

## EFFECT OF EXTRACTION METHOD OF *TITHONIA DIVERSIFOLIA* LEAVES AGAINST *SPODOPTERA FRUGIPERDA* LARVA MORTALITY

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### ABSTRACT

Currently, corn crop commodities in Indonesia face threats due to the presence of invasive insect pests Fall Armyworm (FAW) *Spodoptera frugiperda*. The bioactive compound derived from plants could affect the insects and it is an alternative to chemical insecticide. The purpose of this study was to evaluate effect of extraction methods of *Tithonia diversifolia* against *S. frugiperda* larvae. This study was conducted in the laboratory and the treatments were crude extract of *T. diversifolia* leaves obtained by maceration extraction, infusa, and Soxhlet extraction. No treatment and water as control in this study. The study arranged as Completely Randomized Design with 4 replication. Ten larvae of instar III and IV were dipped at 0.5% of crude extracts and the mortality the insects were observed. The results showed that leaf crude extract of *T. diversifolia* obtained by soxhlet extraction method had the potential to control *S. frugiperda* larvae with a mortality percentage of 92.50%.

**KEYWORDS:** Extraction method, *Tithonia diversifolia*, *Spodoptera frugiperda*.

### INTRODUCTION

Corn is the third staple food after wheat and rice and the commodity has great demand by the community and high economic value (Kartika, 2019). The need increase both for people consumption and industrial raw materials (Iriany *et al.*, 2011). Currently, corn crops in Indonesia face a threat due to the presence of an invasive pest, Fall Armyworm (FAW) *Spodoptera frugiperda* J. E. Smith. This pest is an insect native to the United States (Nonci *et al.*, 2019), and has been reported to cause significant losses of corn yield in African and European countries (FAO & CABI 2019). Currently, the *S. frugiperda* disperse to 32 provinces including North Sumatra and it is estimated to continue disperse throughout Indonesia (BBPOPT, 2020<sup>a</sup>).

Commonly, farmers use insecticides of emamectin benzoate, siantraniliprol, spinetoram, and tiamectosam to control this insect pest (BBPOPT, 2020<sup>b</sup>). However, it causes negative effects such as resistance, resurgence, threat of natural enemies who are non-target organisms and the impact of residues on the environment (Laba, 2010). As well as the active ingredients of insecticides become a source of toxins that harm human health (Oktofa, 2016).

Botanical insecticides are one of the alternatives to pest control. Some plants contain natural/biological

compounds which are secondary metabolite compounds. *Tithonia diversifolia* contains compounds such as alkaloid group compounds, sesquiterpene lactone, monoterpenes and flavonoids (Pereira *et al.*, 1997). According to Asri and Gunawan (1998) the compounds contained at least 12 terpenoid compounds, 14 flavonoid compounds, and sugars. The compounds cause mortality, physiological and biological activities against insects. There were some studies conducted on *T. diversifolia* leaf extract. Leaf crude extract at 2% of concentration caused 100% mortality against *chrysomya bezziana* fly larvae at 3 days after treatment, whilst at 1% of concentration caused mortality effects increasing day by day (Wardhana & Diana, 2014). Application crude extract on *Scotinophara coarctata* showed of LT<sub>50</sub> value was 16.47 hours at 7% of concentration by direct spraying on insects (Juliani & Yuliani, 2017). Treated *T. diversifolia* leaves crude extract on *Aleurodicus dugesii* mealybug showed of LC<sub>50</sub> value was 3,192 mg.l<sup>-1</sup> and LT<sub>50</sub> value was 4,169 days (Widyastuti *et al.*, 2018).

There are various method extraction to obtain compounds derived from plants. The method extractions ranging from simple to complicated extraction to those that require equipment to obtain compounds contained in the leaves of *T. diversifolia*. The use of improper extraction methods can eliminate the bioactive compounds contained in the plant material which results in a reduced toxicity/biological effect on the target. This

study conducted to evaluate effect of extraction method of *Tithonia diversifolia* leaves against *Spodoptera frugiperda* larvae.

## MATERIALS AND METHODS

### Insect Rearing

The initial population of insects is by taking larvae of *S. frugiperda* directly from corn field. The larvae placed into a plastic box and handled under laboratory environment ( $29 \pm 2^\circ\text{C}$  and  $90 \pm 5\%$  RH). The larvae fed with young corn until the larvae become pupae, When it has become an adult then transferred to a glass box (60 x 25 x 30 cm) with air circulation and fed honey (1 honey : 10 Water) with a cotton swab and hung until the adult lays the eggs. Eggs will hatch 2-3 days into small larvae (instar 1) and then be reared into adult. The rearing was continued until the 4th generation.

### Plant Material Collection and Extraction Method

This research was conducted at the Laboratory of the Faculty of Agriculture, Islamic University of North Sumatra, Medan, from August 2020 to February 2021. Leaves of *T. diversifolia* collected from around Medan city. The leaves were washed thoroughly then air dried for 14 days under shade (room condition). The dried leaves were ground using electric grinding machine and finally passed through a 30 mesh sieve to obtain the powder. Maceration extraction (E1) by soaking 50 g the powder in 70% of 350 ml methanol (Yenie et al., 2013) and placed in a closed room which was not exposed to light for 7 x 24 hours and stirred a day once then filtered with sterile flannel cloth. The remaining powder was macerated again with the addition the 150 ml of 70% methanol for 1 x 24 hours (Anita and Lean, 2016), then the results were combined as the maceration extraction solution. Extraction of infusion (E2) by boiling 50 g of powder in 500 ml of water (aquadest) for 15 minutes with a temperature of  $\leq 90^\circ\text{C}$  then filtered with a sterile flannel cloth (Rheza, 2015). Soxhlet extraction (E3) by taking 50 g of powder then wrapped in filter paper and then put into a soxhlet using a 500 ml acetone as solvent. The process extraction took about 12 hours (Anita and Lean 2016). All crude extract solution were evaporated using Rotary Vacuum Evaporator.

The application of leave crude extracts was by dipping larvae for 10 seconds in 0.5% concentration of each crude extract which was obtained at difference method. Each treatment used 10 of instar 3 or 4 larvae and repeated 4 times (Priyono, 1999). The effect of the treatment is observed by counting the number of dead larvae every day until changed to pupate. The death larva was showed no response when it was touched with fine brushes.

The insect mortality data was normalized using arc sin  $\sqrt{x}$  before analysis. Data were analysed using one-way ANOVA to determine the effect of treatment. If ANOVA result were significant, Duncant Multiple Range Test ( $p < 0.05$ ) were used to separate the means. Probit

analysis (Finney 1971) was used to know the Lethal Time effect. All statistical analysis were run on the IBM SPSS Statistics 20.

## RESULTS AND DISCUSSION

There was a significant effect of *T. diversifolia* leaves crude extract obtained at different method in influencing mortality of *S. frugiperda* larvae ( $F = 61,245$ ;  $df = 4$ ;  $p < 0.05$ ). The mortality of *S. frugiperda* larvae at 8 DAT (Days After Treatment) was showed at Table 1.

**Table 1: Effect of various methods extraction of *T. diversifolia* leaf on larval mortality of *S. frugiperda* at 8 DAT.**

| Treatment                          | Percent Mortality (Mean $\pm$ SEM) |
|------------------------------------|------------------------------------|
| E <sub>0</sub> (-) (Control)       | 2,50 $\pm$ 2,50 c                  |
| E <sub>0</sub> (+) (Water Control) | 5,00 $\pm$ 2,89 c                  |
| E <sub>1</sub> (Maceration)        | 55,00 $\pm$ 2,89 b                 |
| E <sub>2</sub> (Influsa)           | 87,50 $\pm$ 2,50 a                 |
| E <sub>3</sub> (Soxhletation)      | 92,50 $\pm$ 2,50 a                 |

Means in a column followed by different letters are significantly different at  $P = 0.05$  by DMRT.

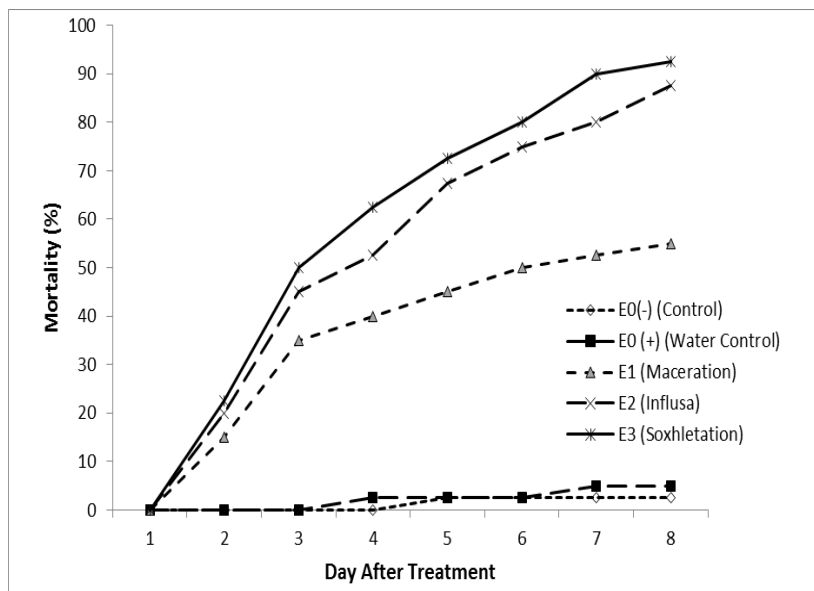
There was no significant difference between negative control E<sub>0</sub>(-) and water control E<sub>0</sub>(+), but both control E<sub>0</sub>(-) and E<sub>0</sub>(+) showed significant different larvae mortality with crude extract obtained by maceration, influsa and soxhletation. The highest larvae mortality showed when *S. frugiperda* larvae treated with crude extract obtain with soxhlet extractor (92,50%) and no significant different with larvae mortality treated with leaf crude extract obtained by influsa extraction (87,50%) (Table 1). Extraction by macerating *T. diversifolia* leaves plant powder (E1) caused moderate *S. frugiperda* larvae mortality (55,00%).

The mortality of *S. frugiperda* larvae was caused by the content of bioactive compounds derived from *T. diversifolia* leaf extract containing alkaloid group compounds, sesquiterpene lactone, monoterpenes and flavonoids. The compounds contain at least 12 terpenoid compounds, 14 flavonoid compounds, and sugars (Pereira, et al., 1997) (Asri and Gunawan, 1998).

The extraction method also affects the content of toxic substances that can be absorbed and taken from *T. diversifolia* leaf extract. It could be the extraction method used heating which can dissolved toxic substances (bioactive) in the leaf extract. The result showed that extraction by heating (soxhlet extraction and influsa) caused highest mortality of *S. frugiperda* larvae compared to no-heating extraction (maceration). It was similarly with other researcher Anita and Lean (2016) who stated in their research that the soxhlet extraction method obtained more flavonoid levels compared to the maceration extraction method.

In addition, the solvent used in the extraction could affect the levels of toxic substances that can be absorbed and taken in the extraction of *T. diversifolia* leaf. Acetone as a solvents has the ability to absorb more toxic substances (bioactives). It was corresponding with the statement of Anam, et al. (2014) that acetone has the

ability to absorb a high bioactive component which produces more yields of 4.11%. This is because the solvent acetone is a polar solvent and has the ability to extract compounds from the polar to semi-polar range. Daily *S. frugiperda* larval mortality showed in Figure 1.



**Figure 1: Effect of various methods extraction of *T. diversifolia* leaf on larval mortality of *S. frugiperda* 1- 8 DAT.**

Figure 1 showed that the mortality rate of *S. frugiperda* in 1-8 DAT due to the application of *T. diversifolia* leaf extract obtained from different extraction methods showed different mortality on each day of observation.

The highest *S. frugiperda* larvae mortality showed at the soxhlet extraction method reach 22.5 – 92.5% at 2 - 8 DAT followed by influsa extraction method reaching 20 - 87.5% at 2 - 8 DAT and the maceration extraction method (extraction) which reaches 15 - 55% at 2 - 8 DAT. The lowest percentage of larval mortality at positive control E0 (+) control was 2.5 – 5% at 4 – 8 DAT and the E0 (-) negative control was 2.5-% at 5 - 8 DAT.

This study showed that there is an increase in the mortality of *S. frugiperda* larvae over time. This is because the longer the larva of *S. frugiperda* feed the treated corn, the more toxins entered its body which caused death of larvae. According to Rustam and Anggita (2021), the higher the content of toxic compounds caused the higher the acceleration of mortality of *S. frugiperda* larvae.

The results of LT<sub>50</sub> data obtained from the mortality of *S. frugiperda* larvae at 1-8 DAT showed in Table 2.

**Table 2: LT<sub>50</sub> of *T. diversifolia* leaf crude extract obtained at various methods extraction Against *S. frugiperda* larvae.**

| Treatment         | LT <sub>50</sub> | 95%FL         | Slope ± SE    |
|-------------------|------------------|---------------|---------------|
| E1 (Maceration)   | 5,960 a          | 3,173 - 9,462 | 3,18 ± 0,40 a |
| E2 (Influsa)      | 3,706 a          | 2,738 - 4,573 | 3,40 ± 0,48 a |
| E3 (Soxhletation) | 3,315 a          | 2,509 - 4,036 | 3,78 ± 0,49 a |

There was no significant difference of LT<sub>50</sub> value of *T. diversifolia* leaf crude extract obtained at various methods extraction Against *S. frugiperda* larvae at observations 1 - 8 DAT. Extraction method by using soxhlet extractor showed the LT<sub>50</sub> level of *S. frugiperda* larvae is 3,315 days, whilst the longer LT<sub>50</sub> level was showed by using maceration extraction with the LT<sub>50</sub> level of *S. frugiperda* larvae was 5,960 days.

**CONCLUSION**

The crude extract of *T. diversifolia* leaf which was obtained by soxhletation extraction method showed good effect on of *S. frugiperda* larvae mortality with the highest mortality was 92.50% at 8 DAT.

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