Research Article

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Stage-Dependent Efficacy of Selected Biopesticides against Beet Armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae), on Welsh Onion under Controlled Greenhouse Conditions

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Abstract

Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae) is a major pest of Welsh onion, and effective biological alternatives to synthetic insecticides are increasingly needed for sustainable pest management. This study evaluated the efficacy of four commercially available biopesticides—Az Tron WG (*Bacillus thuringiensis*), HH-SK3 (*Bacillus thuringiensis* + *Beauveria bassiana* + *Metarhizium anisopliae*), Sokupi 0.5SL (matrine), and Meta Green (*Metarhizium anisopliae*)—against first- through fourth-instar *S. exigua* under controlled greenhouse conditions using a leaf-dip bioassay adapted from the IRAC susceptibility-test method for leaf-eating lepidopteran larvae. Corrected control efficacy was assessed at 24, 48, 72, 120, 168, and 336 hours after treatment (HAT), and treatment effects were analyzed using generalized linear mixed models. Control efficacy increased progressively with exposure time for all products, reaching 93.3–100.0% by 336 HAT across all larval instars. Younger larvae responded more rapidly than older larvae, with first- and second-instar larvae achieving ≥90% control by 120 HAT, whereas third- and fourth-instar larvae generally required 120–168 HAT to reach comparable efficacy. Az Tron WG consistently provided the fastest early suppression, although differences among products diminished with prolonged exposure. These findings indicate that all four commercial biopesticides are effective for managing *S. exigua* and emphasize that early application against young larval instars can accelerate control and strengthen integrated pest management in Welsh onion production.

Keywords: Spodoptera exigua, biopesticides, welsh onion, Matrine, Bacillus thuringiensis, Beauveria bassiana, Metarhizium anisopliae

1. INTRODUCTION

Spodoptera exigua (Hübner) is a widely distributed polyphagous insect pest that infests more than 130 host plant species belonging to at least 30 plant families, making it one of the most important lepidopteran pests in vegetable production systems [1, 2]. The larval stage is responsible for most crop injury because larvae feed directly on foliage, thereby reducing photosynthetic area and market quality. Among *Allium* crops, Welsh onion (*Allium fistulosum*) is particularly vulnerable to repeated infestations of *S. exigua*, especially in Asian production regions where the pest is considered a persistent constraint to cultivation [3, 4]. Surveys conducted in Vietnam further indicated that Welsh onion can be attacked throughout its growth period, although infestation generally increases as plants become larger [4]. Related work on other *Allium* pests also demonstrates that sustained foliar insect pressure can contribute to substantial production losses, reinforcing the need for effective and crop-compatible pest-management strategies [5].

The economic impact of *S. exigua* extends beyond visible leaf damage. Experimental studies on Welsh onion demonstrated that infestations of 40 larvae per 20 plants reduced marketable yield by 36.5–49.2%, depending on planting schedule, corresponding to losses of more than 1.7–2.2 tons per 10 a [6]. More broadly, severe beet armyworm outbreaks have been reported to reduce crop productivity by as much as 75% under conditions favorable for pest development [7]. Despite the widespread use of chemical insecticides, field populations can remain difficult to suppress, and persistent infestations have been reported even in conventionally managed Welsh onion fields [4]. This difficulty is consistent with the increasing occurrence of insecticide resistance, which has become one of the principal challenges in the management of *S. exigua* populations worldwide [1, 8].

Growing concerns regarding insecticide resistance and environmental sustainability have encouraged greater interest in biological alternatives. Current reviews recommend reducing dependence on synthetic insecticides through integrated pest management approaches that incorporate microbial, botanical, and other biologically based control agents [1, 7, 9]. Compared with exclusive reliance on conventional synthetic insecticides, biological control products can be more compatible with sustainable agricultural systems because they offer alternative modes of action and may reduce some non-target and environmental burdens when appropriately selected and applied [10, 11]. A range of microbial agents has demonstrated activity against *Spodoptera* species. For example, *Beauveria bassiana* has been reported to induce larval mortality approaching 88% while also affecting larval development and the reproductive performance of surviving adults [12]. Combinations of *B. bassiana* with botanical extracts such as neem have also reduced the time required to achieve mortality compared with individual treatments [9]. Other biologically derived insecticides, including spinosad, have likewise shown high toxicity against *S. exigua* larvae in laboratory bioassays [8, 10].

The performance of these biological products, however, may vary according to the developmental stage of the target insect. A meta-analysis of *Spodoptera* control studies showed that efficacy assessments are frequently stratified by larval instar, with third instars especially common in the experimental literature [13]. Experimental evidence across noctuid species further indicates that younger larvae often exhibit greater susceptibility than older larvae. In *Spodoptera litura*, nucleopolyhedrovirus pathogenicity was evaluated across second-, third-, and fourth-instar larvae, with early instars showing the highest mortality at comparable viral concentrations [14]. Comparable patterns have been reported for *Spodoptera frugiperda*, in which the efficacy of entomopathogenic agents declined as larval age increased [15]. Earlier observations in *Spodoptera mauritia* likewise demonstrated marked age-dependent responses during the final larval instar [16]. Collectively, these findings indicate that larval developmental stage can influence both the magnitude and the speed of treatment response, an issue that is particularly relevant for leafy vegetables because continued feeding rapidly reduces commercial quality.

Although previous studies have established the importance of *S. exigua* as a pest of Welsh onion and documented the potential of microbial and botanical biopesticides, direct comparisons among commercially available products across several larval instars remain limited. Information describing how larval developmental stage influences the response to different commercial biopesticides under controlled greenhouse conditions is still relatively scarce. Therefore, the present study evaluated and compared the efficacy of four commercially available biopesticides against first- through fourth-instar *S. exigua* larvae on Welsh onion and examined how larval developmental stage affected both the speed and the overall level of pest control.

2. MATERIAL AND METHODS

Welsh onion (*Allium fistulosum* L., cultivar *[specify cultivar]*) was grown in a greenhouse maintained at *27 ± 1°C*, *65 ± 5% relative humidity (RH)*, and a *16:8 h (light:dark) photoperiod*. Fifteen days before planting, the soil was treated with *[specify insecticide and fungicide]* according to the manufacturer's recommendations. Plants were grown individually in plastic pots (20 cm diameter × 30 cm height), with each pot containing one clump of four to five plants. To ensure a continuous supply of experimental material throughout the study, planting was carried out at 7-day intervals, with 20 pots established in each planting batch.

Larvae of *Spodoptera exigua* were collected from commercial Welsh onion fields in Hai Duong Province, Vietnam, and maintained under laboratory conditions of $27 \pm 1°C$, $65 \pm 5\%$ RH, and a 16:8 h light:dark photoperiod. Larvae were continuously reared on insecticide-free Welsh onion leaves until adult emergence. Newly emerged males and females were paired in mesh cages ($30 \times 30 \times 45$ cm) and provided with cotton pads soaked in a 10% honey solution as a food source. Egg masses were collected daily, transferred to separate containers, and monitored until hatching. Newly hatched larvae were subsequently reared on fresh, insecticide-free Welsh onion leaves until they reached the larval instars required for the bioassays.

Four commercially available biopesticides representing microbial and botanical active ingredients were evaluated in this study: Az Tron WG (*Bacillus thuringiensis*), HH-SK3 (*Bacillus thuringiensis* + *Beauveria bassiana* + *Metarhizium anisopliae*), Sokupi 0.5SL (matrine), and Meta Green (*Metarhizium anisopliae*). Each product was prepared immediately before use according to the manufacturer's recommended field application rate. The commercial products, active ingredients, formulations, recommended doses, and modes of action are summarized in Table 1.

Table 1. *Commercial biopesticides evaluated in this study*

Product	Active ingredient	Formulation	Recommended dose	Mode of action
Az Tron WG	Bacillus thuringiensis	WG	[ ]	[ ]
HH-SK3	Bacillus thuringiensis + *Beauveria bassiana* + *Metarhizium anisopliae*	[ ]	[ ]	[ ]
Sokupi 0.5SL	Matrine	SL	[ ]	[ ]
Meta Green	Metarhizium anisopliae	[ ]	[ ]	[ ]

The insecticidal efficacy of the four biopesticides against *S. exigua* was evaluated using a leaf-dip bioassay adapted from Insecticide Resistance Action Committee Method No. 007 for leaf-eating lepidopteran larvae [25].

Welsh onion plants aged 20–25 days were used in all experiments. Leaves were cut into 5-cm segments, rinsed with distilled water, and air-dried at room temperature. Leaf segments were immersed for 30 s in each biopesticide solution prepared at the manufacturer's recommended concentration and were then allowed to air-dry completely. Control leaves were immersed in distilled water only.

Five treated leaf segments were placed in each plastic bioassay container ($4 \times 9 \times 6.5$ cm), which was lined with moistened filter paper and covered with a 50-mesh screen to provide ventilation. Ten healthy larvae of the same instar (first, second, third, or fourth instar) were introduced into each container. Each treatment was replicated three times for each larval instar, providing independent replicate containers for statistical analysis.

Bioassays were maintained under the same environmental conditions used for insect-colony maintenance. Fresh treated leaves were supplied daily throughout the experimental period to maintain food quality and treatment exposure. Larval mortality was recorded at *24, 48, 72, 120, 168, and 336 hours after treatment (HAT).*

To confirm fungal infection in treatments containing entomopathogenic fungi, dead larvae were surface-sterilized by immersion in 95% ethanol for 1 min, rinsed in sterile distilled water for 5 min, air-dried, and transferred to Petri dishes lined with moist filter paper. The dishes were incubated in darkness at room temperature for 3–5 days and were examined for visible fungal outgrowth.

Control efficacy was corrected for natural mortality in the untreated control using Abbott's formula [26]:

$$\text{Corrected efficacy (\%)} = \frac{C_a - T_a}{C_a} \times 100, \tag{1}$$

where:

- C_a = number of surviving larvae in the control treatment;
- T_a = number of surviving larvae in the treated group.

Data were initially organized in Microsoft Excel and analyzed using *R version 4.3.1* (R Foundation for Statistical Computing, Vienna, Austria) with *RStudio version 2023.06.2 Build 561*. Corrected control efficacy was analyzed using generalized linear mixed models (GLMMs) implemented in the *glmmTMB* package with a beta error distribution. Biopesticide treatment, larval instar, and observation time were included as fixed effects, whereas replicate was treated as a random effect. Overall treatment effects were assessed using Type III analysis of variance (ANOVA), and pairwise comparisons among treatments were performed using *Šidák-adjusted* post hoc tests. Statistical significance was declared at $P < 0.05$. Model assumptions and residual behavior were checked before interpreting treatment contrasts.

3. RESULTS

The efficacy of all four biopesticides increased progressively with exposure time across all larval instars of *S. exigua*. However, the rate at which effective control was achieved varied according to larval developmental stage. First- and second-instar larvae responded more rapidly to treatment than third- and fourth-instar larvae, whereas differences among the four biopesticides were generally small during much of the experiment. By the end of the 336-h observation period, all products had achieved high control efficacy (>93%) regardless of larval stage.

Among first-instar larvae, all biopesticides showed rapid activity, with initial efficacy ranging from 43.3% to 50.0% at 24 HAT. Control increased sharply during the first 72 h, reaching 86.7–93.3%, and exceeded 93% for all treatments by 120 HAT. At 168 HAT, Az Tron WG, Sokupi 0.5SL, and Meta Green achieved complete control (100%), whereas HH-SK3 reached 96.7%. These efficacy levels remained unchanged through the final observation at 336 HAT. No statistically significant differences among treatments were detected at any observation time (Table 2).

Table 2. *Control efficacy (%) of selected biopesticides against first-instar Spodoptera exigua under controlled greenhouse conditions*

Products	24 HAT	48 HAT	72 HAT	120 HAT	168 HAT	336 HAT
Az Tron WG	50.00^{a}	83.33^{a}	93.33^{a}	96.67^{a}	100.00^{a}	100.00^{a}
HH-SK3	46.67^{a}	76.67^{a}	86.67^{a}	93.33^{a}	96.67^{a}	96.67^{a}
Sokupi 0.5SL	46.67^{a}	73.33^{a}	90.00^{a}	96.67^{a}	100.00^{a}	100.00^{a}
Meta Green	43.33^{a}	73.33^{a}	86.67^{a}	96.67^{a}	100.00^{a}	100.00^{a}

Values followed by the same letter within a column are not significantly different (Šidák-adjusted post hoc test, P > 0.05; n = 120).

Second-instar larvae exhibited a similar response pattern, although the increase in efficacy during the first 48 h was slightly slower than that observed in first-instar larvae. Initial control ranged from 36.7% to 43.3% at 24 HAT and increased to 60.0–76.7% at 48 HAT. By 120 HAT, all treatments had achieved at least 90% efficacy. Complete control was obtained with Az Tron WG and Sokupi 0.5SL at 168 HAT, whereas Meta Green reached 100% efficacy at 336 HAT. HH-SK3 maintained a final efficacy of 93.3%. As observed in first-instar larvae, statistical analysis showed no significant differences among treatments throughout the experiment (Table 3).

Table 3. *Control efficacy (%) of selected biopesticides against second-instar Spodoptera exigua under controlled greenhouse conditions.*

Products	24 HAT	48 HAT	72 HAT	120 HAT	168 HAT	336 HAT
Az Tron WG	43.33^{a}	76.67^{a}	86.67^{a}	96.67^{a}	100.00^{a}	100.00^{a}
HH-SK3	40.00^{a}	63.33^{a}	76.67^{a}	90.00^{a}	93.33^{a}	93.33^{a}
Sokupi 0.5SL	43.33^{a}	66.67^{a}	86.67^{a}	96.67^{a}	100.00^{a}	100.00^{a}
Meta Green	36.67^{a}	60.00^{a}	83.33^{a}	96.67^{a}	96.67^{a}	100.00^{a}

Values followed by the same letter within a column are not significantly different (Šidák-adjusted post hoc test, P > 0.05; n = 120).

Control efficacy increased more gradually in third-instar larvae. Initial efficacy remained low (30.0–33.3%) at 24 HAT and increased to 53.3–73.3% after 48 h. Among the tested products, Sokupi 0.5SL showed the highest efficacy at 72 HAT (90.0%), whereas Az Tron WG, HH-SK3, and Meta Green achieved 70.0%, 66.7%, and 76.7%, respectively. By 120 HAT, efficacy exceeded 86% for all treatments and approached maximum levels thereafter. At the final assessment, Az Tron WG reached complete control (100%), whereas the remaining treatments achieved 93.3–96.7% efficacy. Unlike the younger instars, statistically significant differences among treatments were detected at intermediate observation times. At 72 HAT, Meta Green differed from the treatments sharing the "a" grouping, and at 120 HAT the letter groupings indicated partial separation among products. These differences were no longer evident by 168 HAT and remained absent at the final observation (Table 4).

Table 4. *Control efficacy (%) of selected biopesticides against third-instar Spodoptera exigua under controlled greenhouse conditions.*

Products	24 HAT	48 HAT	72 HAT	120 HAT	168 HAT	336 HAT
Az Tron WG	30.00^{a}	60.00^{a}	70.00^{a}	96.67^{a}	96.67^{a}	100.00^{a}
HH-SK3	33.33^{a}	66.67^{a}	66.67^{a}	86.67^{ab}	93.33^{a}	96.67^{a}
Sokupi 0.5SL	30.00^{a}	73.33^{a}	90.00^{a}	93.33^{ab}	96.67^{a}	96.67^{a}
Meta Green	30.00^{a}	53.33^{a}	76.67^{b}	90.00^{b}	93.33^{a}	93.33^{a}

Values followed by the same letter within a column are not significantly different (Šidák-adjusted post hoc test, P > 0.05; n = 120).

Fourth-instar larvae were the least responsive during the early stages of exposure. Initial efficacy was below 25% for all treatments and remained at or below 70% through 72 HAT. Az Tron WG consistently showed the highest early efficacy, reaching 50.0% and 70.0% at 48 and 72 HAT, respectively, whereas HH-SK3 showed the slowest response. Control continued to increase with exposure time, and all treatments reached at least 80% efficacy by 120 HAT. At 168 HAT, efficacy ranged from 86.7% to 96.7%, and final control at 336 HAT ranged from 93.3% to 96.7%. Significant differences among treatments were observed at 120 and 168 HAT, but these differences were no longer detected at the final observation, indicating convergence in overall control efficacy after prolonged exposure (Table 5).

Table 5. *Control efficacy (%) of selected biopesticides against fourth-instar Spodoptera exigua under controlled greenhouse conditions.*

Products	24 HAT	48 HAT	72 HAT	120 HAT	168 HAT	336 HAT
Az Tron WG	23.33^{a}	50.00^{a}	70.00^{a}	93.33^{a}	96.67^{a}	96.67^{a}
HH-SK3	20.00^{a}	33.33^{a}	56.67^{a}	80.00^{a}b	86.67^{ab}	93.33^{a}
Sokupi 0.5SL	23.33^{a}	40.00^{a}	60.00^{a}	93.33^{b}	93.33^{ab}	96.67^{a}
Meta Green	20.00^{a}	46.67^{a}	66.67^{a}	86.67^{b}	90.00^{b}	93.33^{a}

Values followed by the same letter within a column are not significantly different (Šidák-adjusted post hoc test, $P > 0.05$; $n = 120$).

Across all larval stages, the speed of control declined as larval age increased. First- and second-instar larvae achieved more than 90% control within 120 HAT for all products, whereas third- and especially fourth-instar larvae required longer exposure to reach comparable efficacy consistently. Az Tron WG generally provided the fastest control during the early observation period, particularly against older larvae, whereas HH-SK3 tended to show slightly lower early efficacy. Nevertheless, by the end of the experiment, all four commercial biopesticides provided similarly high levels of control, with final efficacy ranging from 93.3% to 100% across the four larval instars.

4. DISCUSSION

The present study evaluated the efficacy of four commercially available biopesticides against different larval instars of *S. exigua* on Welsh onion under controlled greenhouse conditions. Four main observations emerged from the results. First, control efficacy increased progressively with exposure time for all products. Second, susceptibility declined with larval development, with first- and second-instar larvae responding more rapidly than older larvae. Third, Az Tron WG generally produced the fastest early suppression, whereas differences among products became smaller with prolonged exposure. Finally, despite variation in the speed of action, all four biopesticides achieved high final efficacy (93.3–100.0%) after 336 h.

The gradual increase in mortality observed throughout the experiment is consistent with the slower action of many microbial and botanical biopesticides. In contrast to insecticides that may produce rapid knockdown, microbial agents often require time for infection, colonization, toxin activity, or progressive physiological disruption, whereas botanical compounds may interfere with feeding, development, or other physiological processes over several days [17, 18]. This temporal pattern resembles previous reports involving *Spodoptera* species. *Beauveria bassiana* caused up to 88% mortality in *S. exigua* while also affecting larval development and adult reproductive performance [12]. Likewise, entomopathogenic fungi produced cumulative mortality of 71.3–93.3% against *S. frugiperda* after extended exposure [19], and SeNPV at sufficiently high concentrations produced nearly complete mortality of *S. exigua* within approximately 7–11 days [20]. Our findings therefore suggest that assessments made shortly after application can underestimate the eventual control potential of biopesticides, particularly formulations based on microbial agents.

Larval developmental stage appeared to influence both the rate and the extent of mortality during the early post-treatment period. Initial efficacy declined from 43.3–50.0% in first instars to only 20.0–23.3% in fourth instars at 24 HAT, and younger larvae reached $\geq$90% control substantially earlier than older larvae. Similar age-dependent susceptibility has been documented across several *Spodoptera* species. The meta-analysis by Sa'adah et al. [13] showed that larval instar is an important experimental dimension in the efficacy literature, while direct experiments on *S. frugiperda* demonstrated progressively lower mortality from younger to older instars following exposure to entomopathogenic agents [15]. Comparable trends have also been reported for *B. bassiana* against *S. littoralis*, where younger larvae were more susceptible than later instars [18], and for *S. litura*, where mortality declined markedly as larval development advanced [21]. Such stage-dependent differences are also consistent with evidence that host immune responses can modulate susceptibility to entomopathogens and viral infection [22]. Together, these studies support the interpretation that older larvae may require longer exposure or higher effective doses to achieve control comparable to that observed in younger instars.

Although all four products ultimately provided comparable control, their early activity differed. Az Tron WG consistently produced the highest or joint-highest efficacy during the first 48–72 h across the larval instars, whereas HH-SK3 generally showed a slower initial response. These differences became less apparent after prolonged exposure, when efficacy converged to more than 90% for nearly all treatment–instar combinations. The relatively rapid response of Az Tron WG is consistent with the ingestion-based activity expected from *Bacillus thuringiensis* toxins, which act on susceptible larval midgut tissues after consumption. Other studies have likewise reported rapid or progressively increasing mortality after exposure to biologically derived products, including spinosad, azadirachtin, *B. thuringiensis*, and entomopathogenic fungi, although the speed of action varies markedly among active ingredients and target species [17, 23, 24]. These early differences may be especially relevant for leafy vegetables because continued feeding during the first few days after infestation can reduce market quality even when larvae eventually die.

The overall efficacy observed in this study also supports the potential role of commercial biopesticides in Welsh onion production. Previous surveys in Vietnam have shown that *S. exigua* remains an important pest of Welsh onion and that conventional insecticide applications do not always provide satisfactory field control [3, 4]. Although the present experiment was conducted under controlled greenhouse conditions, the consistently high final efficacy indicates that these products warrant consideration as components of integrated pest management programs designed to reduce exclusive dependence on synthetic insecticides. Because the products represent different biological active ingredients and modes of action, they may also be useful within carefully designed rotation strategies intended to diversify selection pressure, an increasingly important consideration in *S. exigua* management [1, 2].

From a practical perspective, the results indicate that application timing may be as important as product selection. All four biopesticides eventually achieved high control, but treatments against first- and second-instar larvae reached effective suppression sooner than treatments against older larvae. Earlier intervention is therefore likely to shorten the period of damaging feeding before mortality occurs and may help reduce foliage injury in Welsh onion. Under field conditions, regular crop monitoring and applications initiated soon after egg hatch or the appearance of young larvae may improve overall control while maintaining compatibility with integrated pest management practices.

Several limitations should be considered when interpreting these findings. The study was conducted under controlled greenhouse conditions with relatively constant temperature and humidity, which do not fully represent the environmental variability encountered in commercial production. Only the recommended application rates of four commercial products were evaluated, and additional dose–response experiments could provide further insight into their relative potency and consistency. Furthermore, the assessment focused primarily on corrected larval control efficacy and did not quantify sublethal effects such as feeding reduction, developmental delay, pupal performance, or adult reproduction, all of which may contribute to overall population suppression. Field validation across different growing seasons, climatic conditions, and production systems would therefore be useful for determining how these greenhouse observations translate into practical crop protection.

5. CONCLUSIONS

All four commercial biopesticides provided high levels of control against *Spodoptera exigua* on Welsh onion, although the speed of control depended on larval developmental stage and exposure time. Applications targeting first- and second-instar larvae resulted in more rapid suppression, whereas prolonged exposure enabled all products to achieve similarly high final efficacy. These findings support further evaluation of the tested biopesticides as components of integrated pest management programs for Welsh onion production, particularly when applications are timed to target young larval populations.

AUTHORS' CONTRIBUTIONS

All authors contributed to the development of the study, critically reviewed the manuscript, approved the final version, and agreed to be accountable for all aspects of the work.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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This research received no external funding.

DATA AVAILABILITY

Data supporting the empirical analysis and the findings reported in this study are available from the corresponding author upon reasonable request.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

In the course of preparing this manuscript, the authors employed ChatGPT to improve the linguistic quality and readability of the content. After using this AI tool, the authors conducted a comprehensive review and made the necessary revisions to ensure the accuracy, integrity, and scientific rigor of the manuscript. All authors bear full responsibility for the final content of the published work, including any errors or inconsistencies that may remain.

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