The potential of essential oils \textit{Eucalyptus citriodora} and \textit{Artemisia vulgaris} against \textit{Sitophilus zeamais} (Coleoptera: Curculionidae)

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\textbf{Abstract.} Medicinal plants have been developing as the main material for botanical pesticides. Many medicinal plants can be used as botanical pesticides due to their effectiveness in controlling the pest. This study aims to examine the potential of \textit{Eucalyptus citriodora} and \textit{Artemisia vulgaris} against \textit{Sitophilus zeamais}, which can be used to control the storage pest using these plants without using synthetic pesticide. This study was conducted in the pest laboratory, Indonesian Spice and Medicinal Crops Research Institute (ISMCRI), from January to February 2021. The formula of two essential oils of \textit{E. citriodora} and \textit{A. vulgaris} with different concentrations (1.5\%, 2\%, 2.5\%, and 3\%) were used to control \textit{S. zeamais}. The result showed that the \textit{A. vulgaris} had the most potent efficiency as a botanical pesticide against \textit{S. zeamais}. The highest mortality values of imago \textit{S. zeamais} at 72 hours after applying \textit{E. citriodora} and \textit{A. vulgaris} were 90\% and 93\%, respectively. \textit{Artemisia vulgaris} has the highest reaching repellent percentage of imago \textit{S. zeamais} its 85\% at 1 hour after application for 3\% concentration. Indicates that essential oils, \textit{E. citriodora}, and \textit{A. vulgaris}, possess insecticidal activity against \textit{S. zeamais}.

\textbf{Keywords:} botanical pesticide, mortality, repellent

1. Introduction

Corn or maize (\textit{Zea mays} L.) is a crop that originated from Mexico, but today corn is one of the most widely grown crops because it can be found all over the world. Some countries make corn as their staple food, such as Latin America, Eastern Africa, Central America, and Southeast Asia, including China. Moreover, corn is the major item in the diet of many people in tropical countries. In temperate regions, corn is used as the main grain for animal feed, as well as new materials for many other purposes, including the use as feedstock for biofuels [1].

One of the problems in the cultivation of corn is the pest attack in the field and after harvest or in the storage. The maize weevil, \textit{Sitophilus zeamais} (Coleoptera: Curculionidae), is an economically important plant pest of grains, cereals, and other processed and unprocessed stored products [2]. This pest causes damage to the whole kernel, reduces weight, and increases moisture, causing mold growth. Moreover, extreme cases can cause complete deterioration of the grain mass [3].

Insect pests of stored grains in Indonesia are mostly controlled by using synthetic chemical insecticides, which can cause harmful effects for users due to toxicity effects and residue of pesticide.
Also, it is likely to create a negative impact on beneficial and non-target organisms and increase the risk to workers for regular use. In addition, insecticides are too expensive for their health and environmental concerns. One of the control strategies for stored grains insect pests is fumigation [4]. Organophosphates and pyrethroids were reported as fumigation insecticides for treating different products [5]. Other insecticides, methyl bromide, and phosphine are reported to have a better result for controlling the stored grain insects, although it is believed to have a harmful effect on the environment due to its effects on ozone; thus, the use of methyl bromide is not recommended by some countries [6]. Although there are advantages of the use of fumigation, particularly with a high volume of stored grains, this method requires skilled workers to operate, particularly with toxic phosphine tablets. Moreover, another method is likely enough with a small volume of stored grain. To increase the synthetic pesticide residue-free grains, we need alternative pesticides that are safe, cheap, and use material that is easy to be obtained such as botanical pesticides.

Around 189 plant families with more than 2,400 plant species are rich sources of bioactive organic compounds [7]. A lot of natural compounds collected from plants showed a large spectrum of biological activities. Essential oils from aromatic and medicinal plants contained various natural substances used for insect management as botanical pesticides. Botanical pesticides can control the major pests of crops due to utilizing the secondary plant compounds as the active ingredient [8]. Essential oils contain volatile compounds which are known and used as bioactive agents [9]. Thus, they are mostly used as antifeedants, insecticides, oviposition inhibitors, repellents, and ovicides, which influence the insect biological parameters such as longevity, growth, and reproductivity [10].

Eucalyptus extract was reported to be used to inhibit the bioactivity of several insects [11]. Moreover, eucalyptus oil has the potential as a natural insect repellent to protect against harmful insects such as mosquitoes, harmful arthropods, and other herbivores as a pest because of the pesticidal activity of eucalyptus oils containing 1,8-cineole, citronellal, citronellol, citronellyl acetate, p-cymene, eucamolol, limonene, linalool, a-pinene, g-terpinene, a-terpineol, alloocimene, and aromadendrene [12]. Furthermore, A. vulgaris had the potential to be used as a natural insecticide because it had a toxicity effect on T. castaneum [13].

Artemisia is an herb species belonging to Asteraceae family and is widely distributed in Asia, Europe, and North America, [14]. The use of essential oils derived from Artemesia for controlling insect pests has been reported. These studies showed the potential of Artemisia essential oils as antifeedant, repellent, and insecticidal for several insect pests such as T. castaneum, Callosobruchus maculatus (Bruchidae), Rhyzopertha dominica (Bostrichidae), and Plodia interpunctella (Phycitinae) [15, 16, 17]. Also, artemisia essential oils affect the survival, fecundity, development, and life table parameters of Sitophilus granaries (Curculionidae) [18]. The potential of Artemisia vulgaris (Asteraceae) essential oil as a repellent and insecticidal has been evaluated on Musca domestica (Muscidae), T. castaneum [19] and on C. maculatus, R. dominica, and T. castaneum [20]. The activities of A. vulgaris essential oil as insecticidal and larvicidal due to camphene, a chloro derivative of camphene, and a-thujone [21]. Therefore, this study aims to examine the potential of Eucalyptus citriodora and Artemisia vulgaris against Sitophilus zeamais, as a first step to control the storage pest using these plants.

2. Materials and methods

2.1. Collection and preparation of essential oils

In this study, two essential oils, Eucalyptus citriodora, and Artemisia vulgaris were used. These oils were collected from Manoko experimental farm, Bandung, West Java, Indonesia. Eucalyptus citriodora and Artemisia vulgaris essential oils were tested on five concentrations consisted of 1.5%, 2%, 2.5%, 3%, 3.5%, and control. Each concentration of essential oils was diluted with acetone as a solvent.

2.2. Rearing of S. zeamais pest

Sitophilus zeamais pest was obtained from a corn grain seller in a Bogor traditional market, West Java, Indonesia. Sitophilus zeamais insects were placed in a plastic box with the size of 36.5 cm length,
29 cm width, and 15 cm height. The top of the box was closed and covered with fine gauze for air circulation. *Sitophilus zeamais* insects were reared and provided with 1 kg of shelled corn as food. The box containing *S. zeamais* insects and their feed was stored on a storage rack in the laboratory at 25°C.

2.3. Toxicity test
A toxicity test was conducted by preliminary test to determine the concentration range that can cause toxicity which is capable of killing around 5-99% of *S. zeamais* insects [22]. This test was conducted by using the fumigation method with filter paper [23]. *E. citriodora* oil and *A. vulgaris* oil were tested on five concentrations, which were 1.5%, 2%, 2.5%, 3%, 3.5%, or equal to 0.15, 0.20, 0.25, 0.30, 0.35 ml/L and control. The treatment was repeated five times. Each concentration of essential oils was diluted with acetone as a solvent. The diluted oils were dripped with 0.5 ml volume evenly on each 8 cm diameter of filter paper that had been previously glued to the petri dish. Therefore, the dose for each treatment was 7.5, 10, 12.5, 15, and 17.5 μL of essential oils/treatment. Meanwhile, the control filter paper was dripped with 0.5 ml acetone. The dripping of essential oils was conducted evenly in a spiral from the outside to the inside. After the treatment, the filter paper was left for ± 2 minutes to evaporate the acetone solvent. After drying, around 0.25 g of dry shelled corn and ten imagos of *S. zeamais* were introduced into a petri dish. Then the petri dish was closed tightly. The gap between the lid and the bottom of the petri dish was sealed with plasticine to prevent the leakage of volatile oil vapors. Observations of insect mortality were conducted after 72 hours of fumigations [24].

2.4. Imago *Sitophilus zeamais* repellency test
The observation was conducted to evaluate the repellent properties of *E. citriodora* and *A. vulgaris* oils against *S. zeamais* at different concentration levels, which were 1.5, 2, 2.5, 3%, and control. The study was conducted in the laboratory with 28±2°C temperature and 80±20% relative humidity. The study was conducted by a modified [25] method, which used the choice filter paper method. This study used Whatman no.1 filter paper with a diameter of 8 cm. Each filter paper was divided into two parts, one part was dripped with *E. citriodora*, and another part was dripped with acetone as control with each 0.5ml treatment concentration. The same treatment was conducted for *A. vulgaris*. The filter paper was then aired. Around ten imagos of *S. zeamais* were placed in the middle of the filter paper, and the petri dish was closed. The observation was conducted on the adults of *S. zeamais* that perched on filter paper at 1, 3, 6, and 24 hours after application. Repellency percentage was calculated by the formula of [26]:

\[
PR = \frac{NC - NT}{NC + NT} \times 100%
\]

PR = Repellency percentage  
NC = Total imago of *S. zeamais* in control  
NT = Total imago of *S. zeamais* in treatment

Data were analyzed by variance. If there was a significant difference, it would be continued by the DMRT test at a 5% level.

3. Results and discussion
The results of the study showed that artemisia and eucalyptus essential oils could be used as alternatives to control storage pests, especially *S. zeamais*. They were effective in increasing the mortality percentage and repellency of *S. zeamais*. The result indicated that 3% of *A. vulgaris* was effective against *S. zeamais*, which enhanced the highest adult mortality after 72 hours applications. Moreover, there is no significant difference with 3% of *E. citriodora*.

The highest adult mortality percentage after 72 hours were 93% and 90% for *A. vulgaris* and *E. citriodora*, respectively. The treatments of *A. vulgaris* and *E. citriodora* generated an increase in...
mortality percentage along with the increase of the dosage of treatments. Mortalities percentages on *E. citriodora* treatment were 13, 53, 78, and 90% on 1.5; 2; 2.5; and 3% presentation of concentration, respectively.

Meanwhile, the mortalities percentages on *A. vulgaris* treatment reached 23, 25, 78, and 93% at the concentration of 1.5; 2; 2.5; and 3% dosages, respectively. On the other hand, the control only generated 15% mortality (Figure. 1). [27] reported that artemisia oil with 3% concentration or equivalent to 0.25 ml/l dosage in the air after 72 hours treatment was able to generate the mortality of *S. zeamais* around 45%. At the highest concentration of 11%, the artemisia oil was effective in causing *S. zeamais* mortality of 95%.

[28] showed that *Artemisia vulgaris* essential oil generates significant repellent activity, particularly to the adult insects with 0.6 mL/mL (v/v) and higher with filter test. This is probably due to the steam phase of the active component of artemisia oil, which is capable of entering the respiratory of *S. zeamais* faster than *E. citriodora*. Artemisia oil produces volatile gas, which can enter through the spiracle, furthermore into the trachea, and is circulated throughout the body. Therefore, this poison damages the insect’s respiratory system [29].

![Figure 1. Mortalities average of *S. zeamais* (%) after 72 hours application.](image_url)

The results indicated the repellency percentage of *E. citriodora* and *A. vulgaris* against the *S. zeamais* (Table 1). *A. vulgaris* at the concentration of 1.5% had the highest average repellency percentage of 85% after 1 hour of application. Moreover, after 3 hours of application, the highest average repellency percentage was 90% for *E. citriodora* and *A. vulgaris*, respectively, at 3% concentration. There is no significant difference in repellency percentage observed between *E. citriodora* and *A. vulgaris* in all treatments 24 hours after application.

*E. citriodora* and *A. vulgaris* were toxic to *S. zeamais* adult at the concentration of 3 % after 3 hours of treatment with 90% of the repellency value (Table 1). Moreover, the repellency value of essential oils increases with increasing observation time. Similar to a previous study [30], this study reported that *Eucalyptus* oil had the highest repellency to *Ephestia cautella* adults, with the repellency of 46% at 24 hours after treatments, and 2.0 ml/l *E. citriodora* oil was toxic to *Tribolium castaneum* adults air after 24 hours treatment. Meanwhile, *E. citriodora* was also toxic to *Aedes aegypti* with LC$_{50}$= 104.4 ppm [31]. This is because the content of active compounds such as volatile alcohol needs time to enter the receptor of the insect. *Eucalyptus* oil has the main compound, *globulolspatulenol*, with relative levels in each fraction of 61.60 and 46.12%. Both of these compounds belong to the class of alcohol sesquiterpene compounds. Derivatives of volatile alcohol compounds can affect the chemical receptors of insect pests [32].
Table 1. Average repellency value of *E. citriodora* and *A. vulgaris* on *S. zeamais*.

| Treatment   | Concentration (%) | Average repellency value (%) | Hour after application |
|-------------|-------------------|------------------------------|------------------------|
| *E. citriodora* | 1.5               | 65<sup>a</sup>               | 60<sup>a</sup>         | 65<sup>a</sup>         | 70<sup>a</sup>         |
|             | 2                 | 65<sup>a</sup>               | 75<sup>a</sup>         | 75<sup>a</sup>         | 80<sup>a</sup>         |
|             | 2.5               | 70<sup>a</sup>               | 75<sup>a</sup>         | 80<sup>a</sup>         | 80<sup>a</sup>         |
| *A. vulgaris* | 1.5               | 50<sup>a</sup>               | 65<sup>a</sup>         | 65<sup>a</sup>         | 75<sup>a</sup>         |
|             | 2                 | 60<sup>a</sup>               | 75<sup>a</sup>         | 80<sup>a</sup>         | 75<sup>a</sup>         |
|             | 2.5               | 80<sup>a</sup>               | 75<sup>a</sup>         | 85<sup>a</sup>         | 75<sup>a</sup>         |
|             | 3                 | 85<sup>a</sup>               | 90<sup>a</sup>         | 90<sup>a</sup>         | 90<sup>a</sup>         |
| Control     | 0                 | 0<sup>a</sup>                | 0<sup>a</sup>          | 0<sup>a</sup>          | 0<sup>a</sup>          |

Note: values followed by the same letter are not significantly different.

The result in Table 1 revealed that the percentage of repellency of *E. citriodora* and *A. vulgaris* at a concentration of 3% was increased with the increase of application time from 1 to 3 hours after application, which was 85% and 90% percentage of repellency, respectively. Even though the repellency value of *E. citriodora* at the concentration of 3% looks similar to *A. vulgaris* at the concentration of 3%, but the repellency value of *A. vulgaris* was higher compared to *E. citriodora* at 1 hour and 2 hours after application. This indicates that *A. vulgaris* essential oil is more toxic than *E. citriodora*. [33] reported that *A. vulgaris* was more toxic on *Tribolium castaneum*, which is likely due to the potential compounds which were present in the hexane extract of the *A. vulgaris*.

Figure 2. Several types of grain damages caused by *Sitophilus zeamais* in the storage. (A) an early stage of pest attack, (B) later stage of the attack, damage almost in all part of the grain, powder of the grain can be found inside the affected grains.

Figure 3. The damages of grain associated with fungi in the later infection stage cause the color change of the grain into dark brown.
Therefore, the number of the compounds contained in E. citriodora and A. vulgaris essential oils have the potential for development as insecticidal to control S. zeamais, because it was found to disrupt physiological parameters, which lead to the high mortality and repellency value of S. zeamais. This is similar to [34], which reported that A. vulgaris affect disrupted the reproduction of exuviated adults by extending the preoviposition period and reducing the period of egg-laying and fecundity because fecundated females could not live more than four days compared to the control group. Moreover, the E. citriodora and A. vulgaris essential oils can be alternative insecticides than the synthetic insecticides, which have been widely resistant to pest insects. The essential oils were not toxic to mammals and fishafter toxicity tests. It is also safe for the environment because it contains easily degradable compounds in the environment [35]. However, further investigation is needed to study the components of these plants which are responsible for inhibiting the activities of enzymes in S. zeamais.

The damages of grains caused by S. zeamais were shown on the control treatment. Sitophilus zeamais generated many holes on the surface of the grains (Figure 2). All stages of S. zeamais from egg, larva, and adult (imago of inside the grains) were observed inside the affected grains. Moreover, S. zeamais also caused powder from the grains, which can be found inside the holes. This condition will lead to the growth of the fungi on the surface in the heavy attack, even inside the grains (Figure 3).

4. Conclusion
This study points out that A. vulgaris and E. citriodora have potential as a botanical insecticide against S. zeamais by generating an increase in insect mortality and repellence percentage. Essential oils E. citriodora and A. vulgaris possess insecticidal activity against S. zeamais. Even though both have similar results for generating insect mortality and repellency, A. vulgaris caused a higher trend of insect mortality and repellence than that caused by E. citriodora, particularly at the treatment with the concentration of 2.5 and 3%. This indicates that A. vulgaris has more potential as a botanical pesticide for controlling S. zeamais in storage.

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