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Original Article

Integrated Pest Management Precision: Harnessing the Joint Influence of Entomopathogenic Fungi, Nematodes, and Chlorantraniliprole for Targeted Control of Fall Armyworm (*Spodoptera frugiperda*)

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ABSTRACT

Background: The *Spodoptera frugiperda*, commonly known as the fall armyworm (FAW), is a significant pest impacting agricultural productivity worldwide. Originating from tropical and subtropical regions, FAW has shown a high capacity for migration and destruction across a wide variety of crops. Integrated Pest Management (IPM) strategies offer sustainable approaches to managing pest populations, minimizing environmental impact, and reducing reliance on chemical insecticides.

Objective: This study aimed to evaluate the efficacy of combining biological control agents, specifically *Heterorhabditis bacteriophora* (Hb) and *Beauveria bassiana* (Bb), with the chemical insecticide chlorantraniliprole (Ch) against third and fifth instar larvae of *S. frugiperda*. The study sought to determine the potential synergistic effects of these combinations on larval mortality and developmental disruption.

Methods: The research encompassed laboratory bioassays, greenhouse, and field trials to assess the impact of the biocontrol agents and chlorantraniliprole, both individually and in combination, on FAW larval mortality and development. Larval populations of *S. frugiperda* were collected and reared under controlled conditions. Treatments applied included Hb, Bb, Ch, Hb+Bb, Hb+Ch, Bb+Ch, and Hb+Bb+Ch. Mortality rates were recorded at 3, 5, and 7 days post-treatment, while developmental parameters were assessed through pupation rates, adult emergence, and egg eclosion percentages.

Results: The combined application of Hb, Bb, and Ch significantly increased mortality rates in both the third and fifth instar larvae compared to individual treatments, with the highest mortality observed in the Hb+Bb+Ch group (100% by day 7). Developmental disruptions were also noted, including reduced pupation rates, adult emergence, and egg eclosion, particularly in treatments involving the combined use of biocontrol agents and chlorantraniliprole. Statistical analysis confirmed the significance of these findings ($P<0.01$).

Conclusion: The integration of Hb, Bb, and Ch presents a viable IPM strategy for effectively managing *S. frugiperda* populations. This combination not only enhances larval mortality but also disrupts developmental stages, offering a potential reduction in FAW infestation levels and associated crop damages. These results underscore the importance of adopting sustainable pest management practices that leverage synergistic effects between biological and chemical agents.

Keywords: Integrated Pest Management, *Spodoptera frugiperda*, *Heterorhabditis bacteriophora*, *Beauveria bassiana*, chlorantraniliprole, biocontrol agents, agricultural pest control, sustainable agriculture.

INTRODUCTION

The *Spodoptera frugiperda*, commonly known as the fall armyworm (FAW), is a highly destructive polyphagous pest originating from the tropical and subtropical regions of the Americas. This pest poses a significant threat to agriculture, with over 353 plant species identified as potential hosts, affecting a wide range of crops including rice, maize, sorghum, cotton, and numerous vegetable plants

(1). The FAW's presence was first recorded in Africa in 2016, and it has since emerged as a major agricultural menace across more than 30 African nations, with projections indicating its spread to as many as 44 countries in the near future (2). The pest exhibits two strains, the corn strain and the rice strain, targeting specific crops according to their strain. The FAW's lifecycle encompasses four stages, and its presence can be discerned through both phenotypic characteristics and damage patterns, with genetic analysis also playing a crucial role in its identification (3).

Entomopathogenic fungi (EPF) have gained global recognition for their role in controlling various insect pests, including the FAW. Over 12 species of EPF have been identified, leading to the development of more than 170 biocontrol products targeting insects such as mites and ticks. *Metarhizium anisopliae* and *Beauveria bassiana* are the primary species of EPF utilized against FAW, showcasing a broad spectrum of infectivity and high virulence across different life stages of lepidopterans, both in field and laboratory settings (6,7).

Entomopathogenic nematodes (EPNs), particularly those from the Steinernematidae and Heterorhabditidae families, have been successfully used against soil-dwelling insect pests. EPNs are capable of infecting, killing, and reproducing within certain species of the Spodoptera genus, including *S. frugiperda*, under both laboratory and field conditions. The effectiveness of EPNs is influenced by environmental and biotic factors such as soil type, moisture, temperature, and the specific characteristics of the EPN and the target insect (8,9,10). The infective juveniles (IJs) of EPNs, which harbor symbiotic bacteria in their intestines, penetrate the host's body through natural openings, releasing the bacteria into the hemocoel and resulting in the insect's death within 24-48 hours due to septicemia and toxemia (11,12).

Chlorantraniliprole, a widely used insecticide, has been evaluated for its efficacy against FAW when used alone and in combination with EPN and EPF. The study aims to assess the singular and synergistic effects of chlorantraniliprole, EPN, and EPF on various developmental stages of *S. frugiperda* under laboratory, greenhouse, and field conditions. This integrated pest management approach seeks to harness the combined knockdown effects of these agents, offering a targeted control strategy for mitigating the impact of FAW on agricultural crops. The integration of chemical and biological control methods represents a promising avenue for sustainable pest management, potentially reducing reliance on chemical pesticides and mitigating environmental impact.

MATERIAL AND METHOD

The research on the integrated pest management precision focusing on the control of *Spodoptera frugiperda*, entailed a comprehensive study that utilized a multidisciplinary approach encompassing entomology and microbial control. The larval population of *S. frugiperda* was meticulously collected from various maize fields and was subsequently maintained under optimal conditions in the Integrated Pest Management (IPM) Microbial Control Laboratory at the University of Agriculture, Faisalabad. This ensured a consistent and reliable source of larvae for the experiments (Abbott, 1925).

For the purpose of the study, *Beauveria bassiana* strain GHA was cultivated on *potato dextrose agar* (PDA) plates. These plates were securely sealed with parafilm to prevent any contamination and incubated at a controlled temperature of 25°C with a 14:10 hour light-dark photoperiod. Following a 7-10 day incubation period, the resulting conidia were harvested using sterile techniques and suspended in a solution for further application in the experiments.

Entomopathogenic nematodes (EPNs), specifically *Heterorhabditis bacteriophora*, were propagated using the last instar larvae of the greater wax moth, Galleria mellonella, adhering to standard in vivo production protocols within the same laboratory facilities. Alongside, Chlorantraniliprole, a novel insecticide under the brand *Coragen*® 200 SC, was procured for the experiment.

The experimental design included assessing the efficacy of chlorantraniliprole, *B. bassiana*, and *H. bacteriophora* against the third and fifth larval instars of *S. frugiperda*. This evaluation was conducted under laboratory conditions at the IPM Microbial Control Laboratory, Department of Entomology, University of Agriculture Faisalabad. The study design encompassed four treatments and a control group, each subjected to either individual agents or their combinations, with the intention of observing the mortality rates across different time intervals.

A separate bioassay investigated the impact of sub-lethal doses of the agents on the developmental stages of *S. frugiperda*. This included recording various parameters such as larval duration, pre-pupal duration, and adult longevity, with daily observations until the death of the last adult.

In the greenhouse, the effectiveness of the treatment combinations was tested under controlled conditions using a randomized complete block design (RCBD). Maize variety "BF-92" was cultivated in pots within a structured environment, ensuring that the applied treatments could be accurately assessed for their efficacy against the target pest.

Field experiments were conducted across two different seasons using maize varieties "BF-92" and “BF-101”. The experimental plots were treated with combinations of the agents to evaluate their effectiveness in a natural setting. Pre and post-treatment assessments were meticulously carried out to gauge the impact on larval numbers and adult emergence.

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All collected data underwent statistical analysis using SPSS version 25, adhering to rigorous standards to ensure the integrity and reliability of the findings. The mortality data were corrected using Abbott's formula, while the development data and the results from the greenhouse and field bioassays were subjected to Analysis of Variance (ANOVA) within a RCBD framework. Mean separations were performed using Tukey's test, with significance determined at $p<0.05$.

Throughout the study, ethical considerations were paramount, with all methodologies aligning with the Helsinki Declaration to ensure the ethical treatment of the insect subjects. This adherence to ethical standards ensured that the study's findings could contribute valuable insights into the targeted control of *S. frugiperda*, offering potential strategies for effective pest management in agricultural settings.

RESULTS

In the comprehensive study aimed at evaluating the efficacy of various treatments against the *Spodoptera frugiperda* (FAW), distinct mortality rates were observed across different instars and treatment regimens over a period of 3, 5, and 7 days. For the 3rd instar larvae, the combination of *Heterorhabditis bacteriophora* (Hb), *Beauveria bassiana* (Bb), and Chlorantraniliprole (Ch) exhibited a progressively significant increase in mortality rates, culminating in a 100% mortality rate by day 7. This treatment demonstrated the highest effectiveness with an F2 17 value of 204 and was statistically significant ($P<0.01$), as detailed in Table 1. Contrastingly, the control group maintained negligible mortality throughout the observation period, underscoring the potency of the combined treatment regimen.

Similarly, the 5th instar larvae showed a marked sensitivity to the treatments, particularly the Hb+Bb+Ch combination, which achieved an 89.20±2.03Aa mortality rate by the 7th day. This combination outperformed the single-agent treatments and other combinations, with a significant F value of 79.9 and $P<0.01$, indicating a robust interaction effect between the agents in targeting the FAW larvae (Table 1).

Table 1: Mortality Rates (%) of *S. frugiperda* Larvae Treated with Various Agents

Instar	Treatment	3 Days	5 Days	7 Days	F2 17	P
3rd	Hb	13.43±2.93Ce	22.66±2.00Bg	36.29±2.02Af	23.7	<0.01
	Bb	21.46±1.37Cd	35.16±1.63Bf	43.67±1.84Ae	47.2	<0.01
	Ch	37.25±0.92Cc	48.83±1.37Bd	59.06±1.59Ad	67.4	<0.01
	Hb+Bb	32.13±1.63Cc	41.02±0.88Be	52.20±1.61Ad	49.7	<0.01
	Hb+Ch	47.51±1.51Cb	56.80±1.67Bc	67.03±1.65Ac	36.5	<0.01
	Bb+Ch	52.52±1.21Cb	74.43±1.09Bb	86.37±1.21Ab	214	<0.01
	Hb+Bb+Ch	66.05±1.42Ca	87.46±1.52Ba	100.00±0.00Aa	204	<0.01
	Control	1	2	2	-	-
5th	Hb	9.52±1.96Bf	15.17±1.78Bf	28.38±1.27Af	32.4	<0.01
	Bb	16.34±1.24Ce	27.05±1.28Be	39.66±2.22Ae	50.0	<0.01
	Ch	33.86±0.95Cc	42.86±1.48Bd	51.11±1.24Ade	47.9	<0.01
	Hb+Bb	25.39±1.31Cd	36.12±1.19Bd	45.39±1.56Ad	53.9	<0.01
	Hb+Ch	41.20±1.82Cb	52.48±1.51Bc	63.01±1.35Ac	47.8	<0.01
	Bb+Ch	46.30±1.13Cb	63.75±1.97Bb	77.78±1.63Ab	95.3	<0.01
	Hb+Bb+Ch	57.52±1.90Ca	76.83±1.34Ba	89.20±2.03Aa	79.9	<0.01
	Control	1	1	2	-	-

Table 2: Effects on Developmental Stages of *S. frugiperda* (%)

Treatment	L3 Pupation	L3 Adult Emergence	L3 Egg Eclosion	L5 Pupation	L5 Adult Emergence	L5 Egg Eclosion
Hb	46.66±2.27b	51.66±2.06b	45.00±1.66b	47.22±2.00b	54.44±1.85b	51.66±2.54b
Bb	38.88±1.40bc	47.22±2.00bc	41.66±2.54bc	42.77±2.34bc	49.44±1.59bc	44.44±0.70bc
Ch	31.11±1.11cde	39.44±1.59cd	32.77±1.33de	33.88±1.59de	41.66±1.87de	35.00±1.13de
Hb+Bb	34.44±1.64cd	42.77±2.18c	36.11±1.02cd	38.33±2.39cd	44.44±1.40cd	42.77±1.02cd
Hb+Ch	28.88±1.64de	33.88±2.77de	25.00±3.07ef	31.66±1.13de	35.00±1.66ef	27.22±2.00ef
Bb+Ch	23.33±2.27ef	27.22±1.02ef	22.77±1.59fg	26.11±2.00ef	28.33±1.42fg	23.88±2.00f

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Treatment	L3 Pupation	L3 Adult Emergence	L3 Egg Eclosion	L5 Pupation	L5 Adult Emergence	L5 Egg Eclosion
Hb+Bb+Ch	17.77±0.70f	21.66±0.74f	14.44±1.40g	18.33±1.42f	22.77±1.59g	15.00±0.74g
Control	91.11±2.22a	86.11±1.33a	89.44±2.00a	93.88±2.00a	95.00±1.66a	91.66±2.54a

Table 3: ANOVA Summary for Mortality, Pupation, Adult Emergence, and Egg Eclosion

Source	df	Mortality %	Pupation %	Adult Emergence %	Egg Eclosion %	F	P
Instar	1	197.02	7.47	9.23	10.98	-	<0.01
Interval	2	825.46	-	-	-	-	<0.01
Treatment	6	1014.23	314.26	293.56	317.55	-	<0.01
Instar*Interval	2	1.30	-	-	-	-	0.27
Instar*Treatment	6	1.92	0.25	1.12	0.80	-	0.07
Interval*Treatment	12	8.48	-	-	-	-	<0.01
InstarIntervalTreatment	12	0.55	-	-	-	-	0.87
Error	210	-	-	-	-	-	-
Total	251	-	-	-	-	-	-

Table 4: Daily Plant Assessment (DPA) Scores for *S. frugiperda*

Treatment	1 DPA	2 DPA	3 DPA	4 DPA	5 DPA	6 DPA	7 DPA	F	P
Hb	0.93±0.02Ab	0.94±0.01Ab	0.86±0.04Bb	0.77±0.01Cb	0.65±0.06Db	0.58±0.08Eb	0.51±0.02Fb	154.96	<0.01
Bb	0.81±0.01Ac	0.84±0.01Ac	0.73±0.08Bc	0.64±0.09Cc	0.43±0.07Dc	0.34±0.05Ed	0.25±0.02Fc	403.00	<0.01
Ch	0.00±0.00Eg	0.00±0.00Eg	0.00±0.00Ef	0.05±0.08De	0.23±0.08Cd	0.42±0.07Bc	0.53±0.01Ab	984.42	<0.01
Hb+Bb	0.37±0.07Bd	0.45±0.01Ad	0.26±0.06Cd	0.18±0.08Dd	0.06±0.05Ee	0.00±0.00Fe	0.00±0.00Fd	675.52	<0.01
Hb+Ch	0.20±0.08Ae	0.17±0.08Be	0.08±0.05Ce	0.00±0.00Df	0.00±0.00Df	0.00±0.00De	0.00±0.00Dd	317.63	<0.01
Bb+Ch	0.14±0.05Af	0.06±0.06Bf	0.00±0.00Cf	0.00±0.00Cf	0.00±0.00Cf	0.00±0.00Ce	0.00±0.00Cd	338.90	<0.01
Hb+Bb+Ch	0.00±0.00g	0.00±0.00g	0.00±0.00f	0.00±0.00f	0.00±0.00f	0.00±0.00e	0.00±0.00d	-	-
Control	1.78±0.09Ga	1.97±0.01Fa	2.25±0.08Ea	2.46±0.01Da	2.58±0.01Ca	2.65±0.07Ba	3.16±0.05Aa	167.86	<0.01

The developmental stages of *S. frugiperda* were also profoundly affected by the treatments. In terms of pupation, adult emergence, and egg eclosion rates, the control group exhibited the highest percentages, signifying minimal developmental disruption in the absence of the treatments. In contrast, the Hb+Bb+Ch treatment significantly reduced these rates to the lowest across both the L3 and L5 stages, indicating a substantial inhibitory effect on the pest's life cycle (Table 2). These findings were statistically significant, with P values <0.01 across all developmental parameters, illustrating the potent biocidal effects of the combined treatments on *S. frugiperda's* ability to complete its life cycle.

Statistical analysis further elucidated the impact of the treatments, with the ANOVA summary revealing significant differences across treatments, instars, and intervals. The interaction between instar and treatment yielded an F value of 1.92 and a P value of 0.07, suggesting a nuanced effect of the treatments across different developmental stages of the larvae. Moreover, the interval and treatment interaction was significant (P<0.01), indicating that the time course over which the treatments were applied played a critical role in determining their efficacy (Table 3).

Daily Plant Assessment (DPA) scores further corroborated the effectiveness of the treatments, with the Ch treatment showing a remarkable recovery from an initial score of 0.00±0.00Eg on day 1 to 0.53±0.01Ab by day 7, reflecting the delayed action of the

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chemical agent. Conversely, the Hb+Bb+Ch treatment maintained a consistent score of 0.00 across the assessment period, underscoring the enduring impact of the combined treatment on FAW activity and survival (Table 4).

Collectively, these results underscore the profound impact of integrating Hb, Bb, and Ch in managing *S. frugiperda* infestations. The synergistic effect of the combined treatments not only heightened mortality rates across various larval stages but also significantly disrupted the pest's developmental processes, offering a compelling strategy for sustainable pest management in agricultural settings.

DISCUSSION

The investigation into the efficacy of combining *Heterorhabditis bacteriophora* (Hb), *Beauveria bassiana* (Bb), and chlorantraniliprole (Ch) against the *Spodoptera frugiperda* (FAW) revealed significant insights into integrated pest management (IPM) strategies. This study, set against the backdrop of the FAW's devastating impact on a broad spectrum of economically significant crops across regions like India, Thailand, and Myanmar, aimed to explore environmentally sustainable alternatives to conventional insecticides. The methodology involved both laboratory and field trials, examining the effects of these agents individually and in combination on the mortality and developmental stages of third and fifth larval instars of FAW.

The results demonstrated that the sequential application of these biocontrol agents and chlorantraniliprole significantly increased larval mortality and impacted larval development, more so than when these agents were applied in isolation. This suggests a potential synergistic interaction, wherein the initial application of EPNs may weaken the larvae, making them more susceptible to subsequent EPF infection. This aligns with prior findings that stressed insects exhibit increased vulnerability to pathogens, thereby enhancing mortality rates or facilitating a more rapid kill (15). Moreover, the study confirmed the potent entomopathogenic capabilities of *B. bassiana* against FAW, resonating with earlier research that highlighted its efficacy in controlling lepidopteran pests (14).

Contrastingly, previous research on the Mediterranean fruit fly indicated that the combined application of different species of entomopathogens did not necessarily result in higher mortality than individual applications (15). This discrepancy underscores the complexity of biological interactions and the influence of host specificity, suggesting that synergistic effects may not be universally applicable across all pest-pathogen combinations. Nonetheless, the integrated application of *S. glaseri* or *H. megidis* with *M. anisopliae* against *H. philanhus* both in vitro and in greenhouse conditions corroborates the potential of combining biocontrol agents to enhance pest management efficacy (18).

One notable strength of this study is its comprehensive approach, encompassing laboratory bioassays, greenhouse, and field trials, which provides a robust evaluation of the treatments' effectiveness. However, this research is not without limitations. The variability in response to combined treatments observed across different studies suggests that outcomes may be contingent on specific ecological conditions and pest-pathogen dynamics. Thus, while the findings present a compelling case for the integrated use of Hb, Bb, and Ch, further research is necessary to elucidate the mechanisms underpinning the observed synergistic effects and to determine their applicability across diverse agricultural contexts (19, 20).

The study's implications extend beyond immediate pest control outcomes, highlighting the potential of integrated pest management strategies to reduce reliance on chemical insecticides. This approach not only mitigates the environmental impact but also addresses concerns regarding human health and food safety by minimizing the presence of harmful residues on crops. Looking forward, it is recommended that future research explore the environmental and ecological ramifications of widespread biopesticide use, including potential impacts on non-target species and the risk of resistance development in pest populations.

CONCLUSION

In conclusion, the integration of chlorantraniliprole with biological control agents like *H. bacteriophora* and *Beauveria bassiana* offers a promising strategy for sustainable pest management. By harnessing the unique modes of action of these agents, this study contributes valuable insights to the field of IPM, paving the way for safer, more effective pest control methods that align with environmental and public health objectives.

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