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CORNTRA: Corn (*Zea mays*) Ribosome-Inactivating Protein Spray Against Fall Armyworm (*Spodoptera frugiperda*)

Matteo Gabriel S. Galgo[1*], Rhoda P. Gallemit[1], Prezzi Lah Mikha Lorraine M. Lavador[1], Ray A. Rodriguez[2], Norman C. Leppla[3], Glory Jean B. Leop[4], Annaliza A. Banggud[5], Erick Venn R. Rollon[6]

[1]*Student Researcher, Science, Technology, and Engineering Program, Tagum City National High School, Tagum City, Philippines*
[2]*Chief, Regional Crop Protection Center XII, Department of Agriculture Region XII, Tacurong City, Philippines*
[3]*Professor and Program Director, IPM, Department of Entomology and Nematology, University of Florida Institute of Food and Agricultural Sciences, Gainesville, United States of America*
[4]*Master Teacher II, Department of Science, Tagum City National High School, Tagum City, Philippines*
[5]*Master Teacher I, Department of Science, Tagum City National High School, Tagum City, Philippines*
[6]*Teacher III, Department of Science, Tagum City National High School, Tagum City, Philippines*

***Abstract*—The need for natural biocontrol agents has intensified due to the rising environmental impacts of synthetic insecticides and the increasing resistance of key agricultural pests, including the Fall Armyworm (*Spodoptera frugiperda*). The aim of the current study was to determine the time and effectiveness profile of CORNTRA, a novel maize-derived β-32 Ribosome-Inactivating Protein (RIP) biopesticide. Through solvent extraction and ammonium sulfate precipitation, a concentrated RIP stock was formulated with 5% ethanol and tested against 2nd–4th instar larvae. Bioassays revealed that CORNTRA achieved a 100.00% mean corrected mortality, numerically exceeding the 83.33% mortality of the 0.5% chlorpyrifos benchmark. One-way ANOVA confirmed significant treatment effects ($F (2, 6) = 31.00$, $p = 0.0007$), while Tukey's HSD indicated no significant difference in lethal efficacy between CORNTRA and the synthetic control ($p = 0.4736$), showing their near-equal performance. Crucially, CORNTRA also demonstrated a significantly faster onset of action ($F (2,6) = 61.00$, $p = 0.0001$) in comparison to the synthetic control, greatly outperforming it ($p = 0.0064$). Overall, CORNTRA showed a superior performance profile, combining rapid activity with high efficacy, highlighting its potential as a sustainable and effective biopesticide for the control of *S. frugiperda*.**

***Index Terms*—crop protection biotechnology, fall armyworm (*Spodoptera frugiperda*), maize ribosome-inactivating protein, protein-based biopesticide, sprayable botanical insecticide, sustainable agriculture, *Zea* mays defense mechanisms.**

1. Introduction

Spodoptera frugiperda (also known as Fall armyworm, or locally harabas na mais) is an American native pest. It affects more than 353 plant species, with the key ones being maize, sorghum, and sugarcane (Tay et al., 2022). The larvae hide in the stems of maize during the day and feed at night on whorls, foliage, silks, and tassels, creating significant damage. This feeding behavior increases the vulnerability of plants to fungal infection, aflatoxin contamination, and leads to significant deterioration of grain.

A major global agricultural threat is the fall armyworm (*Spodoptera frugiperda*), which is estimated to cause US$2.5–US$6.2 billion in annual losses to maize production in sub-Saharan Africa alone (Kasoma et al., 2020), a 36% annual maize production loss valued at about US$200 million in Ethiopia in 2021 (Abro et al., 2021), and a 17% increase in hunger probability in Zimbabwe (Tambo et al., 2021). The pest later spread to the Philippines, where a 2024 outbreak destroyed corn plantations in twelve provinces and caused a net economic loss of over ₱57 million (Gomez, 2024). Although integrated pest management (IPM) and synthetic insecticides are the most widely used control strategies, *S. frugiperda* populations rapidly develop resistance and exhibit adaptive changes (Gutiérrez-Moreno et al., 2018), which underscores the urgency of identifying alternative control methods. It is also worth noting that *Zea mays* contains a ribosome-inactivating protein (RIP), maize b-32, which interferes with protein synthesis and can cause rapid death in pests (Bertholdo-Vargas et al., 2009).

The current experiment compared a partially purified maize b-32 extract as a biopesticide spray against fall armyworm larvae, which are currently causing significant damage to maize production in the Philippines. Efficacy was evaluated using corrected mortality rates and a time-based rating scale. The aim is to provide a viable, economical, environmentally friendly, and sustainable solution to the problem of excessive dependence on synthetic chemical pesticides in farming.

2. Research Questions

Considering issues presented, this study aimed to address the

*Corresponding author: matteogalgo149@gmail.com

following inquiries:

1. Does the maize-derived biopesticide significantly affect the mortality rate of *S. frugiperda* larvae?
2. What are the specific effects of the maize-derived biopesticide on the mortality rate of *S. frugiperda* after exposure?
3. How does the effectiveness of CORNTRA compare with that of a commercially available 31.5 EC pesticide in controlling *S. frugiperda* populations after preliminary exposure?

3. Significance of the Study

This project evaluated CORNTRA through the efficacy of CORNTRA as an alternative off-pesticide that can be used to manage the spread of the fall armyworm (FAW) because it is an effective and sustainable agent based on maize b-32 rather than synthetic pesticides. The creation of this RIP biopesticide, along with the results of trial comparisons, contributed to a better understanding of how plant-based compounds can help satisfy the environmental need for sustainable agricultural inputs, reducing reliance on conventional chemical agents. Through the achievement of the study objectives, the sectors positively affected included:

A. Integrated Pest Management (IPM) Sector

The research supported both national and international programs in reducing crop loss and stabilizing the food supply in the event of FAW infestations. It provided strong statistical evidence of a non-synthetic, biodegradable tool that can be integrated into modern IPM guidelines. The methodology reduced operational costs for various agencies such as the Philippine Department of Agriculture (DA), National Food Authority (NFA), and Food and Agriculture Organization (FAO).

B. Cereal Crop and Corn Industries

The study addressed the significant economic challenge of FAW. It provided local industries and farmers with a cost-effective, natural solution derived directly from the corn plant. This contribution made crop protection applications financially accessible to farmers, cooperatives, and other stakeholders in the agricultural sector. In addition, it met global needs for a residue-free approach and strengthened agricultural stability.

C. Environmental and Public Health Control

The study aimed to confirm the effectiveness of a cheaper yet efficient substitute for traditional pesticides. This initiative significantly minimized the risks of chemical contamination associated with chlorpyrifos and its toxicity. These outcomes are especially relevant to agencies responsible for safeguarding public health and environmental standards, namely the Philippine Department of Health (DOH), World Health Organization (WHO), and the Philippine Department of Environment and Natural Resources (DENR).

4. Review of Related Literature

A. Fall Armyworm (S. Frugiperda)

Spodoptera frugiperda (Fall armyworm or FAW, locally referred to as harabas) was an invasive pest that affected over 353 plant species across 76 families. Classified into corn (sfC) and rice (sfR) strains (Tay et al., 2022) in 1985, the sfC variant caused most of the global outbreaks and led to more than ₱57 million in net losses in the Philippines (Gomez, 2024). FAW larvae aggressively fed on maize whorls, producing yellowish-brown frass as they burrowed. At this stage, many pesticides proved to be ineffective (Bessin, 2019). Although biopesticides using *Bacillus thuringiensis* and *Metarhizium anisopliae* showed promise, other methods were still necessary to improve sustainability and reduce dependence on microbial imports (Kafle, 2025).

B. Ribosome-Inactivating Proteins (RIPs)

Ribosome-inactivating proteins (RIPs) were plant-derived RNA N-glycosidases that depurinated adenine (A4324) on 28S rRNA, halting protein synthesis. They occurred as type I (single-chain), type II (with a lectin-B chain), and the less common type III, which had an extended C-terminal domain of unknown function but shared the endocytotic entry of type I RIPs (Stirpe, 2004). Notably, RIPs have shown prior significant biomedical potential; in one example, curcin from *Jatropha curcas* inhibited mouse sarcoma-180 growth by over 40% at 100 µg/mL (Zhao et al., 2012), highlighting their prominent versatility and cytotoxic capacity beyond medicine.

C. Maize-Derived b-32 RIP

The maize-derived b-32 RIP was a moderately cytotoxic, underutilized type III RIP with a resolved crystal structure bound to adenine (A4324) (Stirpe, 2004). It was first encoded in the endosperm as a silent form of the molecule (pI ≈ 6), which was then activated to form an A–B dimer that depurinated A4324 of the rRNA, thereby inducing apoptosis in the cells. Activated b-32, when ingested in the diet at a ratio of 1 mg/g, caused nearly 70 percent mortality in the cabbage looper (*Trichoplusia ni)* and caused the sap beetle (Nitidulidae) and the maize weevil (*Sitophilus zeamais*) to feed at a rate approximately sixfold slower (Bertholdo-Vargas et al., 2009). In addition, empirical studies showed that b-32 blocked the growth of fungal hyphae (Lanzanova et al., 2011) and provided resistance to fall armyworm larvae feeding on transgenic maize leaves that expressed b-32 (Dowd et al., 2012). These observations support the potential of using b-32 as a biocontrol agent in biopesticide formulations.

D. Comprehensive Tests for the Biopesticide Spray

In order to come up with an inference of the effectiveness of the engineered biopesticide, a series of tests was conducted, comprising the following:

1. Mortality Rate. The larvicidal efficacy against *S. frugiperda* was evaluated on seven drop applications on maize b-32 extract (experimental), chlorpyrifos (positive control), and distilled water (negative control). Death was assumed as immobility, which

was corrected using the formula of Abbott (Campbell and Miller, 2017). Statistical data was analyzed by one-way ANOVA ($p < 0.05$) to identify treatment differences.

2. Rating Scale. The rating scale test commenced immediately after treatment application. The parameter used to measure biopesticide efficacy was the time taken to induce *S. frugiperda* death (TTD, or Time to Death). Death occurring within 1–20 minutes was rated 5, 20–40 minutes as 4, 40–60 minutes as 3, 60–80 minutes as 2, and over 80 minutes as 1, providing a standardized comparison between effectiveness and time to action.
3. Comparative Efficacy. The biopesticide made from maize was evaluated on the basis of larval mortality, feeding repulsion, and mass suppression data. To determine significance and decrease the chance of Type I (false positive) error, significance was measured in comparison of the treatment differences against the chlorpyrifos control using one-way ANOVA and Tukey post-hoc test ($p < 0.05$) (Chmiel et al., 2022).

5. Theoretical Framework

The activities that led to the creation of a biopesticide called CORNTRA, which is based on *Zea mays*, were founded on the notion that some plant RIPs can inhibit protein synthesis in insect pests. Maize RIP b-32 deaminated 28S rRNA, blocking translation and inducing apoptosis (Stirpe, 2004). Controlled by the Opaque-2 gene and synthesized as an inactive precursor, it was activated by proteolytic cleavage and then targeted the midgut epithelium of lepidopteran pests such as *S. frugiperda*. As a type III RIP, b-32 gained access to cells via passive uptake through the midgut, enabling direct, non-GMO, and sprayable application. CORNTRA uses this mechanism as a fast, innovative, and eco-friendly way of controlling pests.

6. Methodology

A. Preparation of Materials

1) Collection and Soaking of Biological Samples

Fig. 1. Extracting the maize endosperm

Zea mays kernels totaling 200 grams were obtained from a local farm (7°26′29.0″N, 125°46′32.0″E; Purok 8, Brgy. San Miguel, Tagum City). To facilitate pericarp removal, the kernels were blanched in boiling water for 5–10 seconds and then immersed in an ice bath at 4°C for 5 seconds. Subsequently, they were stored in a chilled refrigerator at 4°C. Live FAW larvae (2nd to 4th instar) were collected from a corn farm with the appropriate permits (7°29′56.0″N, 125°47′46.0″E; Cuambogan, Tagum City) and acclimated for 24 hours prior to the bioassay.

2) Labeling and Setup of Equipment

All reusable tools and equipment were extensively sterilized (a knife, containers, and a blender, strainers of stainless steel, stirring rod, centrifuge tubes, spray bottle and petri plates) and wiped with 70% ethanol as well as labeled accordingly, should they be applicable.

B. Extraction and Concentration

1) Homogenization and Initial Filtration

The 200 grams of corn kernels were mixed with 310 mL of distilled water and blended for 5 minutes to create a fine, slightly thick slurry. It was then filtered through a fine 0.5 mm, 200 mesh opening using a SUS304 stainless-steel strainer into a sterilized glass beaker. The ~380 mL crude extract was then collected and cooled in an ice bath at 4°C before the next step.

Fig. 2. Creation of the initial slurry (Pre-salinization)

2) Protein Precipitation

The homogenate was mixed with about 215.0 grams of thoroughly filtered ammonium sulfate powder gradually while stirring with a rod to ensure it dissolved completely. The mixture was then stored in a sterilized glass bottle, and was sealed tightly, then placed in an ice bath at a steady 4°C to help the maize proteins precipitate.

Fig. 3. Protein precipitation and preservation

3) Sedimentation

The chilled mixture was removed from the ice bath and transferred partially into 16 5 mL centrifuge tubes. These tubes were spun in a centrifuge at 10,000 rpm for 15 minutes, which compelled the precipitated proteins to form a protein pellet at the bottom of each tube. The clear supernatant was then carefully poured off into a sterile stainless-steel bowl and discarded appropriately, and then all 16 of the pellets were first individually resuspended in 0.5 mL of water to liquify the pellet, then scooped out and placed in a sterile glass jar. The combined protein pellets were resuspended in a measured volume of distilled water using a 250 mL graduated cylinder and gently stirred until fully dissolved, yielding about 150 mL of concentrated protein stock.

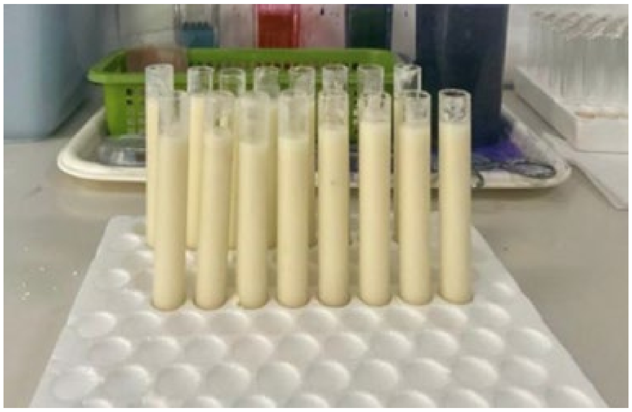

Fig. 4. Pre-centrifugation

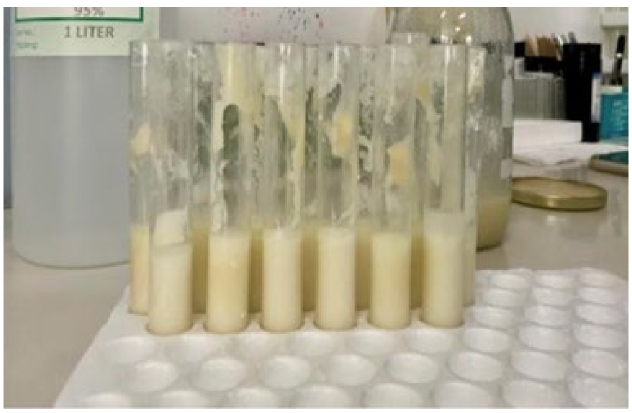

Fig. 5. Post-centrifugation

C. Pesticide Formulation

A concentrated protein stock was then diluted with an optimal volume of the sterile distilled water, then ethanol was added, and subsequently 39mL of sterile distilled water was added to the protein stock. Then, 11 mL of 95% ethanol totaling 5% of the final 200ml was added. The mixture that was then obtained was placed in a clean plastic spray bottle and kept at 4°C in an ice bath until it was used in the bioassay.

D. Bioassay Setup and Efficacy Testing

1) Establishment of Treatment Groups

Nine sterile petri dishes were used, with three replicates for each treatment group. The three treatment groups were as follows:

Table 1
Treatment groups and description

Treatment	Treatment Description
T1: Negative control	Distilled water (no active ingredients)
T2: Positive control	0.5% chlorpyrifos EC
T3: Experimental group	Final maize b-32 extract

E. Application and Larvae Housing

Fig. 6. Larval Transfer and Housing

Two *Spodoptera frugiperda* larvae in the middle of each replicate of every experimental group were placed on opposite sides of a petri dish using clean forceps. Afterwards, seven drops of the selected treatment solution were applied to every larva using a sterile pipette, thus guaranteeing the uniformity of the application and reducing the flaws in delivering the application by an inaccurate spray.

F. Observation and Data Analysis

The petri dishes were kept in a cool and well-ventilated area. Immediately after treatment, larvae were observed, and the time to death was recorded and later compared using a standardized rating scale. One-way ANOVA and the post-hoc Tukey significance test were used for statistical analysis of categorical variables (mortality, rating scale).

Fig. 7. Pre-treatment (T1, T2, T3)

Fig. 8. Post-treatment (80 min. threshold)

G. Testing and Data Collection

1) Larvicidal Efficacy and Biopesticide Performance

Larval mortality rates and a time rating scale were used to gauge how effective the maize biopesticide was. Three replicates of the experiment were conducted, using two larvae each. From T1 to T3, the treatments were administered in a sequential order. The mean and standard deviation of each treatment group were determined by summing the outcomes of all replicates. One-way ANOVA with Tukey's Honestly Significant Difference test was used as the statistical measure to compare treatment effects and identify significant differences.

Mortality Rate. Two sets of *Spodoptera frugiperda* larvae were exposed to drops of either CORNTRA extract (T3), 0.5 percent chlorpyrifos (T2), or 0.7 mL (seven drops) of distilled water (T1). Following treatment, the larval stage was observed, and mortality rates were noted until the very end. The corrected mortality rate, which accounts for treated deaths that have been adjusted for natural mortality, was determined using Abbott's formula (Campbell & Miller, 2017):

$$\text{Corrected Mortality Rate } (\%M_{corr}) = \frac{(\%\text{ Treated Mortality} - \%\text{ Control Mortality})}{100 - \%\text{ Control Mortality}}$$

All treatments underwent additional analysis to ascertain efficacy, with significant differences between groups being identified using one-way analysis of variance (ANOVA). Tukey's Honestly Significant Difference (HSD) post-hoc test was then used to adjust the *p*-values and lessen the likelihood of Type I errors.

Rating Scale. Two-larva groups of *S. frugiperda* were tested using T1, T2, and T3. The time to mortality was measured using a digital stopwatch, and death was confirmed by the absence of locomotor activity and righting reflex. The mean rating per group represented overall efficacy, as shown in the table below:

Table 2
Rating scale

Rating	Duration to Mortality
5	1-20 minutes
4	20-40 minutes
3	40-60 minutes
2	60-80 minutes
1	Above 80 minutes

Relative Efficacy. Comparison between each experimental biopesticide tier and the positive commercial control (0.5% concentrated Brodan 31.5 EC pesticide, prepared by dilution of 1 mL in 356 mL of water) was determined by integrating the above metrics, with a one-way ANOVA test conducted preceding a Tukey's HSD post hoc test to determine any treatments that differ significantly.

H. Waste Disposal

Used petri dishes were cleaned and immersed overnight in boiling water. Non-biodegradable waste materials were classified and transferred to the general, recyclable, and hazardous containers. Traces of chemical waste consisting of chlorpyrifos (31.5 EC) and purification reagents were marked clearly and sent to the appropriate authorities to be disposed under relevant regulations.

I. Data Analysis

The data analysis that was done in this study focused on evaluating the larvicidal ability of CORNTRA, a maize b-32 derived biopesticide, on the larvae of *Spodoptera frugiperda*. There were three major frameworks studied, including mortality rate, a rating scale, and comparative efficacy.

Mortality Rate. Direct mortality was measured as the number of larvae that died in each treatment group after exposure. The raw mortality rate (%) was then calculated using Abbott's formula, and the standard deviation was determined.

Rating Scale. A digital stopwatch was used to measure biopesticide efficacy ± 1 s to determine the precise time required for the biopesticide to induce death of the *S. frugiperda* larvae. The rating was then determined through Table 2.

Comparative Efficacy. CORNTRA performance was determined by combining the above measures and comparing the results of both Tukey HSD tests to identify treatments with significant differences after correcting false p-values caused by Type I errors. All statistical analyses and graphs were created using *astatsa.com*.

7. Results and Discussion

A. Results

This section presents the main findings related to the study's goals and engineering aims. The experiment focused on identifying precise statistical significance between Treatment 3 (CORNTRA) and Treatment 2 (Positive Control). The differences in mortality within the Positive Control were due to different larval tolerance to the Brodan pesticide, an expected source of variation in biopesticide tests.

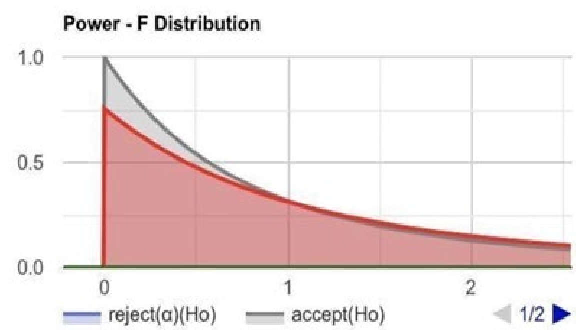

Fig. 9. Probability of rejecting mortality rate H_0

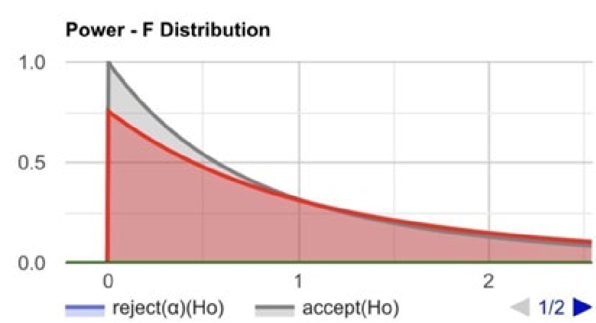

Fig. 10. Probability of rejecting rating scale H_0

Table 3
Pre-ANOVA data

Grp.	Iteration	No. of Larvae	MR[a]	TTD[b]	Final Rating
CRA[c]	1st	2	100%	<40min.	4
CRA	2nd	2	100%	<40min.	4
CRA	3rd	2	100%	<40min.	4
PC[d]	1st	2	100%	>40 min.	3
PC	2nd	2	50%	1st larvae > 40min., 2nd larvae >80min.	2
PC	3rd	2	50%	>40min.	3
NC[e]	1st	2	100%	>80min.	1
NC	2nd	2	0%	>80min.	1
NC	3rd	2	0%	>80min.	1

[a]MR: Abbreviation for "mortality rate" (measured in percent).
[b]TTD: Abbreviation for "time to death" (measured in min.).
[c]CRA: Abbreviation for CORNTRA (experimental group).
[d]PC: Abbreviation for positive control.
[e]NC: Abbreviation for negative control.

Table 4
ANOVA Results for the mortality rate

Source	SS	df	MS	F	*p*
Treatment Between Groups	17,222.2222	2	8,611.1111	31.0000	0.0007
Error Between Groups	1,666.6667	6	277.7778		
Total	**18,888.8889**	**8**			

Table 5
ANOVA Results for the rating scale

Source	SS	df	MS	F	*p*
Treatment Between Groups	13.5556	2	6.7778	61.0000	0.0001
Error Between Groups	0.6667	6	0.1111		
Total	**14.2222**	**8**			

Table 6
Tukey's HSD results for the mortality rate

Treatment Comparison	Tukey HSD Q statistic	Tukey HSD *p*-value	Tukey HSD inference
CRA vs PC	1.7321	0.4736095	insignificant
CRA vs NC	10.3923	0.0010053	** p<0.01
PC vs NC	8.6603	0.0020934	** p<0.01

Table 7
Tukey's HSD results for the rating scale

Treatment Comparison	Tukey HSD Q statistic	Tukey HSD *p*-value	Tukey HSD inference
CRA vs PC	6.9282	0.0064592	** p<0.01
CRA vs NC	15.5885	0.0010053	** p<0.01
PC vs NC	8.6603	0.0020934	** p<0.01

Fig. 11. Visual Confirmation of Mortality Rate H_0 Rejection

Fig. 12. Visual confirmation of rating scale H_0 Rejection

B. Discussion

In this section, a comprehensive analysis of the larvicidal efficacy and speed of action findings and their broader implications was explored, offering insights into the many interpretations that arise from the bioassay results. This exploration seeked to establish the potential of CORNTRA to function as a viable, sustainable alternative to synthetic chemical controls against FAW infestation.

1) Mortality Rate Results

CORNTRA showed impressive larvicidal effectiveness against *S. frugiperda*. ANOVA confirmed a significant difference among the treatments ($F(2,6) = 31.00$, $p = 0.0007$; Fig. 9, 11; Table 4). A perfect average mortality was achieved (100.00%, SD = 0.00), outperforming the Positive Control (83.33%, SD = 28.87) and Negative Control (0.00%, SD = 0.00). Tukey's HSD (Table 6) showed CORNTRA was statistically similar to the Positive Control ($p = 0.4736$), but there were significant differences compared to the Negative Control ($p < 0.01$), confirming CORNTRA achieved a same-standard mortality rate as chlorpyrifos.

2) Rating Scale Results

Rating Scale analysis showed CORNTRA's quick, superior action. ANOVA showed a significant difference among the means ($F(2,6) = 61.00$, $p = 0.0001$; Fig. 10, 12; Table 5). CORNTRA received the highest score of 4.00 (mortality < 40 minutes, SD = 0.00), outperforming the Positive Control (2.67, SD = 0.58) and Negative Control (0.00, SD = 0.00). Tukey's HSD (Table 7) confirmed significant differences between CORNTRA and both controls ($p = 0.0064$ vs. Positive; $p = 0.0001$ vs. Negative), showing maize b-32 has a faster speed of action than chlorpyrifos and confirming the study's frameworks.

3) Assessed Comparative Efficacy

CORNTRA achieved a similar lethal total ($p = 0.4736$) as the synthetic control but operated much faster ($p = 0.0064$; Table 8). This proved it to be an efficient and sustainable biopesticide with a 100% mortality rate, no variability, rapid action, and provided synthetic-level performance with an all-natural rapid mechanism.

8. Conclusion

CORNTRA was found to be as promising as chlorpyrifos against FAW with an average corrected mortality of 100.00, statistically the same as that of the Positive Control, 83.33 ($p = 0.4736$). Notably, pre-eminently, the data provided by the Rating Scale indicated that CORNTRA was much faster than the control ($p = 0.0064$), i.e., they had the same standard of mortality rate, but CORNTRA induced it quicker. Altogether, CORNTRA was as effective as synthetic pesticides and induced death faster, warranting its consideration as a feasible option to use in IPM programs against FAW.

9. Recommendations

Further research in the same field should use more effective mechanisms of drawing accurate statistics. The replication should be increased to at least five per treatment to enhance the statistical power and reduce intergroup variability. Standard protocols are recommended to be used to model survival curves, which could give a better understanding of the dose-response action of Maize b-32 RIP.

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