The synergy of Calotropis gigantea and Cresscentia cujete plant extracts as an inhibitor of egg hatching and antifeedant against Spodoptera frugiperda

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Abstract. Spodoptera frugiperda is an invasive pest that has spread in various countries including Indonesia. S. frugiperda has many hosts but a high interest in maize. This study for determining the ability of Calotropis gigantea extract, Cresscentia cujete extract and mixed extracts in inhibiting the ability of S. frugiperda eggs to hatch and inhibiting the eating activity of S. frugiperda. The research has been done at the Pesticides and Natural Materials Laboratory, Faculty of Agriculture, Hasanuddin University. Observations were carried out by examination various concentrations (2.5%, 5%, 7.5%, and 10%) extracts of C. gigantea and C. cujete, while mixed extract with a concentration of 7.5% on the ovicidal ability and feeding inhibition of S. frugiperda larvae. The results showed that treatment with a concentration of 10% C. gigantea extract had an ovicidal effect percentage of 90.99% while the antifeedant percentage was 70.05%. Treatment extract of C. cujete with a concentration of 10% had an ovicidal effect percentage of 91.7% while the antifeedant percentage was 85.26%. The conclusion is the existence synergy between C. gigantea and C. cujete extracts had showed that the ovicidal effect and antifeedant activity will be even higher to S. frugiperda.

1. Introduction
Spodoptera frugiperda is an invasive pest that causes major damage in various regions in America, especially for maize in tropical and subtropical areas [1, 2], then on 2016 the first reported S. frugiperda in West Africa [3] and spread rapidly to Saharan Africa on 2017 [4] because S. frugiperda can migrate over long distances [5] with wind assistance [6]. In 2019 S. frugiperda was reported that in Indonesia, to be precise in West Pasaman Regency, West Sumatra. S. frugiperda is destructive to maize crops with a severe level of attack [7]. This pest has also spread to Aceh, Lampung, Banten, Java, Kalimantan, Gorontalo, Bengkulu, NTB, South Sulawesi, Bali and Maluku which attacks almost all maize crops.

As a result of S. frugiperda attacks maize yields decreased by 39% in America [8] by 40% in Honduras [9], while in Argentina it reached 72% [10]. As a result of S. frugiperda attacks in African and European countries causes yield losses of between 8.3 to 20.6 million tonnes per year with an economic loss of
between US $ 2.5-6.2 billion per year [11]. *S. frugiperda* is polyphagous and have many host plants from 76 plant families including Poaceae, Solanaceae, Brassicaceae, Cucurbitaceae, Amaranthaceae, Caricaceae, Cyperaceae, Euphorbiaceae, Fabaceae, etc. [12] and causes a decrease in the sale value of plants sugarcane, tomatoes, rice, potatoes, and cotton [4, 13].

Pest control is quite difficult in some African countries. This pest is known to be resistant to many synthetic insecticides. In addition, the use of synthetic insecticides is not recommended because it has a wide spectrum of targets and can cause environmental damage. Therefore, insecticides from plant extracts use as alternative which environmentally friendly and specific to the target pests. Plant extracts make use of secondary metabolites found in plants. Plants that will be used as extracts in this study are *Calotropis gigantea* and *Crescentia cujete*.

*C. gigantea* is a plant that grows wild and is distributed in tropical and subtropical climates [14]. *C. gigantea* is reported to have anti-microbial and cytotoxic activity [15] and also anti-bacterial [16]. *C. gigantea* contains secondary metabolites like the cardenolide compounds, flavonoids, alkaloids, tannins, saponins, and other cytotoxic compounds that can fight pests [17]. At Ladybugs, it has been reported that *C. gigantea* has an antifeedant effect which reduces the eating activity of insects causing faster death [18], while at *Spodoptera exigua*, *C. gigantea* extracts are reported to be able to affect feeding activity and larval mortality [19].

*C. cujete* is a plant that grows in tropical areas including Indonesia [20]. *C. cujete* contains alkaloid compounds, tannins, and flavonoids [21]. Alkaloid compounds have a bitter taste so insects don't like them and also have a repellent effect on insects. Tannin compounds have an antifeedant effect by reducing the activity of digestive enzymes so that the ability of insects to digest food is reduced. The extract *C. cujete* was also able to inhibit egg-laying and reduce egg hatchability [22].

Based on the effectiveness of *C. gigantea* and *C. cujete* which have ovicidal and antifeedant effects on several previously reported insects, a study was carried out by observing the ovicidal and antifeedant activity to *S. frugiperda*.

2. Materials and method

2.1. Collecting insect
Larvae of *S. frugiperda* instar 4-6 were taken and collected from infected maize plants in the Bontonompo area, Gowa Regency of South Sulawesi.

2.2. Rearing insect
Larvae *S. frugiperda* that has been taken are placed in a container with a hole at the lid as air exchange, each cup contains 1 larva and then entered young corn as a food until they turn into pupae. Pupae are transferred to a cylindrical cage with a diameter of ± 30 cm and a height of ± 40 cm and then tightly closed. Pupae in confinement will become imago after 5-6 days. On the sides of the cage there a window covered with a net to make it easier to enter honey as food for imago. In confinement, male and female imago will mate and produce eggs. The newly laid eggs are transferred to a rectangular container with a length of ± 15 cm, a width of ± 10 cm, and a height of ± 5 cm. Each container has been given young corn as food when the eggs hatch into 1st instar larvae. The larvae are used as stocks for examination when they reach instar 3.

2.3. Single extract preparation
As much as 2 kg of fresh and green *C. gigantea* leaves were taken around the Barombong road, Gowa Regency of South Sulawesi, while 2 kg of ripe *C. cujete* fruit were taken on the streets around Bantaeng Regency of South Sulawesi. Each of the leaves of *C. gigantea* and the fruit of *C. cujete* is washed with clean water and then dried. Chopped until smooth then put into a 5 L container then added 2 L of water and 100
ml of molasses. Mix well then closed tightly. Let stand for ± 2 weeks. Each fermentation product of *C. gigantea* and *C. cujete* will be used as a stock extract with a concentration of 100%.

2.4. Mixed extract preparation

*C. gigantea* and *C. cujete* extracts were combined using a 1:1 ratio with a concentration of 7.5%. For example, 1 L of *C. gigantea* extract with a concentration of 7.5% plus 1 L of *C. cujete* extract with a concentration of 7.5%. A concentration of 7.5% was used because based on the preliminary test results, a concentration of 7.5% was able to inhibit the development of *S. frugiperda* to reach 50%.

2.5. Bioassay for single and mixed extract

Every single extract was evaluated for the efficacy level of 2.5%, 5%, 7.5% and 10% concentration, while the mixed extract was evaluated at a concentration of 7.5% for ovicidal activity and antifeedant activity of *S. frugiperda* larvae. Each treatment was repeated 3 times, and each replication contained 5 samples of test larvae.

2.6. Ovicidal effect

The egg group laid by the female in a cage was put into a petri dish containing extract residue (0.3 ml). Each petri dish contained 1 group of *S. frugiperda* eggs. The things observed in this examination were the number of eggs that hatched in the treatment and control, then recorded and calculated the percentage of ovicidal activity with the following equation:

\[
PRC: \frac{\text{The Number of eggs laid by control} - \text{The Number of eggs laid by treated}}{\text{The number of eggs laid by control}} \times 100 \quad (1)
\]

The magnitude of the ovicidal effect of a plant extract can be determined by grouping the calculation results into the following categories [23]:

- **-**: There is no effect
- **+**: < 25% low grade ovicidal effect
- **++**: 25 - 50% middle grade ovicidal effect
- **+++**: 50 - 75% high grade ovicidal effect
- **++++**: > 75% very high grade ovicidal effect

2.7. Anti-feedant effect

Feeding inhibition testing was carried out by weighing the corn leaves 1 gr and then dipping them in the extract as food for *S. frugiperda* larvae. Larvae of *S. frugiperda* instar 3 which had been fasting for 5-6 hours, then infested into a petri dish has contained corn leaves, then observed until the larvae in the control died. Observations were made by calculating the percentage of antifeedant using the following formula [24]:

\[
PM = \frac{(Bk-Bp)}{(Bk+BP)} \times 100\% \quad (2)
\]

Note:

*PM* = antifeedant activity (%)

*Bk* = The weight of the control leaves eaten

*Bp* = The weight of the treatment leaves eaten
3. Results

3.1. Ovicidal effect by single extract

Observation of the ovicidal effect of a plant extract was carried out by observing the number of eggs that hatched and did not hatch after being given the extract treatment and in the control treatment. Based on the formula used to calculate the percentage of ovicidal activity, data on the ovicidal activity of *C. gigantea* extract against *S. frugiperda* eggs were obtained as shown in table 1. Based on the results obtained, the average non-hatching eggs at a concentration of 7.5% and 10% using *C. gigantea* extract, sequent is 62.83% and 74.58%, both into category high-class ovicidal effect. The 5% concentration is into the middle-class category, and 2.5% concentration is in a low category, while the control has no ovicidal activity.

| Concentration | Average percentage eggs not hatched (%) | Categories |
|---------------|----------------------------------------|------------|
| 0% (control)  | 0                                      | -          |
| 2.50%         | 11.85                                  | +          |
| 5.00%         | 28.05                                  | ++         |
| 7.50%         | 62.83                                  | +++        |
| 10.00%        | 74.58                                  | +++        |

The average percentage of non-hatching eggs at a concentration of 5%, 7.5% and 10% using *C. cujete* extract, sequent are 77.73%, 81.44%, and 90.93%, the three concentrations entered to a category of very high-class ovicidal effects. The 2.5% concentration into the middle-class category, while the control does not have ovicidal activity (table 2).

| Concentration | Average percentage eggs not hatched (%) | Categories |
|---------------|----------------------------------------|------------|
| 0% (control)  | 0                                      | -          |
| 2.5%          | 47.95                                  | ++         |
| 5.0%          | 77.73                                  | +++        |
| 7.5%          | 81.44                                  | +++        |
| 10.0%         | 90.99                                  | +++        |

3.2. Ovicidal effect by mixed extract

The mixing of *C. gigantea* and *C. cujete* extracts aims to determine the activity of the mixture of the two extracts if applied together. Usually, mixing of extracts is used as an alternative due to deficiency of raw materials at the farm level [25] and it is hoped that it will increase the effectiveness of botanical insecticides to control pests. The average percentage of ovicidal using a mixed extract with a concentration of 7.5% reached 91.7% with a very high category of ovicidal effect class (table 3).
Table 3. Average percentage ovicidal effect mixed extract against *S. frugiperda*.

| Concentration  | Average percentage egg not hatched (%) | Categories |
|----------------|----------------------------------------|------------|
| 0% (control)   | 0                                      | -          |
| 7.50%          | 91.7                                   | ++++       |

3.3. Anti-feedant effect by single extract

The treatment of *C. gigantea* leaf extract as antifeedant to *S. frugiperda* larvae showed a significant effect compared to the control treatment. This effect was seen in differences in leaf weight eaten by larvae. In the treatment given *C. gigantea* extract with a concentration of 10% was able to inhibit eating up to 64.40%, while at a concentration of 7.5%, 5%, and 2.5% are sequent can be inhibited eating of larvae by 39.86%, 18.11 %, and 9.9%. As for the control treatment, there was unseen inhibition of eating on *S. frugiperda* larvae (table 4).

Table 4. Average percentage antifeedant of *C. gigantea* extract against *S. frugiperda* larvae.

| Concentration | Average percentage antifeedant (%) |
|---------------|--------------------------------------|
| 0% (control)  | 0a                                   |
| 2.5%          | 9.90b                                |
| 5.0%          | 18.11c                               |
| 7.5%          | 39.86d                               |
| 10.0%         | 64.40e                               |

Note: The number followed by the same letter in the same column shows an insignificant difference according to the LSD test at 0.05 level.

Like the treatment given *C. gigantea* extract, the treatment with *C. cujete* extract also showed a significant effect compared to the control treatment on feeding inhibition of *S. frugiperda* larvae. This effect was seen in differences in leaf weight eaten by larvae. In the treatment given the extract of *C. cujete* with a concentration of 10% was able to inhibit up to 70.05%, while at a concentration of 7.5%, 5%, and 2.5% are sequent can inhibited eating of larvae are 48.52%, 40.19 %, and 8.55%. As for the control treatment, there was unseen inhibition of eating on *S. frugiperda* larvae (table 5).

Table 5. Average percentage antifeedant of *C. cujete* extract against *S. frugiperda* larvae.

| Concentration | Average percentage antifeedant (%) |
|---------------|--------------------------------------|
| 0% (control)  | 0a                                   |
| 2.50%         | 8.55b                                |
| 5.0%          | 40.19c                               |
| 7.50%         | 48.52d                               |
| 10.0%         | 70.05e                               |

Note: The number followed by the same letter in the same column shows an insignificant difference according to the LSD test at 0.05 level.
3.4. Anti-feedant effect by mixed extract

The synergy between \( C. \) gigantea and \( C. \) cujete extracts showed a very high effect on feeding inhibition against \( S. \) frugiperda larvae. This is because the secondary metabolites in \( C. \) gigantea and \( C. \) cujete are combine to make it stronger. It can be seen that the concentration of 7.5% mixed extract was able to inhibit 85.26% (table 6).

| Concentration | Average percentage antifeedant (%) |
|---------------|-----------------------------------|
| 0% (control)  | 0a                                |
| 7.5%          | 85.26b                            |

Note: The number followed by the same letter in the same column shows an insignificant difference according to the LSD test at 0.05 level.

4. Discussion

The ovicidal effect of \( C. \) gigantea extract is due to the existence of several inhibiting compounds such as flavonoids, tannins, alkaloids, saponins, glycosides, steroids and phytosterols [26, 27]. This is the reason \( S. \) frugiperda eggs cannot hatch into larvae. \( C. \) gigantea has an ovicidal effect on \( H. \) armigera eggs by inhibiting egg hatching up to 100% [17].

\( C. \) gigantea Contained steroid compounds (\( \beta \)-sitosterolacetate) in that can cause growth inhibition (anti-juvenile hormone) by inhibiting the development of embryonic eggs [28]. In general, steroid compounds in some plant extracts will inhibit protein by blocking sterol carrier proteins [26], so that insects grow abnormally.

The bioactive compounds contained in \( C. \) gigantea such as alkaloids and glycosides have toxic activity and can influence insect growth [29]. \( C. \) gigantea also contains tannin compounds, steroids, terpenoids, saponins, and flavonoids. Saponin compounds can interfere the food absorption and also play a role in reducing the activity of the protease enzyme in the digestive tract. Tannin and flavonoid compounds are also reported to reduce the activity of amylase and protease enzymes so that the ability of food digest also decreases, which causes the development of insects to be disrupted [30]. The feeding activity of \( S. \) exigua is inhibited due to the influence of \( C. \) gigantea extract [19].

Contained secondary metabolites in \( C. \) cujete like flavonoid compounds can be insecticidal and antimicrobial, besides that, it is also toxic and can inhibit insect appetite [31]. The tannin and saponin compounds in \( C. \) cujete cause a bitter taste, which makes insects don't like it [32]. Tannins can interfere with the digestive process in insects. Likewise, saponin compounds can interfere with the process of absorption of food [33].

The tannin and flavonoid compounds in \( C. \) gigantea can inhibit the eating activity of insects, while the tannin, saponin, and flavonoid compounds in \( C. \) cujete are also reported to be able to interfere with the digestion process and absorption of food in insects so that the synergy between of two extracts can strongest the antifeedant effect to \( S. \) frugiperda larvae.

5. Conclusion

Leaf extracts of \( C. \) gigantea and fruit extracts of \( C. \) cujete have ovicidal activity which can reduce egg hatchability and also have antifeedant activity that can inhibit the eating in \( S. \) frugiperda. After mixing \( C. \) gigantea and \( C. \) cujete it can be seen that there is a synergy between the two extracts. It is proven by the higher percentage of ovicidal activity and antifeedant activity in the mixed extract than the single extract. \( C. \) cujete extract contains several secondary metabolite compounds, like alkaloids, phenols, and saponins. Saponins are reported to have a systemic effect on \( S. \) litura larvae, phenols contained toxins and can causes
burn defects, while alkaloids can disrupt the nervous system. These compounds allow changes in the eggs so that *S. frugiperda* eggs are unable to hatch.

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