolepis gracilipes, and the test cockroach used was a German cockroach, namely *Blattella germanica*. Meanwhile, the tools needed consist of a circular trap made of mica plastic (Figure 2), an Eppendorf bottle, a plastic container, glue, vaseline, and a brush.

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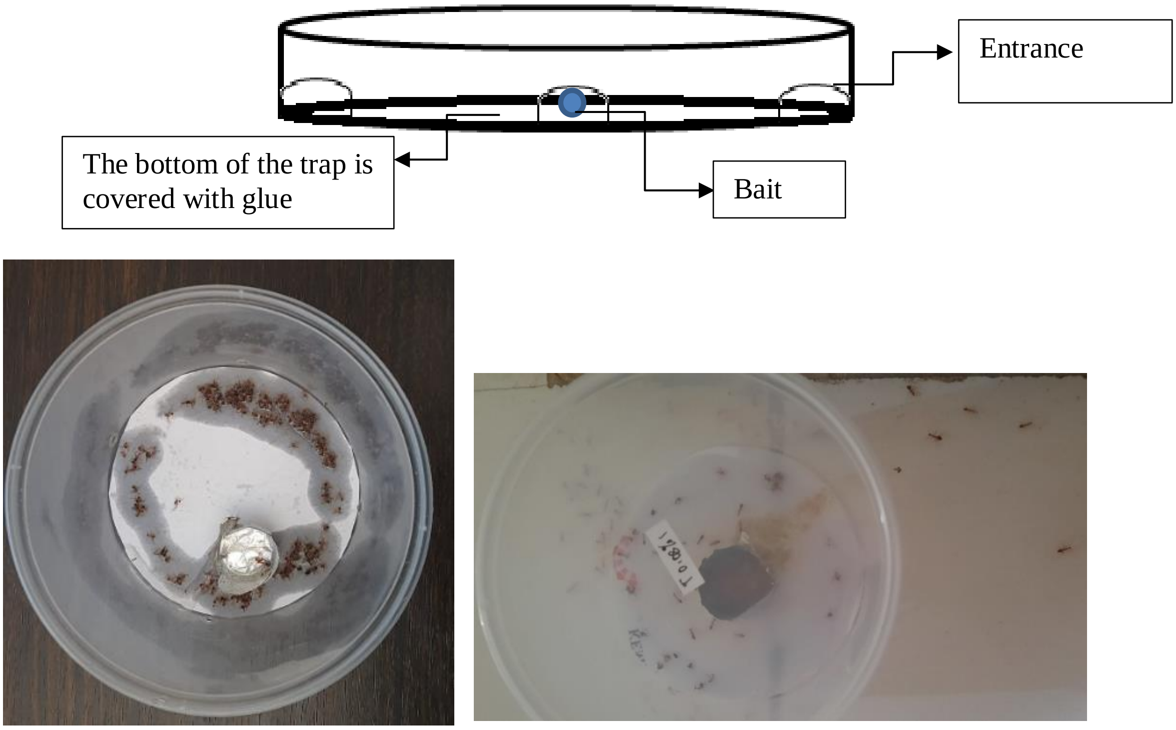


Figure 3. Trap model in the no choice treatment of ant bait

3.2.3 Cockroaches Mass Rearing

Adults of the German Cockroach *B. germanica* were obtained from supermarkets in the Bogor area. The cockroach adults obtained from the field were then taken to the Entomology Laboratory, SEAMEO BIOTROP, and kept in a maintenance container. The feed used during rearing is fresh potatoes. Egg packets (ooteka) produced by cockroaches are periodically removed from the rearing container and transferred in separate containers until they hatch. Furthermore, nymphs that have been released on the same day are transferred back in a separate container every day, so it is expected to obtain cockroaches with a relatively uniform age for use in testing.

3.2.4 No Choice Test Method

Tests were carried out using *A. gracilipes* ants around the SEAMEO BIOTROP area and German cockroaches cultured by the Entomology laboratory, SEAMEO

BIOTROP. Testing is carried out using two methods, namely the choice and no choice methods. Testing with the no-choice method for ants was carried out through trapping. The traps used are made of mica plastic in the shape of a circle with a diameter of ± 9 cm, and there are 4-5 ant inlets. The bottom of the trap is then coated with vaseline to trap ants that have entered the trap (Figure 3). Three different types of bait, namely chicken meat, chicken fat, and sugar solution, were tested singly and in combination (Table 2). Each as much as 1 gram of bait is prepared and placed in the center of the trap. Each bait type treatment was repeated five times.

Table 2. Bait combination used in treatment

No.	Treatment[a]	Treatment code
1.	Chicken meat	D
2.	Chicken fat	L
3.	25% Sugar solution	G
4.	Chicken meat + Chicken fat	DL
5.	Chicken meat + 25% Sugar solution	DG
6.	Chicken fat + 25% Sugar solution	LG
7.	Chicken meat + Chicken fat + 25% Sugar solution	DLG

[a]Each treatment replicated 5 times

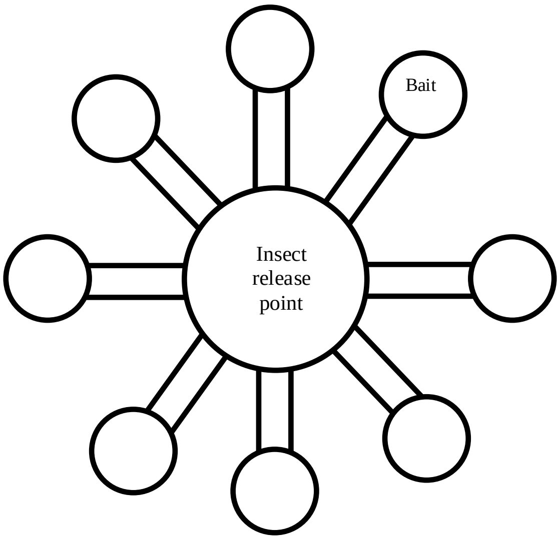
Traps were placed randomly at locations that were thought to be the path of ants for 72 hours. The trapped ants were then counted and put into a collection bottle containing 70% alcohol for identification. In addition, to observing the number and types at 72 hours of trapping, observations were also made on the length of recruitment time and the number of ants that came after 30, 60, and 90 minutes.

Meanwhile, the no-choice test for German cockroaches was carried out using a plastic container whose top was covered by gauze and contained 1 gram of single and combination bait (Figure 4). A total of 5 German cockroaches fasted for one day were then put into a container containing the bait. Observations were made 72 hours after infestation by looking at the cockroach consumption in each bait treatment. Each treatment was repeated five times.

Figure 4. Preference testing of cockroach againts several types of bait with no choice method

3.2.5 Choice Test Method

Testing the preferences of German cockroaches and ants with the choice test method were carried out using the same method, namely using a testing arena in the form of a circular container with eight branches that contained a container containing bait (Figure 5). The plastic container used for releasing insects is a circle with a diameter of 15 cm, connected with eight branches on each side. The branch is made of PVC pipe with a diameter of 1/2 inch, and the feed container at the end of the branch is circular with a diameter of 10 cm, which is covered with gauze at the top for air circulation and makes it easier to observe.



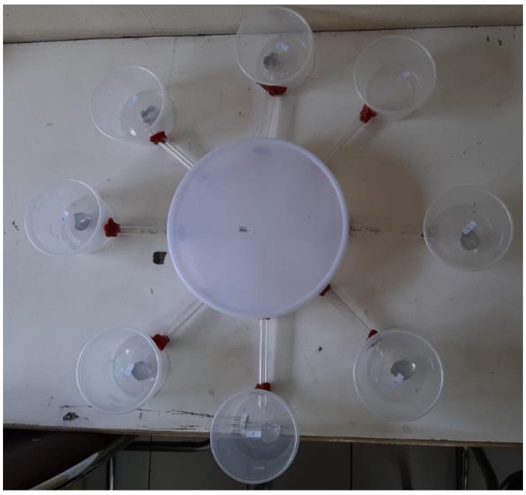


Figure 5. Arena used for preference test of ants and cockroaches using choice test

Each type of bait, either single or in combination, is placed at each end of the branch at random. The test insects prepared, namely cockroaches and ants, are then placed in the center of the testing arena/insect release container separately. Observations were made at 6, 12, 24, and 72 hours after treatment by looking at the frequency of cockroaches and ants in each bait container. Then after 72 hours, the amount of consumption of cockroaches and ants was calculated for each bait combination. Each treatment was repeated five times.

3.2.6 Data Analysis

The data obtained were analyzed using Microsoft Excel 2007 and all data were processed using SAS 9.2 software and then Duncan's multiple comparison test was performed ($\alpha = 0.5$).

3.3 Effectiveness of Botanical Insecticide-Based Toxic Bait against Ants and Cockroaches

3.3.1 Location and Time

Testing the effectiveness of toxic bait were carried out at Entomology Laboratory and SEAMEO BIOTROP office and expected to be completed in 4 months.

3.3.2 Material and Equipment

The test insect used was the adults of the German cockroach (*B. germanica*). The bait used was ground meat + sugar (1:1) which had the highest preference for ants and cockroaches compared to other types of bait. Botanical insecticides were extracted using *C. manghas* and *T. vogelii*. *C. manghas* seeds are obtained from the plant around Bogor. Meanwhile, the leaves of *T. vogelii* were obtained at the Bina Sarana Bakti Foundation (Agatho), Cisarua District, Bogor. The solvents used were methanol and ethyl acetate. The tools needed consist of equipment for extraction, namely filter paper, Erlenmeyer flask, glass funnel, rotary evaporator, scales, blender, and equipment for testing such as petri dishes.

3.3.3 The Extraction of Botanical Insecticide

Extraction is done by the maceration method. The leaves of *T. vogelii* obtained from the field were cut into small pieces and then air-dried in the room. Meanwhile, the seeds of *C. manghas* were peeled, and the seeds were removed first to take the flesh of the seeds and dried for 1 week (Figure 6). The dried leaves of T. *vogelii* and seeds of *C.*

manghas were then ground separately using a blender to a powder and sieved using a 0.5 mm mesh sieve.

Figure 6. The process of separating and chopping the seeds of *Cerbera manghas* (a) and leaves of *Tephrosia vogelii* (b) into powder

T. vogelii leaf powder was then soaked using ethyl acetate solvent at a ratio of 1:10 (w/v), while the *C. manghas* seed powder was soaked using methanol solvent at the same ratio. Soaking for each powder was carried out for 24 hours. After 24 hours, it was filtered using a glass funnel lined with Whatmann No. filter paper. 41 (diameter 185 mm). The results of filtering the ethyl acetate solution of *T. vogelii* leaves and *C. manghas* seed methanol were then evaporated using a rotary evaporator at a temperature of 50 °C to obtain a crude extract (Figure 7). The evaporated solvent was used to re-soak the dregs of each plant, and this step was repeated until the filtering results were close to colorless. The extracts from each immersion were stored in a refrigerator (± 4 °C) in different containers until they were used for testing (Dadang & Prijono 2008).

Figure 7. Maceration process of seed powder of *Cerbera manghas* (left) and *Tephrosia vogelii* (right) in methanol and ethyl acetate (1:10) (a). The process of filtering the solution and the results of filtering the solutions of *C. manghas* (top) and *T. vogelii* (bottom) (b). The process of separating solvents and plant extracts (c).

3.3.4 Testing the Effectiveness of Toxic Bait Against Ant and Cockroach

3.3.4.1 Testing the Toxicity of Botanical Insecticide

Toxicity testing was carried out in two stages, namely a preliminary test and a further test. Preliminary tests were carried out to determine the concentration range of botanical insecticide that causes mortality of the test insects in the range of more than 0% but less than 100%. Each crude extract was mixed with methanol solvent and Tween-80 (5:1) and then diluted by adding distilled water to a certain volume according to the desired concentration. The concentrations used in the preliminary test for each extract were 1%, 2%, 3%, 4%, and 5%, as well as the control treatment (0%). The test was conducted using the cockroach feed dipping method, namely plain bread in each extract solution. A total of 10 cockroaches that had been starved for 24 hours were put in a plastic container, then white bread that had been dipped in each extract solution was put into a plastic container containing the cockroach (Figure 8). Each treatment was repeated five times. Observations will be made at 24, 48, and 72 hours after treatment (HAT) on the number of insect's mortality.

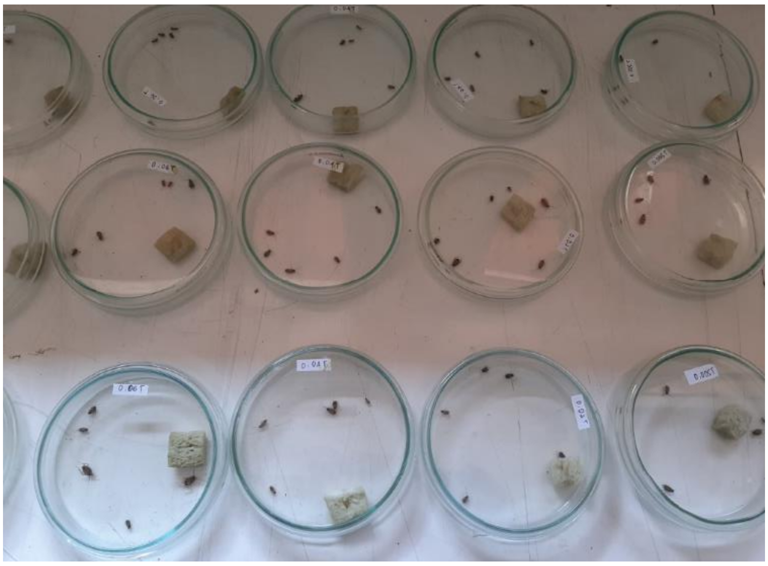


Figure 8. Toxicity tets of *Tephrosia vogelii* and *Cerbera manghas* extracts against the third instar of German cockroach

The preliminary test results were analyzed using the POLO PC program to determine the values of LC_{50} and LC_{95} at 72 HAT for each type of extract. The concentration of each LC analysis result is used as the basis for further tests.

3.3.4.2 Testing the Preference of Cockroaches and Ants against Toxic Bait

The test of bait preference combined with extracts of *T. vogelii* and *C. manghas* was carried out to determine the possibility of rejection of the test insects on baits containing extracts. The test was carried out by the choice method (choice test) using a testing arena in the form of a circular container with three branches which at the end contained a container containing a combination of meat and sugar (1:1), meat and sugar combination bait (1:1) + extracts in the concentration of 3% and an empty container without a feed as a control (Figure 9). The plastic container used for releasing insects is a circle with a diameter of 15 cm, connected with three branches on each side. The branch is made of PVC pipe with a diameter of 1/2 inch, and the feed container at the end of the branch is circular with a diameter of 10 cm, which is covered with gauze at the top for air circulation and makes it easier to observe.

The test insects prepared, namely cockroaches and ants, are then placed in the center of the testing arena/insect release container separately. Observations were made at 1, 4, 6, and 24 hours after treatment by looking at the frequency of cockroaches and ants in each bait container and the number of dead test insects. Each treatment was repeated five times.

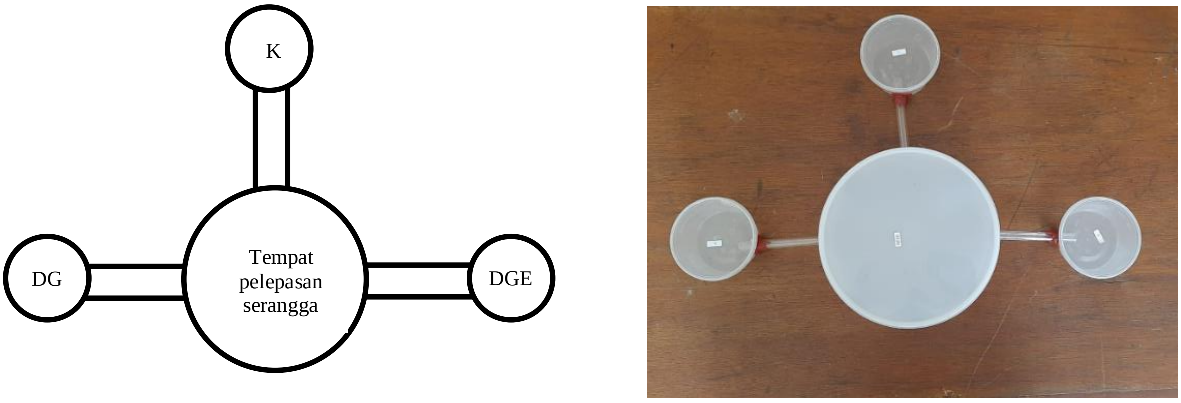


Figure 9. Arena used for preference test of ants and cockroaches to bait combination extracts using choice test method. DG: a container containing a combination of ground chicken meat + 25% sugar solution (1:1). DGE: a container containing a combination of ground chicken meat + 25% sugar solution + extract. K: empty container or no feed

4. RESULTS AND DISCUSSION

4.1 Diversity of Ant Species in Different Urban Areas in Bogor

Analysis of the diversity of ant species in various urban environments in Bogor, namely office environments, bus terminals, malls, and housing estates, was carried out through an inventory activity for five months with ten sampling points at each location so that there were 50 sampling replicates for each location. Based on the results of the inventory and identification, 7532 individual ants were obtained from 4 subfamilies, namely Dolichoderinae, Formicinae, Myrmicinae, and Ponerinae, as well as 28 species from all subfamilies found (Table 3).

There were several species of ants found in all locations, namely *Tapinoma melanocephalum*, *Dolichoderus thoracicus, Anoplolepis gracilipes, Paratrechina longicornis, Pheidole* sp. 01, *Monomorium floricola,* and *Tetramorium* sp. 01 (Figure 10). *T. melanocephalum* and *M. floricola* are groups of tramp ants, while *A. gracilipes* and *P. longicornis* are groups of tramp ants and are invasive. Tramp group ants have a habit of invading other areas, especially areas outside their distribution and their presence is closely related to human life, and their numbers can be very abundant in disturbed habitats, agricultural land, and urban areas (McGlynn 1999, O' Dowd *et al.* 2003, Boss *et al.* 2008). The existence of tramp ant groups has also been known to be very abundant in all types of habitats around the city of Bogor, especially in the yard of the house, where there are generally ornamental flowers and fruit plants that strongly support their habitat. In addition, some tramp ants also interact and associate with other insects such as homopteran, which may be very abundant in fruiting and flowering plants in home gardens (Rizali *et al.* 2008).

The presence of invasive tramp ants, such as *A. gracilipes* and *P. longicornis*, which are highly adaptable to disturbed habitats, also can cause the loss of other ant species from a habitat due to competition (Holway *et al.* 2002). This can be seen in the inventory results in the office habitats, where sampling is carried out in office buildings, gardens, and parks around the offices in the office area. The inventory results at that location showed a very high and quite dominant abundance of tramp ants, namely *A. gracilipes*. This may be one of the reasons for the low number of other ant species found in the area (Table 3).

Table 3. Diversity of ants in different urban areas in Bogor.

No.	Species	BIO[a]		BS[a]		ELS[a]		PK[a]	
		M1	M2	M1	M2	M1	M2	M1	M2
Dolichoderinae									
1.	*Tapinoma melanocephalum**	113	3	60	182	75	57	124	-
2.	*Tapinoma* sp. 01	-	40	4	22	3	57	33	-
3.	*Dolichoderus thoracicus*	72	404	128	102	223	110	-	219
4.	*Technomyrmex* sp.	-	2	-	-	-	-	-	-
5.	*Leptomyrmex* sp.	-	-	-	5	-	-	-	-
Formicinae									
6.	*Anoplolepis gracilipes***	180	251	4	46	91	37	118	97
7.	*Camponotus* sp.	-	-	1	-	2	-	-	-
8.	*Nylanderia* sp. 01	-	17	11	2	-	8	2	-
9.	*Nylanderia* sp. 02	-	7	6	-	-	8	-	-
10.	*Nylanderia* sp. 03	-	-	15	5	1	1	11	3
11.	*Oecophylla smaragdina*	-	-	-	-	-	-	-	19
12.	*Paraparatrechina* sp.	-	-	13	-	-	-	-	6
13.	*Paratrechina longicornis***	-	71	66	101	10	142	209	152
14.	*Polyrhachis amana*	-	-	-	-	-	-	-	8
Myrmicinae									
15.	*Cardiocondyla sp.*	-	8	-	-	-	-	-	-
16.	*Lophomyrmex birmanus*	-	-	33	-	-	-	-	-
17.	*Monomorium floricola**	-	10	51	35	-	19	155	7
18.	*Monomorium* sp. 01	-	-	-	-	-	-	40	-
19.	*Monomorium* sp. 02	35	-	21	30	1	-	35	-
20.	*Pheidole* sp. 01	-	38	-	-	15	-	21	-
21.	*Pheidole* sp. 02	1	51	-	721	1	53	225	175
22.	*Pheidole* sp. 03	111	6	4	160	42	75	244	260
23.	*Pheidole* sp. 04	-	-	59	-	370	118	-	-
24.	*Tetramorium lanuginosum*	101	-	28	84	-	-	-	-
25.	*Tetramorium* sp. 01	1	66	21	45	43	20	-	62
26.	*Monomorium* sp. 03	-	-	5	21	6	-	31	-
Ponerinae									
27.	*Odontoponera* sp. 01	1	-	-	-	-	-	5	-
28.	*Odontoponera* sp. 02	-	-	2	-	-	1	-	-

[a]BIO: Office, BS: Bus Terminal, ELS: Mall, PK: Housing. Inventory data from 10 sampling points for five months of observation. M1: Dry season (July – August). M2: Rain season (September – October).*: group of tramp ant. **: Group of tramp ant and invasive

The most common ant species found were in bus terminal areas, which were 21 species, while the areas with the least number of species were found in offices, 17 species. Based on the results of the analysis of significant differences in the diversity of ant species at each location, which was carried out through the Tukey test with a 95% confidence level, it showed that the diversity of ant species in the bus terminal habitat was not significantly different from the mall area. However, the diversity of ant species in three locations, namely bus terminals, malls, and housing estates, showed significant differences with the diversity of ant species in office habitats.

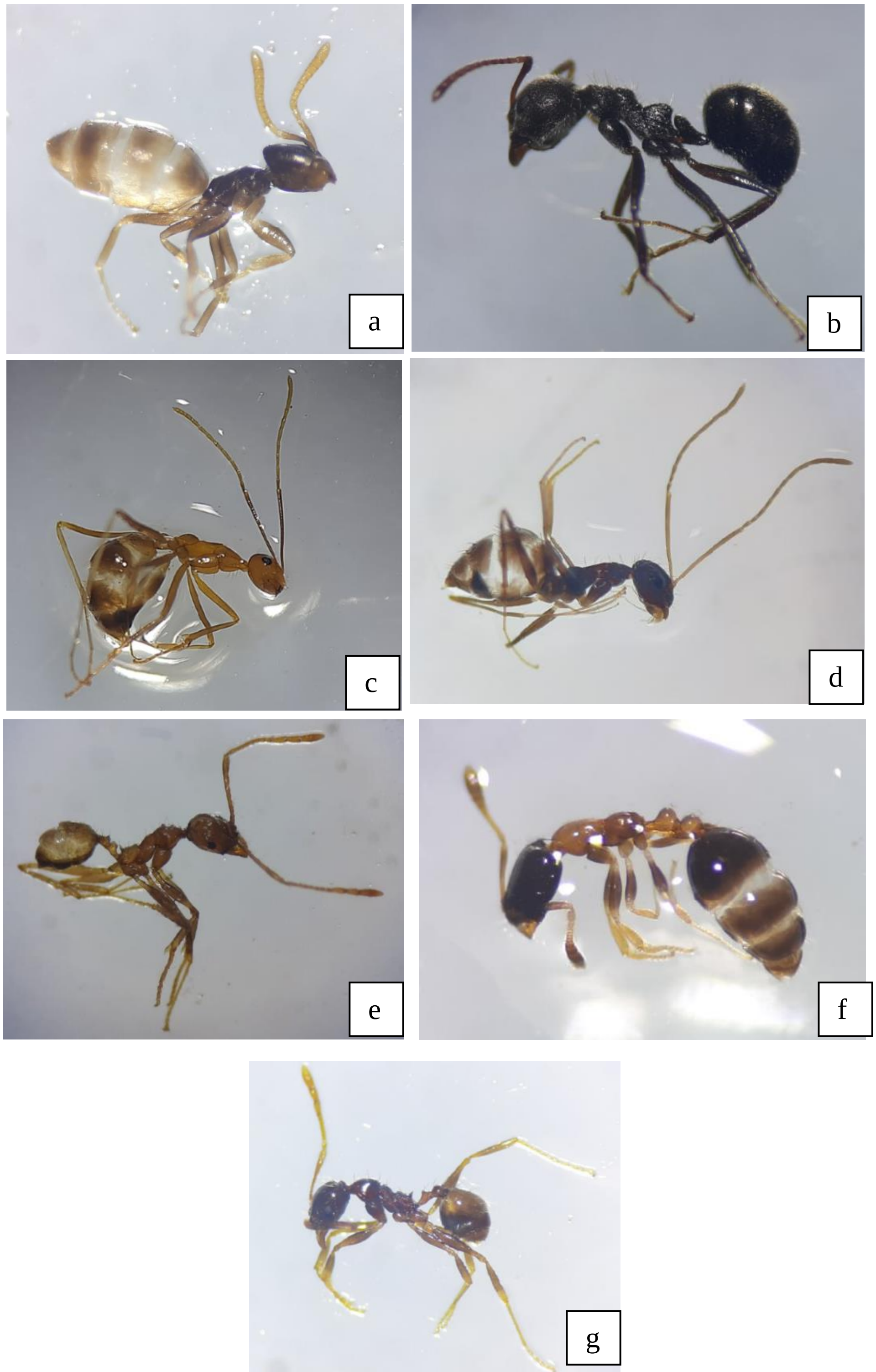


Figure 10. The types of ants found in all sampling locations, namely offices, malls, housing, and bus terminals in Bogor *Tapinoma melanocephalum* (a), *Dolichoderus thoracicus* (b), *Anoplolepis gracilipes* (c), *Paratrechina longicornis* (d), *Pheidole* sp. 03 (e), *Monomorium floricola* (f), and *Tetramorium* sp. 01 (g)

The results of Chao's estimation analysis on the total number of species in office areas, bus terminals, malls, and housing estates indicate that the collection method used is appropriate and successful because most of the ant species are found in each location have been successfully collected. Based on Chao's estimation value, the number of ant species that have been collected in office locations, bus terminals, malls, and housing estates was 85%, 100%, 95%, and 82%, respectively (Table 4). The lowest estimates for residential and office habitats indicate that the potential for ant diversity in these two locations should be higher than the ant diversity obtained. The species accumulation curve also shows that the number of species found during the five months of observation shows an ongoing increase (Figure 11).

Table 4. Number and estimated number of ant species (based on Chao estimates) from different urban areas in Bogor

Habitat	Number of Species[a]	Estimation (Chao)
Office	17 b	20 (85%)
Bus Terminal	21 a	21 (100%)
Mall	19 a	20 (95%)
Housing Area	18 ab	22 (82%)

[a]The numbers in the same column and followed by the same letter show results that are not significantly different based on the Tukey multiple interval test at the level = 5%

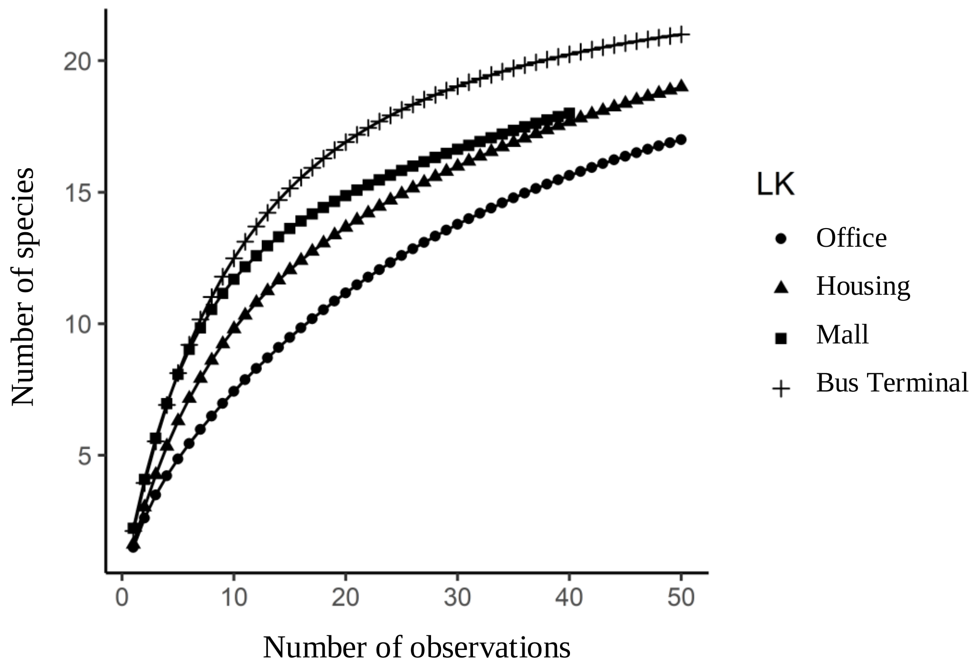


Figure 11. Accumulation curves of ant species found in different urban areas in Bogor

An increase in the number of species for 50 times of sampling indicates that additional species have not been obtained in the previous inventory activities. In addition, the results of the analysis of variance indicate the influence of habitat conditions on the diversity of ant species. The analysis of variance on the number of ant species found in 4 different habitats was significant, with a value of F_{hit} (3.36) 0.0002***. Based on the boxplot, the locations with different ant species are office areas (Figure 12).

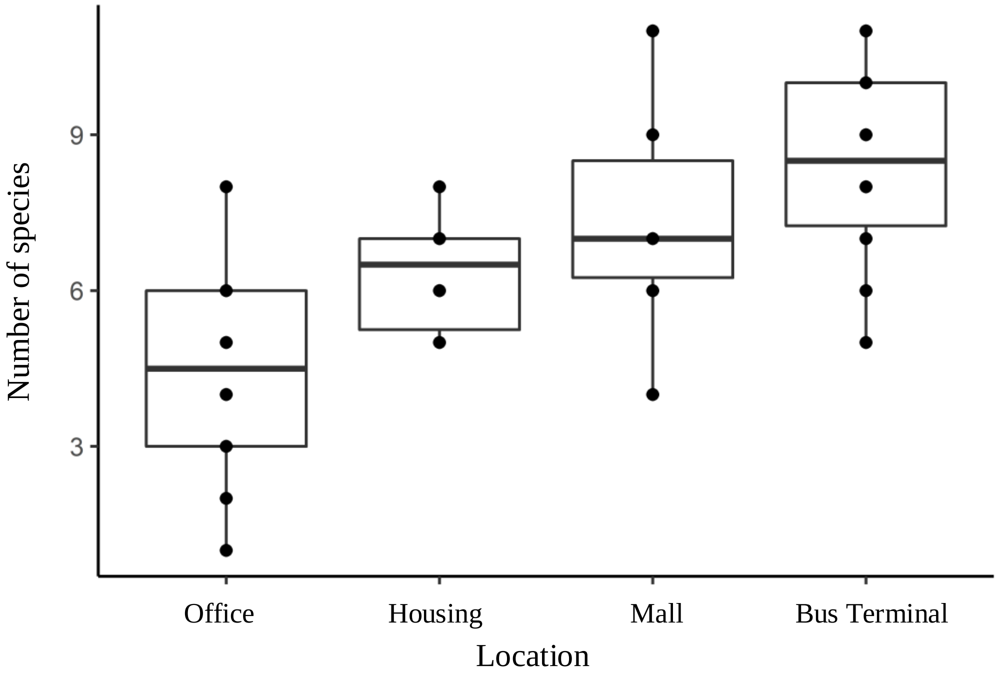


Figure 12. The diversity of ants found in each type of urban area. Data using the same number of plots (n=10)

This is also supported by the results of the similarity analysis, which was carried out through a nonsimetric multidimensional scale (NMDS) analysis, where the ordinance was obtained from the data of existing and non-existent species (not obtained) in each plot using the Bray Curtis index. The results of the similarity analysis at the four locations showed a significant difference, which means that differences in species composition were detected between each area (ANOSIM, R: 0.1875. $P < 0.001$, Significance: 2e-04) (Figure 13). In general, ant species diversity in the habitat is strongly influenced by the level of disturbance of the habitat. The higher the level of habitat disturbance, the greater the diversity, and the presence of ant species tends to increase. The habitats with high disturbance intensity are generally only able to facilitate the existence of tramp ant species that can adapt to human disturbances (Hasriyanty *et al.* 2015, Gibb & Hochuli 2003).

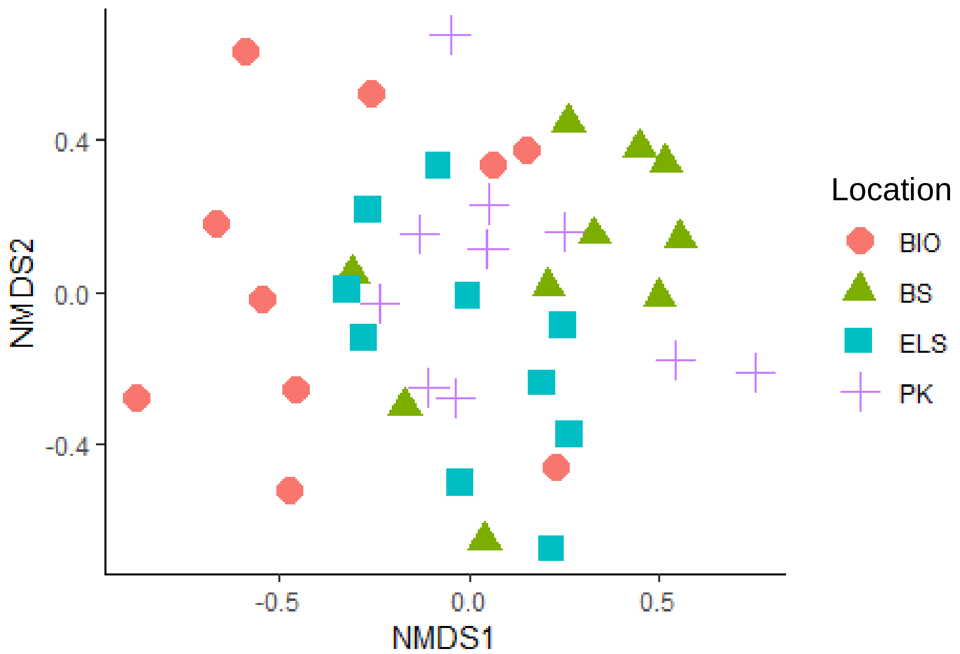


Figure 13. NonMetric Dimensional Scale (NMDS) ordination from ant species composition at four different area. BIO: Office. BS: Bus Terminal. ELS: Mall. PK: Housing

In this study, the level of disturbance at the four sampling locations tends to be the same. All of these locations are urban areas with relatively high human activities. However, the differences in the number and composition of species found at the four locations may be due to the condition of the trap placement points or the microclimate at each ant sampling point in each location. A sampling at the location with the highest number of species, namely the bus terminal, was carried out around the bus parking area, a very open area, and the sidewalk area along with the bus terminal (Table 1). Along the side of the bus terminal parking area, there are large trees that are pretty shady. In addition to the bus terminal sidewalk area, there is a garden with various ornamental plants. This condition is then suspected to be suitable for various ant species, thus causing the highest diversity of ants at this location. Meanwhile, sampling in office areas that tend to be dominated by tramp ant groups was carried out inside the office, parks, parking areas, teak gardens, and citrus gardens. The low diversity of ant species at this location, apart from the high dominance by tramp ants, also most of this area is in the form of buildings which may not be suitable for ant habitat.

In addition to the tramp and invasive ant groups, a relatively high abundance of ant species was found in the *D. thoracicus*. This type of ant is found in all urban areas with a quite high abundance, especially in the office area. The *D. thoracicus* ant is a common ant species in the agroecosystems in Southeast Asia. These ants are known as one of the natural enemies that can be used to control *Conopomorpha cramerella* and *Helopeltis sp.* (Saleh 2013, Philpott & Armbrecht 2006). Meanwhile, the highest abundance of tramp and invasive ant species in the office area was *A. gracilipes.*

However, there are differences in the abundance of the two types of ants in two different seasons. In the dry season, the abundance of *A. gracilipes* ants was higher than *D. thoracicus* ants. In contrast, in the rainy season, the opposite occurred, where the abundance of *D. thoracicus* ants was higher than *A. gracilipes*. The difference in abundance of the two types of ants in the dry and rainy seasons is almost doubled on average. This may indicate the existence of competition between the two species. When one species has already mastered the habitat with the support of suitable environmental conditions such as temperature and humidity, the other species will tend to lose, and their abundance decreases. *D. thoracicus* ants will usually become immobile and open their jaws wide as a form of defense when interacting with *A. garcilipes*. In addition, *A. gracilipes* ants can eliminate up to 40% of the population of other ant species, such as *Oechophylla smaragdina*, *P. longicornis,* and *D. thoracicus,* due to the influence of formic acid and are killed by *A. gracilipes* ants (Chong & Lee 2010). The pattern of ant species diversity in two different seasons, namely the dry season (July – August) and the rainy season (September – October) at each location, did not show any difference with the overall analysis results (Figure 14).

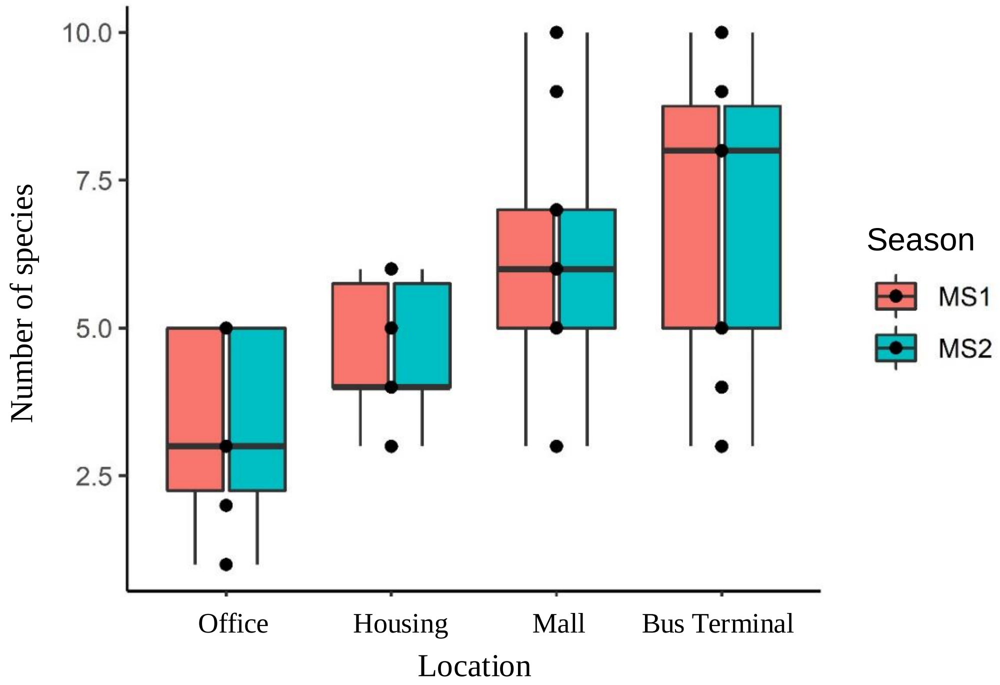


Figure 14. Diversity of ant species at different urban area in two different seasons

The highest number of species for each season is found in the bus terminal habitat and the lowest in the office area. The results of the analysis of variance on the number of ant species found in 4 habitats in two different seasons were significant, with a value of F_{hit} (3.36) 0.0004***. Based on the boxplot, the location with the highest number of ant species is the bus terminal, significantly different from housing and office habitats in each season. Meanwhile, the number of ant species in the terminal environment was not significantly different from the mall environment, and the number of ant species in the mall environment was not significantly different from the residential environment.

This is also supported by the results of the similarity analysis at each location in two different seasons, which was carried out through a nonsymmetric multidimensional scale (NMDS) analysis. The ordinance was obtained from the existing and non-existent species (not obtained) data in each plot using the Bray Curtis index. The results of the similarity analysis at the four locations in two different seasons showed a significant diversity of ant species in each season. This shows that in both the dry and rainy seasons, the composition of ant species in each area is different with the ANOSIM value, R: 0.1125. $P < 0.001$, Significance: 0.0121 in both dry and rainy seasons (Figure 15, Figure 16).

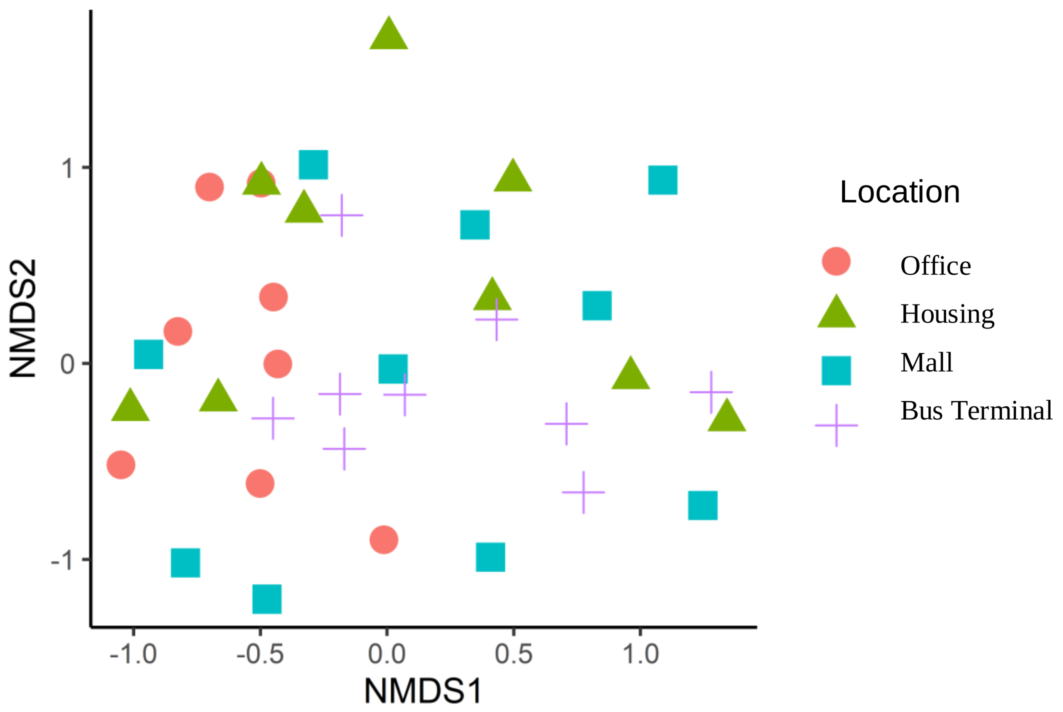


Figure 15. NonMetric Dimensional Scale (NMDS) ordination from ant species composition at four different area in dry season. BIO: Office. BS: Bus Terminal. ELS: Mall. PK: Housing

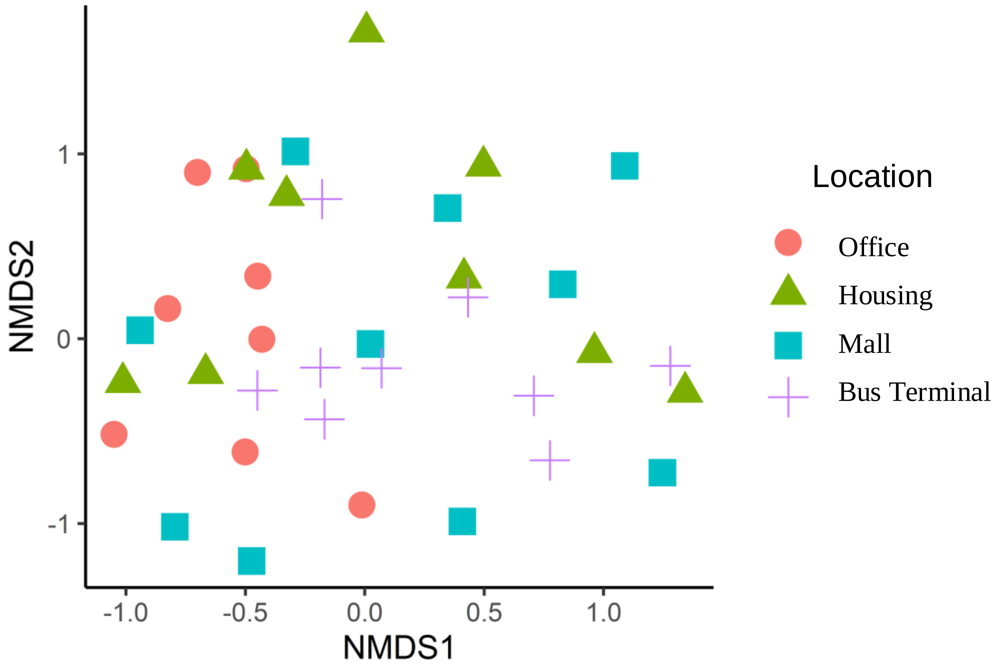


Figure 16. NonMetric Dimensional Scale (NMDS) ordination from ant species composition at four different areas in rainy season. BIO: Office. BS: Bus Terminal. ELS: Mall. PK: Housing

4.2 Preference of Cockroaches and Ants to Protein, Lipids and Sugar Based Bait

4.2.1 Preference of *Blattella germanica* Cockroach to Baits Through Chice and No Choice Test

Consumption of *B. germanica* cockroaches on several bait types, including protein, lipid, and sugar, showed a higher interest in combination baits than single baits (Table 5). The results showed that German cockroaches had a higher interest in the combination bait of ground chicken meat + 25% sugar solution (1:1) with an average feed consumption of 0.35 g (choice method) and 0.55 g (no choice method). The amount consumption of ground chicken meat + sugar in the test with the no-choice method was not different from the amount of sugar feed consumption, which was 0.54 g for 72 hours of feeding. The same thing also happened to the test results with the choice test method where the amount of feed sugar consumption was not much different from the combination of ground chicken + sugar, which was 0.31 g.

Meanwhile, the consumption of ground chicken meat in the choice and no-choice method was lower than sugar solution bait and a combination of ground chicken meat + sugar solution. The amount of ground chicken meat consumption in the test with the choice method was 0.19 g, while in the no-choice method, the amount was 0.45 g. This amount was not significantly different from the amount of sugar solution consumption and the combination of ground chicken meat + sugar solution based on Duncan's significant difference test results with a 95% confidence level. Based on these data, it was seen that *B. germanica* cockroaches had a higher preference for baits containing protein and sugar than baits containing lipids. The results of Duncan's significant difference test conducted with a 95% confidence level showed that the total consumption of all types of bait containing lipids in the no-choice treatment showed a significant difference with the bait containing protein and sugar. Thus, it appears that the *B. germanica* cockroach is less attracted to baits containing lipids (fats).

Table 5. Consumption of cockroaches on several types of bait either single or in combination with the method choice and no-choice for 72 hours of incubation.

Type of Bait	**∑ consumption (g)[a]**	
	Choice method	**No-choice method**
Chicken fat	0.07 c	0.39 bc
Ground chicken meat	0.19 abc	0.45 ab
25% sugar solution	0.31 ab	0.54 a
Chicken fat + Ground chicken meat (1:1)	0.21 abc	0.37 bc
Chicken fat + 25% sugar solution (1:1)	0.24 abc	0.34 bc
Ground chicken meat + 25% sugar solution (1:1)	0.35 a	0.55 a
Chicken fat + Ground chicken meat + 25% sugar solution (1:1)	0.26 ab	0.28 c

[a]The numbers in the same column followed by the same letter show results that are not significantly different based on Duncan's multiple interval test at the level of = 5%

The higher consumption of sugar bait compared to ground chicken meat bait, both in the treatment with choice and no-choice, indicated that *B. germanica* cockroaches preferred baits containing sugar over protein baits. Sugar which is one type of bait with high carbohydrate content, has a high enough effect on stimulating the feeding activity of German cockroaches. This is because sugar is straightforward to find around the environment or cockroach habitat and is contained in various types of food. In addition, to meet their nutritional needs, German cockroaches prefer the type of feed or bait that contains high carbohydrates compared to fat and protein. Carbohydrates are needed as the primary energy source for German cockroaches (Lauprasert et al. 2006, Carrel & Tanner 2002, Wada). - Katsumata & Schal 2021).

However, being an omnivorous insect, the German cockroach will eat almost any type of food it finds nearby (Reierson 1995). This can be seen from the consumption of cockroaches in all types of bait given. In addition to nutritional factors, the aroma and texture of the bait or feed given can also significantly affect the interest of German cockroaches. The texture of the baits given in this study was generally almost the same, namely soft and slightly runny except for the sugar bait in the form of a solution, so that all types of bait used in this study were suitable for the cockroach nymph phase. The cockroach nymph phase used in this study generally prefers soft foods in texture and liquid form. In this phase, cockroaches do not yet have a strong mandible to consume foods with solid or complex textures (Amalia & Harahap 2010). Because there was no difference in the texture of the bait used, the bait texture did not significantly affect the attraction of cockroaches to the bait used in this study.

Meanwhile, the aroma of the bait used in the study, both single and combination baits, can influence cockroach preferences. Cockroaches are known to often respond to the smell of food at close range. In tests using an olfactometer, cockroaches often survey the food or bait given to each olfactometer chamber before finally choosing the appropriate food or bait (Reierson 1995, Lauprasert *et al.* 2006). This behavior can also be seen from observing the cockroach's preference for each type of bait, either singly or in combination, based on its presence in each olfactometer chamber in the test using the choice method.

Based on the test results using the choice method, the presence of cockroaches was found in each type of bait on observation 72 hours after the release of cockroaches in the olfactometer. The highest percentage of German cockroach presence at 72 hours of observation was found in the combination bait of ground chicken meat + sugar solution, which was 27.2% and significantly different from other types of bait (Table 6). These results are in line with testing the preference of cockroaches for bait based on the amount of consumption. The type of bait most consumed is a combination of ground chicken + sugar. However, the frequency of the presence of cockroaches in other types of bait was not significantly different based on Duncan's significant difference test with a 95% confidence level.

The regression analysis results between the frequency of the presence of cockroaches and feed consumption resulted in the equation $y = 0.0035x + 0.1905$ and $R^2 = 0.1358$ (Figure 17). The value of R2, indicates no correlation between the frequency of cockroaches' presence and the consumption of each type of bait. According to Herma & Harahap (2010), the combination of baits visited by cockroaches in the test with the chosen method will not necessarily be consumed more. The presence of cockroaches in each type of bait is probably only part of the cockroach's observations of the several types of bait available in response to the aroma.

Table 6. Preference of cockroach on each type of bait combination based on its presence.

Type of bait	**Preference of Cockroach (%)**
Chicken fat	4.5 a
Ground chicken meat	3.5 b
25% sugar solution	5.1 b
Chicken fat + Ground chicken meat (1:1)	3.0 b
Chicken fat + 25% sugar solution (1:1)	1.0 b
Ground chicken meat + 25% sugar solution (1:1)	27.2 b
Chicken fat + Ground chicken meat + 25% sugar solution (1:1)	7.9 ab

[a]The numbers in the same column followed by the same letter show results that are not significantly different based on Duncan's multiple interval test at the level of = 5%

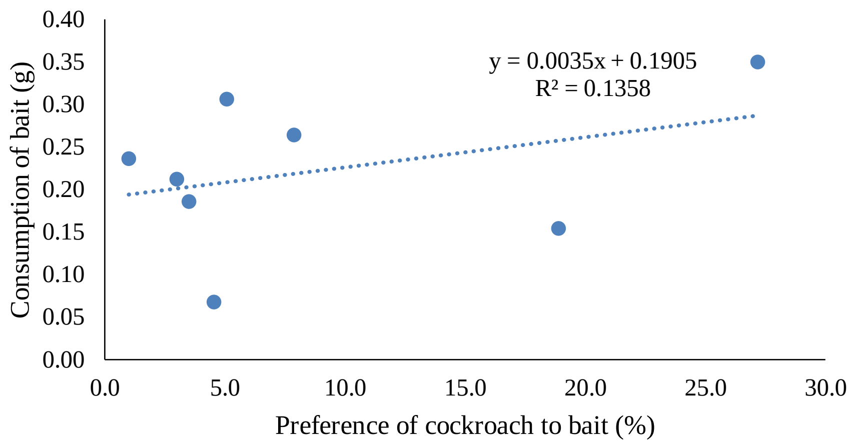


Figure 17. The results of the regression analysis between the frequency of the presence and the amount of consumption

4.1.1 Preference of *Anoplolepis gracilipes* Ants to Baits Through Choice and No Choice Test

Testing the preference of ants on several types of bait through the choice and no-choice was carried out using *A. gracilipes* ants. This type of ant was chosen because of its high abundance in the office area and its availability throughout the season (dry season and rainy season). Testing of ant preferences with the choice method was carried out through trapping and the observation was conducted by recording the recruitment time or the time of the first arrival of ants on each bait. In addition, the level of ant preference to bait was evaluated based on the number of ants trapped on each type of bait for 72 hours of trapping.

The trapping results showed that *A. gracilipes* ants needed the shortest time to find the combination bait of ground chicken meat + sugar solution, which was about 30 minutes. Then followed by the bait of chicken fat + ground chicken meat for 56 minutes, and the combination bait of chicken fat + meat ground chicken + sugar for 72 minutes. Meanwhile, for other types of bait, both singly and in combination, the arrival of *A. gracilipes* ants was recorded more than 90 minutes after baited traps were placed.

Based on the data in Table 7, the trap with the highest number of ants was a trap containing a sugar solution bait and a combination of ground chicken meat + sugar bait. However, the results of Duncan's significant difference test conducted at a 95% confidence level showed no significant difference in ant preferences for each type of bait assessed based on their presence, both before and after the data was transformed (Appendix 4). This is thought to have occurred because there were several repetitions of traps on each type of bait that ants did not visit for 72 hours of trapping.

Table 7. The number of *Anoplolepis gracilipes* ants trapped in each type of bait, either singly or in combination with the no-choice test method for 72 hours of trapping.

Type of bait	**∑ ants (individu)[a]**
Chicken fat	33.0 a
Ground chicken meat	20.3 a
25% sugar solution	52.2 a
Chicken fat + Ground chicken meat (1:1)	1.0 a
Chicken fat + 25% sugar solution (1:1)	17.4 a
Ground chicken meat + 25% sugar solution (1:1)	47.0 a
Chicken fat + Ground chicken meat + 25% sugar solution (1:1)	28.9 a

[a]The numbers in the same column followed by the same letter show results that are not significantly different based on Duncan's multiple interval test at the level of = 5%

Meanwhile, the ant preference test conducted with the choice method showed slightly different results from the no-choice method. The presence of ants and the highest bait consumption were found in sugar solution bait, followed by chicken fat + sugar bait (Table

8). In the no-choice test, the average presence of *A. gracilipes* ants on chicken fat + sugar bait was only 17.4 or the second lowest compared to other baits. This result was inversely proportional to the results of the choice method. The regression analysis results on the number of ant presence and the amount of bait consumption in the test with the choice method resulted in the equation y = 0.0071x + 0.373 and the value of R2 = 0.705 (Figure 18). This shows that 70.5% of ant bait consumption is influenced by the presence of ants in the bait.

Table 8. The preference of ants on each type of bait combination was based on the percentage of their presence and the number of baits consumed at 72 hours of observation.

Type of bait	Preference (%)[a]	∑ Consumption of bait (g)
Chicken fat	2.56 c	0.43 bc
Ground chicken meat	5.28 bc	0.48 b
25% sugar solution	46.32 a	0.74 a
Chicken fat + Ground chicken meat (1:1)	2.32 c	0.43 bc
Chicken fat + 25% sugar solution (1:1)	22.8 b	0.47 b
Ground chicken meat + 25% sugar solution (1:1)	6.24 bc	0.47 b
Chicken fat + Ground chicken meat + 25% sugar solution (1:1)	7.52 bc	0.39 bc

[a]The numbers in the same column followed by the same letter show results that are not significantly different based on Duncan's multiple interval test at the level of = 5%

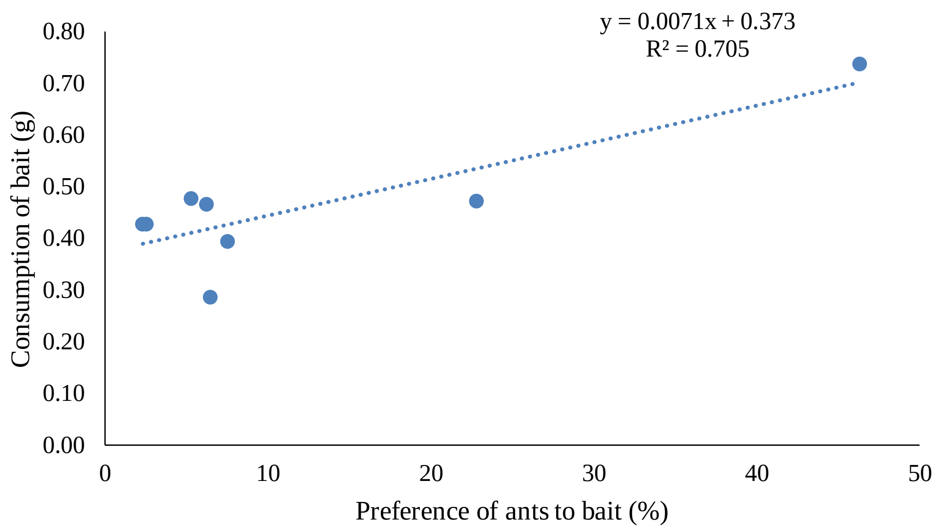


Figure 18. The results of the regression analysis between the frequency of the ant presence and the amount of consumption

In general, ants have a high preference for food with carbohydrate content, which can vary temporally and spatially. Several factors that can influence ant preferences in choosing their food include eating habits or nutritional history that are commonly consumed previously and the presence of alternative feed foods (Cook *et al*. 2011, Abbott *et al*. 2014). The critical food sources for invasive ants are protein which is generally obtained from other prey animals, and liquid carbohydrates, which can be obtained from honeydew, nectar, and extrafloral nectar (Holway *et al.* 2002, Helms & Vinson 2008).

4.2 Toxicity of *Cerbera manghas* Seed Extract and *Tephrosia vogelii* Leaf Extract against Cockroach *Blattella germanica*

Testing the effectiveness of *T. vogelii* leaves and *C. manghas* extracts was conducted against the third instar of *B. germanica* cockroaches. Extraction of *T. vogelii* leaves using ethyl acetate resulted in a dark green extract with a slightly sticky texture like a paste. Meanwhile, the extraction of *C. manghas* carried out using methanol as a solvent produced light brown and dark brown extracts with a more liquid and oily extract texture (Figure 19).

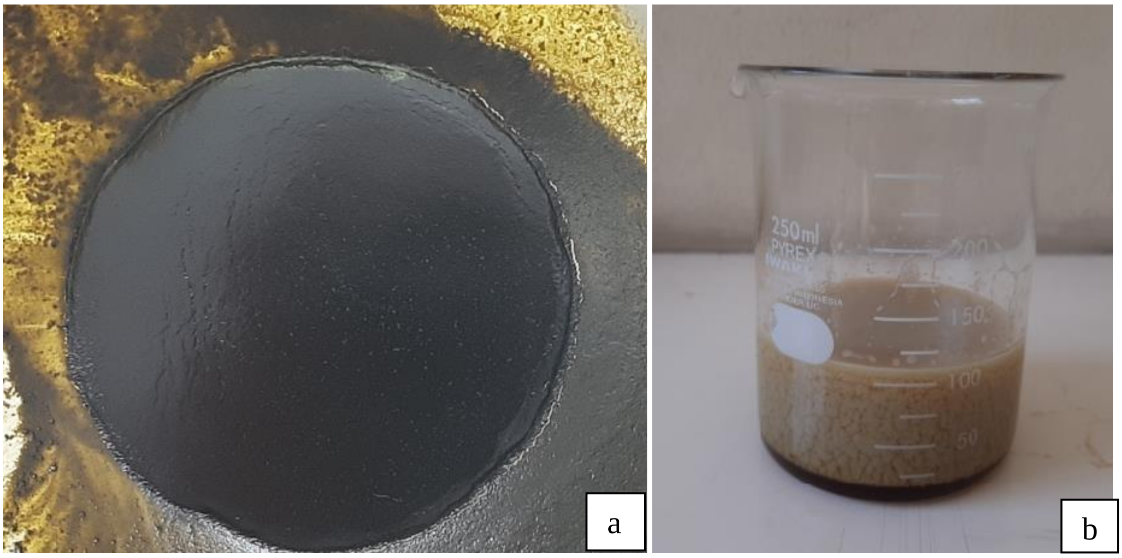


Figure 19. Extract of *Tephrosia vogelii* leaves (a) and *Cerbera manghas* seeds (b)

The test results showed that both extracts had fairly good toxicity against the third instar German cockroach. The LC_{50} and LC_{95} values of the *T. vogelii* extract against the third instar German cockroach were 0.023% and 0.058%, respectively. Meanwhile, the toxicity of *C. manghas* extract was lower than that of *T. vogelii* against the third instar German cockroach. The LC_{50} and LC_{95} values of *C. manghas* extract against the third instar German cockroach were 0.027% and 0.073%, respectively (Table 9). The high toxicity of *T. vogelii* extract against the third instar German cockroach was also indicated by the higher probit regression intercept value (a value) and higher b value. A higher b value will result in a steeper regression graph. This indicates that adding a certain amount of concentration will kill the test insects more in the treatment with *T. vogelii* extract compared to the treatment with *C. manghas* extract.

Table 9. Toxicity parameter estimation of *Tephrosia vogelii* and *Cerbera manghas* extracts against *Blattela germanica* cockroaches at 72 hours after treatment

Extract[a]	*a* ± GB[b]	*b* ± GB[b]	LC_{50} (SK 95%)(%)[a]	LC_{95} (SK 95%)(%)[a]
TV	6.687 ± 4.079	1.118 ± 0.692	0.023 (0.015 – 0.031)	0.058 (0.040 – 0.228)
CM	5.932 ± 3.779	1.026 ± 0.649	0.027 (0.020 – 0.036)	0.073 (0.049 – 0.269)

[a]TV: *T. vogelii*. CM: *C. manghas*
[b]*a*: probit regression intercept. *b*: probit regression slope. GB: standard error. SK: confident level. LC: Lethal concentration.

However, further testing related to the toxicity of the extracts of *T. vogelii* and *C. manghas* against German cockroaches with higher instars showed a decrease in the toxicity of the two extracts. Toxicity testing of *T. vogelii* and *C. manghas* extract at LC_{50} and LC_{95} values did not cause mortality in German cockroaches with 5-6 instars and imago. Two things probably caused the absence of mortality in German cockroaches with higher instars. The first one is the insect's rejection of the treatment given so that the cockroaches did not want to eat and remained alive for 72 hours of observation. Another possibility is that the concentrations contained in the extract, namely LC_{50} and LC_{95} for the third instar german cockroaches, are not toxic enough to 5-6 instar german cockroaches or imago.

In this case, it is necessary to carry out further testing to make the toxicant contained in the bait will not cause a rejection effect (repelent) but remain at a level that can be lethal (LC_{95}). However, suppose this is difficult to achieve. In that case, another alternative is applying a combination of bait with vegetable extracts focused on cockroach nymphs that have just hatched until they are around third instars. Several extracts and essential oils have been known to be lethal or only as a repellant to German cockroaches. Leaf and fruit extract of *Schimus mole* L. is known to repel German cockroaches and kill cockroaches when applied topically (Ferreo *et al.* 2007). Sitticok *et al.* (2013) test results showed that eight types of essential oils could cause a knockdown effect at a dose of 0.24 ul/cm^2 at one hour after treatment and 100% mortality at 24 hours after treatment. However, the application of these essential oils could not prevent the hatching of the ooteka, so repeated applications were needed to prevent reinfestation (Phillips & Apple 2010).

4.3 Preference of the Cockroach *Blattella germanica* and the Ant *Anoplolepis gracilipes* to the Combination Baits of Botanical Insecticides

The preference test of *B. germanica* and *A. gracilipes* to baits that have been combined with extracts aims to assess the possibility of rejection of the two types of insects to baits containing toxicants. The test was carried out using a combination bait of ground chicken meat + sugar solution (DG) which was the type of bait most preferred by cockroaches based on the results of the previous preference test. The interestingly results showed on the preference test of the cockroach which were calculated based on the number of cockroaches present in each type of bait used, includes a combination of ground chicken meat + sugar solution (DG), a combination of DG bait mixed with extract (DGE), and an empty container without bait as a control. Most of the cockroaches chose to enter the control box or without bait (Figure 20).

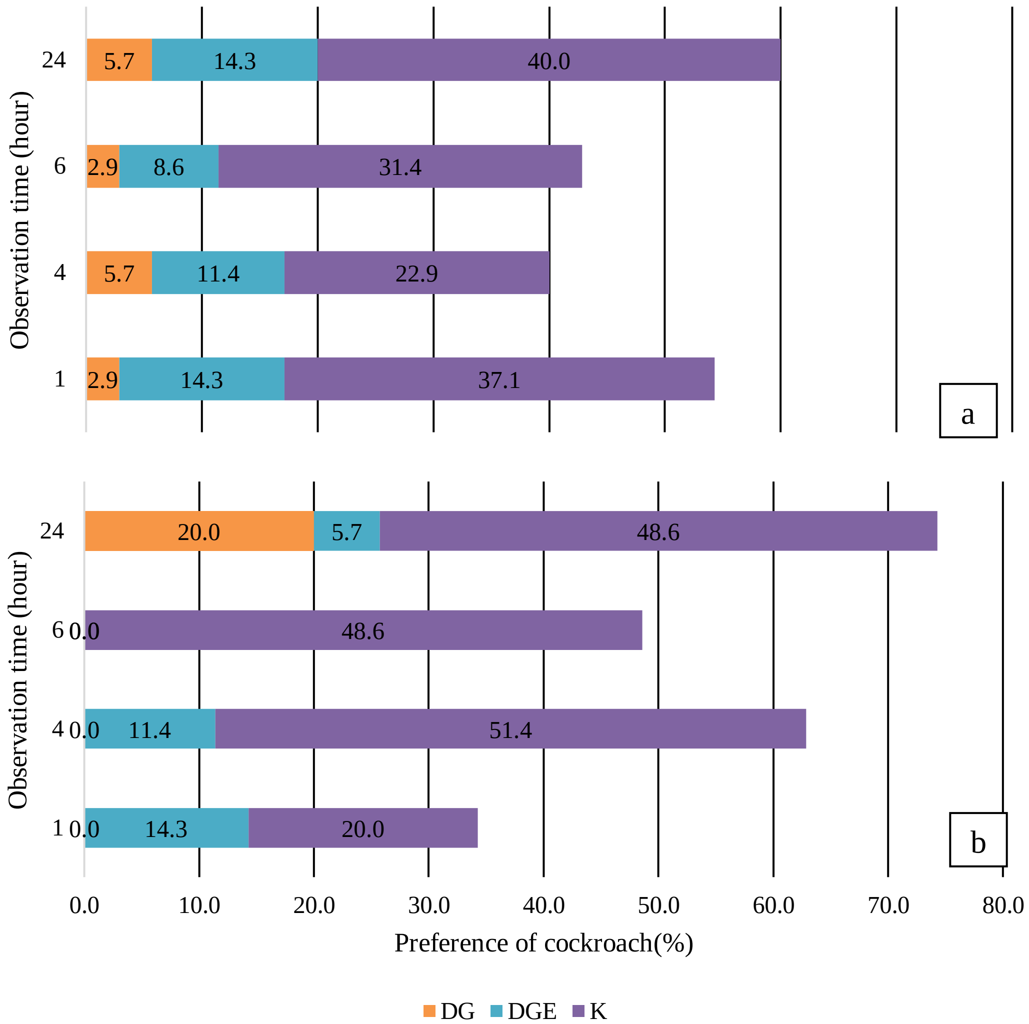


Figure 20. The preference of the *Blattella germanica* cockroach to the combination of ground chicken meat and 25% sugar solution (DG), and the combination of DG bait with extracts (DGE) of *Tephrosia vogelii* (a) and *Cerbera manghas* (b)

However, when compared between the DG and DGE treatment containers, it was seen that German cockroaches tended to visit the DGE container compared to the DG container during 24 hours of observation, except for the *C. manghas* extract with a 24-hour observation time. This can predict that the possibility of bait mixed with extract in the ratio (bait: extract; 2: 1) does not cause a repellent effect on a cockroach. So, it has the potential to be used as a toxicant in the bait used. Further testing needs to be done by observing the amount of bait consumed by German cockroaches over a certain period.

The opposite occurred in testing the ants' preferences for bait combinations with botanical insecticide. The test results showed that the presence of ants on baits combined with both types of extracts was much lower than ants on baits without extract and control (Figure 21). This indicates that there is a potential ant repulsion effect on baits combined with extracts, so the addition of extracts to baits with these compositions needs to be re-evaluated

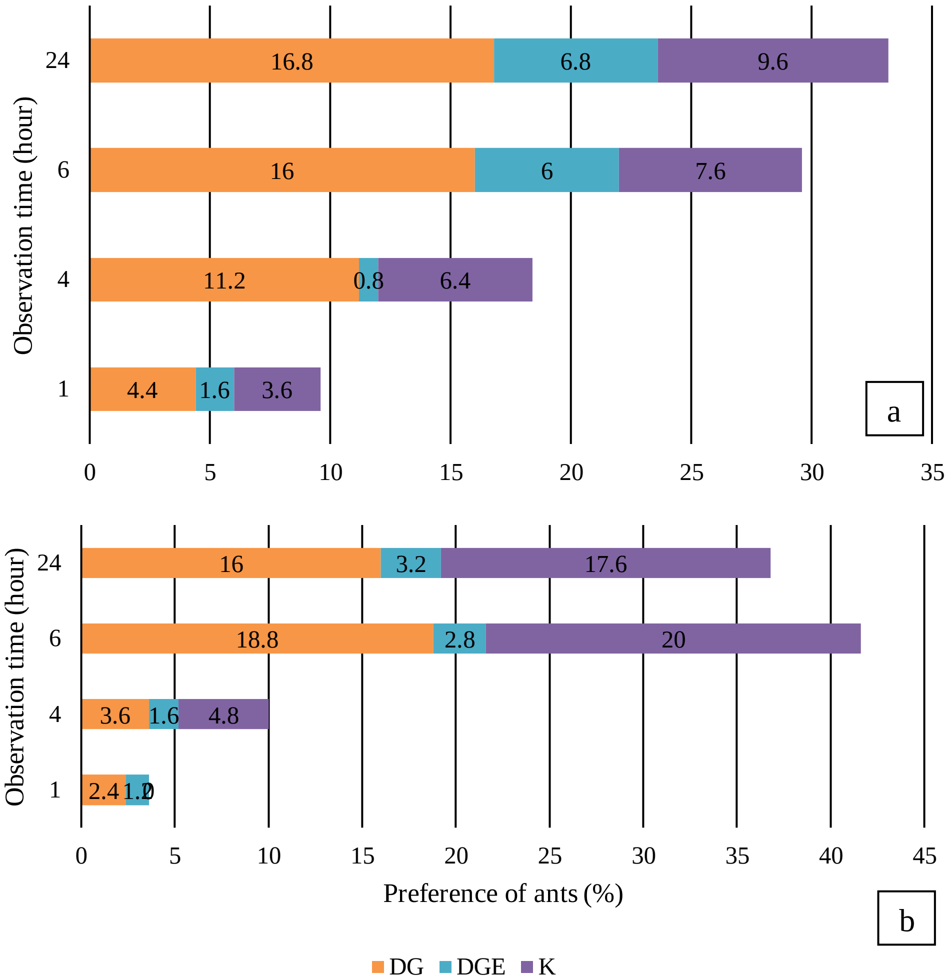


Figure 21. The preference of the *Anoplolepis gracilipes* ants to the combination of ground chicken meat and 25% sugar solution (DG), and the combination of DG bait with extracts (DGE) of *Tephrosia vogelii* (a) and *Cerbera manghas* (b)

5. CONCLUSION

The inventory and identification obtained 7532 individual ants from 4 subfamilies, namely Dolichoderinae, Formicinae, Myrmicinae, and Ponerinae, as well as 28 species. The analysis of the diversity of ant species at each location showed that the ant species in the bus terminal habitats were not significantly different from the mall area. However, the diversity of ant species in three locations, namely bus terminals, malls, and housing estates, showed significant differences with the diversity of ant species in office habitats. The study of seasonal differences did not show any effect on the diversity of ant species in each location. In the bait preference test, the cockroach *Blattella germanica* and the ant *Anoplolepis gracilipes* showed different preferences for the type of bait tested. The *B. germanica* cockroach showed a high preference for the combination of ground chicken meat + 25% sugar solution (1:1). In addition, the *A. gracilipes* ants preferred carbohydrate (sugar) and lipid-based baits, where the highest preference was found in 25% sugar solution baits followed by a combination of sugar + chicken fat solution (1:1). The LC_{50} and LC_{95} values of the *T. vogelii* extract against the third instar German cockroach were 0.023% and 0.058%, respectively. Meanwhile, the toxicity of *C. mangha*s extract was lower than that of *T. vogelii* against the third instar German cockroach. The LC_{50} and LC_{95} values of *C. manghas* extract against the third instar German cockroach were 0.027% and 0.073%, respectively. The addition of *T. vogelii* and *C. manghas* extracts to the bait indicated a potential repellent effect by both types of insects. Thus, the addition of extracts to baits with these compositions needed to be re-evaluated.

6. PRINCIPAL INVESTIGATOR AND OTHER RESEARCHERS

Research Coordinator	: Dr. Idham Sakti Harahap
Research Members	: Trijanti A. Widinni Asnan, SP. M,Si. Herni Widhiastuti, S.Si.
Assistant	: Heri Yanto

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APPENDICES

Appendix 1. Analysis of variance of cockroach preference testing with no-choice method

The SAS System 13:50 Thursday, November 18, 2021 1

The GLM Procedure
Class Level Information

Class	Levels	Values
PERL	7	D DG G L LD LDG LG

Number of observations 35

The GLM Procedure

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.30948000	0.05158000	6.59	0.0002
Error	28	0.21916000	0.00782714		
Corrected Total	34	0.52864000			

R-Square	Coeff Var	Root MSE	Y Mean
0.585427	21.26710	0.088471	0.416000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PERL	6	0.30948000	0.05158000	6.59	0.0002

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PERL	6	0.30948000	0.05158000	6.59	0.0002

The GLM Procedure
Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	28
Error Mean Square	0.007827

Number of Means	2	3	4	5	6	7
Critical Range	.1146	.1204	.1242	.1269	.1289	.1305

Means with the same letter are not significantly different.

```
Duncan Grouping        Mean     N   PERL
              A      0.54600    5   DG
              A
              A      0.54200    5   G
              A
         B    A      0.44800    5   D
         B
         B    C      0.38800    5   L
         B    C
         B    C      0.37200    5   LD
         B    C
         B    C      0.34000    5   LG
         C
         C       0.27600    5   LDG
```

Appendix 2. Analysis of variance of cockroach preference testing with choice test method

The SAS System 13:50 Thursday, November 18, 2021 4

The GLM Procedure

Class Level Information

Class	Levels	Values
PERL	8	B D DG G L LD LDG LG

Number of observations 40

The GLM Procedure

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	0.27568000	0.03938286	2.47	0.0383
Error	32	0.51096000	0.01596750		
Corrected Total	39	0.78664000			

R-Square	Coeff Var	Root MSE	Y Mean
0.350453	56.92008	0.126363	0.222000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PERL	7	0.27568000	0.03938286	2.47	0.0383

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PERL	7	0.27568000	0.03938286	2.47	0.0383

The GLM Procedure

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	32
Error Mean Square	0.015968

Number of Means	2	3	4	5	6	7	8
Critical Range	.1628	.1711	.1765	.1804	.1833	.1856	.1874

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	PERL
A	0.35000	5	DG
A			
B A	0.30600	5	G
B A			
B A	0.26400	5	LDG
B A			
B A C	0.23600	5	LG
B A C			
B A C	0.21200	5	LD
B A C			
B A C	0.18600	5	D
B C			
B C	0.15400	5	B
C			
C	0.06800	5	L

Appendix 3. The frequency of the cockroaches presence in preference testing with choice test method

The SAS System 13:50 Thursday, November 18, 2021 7

The GLM Procedure

Class Level Information

Class	Levels	Values
PERL	8	B D DG G L LD LDG LG

Number of observations 40

The GLM Procedure

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	2970.40749	424.34393	1.70	0.1433
Error	32	7967.72501	248.99141		
Corrected Total	39	10938.13249			

R-Square	Coeff Var	Root MSE	Y Mean
0.271564	177.8003	15.77946	8.874825

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PERL	7	2970.407486	424.343927	1.70	0.1433

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PERL	7	2970.407486	424.343927	1.70	0.1433

The GLM Procedure

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	32
Error Mean Square	248.9914

Number of Means	2	3	4	5	6	7	8
Critical Range	20.33	21.37	22.04	22.52	22.89	23.17	23.41

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	PERL
	A	27.192	5	DG
A				
B	A	18.820	5	B
B	A			
B	A	7.872	5	LDG
B				
B		5.076	5	G
B				
B		4.538	5	L
B				
B		3.500	5	D
B				
B		3.000	5	LD
B		1.000	5	LG

Appendix 4. The presence of ants in traps of various bait with no choice method

The SAS System 13:50 Thursday, November 18, 2021 10

The GLM Procedure

Class Level Information

Class	Levels	Values
PERL	8	D DG G K L LD LDG LG

Number of observations 40

The GLM Procedure

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	9120.17500	1302.88214	0.58	0.7630
Error	32	71281.60000	2227.55000		
Corrected Total	39	80401.77500			

R-Square	Coeff Var	Root MSE	Y Mean
0.113433	203.6545	47.19693	23.17500

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PERL	7	9120.175000	1302.882143	0.58	0.7630

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PERL	7	9120.175000	1302.882143	0.58	0.7630

The GLM Procedure

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	32
Error Mean Square	2227.55

Number of Means	2	3	4	5	6	7	8
Critical Range	60.80	63.91	65.92	67.37	68.46	69.32	70.01

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	PERL
A	52.20	5	G
A			
A	35.00	5	DG
A			
A	28.80	5	LDG
A			
A	26.60	5	L
A			
A	17.40	5	LG
A			
A	17.20	5	D
A			
A	5.00	5	K
A			
A	3.20	5	LD

The GLM Procedure

Class Level Information

Class	Levels	Values
PERL	8	D DG G K L LD LDG LG

Number of observations 40

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	68.6768975	9.8109854	0.77	0.6169
Error	32	407.9856000	12.7495500		
Corrected Total	39	476.6624975			

R-Square	Coeff Var	Root MSE	Y Mean
0.144079	104.0930	3.570651	3.430250

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PERL	7	68.67689750	9.81098536	0.77	0.6169

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PERL	7				

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	32
Error Mean Square	12.74955

Number of Means	2	3	4	5	6	7	8
Critical Range	4.600	4.835	4.987	5.096	5.179	5.244	5.296

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	PERL
A	5.466	5	G
A			
A	4.568	5	DG
A			
A	4.252	5	LDG
A			
A	3.840	5	D
A			
A	3.468	5	L
A			
A	2.712	5	LG
A			
A	1.578	5	K
A			
A	1.558	5	LD

Appendix 5. Testing the preference of ants on various types of bait with the choice test method

The SAS System 15:32 Saturday, November 20, 2021

The GLM Procedure

Class Level Information

Class	Levels	Values
PERL	8	B D DG G L LD LDG LG

Number of observations 40

The GLM Procedure

Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	8023.23200	1146.17600	7.34	<.0001
Error	32	4997.82400	156.18200		
Corrected Total	39	13021.05600			

R-Square	Coeff Var	Root MSE	Y Mean
0.616174	100.4604	12.49728	12.44000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PERL	7	8023.232000	1146.176000	7.34	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PERL	7	8023.232000	1146.176000	7.34	<.0001

The GLM Procedure

Duncan's Multiple Range Test for Y

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	32
Error Mean Square	156.182

Number of Means	2	3	4	5	6	7	8
Critical Range	16.10	16.92	17.46	17.84	18.13	18.35	18.54

Means with the same letter are not significantly different.

Duncan Grouping		Mean	N	PERL
	A	46.320	5	G
	B	22.800	5	LG
	B			
C	B	7.520	5	LDG
C	B			
C	B	6.480	5	B
C	B			
C	B	6.240	5	DG
C	B			
C	B	5.280	5	D
C				
C		2.560	5	L
C				
C		2.320	5	LD

Appendix 6. The data of ant bait consumption on preference testing with choice test method

The SAS System 15:32 Saturday, November 20, 2021

The GLM Procedure
Class Level Information

Class	Levels	Values
PERL	8	B D DG G L LD LDG LG

Number of observations 40

The GLM Procedure
Dependent Variable: Y

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	0.56533750	0.08076250	7.22	<.0001
Error	32	0.35784000	0.01118250		
Corrected Total	39	0.92317750			

R-Square	Coeff Var	Root MSE	Y Mean
0.612382	22.90143	0.105747	0.461750

Source	DF	Type I SS	Mean Square	F Value	Pr > F
PERL	7	0.56533750	0.08076250	7.22	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
PERL	7	0.56533750	0.08076250	7.22	<.0001

The GLM Procedure

Duncan's Multiple Range Test for Y
NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	32
Error Mean Square	0.011183

Number of Means	2	3	4	5	6	7	8
Critical Range	.1362	.1432	.1477	.1509	.1534	.1553	.1569

Means with the same letter are not significantly different.

Duncan Grouping	Mean	N	PERL
A	0.73800	5	G
B	0.47800	5	D
B			
B	0.47200	5	LG
B			
B	0.46600	5	DG
B			
C B	0.42800	5	LD
C B			
C B	0.42800	5	L
C B			
C B	0.39400	5	LDG
C			
C	0.29000	5	B

Appendix 7. The diversity analysis results of ant species diversity in four different habitats

S	std	r	Min	Max	
Mall	6.3	2.057507	10	3	10
Perkantoran	3.3	1.636392	10	1	5
Perumahan	4.6	1.074968	10	3	6
Terminal	6.9	2.424413	10	3	10

Alpha: 0.05 ; DF Error: 36

Critical Value of Studentized Range: 3.808798

Minimun Significant Difference: 2.24884

Treatments with the same letter are not significantly different.

	S	groups
Terminal	6.9	a
Mall	6.3	ab
Perumahan	4.6	bc
Perkantoran	3.3	c

Appendix 8. Estimator of toxicity parameter of *Tephrosia vogelii* leaf extract against the third instar *Blattella. germanica* cockroach

```
POLO-PC
(C) Copyright LeOra Software 1987

Input file >
input: = uji insektisida tep terhadap kecoa B. germanica
input: = lima taraf dosis plus kontrol
input: = lima ulangan per perlakuan, 5 imago per perlakuan
input: = data mortalitas 72 jam setelah perlakuan
input: = Konsentrasi (%), jumlah serangga uji, jumlah serangga
   mati
input: *insecta
input: 0 25 0
input: 0.0125 25 5
input: 0.02 25 9
input: 0.03 25 15
input: 0.04 25 20
input: 0.05 25 25

preparation     dose      log-dose     subjects  responses
   resp/subj
insecta        .00000      .000000        25.         0.
   .000
               .01250    -1.903090        25.         5.
   .200
               .02000    -1.698970        25.         9.
   .360
               .03000    -1.522879        25.        15.
   .600
               .04000    -1.397940        25.        20.
   .800
               .05000    -1.301030        25.        25.
   1.000

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
   you want the likelihood function to be maximized [Y] ? LD's to
   calculate [10 50 90] > Do you want to specify starting values of
   the parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
   function

Maximum log-likelihood  -61.296183

            parameter       standard error      t ratio
insecta     6.6875473        1.0574493        6.3242253
SLOPE       4.0787653        .65979068        6.1819081

Variance-Covariance matrix
                  insecta       SLOPE
  insecta         1.118199      .6924354
  SLOPE           .6924354      .4353237


Chi-squared goodness of fit test
```

```
preparation   subjects   responses   expected    deviation
   probability
insecta          25.         5.         3.531       1.469
   .141252
                 25.         9.        10.108      -1.108
   .404331
                 25.        15.        17.075      -2.075
   .682992
                 25.        20.        20.946       -.946
   .837854
                 25.        25.        22.909       2.091
   .916353

chi-square 4.2564        degrees of freedom   3    heterogeneity
   1.4188

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near
   0 or 1
are especially troublesome.  A plot of the data should be
   consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
    g(.90)=.20562            g(.95)=.37601
   g(.99)=1.2666

"With almost all good sets of data, g will be substantially
   smaller than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5


Effective Doses
                     dose    limits       0.90        0.95
   0.99
LD50  insecta       .02293  lower      .01754      .01482
                            upper      .02828      .03096
LD95  insecta       .05803  lower      .04264      .03954
                            upper      .12049      .22764


 uji insektisida tep terhadap kecoa B. germanica
   insecta    subjects 125   controls 25
     log(L)=-61.30   slope=4.079+.660   nat.resp.=.000+.000
     heterogeneity=1.42   g=.376
     LD50=.023   limits: .015 to .031
     LD95=.058   limits: .040 to .228

Stop - Program terminated.
```

Appendix 9. Estimator of toxicity parameter of *Cerbera manghas* extract against the third instar *Blattella germanica* cockroach

```
POLO-PC
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Input file >
input: = uji insektisida c. manghas terhadap kecoa B. germanica
input: = lima taraf dosis plus kontrol
input: = lima ulangan per perlakuan, 5 imago per perlakuan
input: = data mortalitas 72 jam setelah perlakuan
input: = Konsentrasi (%), jumlah serangga uji, jumlah serangga
   mati
input: *insecta
input: 0 25 0
input: 0.0125 25 3
input: 0.02 25 9
input: 0.03 25 11
input: 0.04 25 18
input: 0.05 25 23

preparation     dose      log-dose     subjects  responses
   resp/subj
insecta        .00000      .000000        25.         0.
   .000
               .01250    -1.903090        25.         3.
   .120
               .02000    -1.698970        25.         9.
   .360
               .03000    -1.522879        25.        11.
   .440
               .04000    -1.397940        25.        18.
   .720
               .05000    -1.301030        25.        23.
   .920

Number of preparations:  1
Number of dose groups:   5
Do you want probits [Y] ? Is Natural Response a parameter [Y] ? Do
   you want the likelihood function to be maximized [Y] ? LD's to
   calculate [10 50 90] > Do you want to specify starting values of
   the parameters [N] ?
The probit transformation is to be used
The parameters are to be estimated by maximizing the likelihood
   function

Maximum log-likelihood  -66.127880

            parameter       standard error      t ratio
insecta     5.9321764        1.0127677        5.8573910
SLOPE       3.7791174        .64608126        5.8492912

Variance-Covariance matrix
                  insecta       SLOPE
  insecta         1.025698      .6492946
  SLOPE           .6492946      .4174210


Chi-squared goodness of fit test
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```
preparation   subjects   responses   expected    deviation
   probability
insecta          25.         3.        2.597        .403
   .103866
                 25.         9.        7.816       1.184
   .312622
                 25.        11.       14.257      -3.257
   .570261
                 25.        18.       18.547       -.547
   .741894
                 25.        23.       21.126       1.874
   .845050

chi-square 3.1971        degrees of freedom   3    heterogeneity
   1.0657

A large chi-square  indicates a poor fit of the data by the probit
analysis model.  Large deviations for expected probabilities near
   0 or 1
are especially troublesome.  A plot of the data should be
   consulted.
See D. J. Finney, "Probit Analysis" (1972), pages 70-75.

Index of significance for potency estimation:
    g(.90)=.17251            g(.95)=.31547
   g(.99)=1.0627

"With almost all good sets of data, g will be substantially
   smaller than
1.0, and seldom greater than 0.4."
         - D. J. Finney, "Probit Analysis" (1972), page 79.

We will use only the probabilities for which g is less than 0.5


Effective Doses
                     dose    limits       0.90        0.95
   0.99
LD50  insecta         .02693  lower      .02180      .01956
                              upper      .03283      .03597
LD95  insecta         .07337  lower      .05298      .04896
                              upper      .15221      .26870


 uji insektisida c. manghas terhadap kecoa B. germanica
   insecta    subjects 125   controls 25
     log(L)=-66.13   slope=3.779+.646   nat.resp.=.000+.000
     heterogeneity=1.07   g=.315
     LD50=.027   limits: .020 to .036
     LD95=.073   limits: .049 to .269

Stop - Program terminated.
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