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Phytochemical Analysis Of Babadotan Leaves Extract In Flour Beet Control

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Abstract

Tribolium castaneum is one of the warehouse insects that attack post-harvest products. More flour beetles are found in processed post-harvest products such as wheat flour and rice flour. this is because wheat flour has a higher protein content. This study aims to determine the effect of Ageratum conyzoides leaf extract in controlling flour beetles. The design of this study is a complete one-way random design. This study uses 5 different concentrations. Namely 0%, 25%, 50%, 75% and 100%. Babadotan leaf extract was macerated using 96% ethanol. There are 10 flour beetles. To determine the effect of babadotan leaf extract, this observation was carried out using a Y-olfactometer. In addition to knowing what anti-insecta compounds contained in babadotan leaf extract, the extract was analyzed by phytochemical analysis by GC-MS method. The results of the analysis of the effects of babdotan leaf extract were analyzed by Anova one-way statistics and advanced LSD test. One-way Anova results showed a difference in the effect of the concentration of babadotan leaf extract. increasing the amount of concentration shows different effects of repellent. Based on the results of the study, the use of 100% babadotan leaf extract concentration gives a high repellent effect in controlling flour beetles.

Abstrak

Kumbang tepung (*Tribolium castaneum)* adalah salah satu serangga gudang yang menyerang produk pasca panen. Kumbang tepung lebih banyak ditemukan pada produk pasca panen yang sudah diolah seperti tepung gandum dan tepung beras. hal ini dikarenakan tepung gandum memiliki kandungan protein yang lebih tinggi. penelitian ini bertujuan untuk mengetahui efek dari ekstrak daun babadotan dalam mengendalikan kumbang tepung. Rancangan penelitian ini adalah rancangan acak lengkap satu arah. Penelitian ini menggunakan 5 konsentrasi yang berbeda. Yaitu 0%;25%;50%;75% dan 100%. Esktrak daun babadotan di maserasi dengan menggunakan etanol 96%. Kumbang tepung yang digunakan sebanyak 10 ekor. Untuk mengetahui efek dari ekstrak daun babadotan maka pengamatan ini dilakukan dengan menggunakan olfaktometer bentuk Y. Selain itu untuk mengetahui senyawa anti insecta apa yang terkandung dalam ekstrak daun babadotan, maka ekstrak di analisis fitokimia dengan metode GC-MS. Hasil analisis efek ekstrak daun babdotan dianalisis statistika Anova satu arah dan uji lanjut BNT. Hasil Anova satu arah menunjukkan adanya perbedaan pengaruh konsentrasi ekstrak daun babadotan. pertambahan jumlah konsentrasi menunjukkan efek repelen yang berbeda-beda. Berdasarkan hasil penelitian, penggunaan konsentrasi ekstrak daun babadotan seesar 100% memberikan efek repelen tinggi dalam mengendalikan kumbang tepung.

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PRELIMINARY

The main problem in storage technology for agricultural food stuffs is the difficulty in controlling the presence of destructive agents that can reduce the quantity and quality of stored materials. Agents that cause damage to stored food include microorganisms (especially bacteria, molds and yeasts), warehouse insects, rodents and several types of birds. Among the warehouse pests that cause the most damage are insects (Jusuf, 2015). Warehouse insects can cause up to 10% loss of stored materials, even in some tropical and subtropical countries yield losses can reach up to 50% (Sjam, 2014). One of the warehouse insects that contribute to damage to rice and post-harvest products is the type of flour beetle (Tribolium castaneum) (Subagiya et al, 2018). According to Zapata et al., (2010) Tribolium castaneum is one of the most destructive warehouse insects in the world. The flour beetle (Tribolium castaneum) is one of the fast reproducing warehouse pests. This is due to the ability of female insects to lay eggs in numbers with a relatively short life cycle. A female flour beetle can produce more than one million in just 150 days (Hanum, 2012).

Barn pests also have a good sense of smell to detect food sources. The nutrients contained in wheat flour finally attracted the attention of Tribolium castaneum warehouse pests. If wheat flour loses its nutritional content, it can cause the weight of the flour to shrink. This is because Tribolium castaneum is able to bore flour grains into finer grains. If this is allowed to do so, the flour will experience a change in odor and aroma due to the benzokuinone compound excreted by Tribolium castaneum.

Preventive efforts in controlling warehouse insects are currently still dominated by the use of chemical insecticides (synthetic) with the fumigation method. Fumigation is the act of spraying with a volatile liquid material using certain fumigants in an airtight space, as well as at certain temperatures and pressures. Fumigant is a type of insecticide which at a certain temperature and pressure is in the form of a gas and in a certain concentration and time can kill pests (Acker, 2009). Synthetic insecticides in the control of warehouse insects that are still used include fumigants made from methyl bromide (MB) and sulfuryl fluoride. The use of chemical pesticides such as methyl bromide as post-harvest fumigant is also very dangerous, this is because methyl bromide is a substance that can damage the ozone layer (Zhang et al., 2004). Some of the newer generation insecticides that are frequently used include deltamethrin, methyl pyrimiphos, methyl chlorphyrifos, ciflutrin, S-bioalletrin, and bifentrin. The results of research by Rasipin et al. (2012) showed that the intensive use of pesticides has an effect on the increase in cases of swelling of the thyroid gland (goiter) in children in agricultural production centers. In its application, synthetic insecticides still have weaknesses, including being toxic to the environment. If used continuously, it can cause resistance to insects, causing residues that pollute the environment (Haryadi, 2010). According Emecki (2010), methyl bromide can damage the ozone layer, while the use of sulfuryl fluoride gas can increase the effect of greenhouse gases.

Various types of plants, both cultivated and wild plants, are known to contain active compounds that are insect repellent, such as essential oils, alkaloids, glycosides and other active compounds that are toxic (Wahyuni, 2016). Babadotan plant (Ageratum conyzoides) is one of the plants that has been studied and has the potential as a source of bioinsecticide. The insect repellent compound in babadotan has been proven through several studies. Eka et al., (2017) tested the effect of babadotan leaf extract on the mortality of soybean pods sucking insects. The chemical content in the bandotan plant is very possible to be used as environmentally friendly vegetable pesticides (Grainge and Ahmed in Astriani, 2010). The chemical content contained in babadotan are saponins, flavonoids, polyphenols, eugenols, and babadotan roots contain essential oils, so that babadotan weeds can be used as environmentally friendly pesticides (Sultan, et al., 2016). The presence of insecticidal compounds in the extract of the Ageratum conyzoides plant can control the larvae of Spadoptera litura (Lumowa, 2011).

METHOD

Flour beetles are obtained from various traditional markets in Semarang for further reproduction. This research was conducted in July-August 2019 at the Ecology laboratory, FMIPA Semarang State University. Bioinsecticide, namely Ageratum conyzoides, is obtained from rice fields and plantations around the campus of the State University of Semarang. The design in this study was a one-factor completely randomized design. The study used 5 concentrations of extract and 5 repetitions. The flour beetles that have been obtained are bred in clean jars that have been given flour and also yeast as additional nutrients. The temperature used is 27 ° C. The beetles were separated to obtain F1 offspring once every 14 days. The babadotan plant used is the part of the leaves that will be extracted using 96% ethanol as a dilute. This study also used phytochemical analysis to determine the content of anti-insect compounds. To determine the effect of the ababdotan leaf extract, observations were made using the Y olfactometer. The independent variable in this study was the concentration of babadotan leaf extract which was determined and will be tested in 5 concentration treatments (0%, 2 5%, 50%, 75% and 100 %). The dependent variable / dependent, namely the preference index and the percentage of respondence. The control variable in this study was beetle age.

Flour Beetle Breeding (Tribolium castaneum)

Cultured broodstock of flour beetles is obtained from contaminated rice and wheat flour. Each adult male and female flour beetles are selected approximately 25 tails, while the male is approximately 25 tails. The difference between male and female beetles is that in male beetles, there is a genitalia pupa. Breeding is done using a 1: 1 ratio. Breeding is carried out in 10 jars containing 10 pairs of flour beetles each. The culture medium used was 90 grams of wheat flour mixed with 10 grams of yeast extract. Every 14 days, all the imago are removed from the jars and transferred to new jars containing the same feed for further propagation. Larvae that emerge from the breeding process are reared until they are adults. This cultured Imago F1 is used for the preference test. This FI generation is assumed to be a sample of experimental animals with a relatively uniform age (Sunaryo et al., 2017).

Making Babadotan Leaf Extract

The extract is made from the babadotan leaf part only. Extraction was carried out by maceration method and the type of solvent used was ethanol 96%. 500 grams of fresh babadotan leaves, which have been pulverized to reduce water content, are chopped and then extracted with 100 ml of 96% ethanol for 12 hours. Furthermore, the solution is distilled at 60-78o C to obtain a concentrated liquid of babadotan leaf extract. This concentrated babadotan extract is assumed to be a 100% concentration extract.The dilution of babadotan extract in order to obtain a concentration of 75%, 50%, and 25% is carried out by the dilution procedure is 100 µL extract, 100% concentration = A µLE extract concentration of 75% = (A-25µl) + 25µl diluent solution (70% alcohol). Extract concentration of 50% = (A-50 µl) + 50 µl of diluent solution. Extract concentration of 25% = (A-75 µl) + 75 µl of diluent solution. Extract concentration 0% = 100 µl diluent solution without extract.

Phytochemical Compound Analysis

Phytochemical analysis aims to qualitatively detect the content of secondary metabolites such as alkaloids, phenolics, flavonoids, steroids, triterpenoids, and saponins in extracts (Guswenrivo et al. 2013). Some of the concentrated extract samples were analyzed for phytochemical components using the Perkin Elmer GC-MS tool in the Instrumentation Laboratory, Chemical Engineering, Semarang State University. The results of the analysis were then identified to determine which compounds were categorized as anti-insect compounds. Anti-insect compounds include dynamic alcohol, eugenol, glycosides, hydrocyanic acid, alkaloids, sinamaldehyd, citronella, geraniol, saponins, flavonoids, polyphenols, methylheptenol and other essential oils (Idris, 2014).

Repelen test

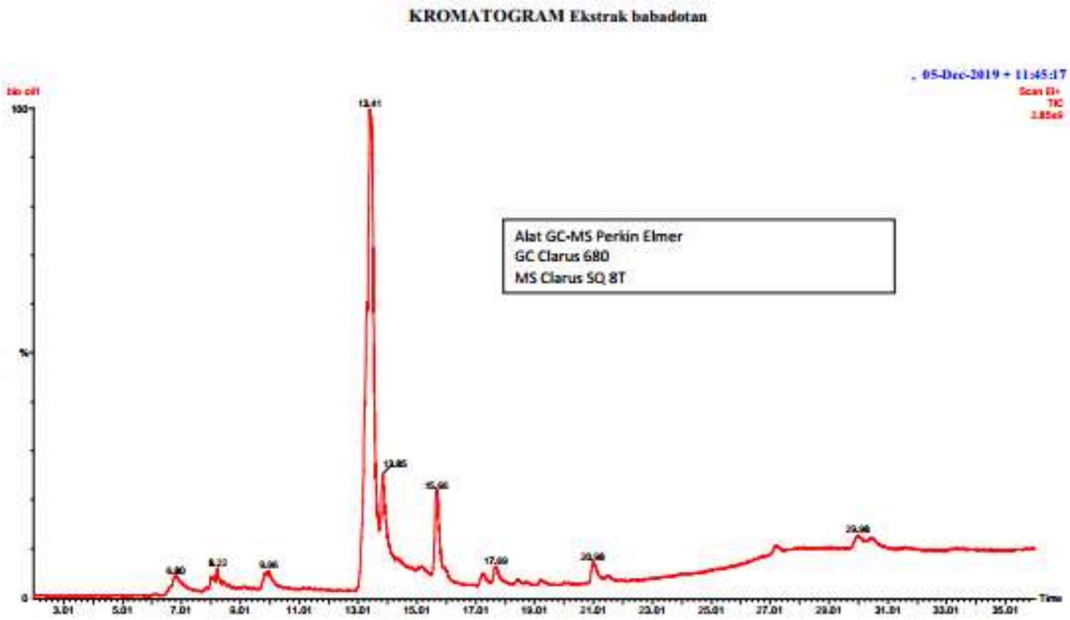
To find out whether babadotan extract has a repellency effect on flour beetles, a preference test was carried out using a Y-shaped olfactometer as seen in Figure 6.The end of arm A is a tunnel for entering experimental animals (adult flour beetles), the end of arm B is used as a control tunnel. and the C end is used as a treatment passageway. Each end is equipped with a glass bottle that can be opened and closed. With this tool, the insects to be tested for their olfactory power only have a two-way orientation when inserted into them, namely towards the treatment hall containing babadotan extract or turning toward the control tunnel which is not given the extract. The preference test was carried out in 5 treatments of extract concentration, namely 100%, 75%, 50%, 25%, and 0%. Each treatment was repeated 5 times using 5 units of the olfactometer simultaneously. Each extract to be tested is dropped on filter paper with a diameter of 2.5 cm, then aerated for 10 seconds to evaporate the remaining ethanol. The filter paper is then placed in a bottle at the end of the C tunnel with flour as much as 5 g as a stimulus for the insects to approach. At the end of the corridor B, only 5 g of non-extracted filter paper was placed as a control. The bottles at the end of aisle B and C are then installed without a lid. The testing mechanism begins by inserting 10 flour beetles through the base of the olfactometer (A), then allowed to walk to both aisles B and C. To anticipate the insects from turning around, a tool in the form of a plug is used which can be pushed into tunnel A slowly until it reaches the junction of passageways B and C. The repellency effect of babadotan extract was observed based on the rejection response shown by flour beetles. If the flour beetle continues to walk towards tunnel C and persists (does not reverse direction) for a set time (one hour), then the insect is assumed to be undisturbed by the presence of the aroma of the babadotan extract. Conversely, if the insects reverse direction or choose tunnel B, it is assumed that the insect's olfactory power is disturbed by the presence of the aroma from the babadotan extract

RESULTS AND DISCUSSION

Phytochemical compound analysis of babadotan leaf extract was carried out using GC-MS (Gas Chromatography Masa Spectrum), at the Instrumentation Laboratory, Faculty of Engineering, Semarang State University. The results of the complete phytochemical compound analysis are presented in Table 1 and depicted in the form of a chromatogram graph as shown in Figure 4

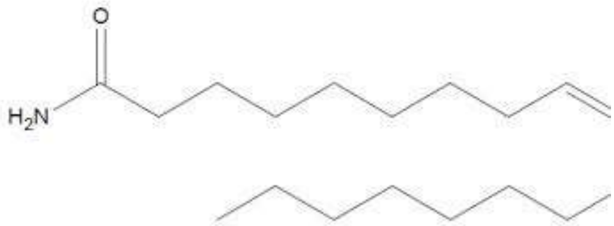
Table 1 . The results of the complete phytochemical compound analysis

No	RT	%Area	Komponen	Nama Lain	Golongan
1	6.799	3.99	Hydroquinone	1-4 benzenediol	Fenol
2	8.22	3.45	Palmitic acid	Asam palmitat	Asam lemak
3	9.96	3.20	α-D-Glucopyranoside, methyl 4,6-O nonylidene-	Catechin	
4	13.411	75.62	cis-Vaccenic acid	Asam Vaksenat	Asam lemak
5	15.661	7.61	Oleic Acid	Asam Oleat	Asam lemak
6	17.251	1.08	cis-13-Eicosenoic acid	Asam Eosin	Asam lemak
7	17.692	1.72	Eicosanoic acid	Asam Eosin	Asam lemak
8	20.982	3.32	Dasycarpidan-1-methanol,acetate (ester)	Metanol	Alkohol
9	17.069	1.08	9-octadecanamide	Oleamdi	Asam lemak



Picture 1. Kromatogram Babadotan Leaf Extract

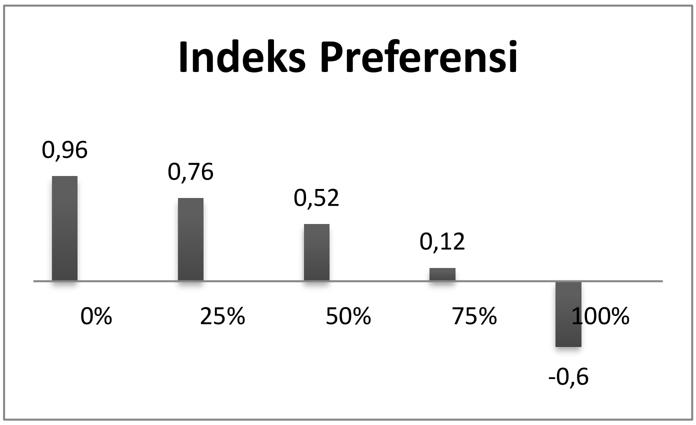
The purpose of this phytochemical analysis of babadotan leaf extract is to determine the content of secondary metabolites in babadotan leaves. Based on the results of phytochemical analysis, babadotan leaf extract contains several fatty acids. These fatty acids include palmitic acid, oleic acid, and elchosenoid acid. Where palmitic acid and oleic acid are saturated fatty acids from secondary metabolites or aleochemicals produced in plant tissue that can be toxic, and can function as stomach and respiratory toxins (Yeni, 2008). In addition to fatty acids, babadotan leaf extract also contains 9-octadecanamide (oleamide) compounds which are included in the fatty acid group as shown in the following figure:



Picture 2. Structure 9-Octadecanamide

The 9-octadecanamide compound is an oleamide compound that can cause motor nerve disruption (Fedorova, et, al., 2001). This causes disruption of the behavior of the flour beetles while in the treatment area. Benzofuran compounds are also found in babadotan leaf extract. where the benzofuran compound is toxic to insects. Babadotan leaf extract also contains hydroquinone compounds. This hydroquinone compound is a phenol group that has a cool taste so that it can function as a natural pesticide (Linggawati, 2002). The chromatogram graph of babadotan leaf extract (Figure 1) shows that the babadotan leaf extract in this study was dominated by vaccinated acid, and the lowest content in the babadotan leaf extract was eosin acid. Where the two compounds are included in the fatty acid group. According to Hikal et al., 2017, fatty acids are classified as anti-insect compounds. Phytochemical analysis of babadotan leaf extract shows that the extract contains secondary metabolites in the form of fatty acids which can be toxic or neurotoxin. In addition, babadotan leaves also contain flavonoids and alkaloids. However, in this study, based on phytochemical analysis, flavonoid compounds were not detected. This is because the extraction method used is distillation. So that the results obtained are less than optimal. Where flavonoid compounds can be used as active ingredients in making vegetable insecticides (Lumowa, 2011). As for alkaloid compounds which can be bitter and poisonous compounds, causing dizziness and refusing to eat until the insects die (Astriani, 2010). According to Nurhudiman et al., (2018), babadotan leaf extract contains saponin compounds. These saponin compounds affect insect mortality because they can cause haemolysis of red blood cells and weaken nerves. Damage to nerve cells results in decreased appetite and eventually the insect's body weakens and experiences death.

The preference test is a test used to determine the repellency effect of a compound. The preference test in this study used the babadotan leaf extract tested against flour beetles. This test uses an olfaktometer. The extract was considered to have a repellant effect if during 1 hour of observation, the flour beetles showed a tendency to avoid the treatment tunnel (entering the control tunnel) much more than the number that entered the treatment tunnel. The results of the calculation of the preference index using the 2018 Chaubey formula are displayed in the form of a bar graph as shown in Figure 1.3 below:



Picture 3. Response Effect Diagram Based on Preference Index

Based on the IP value presented in Figure 1.3, it can be seen that the negative preference index value indicates a repellency effect on flour beetles (Rinaldi et al., 2016). At the five concentrations in this study that had a repellency effect based on the IP value was babadotan leaf extract with a concentration of 100%. This is indicated by a negative IP value, namely -0.6. While the extract with a concentration of 75% showed an IP value of 0.12. The positive preference index number shows that flour beetles choose flour without treatment (control) compared to flour with babadotan leaf extract (treatment). At a concentration of 25% indicates an IP value of 0.76, a concentration of 50% indicates an IP value of 0.52, and a concentration of 75% indicates an IP value of 0.12.

Based on the results of the calculation of the preference test, Table 1.2 shows the number of flour beetles that entered the control hall due to the presence of babadotan leaf extract in the treatment hallway. The number of flour beetles that entered the treatment aisle were counted. So that the results obtained from the preference of babadotan leaf extract against flour beetles. The results of the calculation of the percentage of babadotan leaf extract response to flour beetles were calculated using the Chaubey 2018 formula.

The results of the research on the repellency effect of babadotan leaf extract in the control of flour beetles are presented in Table 13. After being analyzed using one-way Anava, the only parameter that had a significant effect was the increase in extract concentration, then the LSD test was carried out (Table 2).

Table 2. Summary of LSD further test results on the effect of concentration on flour beetles

Konsentrasi (%)	**PR**					**Rata-rata PR(%)**
	1	**2**	**3**	**4**	**5**	
0	0	0	10	10	0	4[a]
25	10	0	20	20	10	12[b]
50	20	20	70	70	20	24[c]
75	60	60	0	60	0	44[d]
100	20	70	20	10	20	80[e]

The LSD test results show that there are differences in each treatment group concentration. Where at a concentration of 0% significantly different from a concentration of 25% by 8%. This is because at a concentration of 0% there are no extracts and babadotan. Furthermore, the concentration of 25% was significantly different from the concentrations of 50%, 75%, and 100% with successive differences, namely 12%; 32%; 68%. This is because at a concentration of 25% the flour beetles can still move actively until they reach the end of the treatment tunnel in the olfactometer. Then at a concentration of 50% it was also significantly different from a concentration of 75% and 100%. This is because the flour beetles are still tolerant of the presence of 50% concentration of babadotan leaf extract. This is also significantly different from the concentration of 75%. Where the number of flour beetle mortality was 44%. This is due to an increase in the concentration of babadotan leaf extract. Furthermore, the concentration of 100% is also significantly different from the concentration of 75% this is because the extract with a concentration of 100% is a high concentration in this treatment. Where the active ingredients in the babadotan leaf extract are getting more concentrated, so this makes it difficult for the flour beetles to enter the control hallway or their food source. The LSD test results showed a tendency for the percentage of repellents to get higher in line with the concentration level of babadotan leaf extract. The highest percentage of repellents occurred in treatment with a concentration of 100% by giving a percentage of 80% of the repellents. According to Rinaldi et, al., (2016), the percentage of repellents can be categorized into five criteria, namely:

1. The repellent effect is very low when the PR value is <20%
2. Low repellent effect when 20% ≤ PR <40%
3. Moderate repellent effect if 40% ≤ PR <60%
4. High repellent effect if 60% ≤ PR <80%
5. The repellent effect is very high if the PR value is ≥ 80%

Based on these criteria, the babadotan leaf extract began to give a repellant effect at the extract concentration of 75% in the moderate category. Furthermore, the PR value at a concentration of 100% shows a result of 80%, so that the resulting repellency effect is included in the high criteria. Based on research (Carino, 1981 in Prakas and Rao, 1997), babadotan plant extracts can cause death in several insects, one of which is the flour beetle. Babadotan leaf extract affects the work of insect hormones, interferes with communication between insects and can cause death (Baharudin, 2015). The response of the flour beetle that prefers to go to the control hall, or the behavior of the flour beetle that immediately reverses direction indicates a disturbing smell or aroma.

CONCLUSION

The anti-insect compounds contained in babadotan leaf extract based on GC-MS phytochemical analysis are oleic acid, palmitic acid, hydroquinone, and vaccinated acid. The preference index of babadotan leaf extract has a moderate repellency effect at a concentration of 75% and high at a concentration of 100%. And babadotan leaf extract has a strong repellency effect against flour beetles at a concentration of 100%.

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