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Potential of Ethanol Extract of Kirinyuh Leaves (*Chromolaena odorata* L.) as a Botanical Biopesticide for Controlling Fall Armyworm (*Spodoptera frugiperda* J.E. Smith) (Lepidoptera: Noctuidae) on Maize Leaves (*Zea mays* L.) under Laboratory Conditions

Ni Putu Sinta Pramita, Dwi Widaningsih*, Ni Nengah Darmiati, Ketut Ayu Yuliadhi, I Nyoman Wijaya, I Dewa Putu Singarsa

Agroecotechnology Study Program, Faculty of Agriculture, Udayana University,
Jln. PB. Sudirman Denpasar Bali 80232, **Indonesia**

**Coresponding author:* sintadeva29@gmail.com

ABSTRACT

Maize (Zea mays L.) is a strategic cereal crop with high economic value in Indonesia. However, its productivity is frequently constrained by fall armyworm (Spodoptera frugiperda J.E. Smith), an invasive pest that causes severe leaf damage and yield losses. Excessive reliance on synthetic insecticides may increase pest resistance, environmental contamination, and risks to human health; therefore, botanical insecticides are increasingly explored as safer alternatives. This study evaluated the potential of ethanol extract of kirinyuh leaves (Chromolaena odorata L.) as a botanical biopesticide against second-instar S. frugiperda larvae on maize leaves under laboratory conditions. The experiment was arranged in a completely randomized design with six treatments, consisting of a control and kirinyuh leaf extract concentrations of 2%, 4%, 6%, 8%, and 10%, with four replications. Maize leaves were treated using a leaf-dip bioassay, and larval mortality was observed every 24 h for 168 h after application. The effects of the extract on pupal and adult abnormalities were also recorded. The results showed that kirinyuh leaf ethanol extract significantly affected larval mortality and insect development. The highest larval mortality was obtained at 10% extract concentration, reaching 50% at 168 h after application. The extract also disrupted metamorphosis, with the 2% concentration producing the highest pupal abnormality (47.5%), whereas the 4%, 8%, and 10% concentrations produced 2.5% abnormal adults. These findings indicate that kirinyuh leaf ethanol extract has moderate insecticidal and developmental-disrupting activity against S. frugiperda, but further optimization of concentration, formulation, and application frequency is required before practical field use.

Keywords: botanical insecticide, Chromolaena odorata, fall armyworm, maize, Spodoptera frugiperda

INTRODUCTION

Maize (Zea mays L.) is a strategic cereal crop with high economic value and substantial potential as a major source of carbohydrates and protein after rice. In Bali, maize production in Tabanan Regency fluctuated from 2016 to 2020, with annual production of 7,265, 8,664, 4,889, 11,150, and 7,249 tons, respectively (Dinas Pertanian dan Ketahanan Pangan Provinsi Bali, 2021). Such production variability is influenced by several factors, including pest pressure in maize-growing areas.

One of the major pests attacking maize is the fall armyworm, Spodoptera frugiperda J.E. Smith (Lepidoptera: Noctuidae), an invasive pest that has become an important constraint in maize cultivation in Indonesia (Lubis et al., 2020). The larvae can attack the growing point of maize plants and may disrupt the formation of young leaves and ears. S. frugiperda larvae have a high feeding capacity, enter the whorl of maize plants, and actively consume plant tissues during the day and night. When food becomes limited, larvae may exhibit cannibalistic behavior (Sukmanto, 2019).

Farmers commonly manage S. frugiperda using synthetic chemical insecticides because they provide rapid effects, flexible application, diverse formulations, and relatively efficient pest suppression (Sukmanto, 2019). However, repeated and excessive use of synthetic insecticides can cause pest resistance, pest resurgence, secondary pest outbreaks, chemical residues, human poisoning, and environmental contamination of soil and water. Therefore, environmentally safer alternatives are needed to support integrated pest management.

Botanical pesticides are considered promising because they are generally more selective and environmentally friendly than many synthetic insecticides (Untung, 2006). Kirinyuh or Siam weed (Chromolaena odorata L.) is one plant species with potential as a botanical pesticide (Wijaya et al., 2018). Kirinyuh leaves contain bioactive compounds, including flavonoids, alkaloids, tannins, triterpenoids, and saponins (Siharis et al., 2018), which may affect insect nervous and muscular systems, reproduction, feeding behavior, repellency, and respiration.

This study aimed to identify the bioactive compounds contained in ethanol extract of kirinyuh leaves, evaluate the most effective extract concentration for inducing mortality in S. frugiperda larvae, and determine the effect of different extract concentrations on the metamorphosis of S. frugiperda under laboratory conditions.

MATERIALS AND METHODS

Time and Place of Study

The study was conducted from September 2024 to January 2025 at the Plant Pest Laboratory, AG Building, second floor, Plant Protection concentration, Agroecotechnology Study Program, Faculty of Agriculture, Udayana University, Denpasar, Bali.

Materials and Equipment

The materials used in this study included kirinyuh leaves, maize leaves, second-instar S. frugiperda larvae, distilled water, 96% ethanol, Tween 80, gauze, plastic cups, filter paper, tissue paper, labels, plastic jars, and rubber bands. The equipment consisted of stationery, scissors, a blender, measuring cylinders, an evaporator flask, rotary vacuum evaporator, Buchner funnel, gloves, soft brushes, 3.5 L glass jars, an analytical balance, forceps, and a mobile phone camera for documentation.

Rearing of Test Insects

S. frugiperda larvae were collected from maize fields in Rejasa Village, Penebel District, Tabanan Regency. Larvae were collected manually using forceps from maize plants showing typical feeding damage, such as leaf-surface scraping and coarse frass around the whorl. Collection was conducted in the late afternoon to evening because larvae are more active during this period. The collected larvae were transferred into plastic cups covered with gauze and secured with rubber bands. Larvae were reared on fresh maize leaves, which were replaced daily or whenever consumed. Rearing was continued until 240 second-instar larvae were obtained for the experiment. The F2 generation was used to minimize prior exposure to chemical pesticides.

Preparation of Kirinyuh Leaf Extract

Kirinyuh leaves used for extraction were fully expanded and free from herbivore attack and disease symptoms. The leaves were collected from Jimbaran, South Kuta District, Badung Regency, Bali, at coordinates 8°47'51.1"S and 115°09'57.4"E, approximately 28 m above sea level. Fresh leaves were air-dried for seven days in a shaded room. The dried leaves were weighed and ground using a blender to obtain kirinyuh leaf powder. The powder was macerated in 96% ethanol in glass jars for three consecutive 24 h periods. In this study, 4,650 mL of 96% ethanol was used to macerate 795 g of kirinyuh leaf powder until fully submerged. The macerate was filtered using a Buchner funnel, and the filtrate was evaporated using a rotary vacuum evaporator at 40 °C to obtain a concentrated extract. The extraction procedure was adapted from Barita et al. (2018).

Preparation of Extract Concentrations

The concentrated kirinyuh extract was diluted to obtain concentrations of 0% (control), 2%, 4%, 6%, 8%, and 10%. Tween 80 (5 mL) was added as an emulsifier. For example, to prepare 100 mL of a 2% solution, 2 mL of extract was mixed with 5 mL of Tween 80 and 93 mL of distilled water. The dilution followed the formula described by Permatasari and Asri (2021).

Experimental Design and Bioassay Procedure

The study used an experimental method arranged in a completely randomized design with six treatments: 0% extract (control), 2%, 4%, 6%, 8%, and 10% kirinyuh leaf ethanol extract. Each treatment was replicated four times, resulting in 24 experimental units. Each unit contained 10 second-instar S. frugiperda larvae.

Leaf-Dip Bioassay

Before application, larvae were starved for approximately 2 h. Fresh maize leaves measuring 10 × 5 cm were dipped into the extract solution according to the assigned concentration for 30 s and then air-dried for 30-60 s (Firdaus and Ulpah, 2016). Two maize leaf pieces were provided for 10 larvae in each experimental unit (Riyadi and Pasaru, 2022). The treated leaves were placed into jars together with S. frugiperda larvae. Treated leaves were provided for 48 h (2 × 24 h), after which untreated fresh maize leaves were supplied until larvae reached the sixth instar. Observations were conducted daily for seven days at 24 h intervals after application.

Observed Variables and Data Analysis

The observed variables included larval mortality, percentage of normal pupae, percentage of abnormal pupae, percentage of normal imagoes, and percentage of abnormal imagoes. The variables were calculated as follows:

Larval mortality (%) = (number of dead larvae / total number of tested larvae) × 100
Normal pupae (%) = (number of normal pupae / total number of tested larvae) × 100
Abnormal pupae (%) = (number of abnormal pupae / total number of tested larvae) × 100
Normal imagoes (%) = (number of normal imagoes / total number of pupae) × 100
Abnormal imagoes (%) = (number of abnormal imagoes / total number of pupae) × 100

The collected data were analyzed using analysis of variance (ANOVA). When treatments showed a significant effect, mean separation was performed using the least significant difference (LSD) test at the 5% significance level.

RESULTS AND DISCUSSION

Larval Mortality of S. frugiperda

Application of kirinyuh leaf ethanol extract significantly affected S. frugiperda larval mortality. Mean mortality differed among treatments according to the LSD test at the 5% level (Table 1).

Table 1. Mean mortality of S. frugiperda larvae at different concentrations of kirinyuh leaf ethanol extract

Treatment	24 HAA	48 HAA	72 HAA	96 HAA	120 HAA	144 HAA	168 HAA
EK0 (0%)	0 b	0 b	0 b	0 b	0 c	0 c	0 d
EK2 (2%)	0 b	0 b	2.5 b	2.5 b	2.5 bc	10 bc	15 cd
EK4 (4%)	10 a	10 ab	15 ab	20 a	20 ab	20 abc	25 bc
EK6 (6%)	2.5 ab	5 ab	7.5 ab	10 ab	12.5 abc	30 ab	30 abc
EK8 (8%)	10 a	15 a	20 a	20 a	27.5 a	35 a	45 ab
EK10 (10%)	7.5 ab	10 ab	15 ab	22.5 a	30 a	40 a	50 a
LSD 5%	6.70	8.09	11.96	11.96	14.08	16.91	16.42

Note: HAA = hours after application. Values followed by the same letter in the same column are not significantly different according to the LSD test at the 5% level.

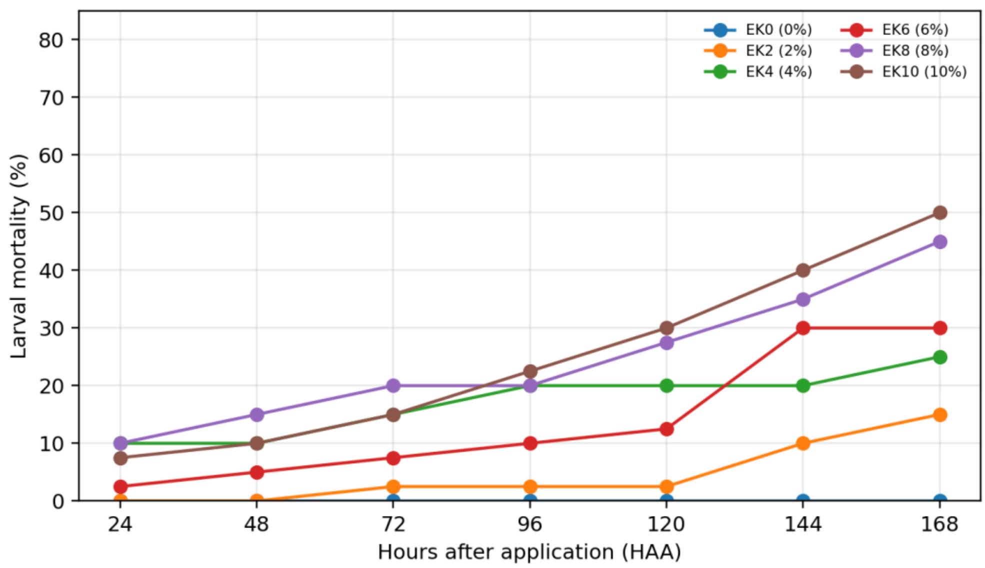

Figure 1. Mortality trend of S. frugiperda larvae after application of kirinyuh leaf ethanol extract

Mortality increased with observation time and extract concentration. At 24 HAA, the 4% and 8% treatments caused 10% mortality, whereas the control produced no mortality. The highest mortality was recorded in the 10% treatment at 168 HAA, reaching 50%. The control treatment consistently produced 0% mortality throughout the observation period. Although the 10% concentration showed the greatest insecticidal activity, it should be interpreted as moderate activity because mortality did not reach the >=80% level commonly used to indicate effective pest control. Therefore, the extract has potential as a botanical biopesticide, but its concentration, formulation, and application interval require further optimization.

Toxicity symptoms observed after extract application included reduced feeding activity, reduced movement, body discoloration from green to pale brown and eventually blackish brown, body shrinkage, and softening of larval tissues. These symptoms suggest physiological disturbance caused by bioactive compounds in the extract, particularly sesquiterpenes and flavonoids, which may act as respiratory and neurotoxic agents.

The ability of kirinyuh extract to suppress S. frugiperda larvae is associated with secondary metabolites in kirinyuh leaves. GC-MS-based studies have reported that C. odorata contains sesquiterpenes and flavonoids that may be toxic to insect larvae. Terpenoids, tannins, saponins, and sesquiterpenes have also been reported as active compounds contributing to pest control activity (Nurhasbah and Safrida, 2017).

Sesquiterpenes are terpenoid compounds that may act as respiratory poisons and have a strong odor. These compounds are presumed to enter the insect body through the spiracles and affect the nervous system. Interference with acetylcholine function can disrupt impulse transmission between nerve and muscle cells, causing spasms, paralysis, respiratory failure, and ultimately larval death (Fitri et al., 2024). Flavonoids may also act as respiratory poisons by entering the tracheal system and disrupting respiration. In addition, flavonoids may inhibit electron transport, leading to impaired mitochondrial energy metabolism, cellular damage, weakness, and death (Permatasari and Asri, 2021).

The relatively low mortality across treatments may be attributed to the tested concentrations, which were insufficient to cause >=80% larval death, and to the single treated-feeding exposure for only 48 h before larvae received untreated leaves. Botanical pesticides often have lower acute toxicity, slower action, and rapid degradation, so repeated application may be necessary to obtain stronger control effects (Anjani et al., 2024).

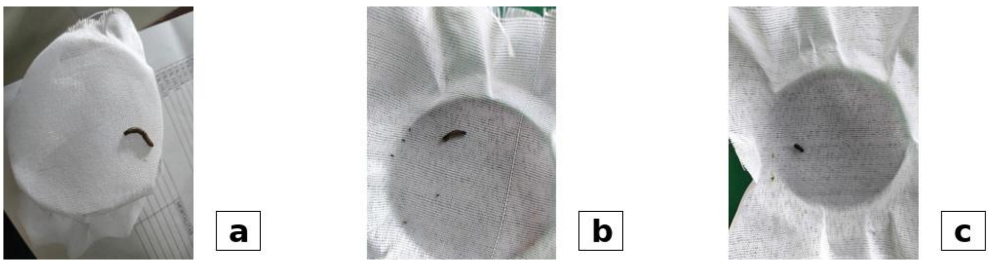

Figure 2. S. frugiperda larvae observed in the study: (a) live larva in the control treatment; (b) larva dead at 24 HAA after exposure to kirinyuh leaf ethanol extract; and (c) larva dead at 72 HAA after exposure to kirinyuh leaf ethanol extract

Effects on Pupal and Adult Development

To determine the effects of treatments on insect development, the percentages of larvae developing into normal pupae, abnormal pupae, normal imagoes, and abnormal imagoes were recorded. The results showed that kirinyuh extract affected the developmental stages of S. frugiperda (Table 2).

Table 2. Mean percentages of normal pupae, abnormal pupae, normal imagoes, and abnormal imagoes of S. frugiperda

Treatment	Normal pupae (%)	Abnormal pupae (%)	Normal imagoes (%)	Abnormal imagoes (%)
EK0 (0%)	100 a	0.00 b	100.00 a	0.00 a
EK2 (2%)	32.5 b	47.5 a	32.5 b	0.00 a
EK4 (4%)	25 bcd	7.5 b	22.5 bc	2.50 a
EK6 (6%)	35 b	2.5 b	35.0 b	0.00 a
EK8 (8%)	7.5 d	17.5 b	5.0 c	2.50 a
EK10 (10%)	10 cd	0.00 b	7.50 c	2.50 a
LSD 5%	19.55	20.75	17.97	4.29

Note: Values followed by the same letter in the same column are not significantly different according to the LSD test at the 5% level.

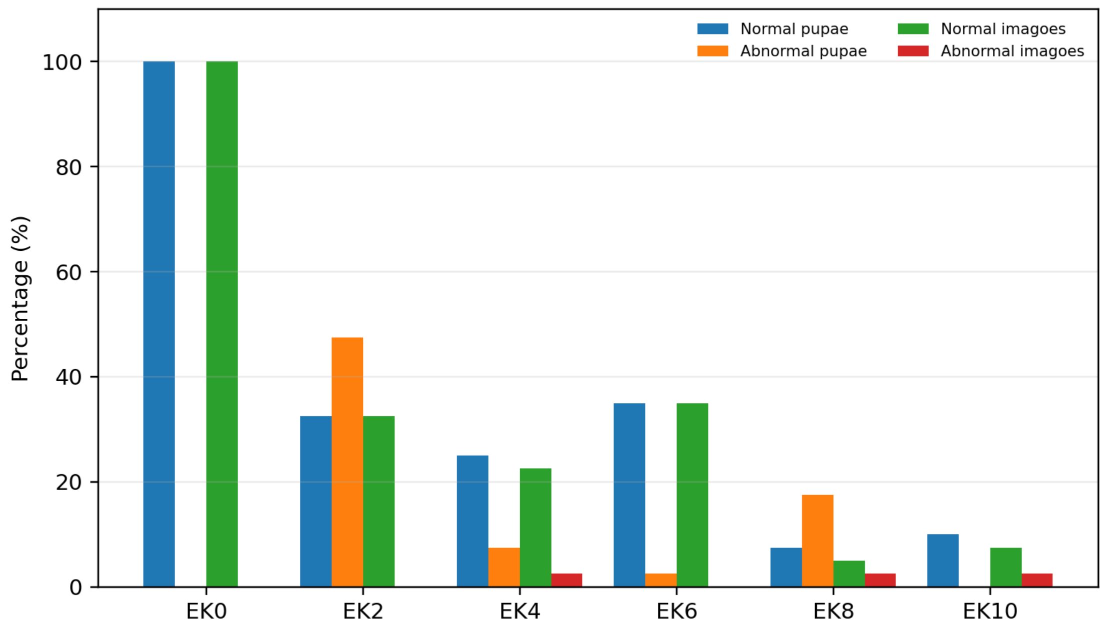

Figure 3. Mean percentages of normal pupae, abnormal pupae, normal imagoes, and abnormal imagoes of S. frugiperda after treatment with kirinyuh leaf ethanol extract

The highest percentage of larvae developing into normal pupae was recorded in the control (100%), with no abnormal pupae. In contrast, the lowest percentage of normal pupae was recorded in the 8% treatment (7.5%), indicating strong developmental disruption. The 2% extract concentration produced the highest percentage of abnormal pupae (47.5%), which was significantly different from the control and other treatments. This response suggests that low-concentration exposure may allow larvae to survive the initial toxic effect but disrupt physiological processes involved in molting and pupation.

Developmental disruption is likely related to bioactive compounds such as flavonoids and sesquiterpenes. Terpenoid compounds may act as antifeedants and interfere with sterol availability in the digestive system. Sterols are precursors of ecdysone, a hormone essential for molting; therefore, reduced free sterol availability may disrupt the molting process (Yogantara et al., 2017). Abnormal pupae generally showed morphological instability and a higher likelihood of failure to emerge as normal adults.

Kirinyuh extract also influenced the formation of normal and abnormal adults. The lowest percentage of normal adults was observed in the 8% treatment (5%), followed by the 10% treatment (7.5%). Abnormal adults were observed at 4%, 8%, and 10% concentrations, each with a mean of 2.5%, although these values were not significantly different from the control. Adult abnormalities included wing deformation, incomplete body development, abnormal coloration, and failure to emerge completely from the pupa.

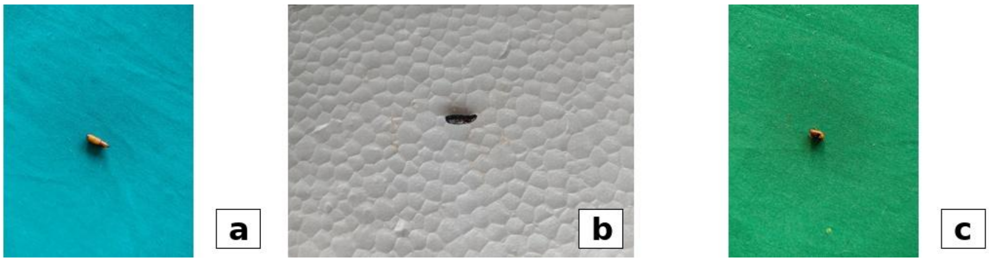

Figure 4. Pupae of S. frugiperda observed in the study: (a) normal pupa; (b) blackened abnormal pupa; and (c) curved abnormal pupa

Figure 5. Imagoes of S. frugiperda observed in the study: (a) normal imago; (b) abnormal imago with wing deformation; and (c) imago failing to emerge completely from the pupa or dying before emergence

Flavonoids in kirinyuh extract may act as stomach poisons that inhibit larval development and disrupt insect hormones, including brain hormone, ecdysone, and juvenile hormone, thereby interfering with larval movement and metamorphosis (Arbit et al., 2024). Sesquiterpenoids may also function as stomach poisons and reduce larval feeding on treated maize leaves (Hartini et al., 2022). These mechanisms explain the observed abnormalities during pupal and adult development.

CONCLUSION

Ethanol extract of kirinyuh leaves (Chromolaena odorata L.) showed potential as a botanical biopesticide against fall armyworm (Spodoptera frugiperda) on maize leaves under laboratory conditions. The 10% concentration produced the highest larval mortality, reaching 50% at 168 h after application. The extract also disrupted S. frugiperda metamorphosis: the 2% concentration produced the highest pupal abnormality (47.5%), while 4%, 8%, and 10% concentrations each produced 2.5% abnormal imagoes. However, because larval mortality did not reach the commonly accepted effective threshold of >=80%, further studies are needed to optimize extract concentration, formulation, application frequency, and field-level performance.

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