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Efektivitas Ekstrak Daun Mimba (*Azadirachta indica* A. Juss) terhadap Mortalitas *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae)

Effectiveness of Neem Leaf Extract (*Azadirachta indica* A. Juss.) on the Mortality of *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae)

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ABSTRACT

Background: Continuous reliance on synthetic insecticides for controlling *Helicoverpa armigera* may lead to pest resistance, resurgence, and environmental residues. Neem (*Azadirachta indica* A. Juss.) leaf extract, which contains the bioactive compound azadirachtin, has potential as a more environmentally friendly botanical insecticide. **Objective**: This study aimed to determine the effective concentration of neem leaf extract against the mortality of H. armigera under laboratory conditions. **Methods**: The experiment was arranged in a Completely Randomized Design (CRD) with five concentrations of neem leaf extract (0, 25, 50, 75, and 100 g/L water) and four replications, resulting in 20 experimental units. Each experimental unit was infested with 10 third-instar *H. armigera* larvae. The observed variables included initial mortality time, lethal time 50 (LT_{50}), daily mortality, total mortality, and ambient temperature and humidity. Data were analyzed using analysis of variance followed by Duncan's New Multiple Range Test (DNMRT) at the 5% significance level. **Results:** Neem leaf extract concentration significantly affected the initial mortality time, LT_{50}, and total mortality of *H. armigera*. The 100 g/L concentration was the most effective treatment, producing an initial mortality time of 12.00 hours, an LT_{50} of 54.00 hours, and a total mortality of 82.50%. However, its total mortality did not differ significantly from that of the 75 g/L concentration. **Conclusions:** Neem leaf extract at 100 g/L water showed the highest effectiveness against *H. armigera* larvae under laboratory conditions. Further field evaluation is required to confirm its effectiveness under field conditions.

Keywords: *Azadirachta indica; botanical insecticide; corn earworm; Helicoverpa armigera; mortality*

ABSTRAK

Pendahuluan: Penggunaan insektisida sintetis secara berkelanjutan dalam pengendalian *Helicoverpa armigera* berpotensi menimbulkan resistensi hama, resurjensi, dan residu di lingkungan. Ekstrak daun mimba (*Azadirachta indica* A. Juss.) yang mengandung senyawa bioaktif azadirachtin berpotensi digunakan sebagai insektisida botani yang lebih ramah lingkungan. **Tujuan**: Menentukan konsentrasi ekstrak daun mimba yang efektif terhadap mortalitas H. armigera dalam kondisi laboratorium. **Metode**: Percobaan disusun menggunakan Rancangan Acak Lengkap (RAL) dengan lima konsentrasi ekstrak daun mimba, yaitu 0, 25, 50, 75, dan 100 g/L air, serta empat ulangan sehingga diperoleh 20 satuan percobaan. Setiap satuan percobaan diinfestasikan dengan 10 larva *H. armigera* instar III. Variabel yang diamati meliputi waktu awal kematian, lethal time 50 (LT_{50}), mortalitas harian, mortalitas total, serta suhu dan kelembapan lingkungan. Data dianalisis menggunakan analisis ragam dan dilanjutkan dengan uji Duncan's New Multiple Range Test (DNMRT) pada taraf 5%. **Hasil**: Konsentrasi ekstrak daun mimba berpengaruh nyata terhadap waktu awal kematian, LT_{50}, dan mortalitas total H. armigera. Konsentrasi 100 g/L merupakan perlakuan yang paling efektif, dengan waktu awal kematian 12,00 jam, LT_{50} sebesar 54,00 jam, dan mortalitas total sebesar 82,50%. Mortalitas total pada konsentrasi 100 g/L tidak berbeda nyata dengan konsentrasi 75 g/L. **Kesimpulan**: Ekstrak daun mimba pada konsentrasi 100 g/L air efektif dalam mengendalikan *H. armigera* pada kondisi laboratorium dan dapat menjadi dasar untuk pengujian lebih lanjut pada kondisi lapangan.

Kata kunci: *Azadirachta indica; botanical insecticide; Helicoverpa armigera; mortality; neem leaf extract.*

BACKGROUND

Corn (*Zea mays* L.) is one of the most important food commodities in Indonesia after rice and wheat, playing a central role in the national economy as a major source of carbohydrates besides rice (Rachman et al., 2023). Expanding corn production on a larger and more productive scale has the potential to raise farmers' income and boost regional economies (Melia et al., 2023). Corn is an important commodity in Riau Province, yet its production there has declined considerably in recent years.

Statistics Indonesia (Badan Pusat Statistik, 2025) reported that corn productivity in Riau Province was 34.64 quintals ha^{-1} in 2022, decreased to 31.83 quintals ha^{-1} in 2023, and reached 34.83 quintals ha^{-1} in 2024. Among the factors that may contribute to reduced corn productivity, infestation by the corn earworm *Helicoverpa armigera* Hubner, a major pest of corn, represents an important biotic constraint. *Helicoverpa armigera* attacks corn ears and prevents proper formation of the tassel, thereby reducing yield (Pramesti, 2019), and continuous larval feeding on the ear progressively reduces the number of kernels formed (Safitri *et al.*, 2013). Forecasted *H. armigera* attack intensity in Riau Province reached 79.86% in March 2023 (BBPOPT, 2023).

Farmers commonly control *H. armigera* using synthetic insecticides. However, continuous and inappropriate use of synthetic insecticides can trigger negative effects such as pest resurgence, resistance, mortality of non-target organisms, and environmental residue (Togola *et al.*, 2018). Botanical insecticides offer an environmentally friendlier alternative to reduce reliance on synthetic products (Setiawan et al., 2021). Botanical insecticides suppress insect feeding activity, thereby limiting plant damage, have a broad control spectrum, and can still act on pests already resistant to synthetic insecticides because their toxicity to mammals is relatively low (Wiranto et al., 2013), although they also degrade quickly, require more frequent application, and have comparatively low toxicity and production capacity (Kusmawati & Istiqomah, 2022). Neem (*Azadirachta indica* A. Juss) is one of the plants with strong potential as a botanical insecticide.

Neem leaves used as a botanical insecticide contain the active compound azadirachtin, which is toxic to insects (Agustin & Rosmini, 2016). Azadirachtin does not kill insects instantly but acts by suppressing feeding activity, inhibiting growth, and reducing reproductive capacity (Ervinatun et al., 2018).

Ma'wa et al. (2023) reported that neem leaf extract applied at 80 g/L water effectively controlled *Spodoptera frugiperda* J.E. Smith larvae on corn, producing a total mortality of 92.50%. Several other botanical insecticides have also been tested specifically against *H. armigera* infesting corn in Riau Province. Rismawanto et al. (2022) reported that tuba root flour extract (*Derris elliptica* Benth.) was effective in controlling *H. armigera* larvae attacking the corn ear. Nofrianti and Salbiah (2022) likewise found that babadotan leaf extract (*Ageratum conyzoides* L.) caused significant mortality of the corn ear borer. More recently, Zahra and Rustam (2025) showed that a 0.75% concentration of forest betel leaf (*Piper aduncum* L.) organic extract produced 85% total mortality of *H. armigera* larvae, with an initial lethal time of 4.75 hours after application. Outside Indonesia, Ali et al. (2021) demonstrated that several botanical plant extracts suppressed *H. armigera* populations and improved tomato yield under field conditions, confirming that botanical extracts are viable substitutes for synthetic insecticides against this pest.

A botanical insecticide is considered effective when it causes at least 80% mortality of the target pest using a water solvent at a concentration not exceeding 10%, or an organic solvent at a concentration not exceeding 1% (Dadang & Prijono, 2008). Regarding neem specifically, Mina et al. (2025) reported that neem leaf extract at a concentration of 40 g/L water effectively reduced *H. armigera* larval populations on tomato, reaching a control effectiveness of 80%, indicating that neem leaf extract is indeed toxic to this insect species. However, this demonstrated efficacy of neem leaf extract against *H. armigera* has so far only been established on tomato and, for other lepidopteran species, on corn — such as against *S. frugiperda* (Ma'wa et al., 2023) — whereas information on the effectiveness of water-based neem leaf extract against *H. armigera* specifically infesting corn remains limited.. Research on neem leaf extract with a water solvent against *H. armigera* has so far been limited. Based on this problem, the present study was conducted to determine the effective concentration of neem leaf extract (*Azadirachta* *indica* A. Juss) against the mortality of *H. armigera* Hubner (Lepidoptera: Noctuidae) under laboratory conditions.

METHODS

Study Site and Time

The experiment was conducted at the Plant Pest Laboratory, Faculty of Agriculture, Universitas Riau, Pekanbaru, from January to March 2025.

Materials and Equipment

The materials used were neem leaves, third-instar *H. armigera* larvae, young corn ears, liquid soap, corn husk, sterile distilled water, sawdust, and water. The equipment used included plastic jars (22 x 20 cm) and cups (4 x 9.5 cm), honey, a brush, an analytical balance, scissors, rubber bands, a stirring rod, cotton, gauze, a beaker glass, a funnel, a blender, a 40-mesh sieve, a thermohygrometer, labels, documentation equipment, and a 250 mL hand sprayer.

Experimental Design

The experiment was arranged in a Completely Randomized Design (CRD) consisting of five treatments with four replications, giving 20 experimental units. Each experimental unit was infested with 10 third-instar *H. armigera* larvae, for a total of 200 larvae. Each replication consisted of 10 larvae maintained individually in separate cups. The treatments consisted of five concentrations of neem leaf extract dissolved in water: A0 = 0 g/L (control), A1 = 25 g/L, A2 = 50 g/L, A3 = 75 g/L, and A4 = 100 g/L.

Insect Rearing

Helicoverpa armigera larvae were collected from a pesticide-free corn field at the experimental garden of the Faculty of Agriculture, Universitas Riau, and reared individually in plastic cups supplied daily with fresh young corn ears until pupation. Pupae were transferred to plastic jars containing sawdust and maintained until adult emergence. Male and female adults were paired in larger jars, provided with cotton soaked in honey solution as food, and given corn husk as an oviposition substrate. Eggs deposited on the husk were transferred to clean jars, and the resulting neonate larvae were reared individually until they reached the third instar used as test insects.

Neem Leaf Extract Preparation

Neem leaves were collected fresh, washed, and air-dried at room temperature for one week before being ground with a blender and passed through a 40-mesh sieve. The resulting powder was weighed according to each treatment concentration (25, 50, 75, and 100 g), dissolved in 1,000 mL of distilled water together with 1 g of liquid soap as a surfactant, and stirred until homogeneous. The solution was left to stand for one hour and then filtered through fine gauze to obtain the neem leaf extract used for application.

Calibration, Infestation, and Treatment Application

Spray volume was calibrated using a 250 mL hand sprayer filled with distilled water and sprayed evenly onto pieces of young corn until wet; calibration was repeated five times, giving an average spray volume of 2 mL of extract per four sprayer strokes. Young corn ears collected from a pesticide-free field were cut into 3 cm segments and placed into plastic cups, and one third-instar *H. armigera* larva was infested per cup and covered with gauze, so that 200 cups were used for the 20 experimental units. Neem leaf extract was applied by spraying the calibrated volume evenly onto the corn segment and the larva in each cup at 17:00 local time.

Observation Variables

The variables observed were initial mortality time (the time required for the first larval death in each treatment, recorded hourly after application), lethal time 50 (LT_{50}, the time required to kill 50% of the larvae in each treatment, also recorded hourly), daily mortality (%, the proportion of larvae that died each day), and total mortality (%, the cumulative proportion of larvae that died by the end of the observation period). Ambient temperature (°C) and relative humidity (%) were recorded daily at 07:00, 12:00, and 17:00 local time using a thermohygrometer.

Statistical Analysis

Mortality data were analyzed using analysis of variance (ANOVA). Initial mortality time and total mortality data were transformed prior to analysis, using square-root and arcsine $\sqrt{(y + 1)}$ transformations, respectively. When treatment effects were significant, means were compared using Duncan's New Multiple Range Test (DNMRT) at the 5% significance level. LT_{50} values were estimated using probit analysis based on mortality-time data. Daily mortality was analyzed descriptively. All statistical analyses were performed using SAS version 9.1.

RESULTS AND DISCUSSION

Initial Mortality Time

Analysis of variance showed that neem leaf extract concentration significantly affected the initial mortality time of H. armigera larvae. The mean initial mortality times are presented in Table 1.

Table 1. Initial mortality time of *H. armigera* larvae following application of neem leaf extract at several concentrations (hours).

Neem leaf extract concentration (g/L water)	Initial mortality time (hours)
0	144.00 a
25	35.50 b
50	25.50 b
75	14.50 c
100	12.00 c

Values followed by the same letter are not significantly different according to DNMRT at the 5% level, following square-root transformation. CV = 5.18%

Neem leaf extract concentration influenced the initial mortality time of *H. armigera* larvae, which ranged from 12.00 to 144.00 hours after application. In the 0 g/L treatment, no mortality occurred within the 144-hour observation period because this treatment contained no neem leaf extract.

The 100 g/L concentration produced the shortest initial mortality time (12.00 hours), which did not differ significantly from the 75 g/L concentration but differed significantly from the lower concentrations. This response may be associated with greater exposure of larvae to the bioactive compounds present in the neem leaf extract at higher concentrations. Sitompul et al. (2014) reported that a higher concentration of botanical insecticide causes faster mortality of the target insect.

Neem leaves contain azadirachtin and other bioactive compounds that can act as antifeedants and growth regulators, thereby reducing feeding activity and disrupting larval development.. Azadirachtin has also been shown to reduce digestive enzyme activity in Lepidopteran larvae, causing them to starve even when food remains available (Koul & Walia, 2020).

The 25 g/L treatment produced the longest initial mortality time (35.50 hours), not significantly different from 50 g/L but significantly different from the other treatments. This slower mortality was attributed to the comparatively low content of azadirachtin at this concentration, consistent with Utami and Cahyati (2017), who reported that a lower concentration contains less active compound and is therefore less effective.

Lethal Time 50 (LT_{50})

Analysis of variance showed that neem leaf extract concentration significantly affected LT_{50} of *H. armigera* larvae, with the 100 g/L concentration killing 50% of the test larvae faster than the other concentrations. The mean LT_{50} values are presented in Table 2.

Table 2. Lethal time 50 (LT_{50}) of *H. armigera* larvae following application of neem leaf extract at several concentrations (hours).

Neem leaf extract concentration (g/L water)	LT_{50} (hours)
0	144.00 a
25	111.75 b
50	89.25 bc
75	71.75 c
100	54.00 d

Values followed by the same letter are not significantly different according to DNMRT at the 5% level, following square-root transformation. CV = 5.23%.

Neem leaf extract concentration influenced LT_{50} of *H. armigera* larvae, which ranged from 54.00 to 144.00 hours. The control treatment (0 g/L) did not reach 50% mortality during the 144-hour observation period; therefore, its LT_{50} exceeded the observation period (>144 h).

The 100 g/L concentration killed 50% of the test population fastest, at 54.00 hours after application, and differed significantly from all other treatments. This was related to the higher amount of active compound delivered to the target insect at this concentration. Hidayati et al. (2013) reported that a higher concentration contains more secondary metabolites, resulting in higher toxicity and faster pest mortality.

The 25 g/L treatment showed the slowest LT_{50}, consistent with its comparatively low active-compound content, which reduced the amount of toxin entering the larval body and delayed mortality (Yunianti, 2016).

The decline in LT_{50} with increasing extract concentration may be related to the greater exposure of larvae to neem bioactive compounds. Azadirachtin is known to act primarily through antifeedant and growth-regulating effects, which can interfere with larval development and ultimately contribute to mortality. Senthil (2016) reported that this hormonal disruption prevents larvae from developing normally and ultimately causes death. Neem leaf extract has also been shown to increase oxidative stress in target insects, damaging tissue and reducing larval resistance, which together explain the shorter LT_{50} values obtained at higher extract concentrations (Isman, 2020)

Daily Mortality

Daily mortality of *H. armigera* larvae was recorded for six days after application and is presented in Figure 1.

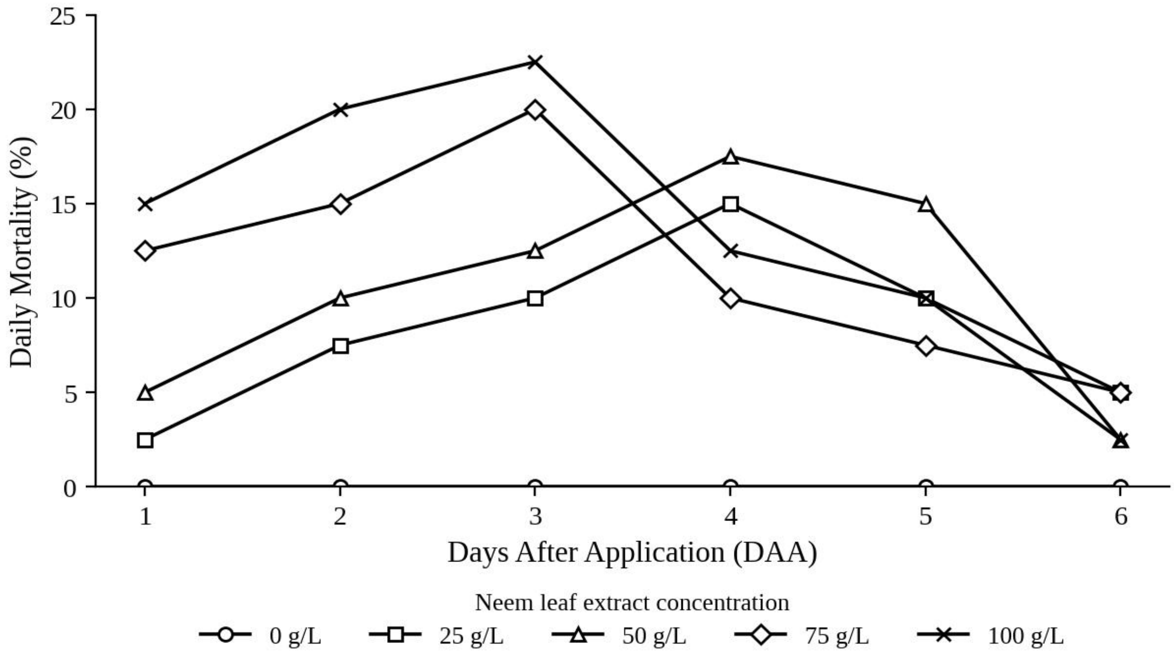

Figure 1. Daily mortality of *H. armigera* larvae after application of neem leaf extract at several concentrations.

The neem extract treatments caused mortality ranging from 2.5% to 15.0% during the first day after application. By the second day, daily mortality across the neem treatments increased to 7.5–20.0%, with higher concentrations generally producing higher mortality. This pattern is consistent with Rahman and Sari (2020), who reported that neem leaf extract effectively increases *H. armigera* mortality, with higher concentrations causing faster larval death owing to their toxic active compounds and disruption of insect physiological processes.

The 100 and 75 g/L treatments reached their peak daily mortality on the third day, at 22.5% and 20.0% respectively, whereas the 50 and 25 g/L treatments peaked later, on the fourth day, at 17.5% and 15.0%. This delay likely reflects the time needed for the active compound to accumulate and act effectively within the larval body; because azadirachtin behaves as a stomach poison and growth inhibitor rather than a fast-acting toxin, its effect typically becomes most pronounced two to three days after application (Tarumingkeng, 1992).

Daily mortality declined in all treatments from the fifth day onward, falling to 7.5-15.0% on day five and to 2.5-5.0% on day six. This decline may be associated with the limited persistence of bioactive compounds in botanical extracts over time. This decline reflects a known limitation of botanical insecticides, whose active compounds degrade relatively quickly in the environment and therefore require more frequent or repeated application (Sutriadi et al., 2019).

Total Mortality

Analysis of variance showed that neem leaf extract concentration significantly affected total mortality of *H. armigera* larvae. The mean total mortality values are presented in Table 3.

Table 3. Total mortality of *H. armigera* larvae following application of neem leaf extract at several concentrations (%).

Neem leaf extract concentration (g/L water)	Total mortality (%)
0	0.00 c
25	50.00 b
50	62.50 bc
75	70.00 ab
100	82.50 a

Values followed by the same letter are not significantly different according to DNMRT at the 5% level, following arcsine $\sqrt{(y + 1)}$ transformation. CV = 1.68%.

Neem leaf extract concentration influenced total mortality of *H. armigera* larvae, which ranged from 0% to 82.50%. The 100 g/L concentration produced the highest total mortality (82.50%), not significantly different from the 75 g/L concentration (70.00%). The tendency toward higher mortality at higher extract concentrations suggests a concentration-dependent response, although several treatment means were not significantly different according to DNMRT, consistent with Sonia et al. (2017), who reported that a higher concentration of botanical insecticide contains more secondary metabolites, producing a stronger toxic effect and greater pest mortality.

The 25 g/L treatment produced the lowest total mortality among the extract treatments (50.00%), not significantly different from 50 and 75 g/L, indicating that increasing the concentration from 25 to 75 g/L did not significantly increase total mortality within that range. This is presumably because the active compound at these three concentrations produced comparatively similar toxicity, resulting in a statistically similar mortality response. Dadang and Prijono (2008) noted that the effectiveness of a botanical insecticide depends on the amount of active compound that enters the target insect's body.

The 100 g/L treatment produced 82.50% mortality, corresponding to 10% (w/v), and therefore met the effectiveness criterion proposed by Dadang and Prijono (2008) for botanical insecticides applied using water as a solvent, proposed by Dadang and Prijono (2008), who considered a botanical pesticide effective when it kills at least 80% of the target pest using a water solvent at a concentration not exceeding 10%.

The decline in larval survival following neem leaf extract application likely reflects the combined effects of reduced feeding activity, disrupted metabolism, and internal tissue damage. Singh et al. (2025) reported that neem extract reduces food-conversion efficiency in Lepidopteran larvae, thereby inhibiting growth and increasing mortality. These findings support the view that neem leaf extract is a more environmentally friendly alternative to synthetic insecticides, being relatively safe for non-target organisms and leaving fewer harmful residues (Campos et al., 2019).

Behavioral and Morphological Changes

Neem leaf extract also affected the behavior and morphology of *H. armigera* larvae. Healthy larvae were characterized by active movement and vigorous feeding, whereas larvae exposed to neem leaf extract showed reduced feeding activity and slower movement compared with the control, consistent with the antifeedant action of azadirachtin (Senthil, 2015).

Body color changed to yellowish-brown within 24 hours after application, consistent with the known effects of azadirachtin in inhibiting molting, disrupting growth, suppressing feeding, and impairing larval development up to death (Qari et al., 2019). At the highest concentration, larvae eventually turned blackish-brown, shriveled, and died by the end of the observation period; some larvae also became rigid and reduced in size as feeding activity declined. These symptoms indicate that neem leaf extract acts as a stomach poison that enters the larval body through ingested food and subsequently disrupts the physiological and metabolic systems of *H. armigera* (Akhtar et al., 2019)..

(a) (b) (c)

Figure 2. Morphological changes in *H. armigera* larvae following neem leaf extract application: (a) healthy larva; (b) larva showing discoloration 24 h after application; and (c) darkened and shriveled larva at 144 h after application

CONCLUSION

Neem leaf extract at 100 g/L water showed the highest effectiveness against *H. armigera* larvae under laboratory conditions, resulting in the shortest initial mortality time (12.00 h), the lowest LT_{50} (54.00 h), and the highest total mortality (82.50%). However, total mortality at 100 g/L was not significantly different from that at 75 g/L. Mortality tended to increase with increasing extract concentration, whereas initial mortality time and LT_{50} decreased. Further field studies are required to evaluate the practical effectiveness of neem leaf extract against *H. armigera* under field conditions.

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