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Summer-Ready Moths: Innovations in Bamboo Borer Breeding Practices

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Abstract

Bamboo borers (*Omphisa fuscidentalis*) are tropical insects highly valued in Thailand, with fried bamboo borers fetching prices of up to THB 3,000 per kilogram. These insects are univoltine, with their life cycle naturally synchronized to the rainy season, as bamboo shoots—their egg-laying substrate—are only available during this time. This study explores the use of *Ocimum sanctum* (holy basil) extract as a natural alternative to juvenile hormone analogs (JHAs) for promoting non-seasonal moth production. Three concentrations of *O. sanctum* extract (10, 100, and 1,000 ppm) were tested, and their effects on development were compared to JHAs. The results demonstrated that 10 ppm extract induced the highest pupation rate (82%), while 1,000 ppm extract yielded the highest adult emergence rate (78%). Both extract and JHA treatments significantly shortened the larval period (92-99 days) compared to the control (270 days), with no significant differences in the pupal period. Preliminary unpublished observations indicate that moths reared under the specified conditions outside the natural rainy season were capable of mating and producing fertilized eggs, suggesting further potential for sustainable non-seasonal production. This study offers an innovative approach to scaling bamboo borer production, enabling local agriculturalists to rear these high-value insects in their bamboo forests without reliance on synthetic chemicals.

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Introduction

The bamboo borer, scientifically known as *Omphisa fuscidentalis* Hampson (Class: Insecta, Order: Lepidoptera, Family: Pyralidae), is a moth found in northern Thailand, Laos, Myanmar, and China. Its larvae feed on the inner pulp of bamboo shoots, including species such as *Dendrocalamus latiflorus* Munro, *Bambusa natans* Wall. ex Munro, *Bambusa polymorpha* Munro, *Bambusa vulgaris* Schrader, *Bambusa tulda* Roxb., and *Thyrsostachys oliveri* Gamble, in tropical highland forests (~500 m above sea level) near Chiang Mai, Thailand (19°N). The bamboo borer undergoes complete metamorphosis, including egg, larva, pupa, and adult stages. Its life cycle spans approximately 12 months [1].

In early August, the adult moth lays eggs over 15–20 days. These eggs hatch into first-instar larvae within 14–20 days. From September to May, the larvae develop through five instars. The fifth-instar larvae enter diapause, a prolonged inactive stage lasting 280–304 days, which is notably longer than the diapause observed in other insects of the same group. Pupation occurs from June to July and lasts 30–40 days [1].

Ocimum sanctum, commonly known as holy basil, is a medicinal plant belonging to the family Lamiaceae. The leaves of O. sanctum contain water-soluble phenolic compounds and various bioactive components. Essential oils extracted from O. sanctum leaves have demonstrated a wide range of insecticidal activities [2, 3]. Some bioactive compounds in the extracts act as insect growth regulators, disrupting growth and development in insects [4]. GC-MS analysis of O. sanctum extract has identified five key compounds: alpha-farnesene, caryophyllene, eugenol, farnesyl acetone, and geranylgeraniol. Among these, caryophyllene (a sesquiterpene) and farnesyl acetone serve as precursors for juvenile hormone synthesis [5].

In insects, metamorphosis is regulated by two hormones: juvenile hormone (JH), secreted by the corpus allatum, and ecdysone, secreted by the prothoracic glands [6]. The regulation of metamorphosis by these hormones is complex. Generally, ecdysteroids trigger pupation, while JH plays a critical role in larval molting [7].

The larval stage is the longest phase in the life cycle of *O. fuscidentalis*, lasting approximately nine months during diapause [8]. During this period, larvae do not feed, and hemolymph ecdysteroid concentrations remain low. However, the application of juvenile hormone analogs (JHAs) can elevate ecdysteroid levels, facilitating pupation [9].

The present study aims to investigate the effects of *O. sanctum* extract on inducing non-seasonal pupation and adult emergence in *O. fuscidentalis* larvae. The research evaluates the impact of different extract concentrations (10, 100, and 1,000 ppm/5 µl) on pupation and adult emergence rates, as well as the characteristics of pupae and adults (complete and incomplete). Additionally, the study examines the morphology of male reproductive structures (testes, seminal vesicles, and sperm bundles) and female reproductive structures (ovaries, number of mature and immature eggs, and protein accumulation) after exposure to the extracts.

Materials and Methods Collection and Maintenance of Larvae

Male and female larvae of *O. fuscidentalis* were collected from a bamboo forest in Mae Wang, Chiang Mai, Thailand, during November. The larvae were maintained in plastic boxes (12×14×8 cm) lined with wet paper at 25°C under continuous darkness. The body size of the larvae was measured, and female larvae, being larger than males, were easily distinguishable based on size [1]. Male and female larvae were separated and placed into separate plastic boxes, with 30 larvae of each sex per box.

Control and Treatment

Control larvae were treated topically with 5 μ l of acetone only. The juvenile hormone analog (JHA) treatment involved methoprene dissolved in acetone to achieve a concentration of 2×10^{-10} ppm/5 μ l, applied dorsally on the larvae.

Preparation of Plant Extract

O. sanctum was procured from a local market in Chiang Mai Province, Northern Thailand. The leaves were washed, dried at 50–60°C for two days, and ground into a fine powder. The extract was prepared using the "cold extraction method" [5]. Fifty grams of powdered leaves were mixed with 500 mL of acetone

(1:10 weight-to-volume ratio) in a beaker at room temperature for 24 hours. The slurry was filtered using Whatman filter paper No. 1, and the acetone was evaporated at 40° C using a vacuum rotary evaporator (Heidolph). The crude extract was stored at 4° C until further use. To prepare treatments, the crude extract was dissolved in acetone to create concentrations of 10, 100, and 1,000 ppm/5 μ l, which were topically applied to the larvae.

Morphological Analysis of the Reproductive System

Male Reproductive System

Adult *O. fuscidentalis* males were anesthetized by cold exposure and secured on an insect tray with the dorsal side up. Dissection was performed through a dorsal longitudinal section. The testes and seminal vesicles were photographed using a Canon IXUS 240 HS camera. The length and width of the testes were measured using image analysis software (ImageJ; Wayne Rasband, NIH). The seminal vesicle was mounted on a microscopic slide and stained with eosin for observation. Sperm bundles were counted, and sperm length was measured under a microscope.

Female Reproductive System

Adult females were anesthetized similarly, and dissection was performed between the 6th and 10th abdominal segments. Fat bodies and tracheae were removed, and the ovaries were washed with insect Ringer's solution. The ovaries were photographed using a Fujifilm X-A5 camera. Mature and immature eggs were counted under a microscope. The length and width of the ovaries were measured using image analysis software.

Ovarian Protein Quantification

Ovaries were homogenized on ice in Ringer's solution (271 mM NaCl, 10 mM KCl, 3 mM CaCl₂) using a microcentrifuge tube until thoroughly mixed. The homogenate was centrifuged at 14,000 rpm for 15 minutes. The supernatant was carefully transferred to a new tube, maintaining a volume of 100 μ l. The solution was spun down again for 3–5 minutes.

For protein quantification, 2 μ l of the supernatant was mixed with 98 μ l of Ringer's solution to create the protein solution. Then, 20 μ l of the protein solution was mixed with 1 ml of Bio-Rad protein assay

reagent. The protein concentration was measured using a spectrophotometer at 595 nm [10].

Homogenized the ovaries on ice with ringer's solution (271mM NaCl, 10mM KCl, 3mM CaCl2) in a microcentrifuge tube until thoroughly. Centrifuged at 14,000 rpm, for 15 minutes. Remove supernatant solution into a new tube volume 100 μ l and spin down for 3-5 minutes. Mix protein volume 2 μ l and ringer's solution volume 98 μ l (protein solution). Mixed protein solution volume 20 μ l and Bio-Rad protein assay reagent volume 1 ml. The protein concentration was determined by spectrophotometer at 595 nm [10].

Statistical Analysis

Data are expressed as means \pm standard deviation. Statistical analysis was conducted using one-way ANOVA, followed by Tukey's post hoc test for multiple comparisons. A significance level of P < 0.05 was considered statistically significant.

Results

Effects of *O. sanctum* Extract on Pupation and Adult Emergence

Fifth-instar larvae treated with *O. sanctum* extract exhibited a concentration-dependent decrease in the percentage of pupation. The pupation percentages for the extract concentrations were as follows: 10 ppm (81.67%), 100 ppm (55%), and 1,000 ppm (51.67%). In comparison, the JHA-treated group showed a pupation percentage of 45%, which was lower than that of the extract-treated groups but higher than untreated controls.

Regarding adult emergence, the group treated with 1,000 ppm of extract had the highest percentage of adults (77.78%), followed by 100 ppm (73.33%) and 10 ppm (12.77%). The percentage of adult emergence in the extract-treated groups was greater than that observed in the JHA group but lower than the control group (Table 1).

Effects of O. sanctum Extract on the Life Cycle of O. fuscidentalis

The effects of *O. sanctum* extract on the life cycle of *O. fuscidentalis* are summarized in Table 2. In the control group, the larval period lasted up to 270 days. In contrast, the *O. sanctum*-treated groups had an average larval period of 92–99 days, which was

not significantly different (P < 0.05). The larvae in the extract-treated groups pupated faster than those in the control group but slower than those in the JHA-treated group.

The pupal period across all groups was similar, averaging 44–46 days. The adult period in the O. sanctum extract-treated and JHA-treated groups ranged from 4 to 5 days, showing no significant difference between them. However, the adult period in the O. sanctum extract-treated groups was significantly different from that of the control group (P < 0.05).

Effects on Pupa and Adult Formation

Pupation was observed after exposure to *O. sanctum* extract. Two types of pupation were identified across the three extract concentrations (10, 100, and 1,000 ppm): complete pupation and incomplete pupation.

Complete pupation was characterized by external morphology resembling naturally developed pupae. In this form, all appendages, such as legs, wings, and antennae, were closely attached to the body and entirely covered by a new pupal cuticle (Fig. 1A-B).

Incomplete pupation, on the other hand, was observed when the larval shape remained unchanged, but the body became darker and harder. In some cases, larvae formed a pupal cuticle but failed to shed the old cuticle from the new pupal body. These incomplete pupae did not transform into adults and died at the pupal stage (Fig. 1C-D).

Similarly, adult formation was categorized into two types: complete adults and incomplete adults. Complete adults exhibited fully developed wings that completely covered the body (Fig. 2A). Incomplete adults, however, had underdeveloped, short wings that did not cover the body entirely (Fig. 2B).

Table 1: Percentage of Pupation and Adult Emergence of *O. fuscidentalis* Treated with *O. sanctum* Extracts at Various Concentrations.

Concentration of	Pupae (%)			Adults (%)
extracts (ppm)	Pupation	Complete pupae	Incomplete pupae	
control	100.00	100.00	0.00	100.00
10	81.67	95.92	4.08	12.77
100	55.00	90.91	9.09	73.33
1,000	51.67	87.10	12.90	77.78
JHA	45.00	92.59	7.41	32.00

Table 2: Life Cycle Duration of *O. fuscidentalis* Treated with *O. sanctum* Extracts, Including Larval, Pupal, and Adult Stages Across Different Concentrations.

oncentration of extracts Life cycle in days			
(ppm)	Larval period	Pupal period	Adult period
control	$270.00 \pm 0.00a$	$45.00 \pm 0.00a$	$6.27 \pm 0.58a$
10	98.60 ± 8.60 b	$45.83 \pm 1.72a$	4.17 ± 0.75 b
100	96.18 ± 12.70b	$44.27 \pm 1.96a$	4.05 ± 0.65 b
1,000	$91.87 \pm 9.09b$	$45.52 \pm 1.75a$	4.05 ± 0.67 b
JHA	$70.41 \pm 8.31c$	$45.88 \pm 1.25a$	4.25 ± 0.46 b

Data (Mean \pm SD) followed by the same letter in a column do not different at P<0.05 (One way ANOVA followed by Tukey's test).

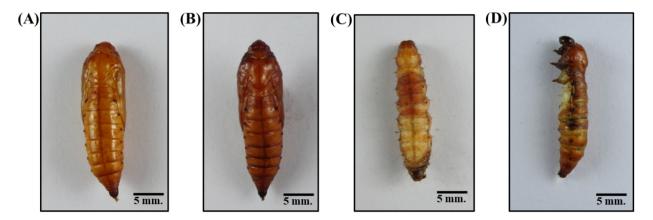


Figure 1: Pupa formation in *O. fuscidentalis* larvae induced by *O. sanctum* extracts. (A-B) Complete pupation, showing fully developed pupae with uniform shape and coloration. (C-D) Incomplete pupation, characterized by larvae retaining their original shape with darkened, hardened bodies.

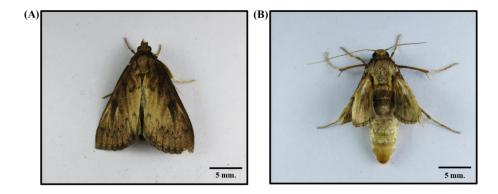


Figure 2: Adult formation in *O. fuscidentalis* larvae induced by *O. sanctum* extracts. (A) Complete adult, characterized by fully developed wings covering the body. (B) Incomplete adult, showing short wings that do not fully cover the body.

Effect on Male Reproductive System of O. fuscidentalis Adults

The male reproductive system of O. fuscidentalis adults comprises round, creamy-white testes. Two long strings extend from the testes, referred to as seminal vesicles, containing sperm (Fig. 3). The average size of the testes (length × width) showed no significant differences among the extract-treated, control, and JHA-treated groups (P < 0.05) (Table 3).

The sperm structure in *O. fuscidentalis* adults generally consists of a head, body, and tail, with all sperm cells packaged in seminal vesicle bags. While their overall morphology includes a smooth, oval head, slim middle section (body), and thinner tail, slight variations in shape and size were observed in specific parts of the cells (head, body, and tail). A normal sperm features a smooth, oval head resembling the shape of an egg, a slim middle section approximately the same length as the head, and a thin tail (Fig. 4).

Treatment with O. sanctum extracts reduced the number of sperm bundles in males. The effectiveness of the extracts followed the order: 1,000 > 10 > 100 ppm. The number of sperm bundles in extract-treated groups was significantly lower than in the control and JHA-treated groups. However, there was no significant difference in the length of sperm bundles between the groups (Table 4).

Effect on Female Reproductive System of O. fuscidentalis Adults

The female reproductive system of *O. fuscidentalis* adults consists of a pair of ovaries, each composed of four ovarioles covered in fat bodies. The average size of the ovaries (length × width) in the extract-treated and JHA-treated groups was significantly smaller than in the control group (Table 3).

Each ovary's four ovarioles are connected to a lateral oviduct by a pedicel. The ovaries are located between the 3rd and 7th abdominal segments. Mature eggs, which are yellow and oblate, are present in the ovarioles and the two lateral oviducts, while immature eggs, whitish and short cylindrical in shape, are located in the common oviduct. The four polytrophic ovarioles of each ovary are fused at the calyx and connected to the lateral oviduct (Fig. 5) [10].

Treatment with O. sanctum extracts reduced the number of mature eggs in females. The effectiveness of the extracts followed the order: 1,000 > 100 > 10 ppm. The number of mature eggs in the extract-treated groups was significantly lower than in the control group. However, there were significant differences in the number of immature eggs between the groups. Additionally, the average protein accumulation in the extract-treated groups showed significant differences compared to the control and JHA-treated groups (Table 5).

Table 3: Average Size (length × width) of Testes and Ovaries in *O. fuscidentalis* Adults Treated with *O. sanctum* Extracts, JHA, and Control.

Concentration of extracts (ppm)	Size of testis		Size of ovaries	
	Length (mm)	Width (mm)	Length (mm)	Width (mm)
control	$1.39 \pm 0.09a$	$1.54 \pm 0.02a$	$41.67 \pm 3.06a$	$1.77 \pm 0.12a$
10	1.27 ± 0.04 a	$1.44 \pm 0.06a$	26.00 ± 1.00 b	$1.37 \pm 0.12a$
100	$1.34 \pm 0.02a$	$1.51 \pm 0.04a$	$27.67 \pm 2.31b$	$1.40 \pm 0.10a$
1,000	1.35 ± 0.09 a	$1.49 \pm 0.03a$	$28.00 \pm 1.00b$	$1.47 \pm 0.25a$
JHA	1.36 ± 0.04 a	$1.53 \pm 0.03a$	27.00 ± 1.00 b	$1.57 \pm 0.12a$

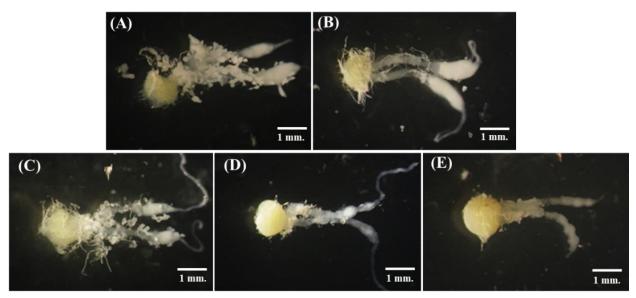


Figure 3: Morphology of testes in *O. fuscidentalis* adult males. (A) Control group, showing normal testis structure. (B) JHA group, illustrating the impact of juvenile hormone analog. (C-E) Effects of *O. sanctum* extracts at 10 ppm, 100 ppm, and 1,000 ppm, showing similar testis morphology across all groups.

Concentration of extracts (ppm)	Number of sperm bundle	Length of sperm bundle		
control	$125.33 \pm 5.03a$	$1.40 \pm 0.01a$		
10	$21.67 \pm 2.31d$	1.40 ± 0.00 a		
100	17.67 ± 2.08d	1.40 ± 0.04 a		
1,000	$73.00 \pm 5.20c$	$1.40 \pm 0.02a$		
THA	98.00 + 3.00b	$1.40 \pm 0.03a$		

Table 4: Number and Length of Sperm Bundles in *O. fuscidentalis* Adults.

Data (Mean \pm SD) followed by the same letter in a column are not significantly different at P < 0.05 (One-way ANOVA followed by Tukey's test).

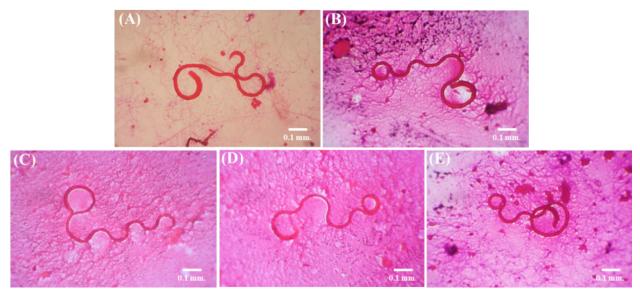


Figure 4: Morphology of sperm bundles in *O. fuscidentalis* adult males. (A) Control group, showing normal sperm bundle structure. (B) JHA group, illustrating the impact of juvenile hormone analog. (C-E) Effects of *O sanctum* extracts at 10 ppm, 100 ppm, and 1,000 ppm, respectively, showing variations in sperm bundle morphology.

Table 5: Number of Eggs and Protein Accumulation in *O. fuscidentalis* Adults.

Concentration of ex-	Number of mature	Number of immature	Protein accumulation
tracts (ppm)	eggs	eggs	(mg/ml)
control	$246.67 \pm 5.69a$	$175.67 \pm 9.29a$	$19.59 \pm 0.10a$
10 ppm	$100.00 \pm 3.46d$	$65.33 \pm 4.73e$	$6.84 \pm 0.13d$
100 ppm	$171.33 \pm 4.04b$	$105.00 \pm 2.65c$	$11.80 \pm 0.23b$
1,000 ppm	179.67 ± 9.24 b	$125.33 \pm 4.73b$	12.09 ± 0.07 b
ЈНА	$154.67 \pm 4.62c$	$85.00 \pm 5.57d$	$9.71 \pm 0.07c$

Data (Mean \pm SD) followed by the same letter in a column are not significantly different at P < 0.05 (One-way ANOVA followed by Tukey's test).

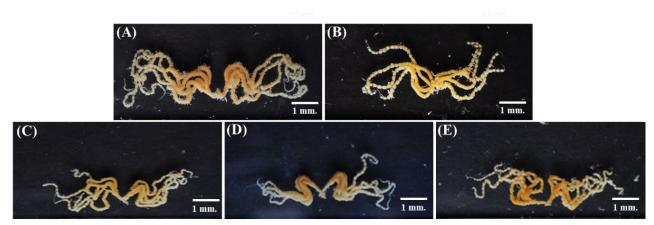


Figure 5: Morphology of ovaries in *O. fuscidentalis* adult females. (A) Control group, showing normal ovarian structure. (B) JHA group, illustrating the impact of juvenile hormone analog. (C-E) Effects of *O. sanctum* extracts at 10 ppm, 100 ppm, and 1,000 ppm, showing similar ovarian structure across all groups.

Discussion

O. fuscidentalis larvae treated with O. sanctum extract showed induced pupation rates ranging from 52-82% in a concentration-dependent manner (highest at 1,000 ppm and lowest at 10 ppm). Conversely, adult emergence rates were inversely proportional, ranging from 12-78%, with higher emergence observed at lower concentrations. This phenomenon may be attributed to bioactive compounds in O. sanctum extracts, such as sesquiterpenes like farnesene and caryophyllene, which are precursors to juvenile hormone (JH) [4, 5, 11]. Juvenile hormone, secreted by the corpus allatum, regulates larval molting and works alongside ecdysone, secreted by prothoracic glands, to trigger pupation [9].

The study demonstrates that *O. sanctum* extract effectively accelerates the development of *O. fuscidentalis* larvae to pupation. Larval development time decreased from 99 days at 10 ppm to 92 days at 1,000 ppm, indicating a dose-dependent effect of the extract on growth and development. Both complete and incomplete pupation were observed at all concentrations (10, 100, and 1,000 ppm), with incomplete pupation characterized by morphological abnormalities.

Morphological examination of *O. fuscidentalis* adults revealed defects in individuals treated with *O. sanctum* extract. Incomplete adults exhibited shorter wings that failed to cover the body (Fig. 2B). By contrast, adults in the control group displayed normal morphology (Fig. 2A) [12]. These abnormalities suggest that bioactive compounds in the extract may disrupt normal development, possibly mimicking the effects of JH.

Reproductive assessments indicated no significant differences in testis size among treatment groups; however, sperm bundle counts were significantly reduced in males treated with *O. sanctum* extract compared to the control and JHA-treated groups. This reduction aligns with findings in *Bombyx mori*, where JH administration inhibited spermatogenesis by reducing spermatid and spermatozoa numbers [13, 14]. Similarly, female reproductive parameters were affected: treated females exhibited smaller ovaries, fewer mature eggs, and significant differences in immature egg numbers compared to the control group. In *Polygonia c-aureum*, exposure to methoprene was found to promote the development of ovaries and male accessory glands. [15]. In addition, the administration of JH to the tobacco cutworm (*Spodoptera litura*) resulted in a reduced number of eggs laid [16]. This may be due to incomplete ovarian development, as observed in female rice moths (*Corcyra cephalonica*). Adult moths of *C. cephalonica* that emerged from fenoxycarb-treated groups exhibited a malformed reproductive system, along with reduced fecundity and hatchability, indicating the gonadotropic effect of this juvenoid [17]. As a result, in accordance with Kayesth et al. (2017), treatment with *Lantana camara* extract in the red cotton bug (*Dysdercus koenigii* Fabricius) also affected reproductive development. [18]. These results suggest that the bioactive compounds in *O. sanctum* extract may function as gonadotropic agents, accelerating

egg maturation while reducing overall fecundity.

Protein accumulation in the ovaries of treated females was significantly lower than in control and JHA groups, implying that O. sanctum extract disrupts oogenesis. This may result from inhibited fat body metabolism, which is critical for synthesizing vitellogenin, the primary yolk protein in insects. The extract can reduce fat body metabolism, limiting the production of proteins and other substances essential for oogenesis. In insects, yolk protein typically consists of 60-90% vitellogenin, depending on the species. Oogenesis in insects is regulated by juvenile hormone, which controls fat body metabolism to produce vitellogenin. This vitellogenin dissolves in the hemolymph and is transported to the oocyte, where it is stored as vitellin protein [19]. Vitellogenin synthesis, regulated by JH, is essential for oocyte maturation. Similar findings have been reported in Plodia interpunctella, where pyriproxyfen treatment reduced ovarian protein accumulation in a dose-dependent manner [20].

These findings highlight the potential of *O. sanctum* extract as a natural insect growth regulator, affecting both development and reproduction in O. fuscidentalis. The presence of bioactive compounds with similar properties to juvenile hormone suggests that O. sanctum extract can be a sustainable alternative to synthetic JH analogs for inducing non-seasonal moth production in bamboo borers. By replacing chemical forms of juvenile hormone with plant-based extracts, this approach offers an environmentally friendly and potentially cost-effective solution for managing the lifecycle of O. fuscidentalis. Future studies should further isolate and identify the active compounds within the extract and explore their precise mechanisms of action, ensuring their efficacy and safety in applied settings.

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