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Effectiveness of Indian Jujube (*Ziziphus mauritiana)* Leaf Extract Using Ethanol Solvent Against Fall Armyworm (*Spodoptera frugiperda*) Under Laboratory Conditions

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ABSTRACT

Maize (*Zea mays* L.) ranks among the major cereal crops globally, after rice and wheat. In Riau Province, a significant decline in maize yield has been observed, mainly due to infestation by fall armyworm (*Spodoptera frugiperda* J.E. Smith). Control of this pest is essential to prevent its population from exceeding economic thresholds. However, the predominant use of synthetic insecticides has led to undesirable consequences, including the development of resistance, pest resurgence, and secondary pest proliferation. As a sustainable alternative, botanical pesticides derived from plant-based compounds are gaining interest. This study investigated the insecticidal potential of Indian jujube (*Ziziphus mauritiana* Lamk) leaf extract prepared with 70% ethanol. This plant is known to contain various bioactive secondary metabolites, including alkaloids, saponins, and flavonoids. The experiment was conducted using a Completely Randomized Design with six extract concentrations (0, 0.2, 0.4, 0.6, 0.8, and 1.0%) and four replications, resulting in a total of 24 experimental units. The results showed that a 1% extract concentration produced the highest efficacy, resulting in 82.50% total larval mortality, with initial mortality observed at 7.25 h post-application. The LT_{50} value was recorded at 52 h, while the LC_{50} and LC_{95} were estimated at 0.74 and 1.62%, respectively. These findings suggest that Indian jujube leaf extract has the potential to serve as an environmentally friendly biopesticide for controlling *S. frugiperda* under controlled conditions.

Keywords: *Zea mays*; Botanical pesticides; Lethal concentration; Lethal time; Probit analysis

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INTRODUCTION

Maize (*Zea mays* L.) is one of the leading cereal crops globally, following rice and wheat, and plays a crucial role in supporting national food security due to its high economic value (Galani et al. 2022). In Riau Province, Indonesia, maize production has experienced a significant decline over recent years. In 2020, total production reached 35,414 metric tons, but dropped to 18,852 metric tons in 2021, and further declined to 8,156 metric tons by 2022 (Indonesian Ministry of Agriculture 2023). One of the primary biological factors contributing to this reduction is the increasing incidence of fall armyworm (*Spodoptera frugiperda* J.E. Smith) infestations (Azwana 2021).

S. frugiperda is a lepidopteran pest originally native to the Americas and has emerged as a significant invasive species in Southeast Asia. The larvae are highly destructive, targeting the apical meristems of maize plants and resulting in the arrested development of the ear and tassel (Maharani et al. 2019). Ragged holes in leaves typically indicate infestation symptoms, frass resembling sawdust, and tunneling into plant tissues. Yield losses attributed to this pest have been reported to range between 15 and 73%, depending on the severity of infestation and control methods used (Nonci et al. 2019).

To manage this pest, most farmers still rely heavily on synthetic insecticides, primarily because of their rapid knockdown effects and accessibility. However, continuous application of chemical pesticides poses several ecological and agronomic concerns, including environmental contamination, residue accumulation, the elimination of beneficial insects, the development of pest resistance, and the emergence of secondary pest outbreaks (Idris 2023). In response to these challenges, the use of botanical insecticides derived from plants rich in secondary metabolites has gained attention. These natural pesticides are biodegradable, environmentally safe, and often readily available (Shaba et al. 2019). One such promising plant is Indian jujube (*Ziziphus mauritiana* Lamk), a hardy shrub with bioactive compounds distributed throughout its roots, stems, leaves, flowers, and fruits (Raharjeng and Masliyah

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2020). Phytochemical analysis has shown that Indian jujube leaves contain approximately 23.53% alkaloids, 5.53% saponins, and 3.03% flavonoids (Hastiana et al. 2022).

Alkaloids in this plant are known to act as both stomach and neurotoxins, interfering with the acetylcholinesterase enzyme activity, which results in neural disruption, paralysis, and ultimately death in target insects (Wahyuni and Loren 2015). Saponins function as digestive toxins, causing irritation and damage to the midgut epithelium, which disrupts nutrient absorption and metabolism (Yanuartono et al. 2017). Meanwhile, flavonoids inhibit molting by interfering with the action of ecdysone, thereby halting larval development (Saputri et al. 2021).

Previous studies support the efficacy of Indian jujube leaf extract in pest management. Asikin et al. (2022) reported that a 70% acetone-based extract of *Ziziphus spina-christi* induced 82.67% mortality in *Plutella xylostella* larvae after 72 h of treatment. According to Dadang and Prijono (2008), botanical pesticides are considered effective when they achieve mortality rates above 80% at a maximum concentration of 1% using organic solvents. The choice of solvent is also a determining factor in extract potency; organic solvents such as methanol offer superior solubility for secondary metabolites compared to water-based solutions (Wahyudi and Minarsih 2023). Based on this background, the present study was conducted to determine the effective concentration of Indian jujube leaf extract, using ethanol as a solvent, for controlling *S. frugiperda* larvae under laboratory conditions.

MATERIALS AND METHODS

This research was conducted from November 2023 to January 2024 at the Plant Pest Laboratory, Faculty of Agriculture, Universitas Riau. The experiment was carried out using a completely randomized design with six treatment concentrations of Indian jujube leaf extract: 0.0 (control), 0.2, 0.4, 0.6, 0.8, and 1.0%, each replicated four times, resulting in a total of 24 experimental units. Each unit consisted of 10 third-instar *Spodoptera frugiperda* larvae.

Indian jujube leaves were collected, air-dried at room temperature, and ground into fine powder. Extraction was performed using the maceration method with 70% methanol at a 1:5 ratio (w/v) for five days. The filtrate was concentrated using a rotary evaporator at 40–45°C to obtain the crude extract. Dilutions for treatment concentrations were prepared using distilled water and applied using a 100 mL hand sprayer.

Larvae of *S. frugiperda* were originally collected from maize fields infested naturally in the Pekanbaru area and reared in the laboratory using pesticide-free young maize cobs as feed. The bioassay used second-generation third-instar larvae to ensure uniformity in development and exposure. Each treatment was applied topically through spraying directly onto larvae and their food substrate under ambient laboratory conditions.

Parameters observed included initial time to mortality (h post-application), lethal time 50% (LT_{50}), lethal concentration 50% (LC_{50}), lethal concentration 95% (LC_{95}), daily larval mortality rate, and cumulative total mortality percentage. Daily mortality was recorded for five consecutive days. Data were organized in Microsoft Excel, and statistical analyses were conducted using analysis of variance (ANOVA), followed by the Honestly Significant Difference (HSD) test at a 5% significance level. Probit analysis for LC_{50} and LC_{95} values was calculated using probit analysis with SPSS version 25.

RESULTS AND DISCUSSIONS

Initial lethal time

The application of various concentrations of Indian jujube leaf extract significantly influenced the initial mortality time of *S. frugiperda* larvae. The time at which the first signs of larval mortality were observed varied across treatment groups, as detailed in Table 1. The application of Indian jujube leaf extract at various concentrations resulted in differences in the time to initial larval mortality (initial lethal time) of *S. frugiperda*, which ranged from 7.25 to 120 h post-treatment. The control treatment (0%) did not induce any mortality within the observation period, as it did not contain any bioactive compounds capable of initiating a toxic response in the larvae. The application of a 0.2% extract concentration resulted in an initial lethal time of 16.25 h, which was not significantly different from the 0.4% concentration, but significantly different from the higher concentrations (0.6, 0.8, and 1.0%). This delay in mortality is likely attributed to the lower quantity of bioactive secondary metabolites present at lower concentrations, which reduced the extract's immediate toxic effectiveness (Nasution and Rustam 2021).

Table 1. Initial lethal time of *S. frugiperda* larvae following treatment with different concentrations of *Z. mauritiana* leaf extract

Concentration of Extract (%)	Initial lethal time (h)
0.0	120.00 ± 0.00[a]
0.2	16.25 ± 0.50[b]
0.4	14.25 ± 0.96[b]
0.6	11.25 ± 0.50[c]
0.8	10.75 ± 0.96[c]
1.0	7.25 ± 0.50[c]

Remarks: Values within a column followed by different lowercase letters indicate significant differences based on the Honestly Significant Difference (HSD) test at the 5% level, after square root transformation (√y) of the data

The highest concentration (1%) induced the fastest lethal response, with initial mortality observed at 7.25 h post-application. This effect was statistically comparable to the 0.6 and 0.8% treatments, but significantly different from the lower concentrations. However, the absence of significant differences among the three highest concentrations suggests a possible plateau effect in toxicity, where increased concentration no longer results in a proportionally faster mortality rate. This may be due to the physiological resilience of the larvae or a saturation point in their sensitivity to the active compounds (Nofrianti and Salbiah 2022).

Phytochemical analyses indicate that the leaves of Indian jujube contain higher concentrations of secondary metabolites compared to the fruit, especially flavonoids, alkaloids, and saponins (Santoso et al. 2023). Among these, flavonoids are believed to be the dominant compounds responsible for early toxicity. These compounds can act as contact poisons, damaging the larval cuticle and disrupting epidermal function. Furthermore, flavonoids interfere with the hormonal regulation of molting by inhibiting the action of ecdysone, thereby preventing successful ecdysis and leading to larval death (Anugraheni and Asngad 2018).

Lethal time 50% (LT_{50})

The application of various concentrations of Indian jujube leaf extract had a statistically significant impact on the LT_{50} (lethal time 50%) of *S. frugiperda* larvae. LT_{50} is a critical toxicological parameter that reflects the time required to induce 50% mortality within a treated population, serving as an indicator of the speed at which the insecticidal compound exerts its toxic effects. Shorter LT_{50} values indicate a faster-acting toxic response, suggesting higher treatment efficacy in a shorter duration. Conversely, longer LT_{50} values imply a slower toxic action, possibly due to lower concentration or reduced bioactivity of the extract. In this study, increasing concentrations of the extract generally led to a decrease in LT_{50}, indicating a dose-dependent response. This pattern suggests that the bioactive compounds in the Indian jujube leaf extract act more rapidly at higher concentrations.

The mean LT_{50} values, based on the Honestly Significant Difference (HSD) test at a 5% significance level (Table 2). The LT_{50} values decreased as the concentration of the extract increased. The three lowest concentrations (0.0, 0.2, and 0.4%) exhibited no significant difference from each other or from the 0.6% treatment, with LT_{50} values remaining above 100 h. This prolonged lethal response at lower concentrations suggests a reduced level of toxicity, likely due to insufficient quantities of active secondary metabolites required to induce rapid mortality (Rustam et al. 2023).

Table 2. Mean lethal time 50% (LT_{50}) of *S. frugiperda* larvae after application of Indian jujube leaf extract

Concentration of Extract (%)	LT_{50} (h) ± SD
0.0	120.00 ± 0.00[a]
0.2	120.00 ± 0.00[a]
0.4	120.00 ± 0.00[a]
0.6	103.25 ± 1.71[a]
0.8	58.00 ± 2.45[b]
1.0	52.00 ± 2.00[b]

Remarks: Means followed by different lowercase letters in the same column indicate significant differences at $p < 0.05$ based on the HSD test after $\sqrt{y}$ transformation

Higher concentrations of 0.8 and 1.0% significantly reduced LT_{50} values to 58.00 and 52.00 h, respectively. Although both treatments were not significantly different from each other, they were statistically different from the lower concentration groups. This indicates that increased levels of active compounds in the extract accelerated the onset of toxic effects on the larvae. This concentration-dependent response aligns with previous studies, which have shown that higher doses of plant-based insecticides tend to cause more rapid mortality due to enhanced biochemical disruption (Adnyana et al., 2012).

Lethal concentration 50 and 95% (LC_{50} and LC_{95})

The estimation of LC_{50} and LC_{95} values for *S. frugiperda* larvae treated with Indian jujube leaf extract was determined using probit regression analysis in SPSS version 25. The analysis revealed that the extract exhibited a median lethal concentration (LC_{50}) of 0.74%, while the concentration required to cause 95% mortality (LC_{95}) was estimated at 1.62%. These values indicate the effective range of the extract in eliciting larval mortality and reflect the dose-dependent toxicity of the bioactive compounds present in the formulation.

Table 3 presents the estimated concentrations of Indian jujube leaf extract required to induce 50% and 95% mortality in *S. frugiperda* larvae. The LC_{50} value was calculated at 0.74%, equivalent to 7.4 $mL \cdot L^{-1}$, with a 95% confidence interval ranging from 0.56 to 0.88%. Meanwhile, the LC_{95} value was determined to be 1.62%, or 16.2 $mL.L^{-1}$, with a wider confidence interval of 1.20 to 5.02%. The width of the confidence interval reflects the precision of the concentration estimates narrower intervals denote higher estimation accuracy, while wider intervals indicate lower precision in prediction (Munandar & Halim 2020). In this case, the LC_{50} value exhibited a narrower confidence range, suggesting a reliable estimate, whereas the broader range for LC_{95} implies greater variability in response among the test population.

Table 3. Estimated LC_{50} and LC_{95} values of *S. frugiperda* larvae after treatment with Indian jujube leaf extract

Lethal concentration	Concentration (%)	CI 95% (%)
LC_{50}	0.74	0.56-0.88
LC_{95}	1.62	1.20-5.02

Remark: CI: Confidence interval

Although the LC_{50} concentration falls within the threshold for botanical pesticide efficacy, the LC_{95} value exceeds the commonly accepted maximum. According to the criteria proposed by Dadang and Prijono (2008), a botanical pesticide is considered effective when it can induce mortality greater than 80% at a concentration of no more than 1%, using an organic solvent as the extraction medium. In this study, the concentration required to achieve 95% mortality (1.62%) surpasses that threshold, indicating that higher concentrations may be less practical or economically feasible for widespread application.

Daily mortality (%)

The daily mortality response of *S. frugiperda* larvae following treatment with various concentrations of Indian jujube leaf extract exhibited dynamic changes over the five-day observation period. The pattern of larval

mortality per day is illustrated in Figure 1. The control treatment (0%) did not induce any larval mortality throughout the observation period, confirming the absence of insecticidal activity in the absence of the extract. On the first day after application, all extract treatments induced relatively low mortality, ranging from 7.5 to 15%. However, larval mortality peaked on the second day, with rates ranging from 10 to 27.5% across concentrations. The highest mortality (27.5%) was recorded at the 1% concentration, suggesting that this phase represented the most active period of the extract's toxic effect.

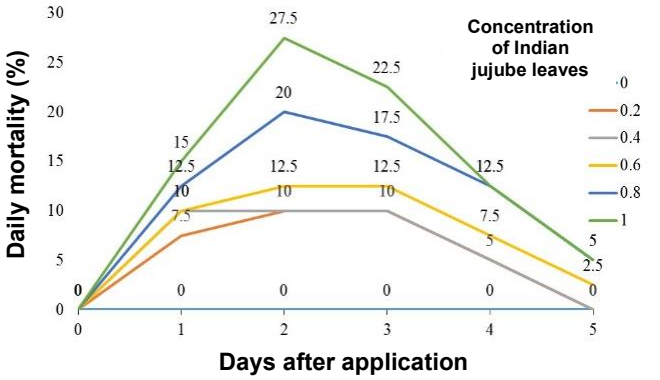

Figure 1. Daily mortality of S. frugiperda larvae after application of Indian jujube leaf extract

This surge in mortality on the second day is likely attributed to the action of alkaloid compounds, which are known to be major bioactive constituents in Indian jujube leaves. Alkaloids act as both stomach poisons and neurotoxins, impairing the nervous system by disrupting hormonal activity, particularly the ecdysone and juvenile hormones that regulate growth and molting (Cania & Setyaningrum 2013; Jemi et al. 2019). Dadang and Prijono 2008) also noted that the maximum bioactivity of plant-based pesticides typically occurs within 2 to 4 days after application, aligning with the mortality peak observed in this study.

In addition to alkaloids, saponins present in the extract may have contributed to larval death by inducing damage to the digestive tract. These compounds disrupt the midgut epithelium, leading to swelling and separation of the peritrophic membrane, which ultimately causes physiological collapse (Wahyuni and Loren 2015). The synergistic effect of these bioactive compounds may explain the rapid escalation in mortality within the first 48 h. From the third to the fifth day of post-treatment, daily mortality rates gradually declined. This reduction likely resulted from the depletion of the larval population due to earlier deaths and the gradual decline in the potency of the extract over time. Botanical insecticides, such as those derived from *Z. mauritiana*, are known to degrade rapidly and possess volatile components, which limit their residual activity beyond a few days (Dadang and Prijono 2008).

Total mortality (%)

The application of Indian jujube leaf extract at varying concentrations had a statistically significant effect on the total mortality of *S. frugiperda* larvae. The mean percentage of larval mortality, as determined by the Honestly Significant Difference (HSD) test, at a significance level of 5%, is presented in Table 4. Total mortality increased with extract concentration, ranging from 30.00% at a 0.2% concentration to 82.50% at a 1.0% concentration. The control group (0%) showed no mortality, indicating that the absence of active compounds rendered it ineffective. The treatments for 0.2, 0.4, and 0.6% did not differ significantly from each other, suggesting similar efficacy levels in the lower concentration range. However, concentrations of 0.8% and 1.0% caused significantly higher mortality rates and were statistically different from the lower concentrations. The 1% concentration proved most effective, achieving a total mortality of 82.50%, which meets the efficacy threshold for botanical pesticides as proposed by Dadang and Prijono (2008), who stated that a botanical pesticide is considered effective if it induces over 80% mortality at a maximum concentration of 1% using organic solvents.

Table 4. Total mortality of *S. frugiperda larvae* following treatment with different concentrations of Indian jujube leaf extract

Concentration of Extract (%)	Total Mortality (%) ± SD
0.0	0.00 ± 0.00^{c}
0.2	30.00 ± 2.16^{b}
0.4	35.00 ± 2.58^{b}
0.6	45.00 ± 3.00^{b}
0.8	72.50 ± 2.38^{a}
1	82.50 ± 2.08^{a}

Remarks: Values followed by different lowercase superscript letters within the column indicate statistically significant differences based on the HSD test at 5% level, after square root (√y) data transformation

The higher efficacy at greater concentrations is attributed to the greater availability of bioactive secondary metabolites. Organic solvents like ethanol (used at 70% concentration in this study) are known to enhance the extraction of active compounds such as alkaloids, flavonoids, and saponins, resulting in higher biological activity compared to water-based solvents (Nihayah et al. 2016). Alkaloid compounds present in the Indian jujube leaf extract likely played a key role in larval mortality. These compounds act as neurotoxins and stomach poisons, interfering with the function of the acetylcholinesterase enzyme in the insect nervous system, ultimately leading to neuromuscular paralysis and death (Wahyuni and Loren 2015).

CONCLUSIONS AND SUGGESTIONS

Based on the study's results, it can be concluded that a 1% concentration of Indian jujube leaf extract was effective in controlling *S. frugiperda* larvae under

laboratory conditions. This concentration induced a total larval mortality of 82.50%, with an initial lethal time recorded at 7.25 h post-application and an LT_{50} value of 52 h. The use of Indian jujube leaf extract at a 1% concentration is therefore recommended as a botanical insecticide for managing *S. frugiperda* larvae. However, further research is necessary to validate its efficacy under field conditions, considering environmental variables and long-term effects on pest populations and non-target organisms.

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