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RESEARCH ARTICLE

Steps Taken to Mitigate the Threats of Maximum Residue Limits (MRL) of Pesticides in Rice and Protecting Guyana Rice Exports Markets: Present and Future Prospects

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ABSTRACT

Governments of many countries are setting Maximum Residue Levels (MRLs) for food and feed being imported. To safeguard the threat of MRLs to rice exported from Guyana, the GRDB embarked on several research projects with the view to reduce MRLs and manage '*Paddy bug*' (*Oebalus poecilus* (Dallas)). Studies included *viz.* identification of biological control agents; screening advance germplasm for tolerance; evaluation of bio-pesticides and synthetic molecules with higher MRLs; pesticide residue studies-growth stages, cut-off points and MRLs between paddy vs. cargo rice. Results from the bioagent survey found relatively low populations of *predators* such as damsel fly, lady bird beetle, spider, and dragon fly in Regions #2, #3, #4, #5, and #6. Also, there were isolated cases of *parasitoids*, *viz. Telenomus podisi* and *Beskia aelops* at very low levels. Results from the tolerance screening revealed that entry FG-18-222 recorded the highest percentage of damage and high incidence of paddy bugs (PB), while significantly ($p < 0.05$) lowest damage was recorded in FG-15-35 (2.13%), G17-109 (3.93%), followed by G-14-10 (4.10%) in 2022. These entries appeared to be the least preferred to PB due to the high presence and relatively low percentage of grain damage. However, all 12 entries were found to be susceptible to paddy bug lighting and feeding. Likewise, *Bio-insecticide* Boom (MRLs 3.0 mg/kg) was evaluated against PB and 162 ml/ac. was the most effective rate. In a separate experiment, Malathion (MRLs of 8.0 mg/kg) at 12–18 ml/ac. was most effective in controlling PB population compared to untreated control. There were no significant differences between these two treatments and the Pronto (Imidacloprid) check. The results from the pesticide residue analysis *viz.* Imidacloprid and Thiamethoxam, showed that systemic A.I. can only be applied once in a rice growing season for PB control no later than 67 DAS. Additionally, results comparing detected MRLs between paddy grains *versus* shelled rice grains revealed >84.41% reduction in the detected MRLs for these systemic A.I. Further research work is recommended in this MS to be undertaken to better understand the PB and mitigate the threat of MRLs with the view of developing an IPM approach to manage PB at the same time protect Guyana rice export markets.

Keywords: Bioagents, Paddy bugs control, Pesticide, Tolerance.

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1. Introduction

Rice is the staple food in Guyana and many parts of the globe [1]. The Guyana's rice industry is currently the largest agricultural industry in the country, benefiting approximately six thousand (6,000) families directly and one hundred and fifty thousand (150,000) persons indirectly [2]. Rice is known as the bedrock of the Guyanese rural economy and by far the most important constituent of the livelihood of small farm families. It is the main contributor to export earnings in Guyana's agriculture sector, which accounted for 18% of Agricultural GDP and 14% of total non-oil exports in 2022 [3].

Rice is the greatest user of arable land, with an annual acreage of approximately 188,000 hectares available for cultivation. The available land is generally grown in two crops annually: the first being the spring crop and the second the autumn crop. The first crop is planted in November-December and harvested in March-April, while the second crop is planted in May-June and reaped between September-October [2]. Rice is cultivated primarily along the coastal belt in Essequibo, Demerara and Berbice, also including rice growing areas in the Essequibo islands of Leguan and Wakenaam and along the banks of the Mahaica, Mahaicony and Abary Rivers.

Increased farmers' performance and continued investments by Government in the rice industry of Guyana have enhanced the output and the ability of the country to compete in the world market. Improved rice varieties, production practices, drainage, and irrigation etc., allow for the rice industry to be competitive and sustainable. Additionally, Guyana continues to have easy access to international markets due to its rice being of the extra-long slender grain type with excellent cooking qualities. In 2020, rice production peaked at 687,539 tons of rice, of which 588,783 tons were exported, earning US $243,239.347. This upward trajectory continued in the first crop of 2021; however, the floods which occurred in May/June of that year resulted in major losses and, consequently, a reduction in production. In 2022, rice was exported to thirty-four (34) countries, including Portugal, France, Jamaica, Trinidad and Tobago, Venezuela, and the United States of America, with the European Market being the largest importer, amounting to 46% [2].

Over the years, pesticides have emerged as one of the most effective methods of pest control [4]. Pesticide residue in rice crops can pose a serious threat to biological diversity and human health [5]. The European Union (EU), which is one of Guyana's largest rice export markets, with more than 46% of Guyana's rice exported in 2022, is setting different MRLs and lowering the previous MRLs for some of the most popular pesticides used in rice for control of a major insect pest of rice, *Oebalus poecilus* (Dallas) commonly called *'Paddy bug'* or *'Stink bug'* [1]. This pest poses a serious threat to the more than 92,000 hectares of rice, which is being double-cropped annually in Guyana due to its ability to cause severe and extensive damage to the developing rice grains if left unmanaged. Two of the recommended pesticides used against the early, mid, and late season pests, especially against the paddy bugs, are *Imidacloprid* (Pronto) and *Thiamethoxam* (Renova). MRLs for these pesticides were revised by the EU and lowered from 1.5 mg/kg to 0.01 mg/kg. It is important to develop alternative strategies to prevent grain damages caused by the PB at the same time ensuring that the pesticide residue levels are maintained below the MRLs of 0.01 mg/kg as set by the importing countries such as the EU. Therefore, it is imperative that companies involved in food and feed products understand and comply with the MRLs standards of their target markets. Reference [6] reported that MRLs of pesticides beyond permissible levels on rice are causing a serious threat to the export competitiveness of rice in major global markets such as the United States, European Union (EU) and the Middle East. During the global Covid-19 crisis, countries are becoming more cognizant of health and safety concerns. Reference [7] found and reported that bio-alternative types of pesticides, including green pesticides, can cancel out the adverse effect of residual chemicals on crops in Farms and stores and so make them more attractive. Likewise, [8] state that the natural biological control of insect pests in the rice agroecosy stem by means of predators and parasitoids is a successful management measure, which can be further strengthened through the rational use of selective insecticides for the species of natural enemies that exist and encourage in the rice ecosystem for the control of the insect pest. Also, [9] conducted a review of natural enemies of stink bugs in the US, noting that the different stages of stink bugs are attacked by parasitoids and predators. He further stated that the effectiveness of stink bugs' natural enemies varies widely with stink bug species and habitats, influencing the biological control of stink bugs across crops. In addition, [10] conducted eco-friendly measures such as host-plant resistance and studied the feeding preference and performance of *O. puganx* on cultivated and non-cultivated rice varieties were examined and found that different rice varieties as being the most preferred and least preferred rice varieties for feeding of paddy bugs.

Despite all of this, the rice industry in Guyana has not been without its challenges, the major one being climate change. Other challenges include insect pests such as the paddy bug, high cost of inputs (fertilizers and pesticides), pesticide residues, labour shortage and the need for more advanced technology in research and development. In view of this, several research was undertaken during 2022 and 2023 aimed at mitigating some of these challenges with the view of lowering the MRLs, protecting rice crops from paddy bug damages as well as safeguarding Guyana's rice export markets. Studies include *viz*. identification and development of suitable biological control agents; screening advance germplasm for tolerance/resistance; evaluation of bio-pesticides and new synthetic molecules with higher Maximum Residue Limits (MRLs); Studies on MRLs of recommended pesticides-[to identify growth stages, suitable cut off points and number of repeat application of pesticides and compare the difference in detected MRLs between paddy and cargo rice gains] to bring awareness of the issues with MRLs and recommend alternative pest management strategies that should be adhered to for effective pest control. Also, this paper will identify the gaps for further research work with the ultimate objective of developing, strengthening and the implementation of an Integrated Pest Management (IPM) for managing the paddy bug (*Oebalus poecilus*) which is one of the major pests of the rice industry in Guyana.

2. Methodology

Studies were carried out through surveys and monitoring exercises. A series of screening trials and evaluation activities, a review of existing literature, and consultations to arrive at a holistic approach that was utilized on how to mitigate the main challenges of pest management (paddy

TABLE I: List of Insecticides Evaluated Against Paddy Bugs

S. n.	Active ingredient (%)	Common name of insecticides	Mode of action	MRLs status on the EU list
1	Malathion-57%	Mal-Shon	Systemic	8.0 (mg/kg)
2	Bio-insecticide (Pyrethrins-7.0%, Soyabean oil-3.0%, Clove oil-2.6%, Peppermint oil-2.6%, Citronella oil-2.2%, Cinnamon oil-0.5%, Cedar oil-0.5%, Thyme oil-0.2%)	Boom	Contact/Repellant	3.0 (mg/kg)

bug) and avoid the issue of Maximum Residue Limits (MRLs) affecting the rice export from Guyana.

2.1. Identification and Development of Suitable Biological Control Agents

A countrywide monitoring and survey exercise was conducted in 2022 to identify what are the biological control agents present within the various rice-growing regions in Guyana. The rice fields in the 35 monitoring zones were monitored using a sweep-net early in the morning before 7:30 am and late afternoon after 4:30 pm. Fifty (50) sweeps were done randomly in nine (9) fields in each rice-growing district/zones. The samples were collected in plastic bags and taken to the Entomology laboratory in Burma. Each sample was sorted, and the number of bio-agents caught from each field was recorded.

2.2. Screening Advanced Germplasm for Tolerance/Resistance

Twelve advanced breeding lines from the 2022 advance yield trials (AYT) were screened for paddy bug preference during the year 2022. The entries were *viz*. G-14-10, FG-15-35, G17-109, G18-05, G18-110, G18-124, UKM-RC-2, UKM-RC-8, FG-17-28, FG-17-94, FG-18-222, and G19-07. The experiment was sown and established under natural field conditions within the Plant Protection Department (Entomology) experimental site at Rice Research Station (RRS), Burma, using the Randomized Complete Block Design (RCBD) layout, with each treatment replicated three times. Plot size 4.5 m × 10 m (45 m^2) and seed rate 120 lbs/acre (132.37 kg/ha) were used. Weed control and fertilizer application were done following the GRDB recommendation (GRDB Rice Farmer's Manuel, 2009). Monitoring to determine the number of bugs present on each entry was done with a sweep net early in the morning, on alternate days from 35 days after sowing (DAS) up to 5% heading and daily from 5% heading up to the time of harvesting. No application of insecticide was done throughout the crop cycle. Paddy bug damage was determined from the harvested grains. Preference was measured based on the level of damage recorded since this reflects the amount of feeding done by the bugs during the susceptible period, which is from flowering to maturity of the grains.

2.3. Evaluation of New Bio-Pesticides and Synthetic Molecules With Higher Maximum Residue Limits (MRLs)

Insecticides were evaluated against paddy bugs in 2022 under field conditions in small plots at the RRS in Burma, Mahaicony. Experimental design utilized were Randomized Complete Block Design (RCBD) with 3 replications for each treatment. Plot size was 4.5 m × 10 m (45 m^2), seed rate used was 120 lbs/acre (132.37 kg/ha), variety sown GRDB 14. Crop was grown following the GRDB recommended crop husbandry practices for weed control and fertilizer application [11]. Each insecticide screened had 5 treatments, including two checks, which were the control (no insecticide) and Pronto (Table I). Monitoring for paddy bugs commenced at 30 DAS and continued alternate days up to the time of 5% flowering and then daily until 10 days before harvesting. Treatments were applied based on the threshold levels for the paddy bugs (i.e., 1 bug per every 2 sweeps).

2.4. Studies on MRLs of Recommended Pesticides

Pronto (Imidacloprid 70 WP) and Renova (Thiamethoxam) are a systemic insecticide, which means that it is taken up by plants and spread throughout the plant's stems, leaves, fruit, and flowers. These two A.I. are widely used by rice farmer/s for pest control in rice, especially against the late season pest-*Oebalus poecilus* (Dallas), commonly called '*Paddy bug*' or '*Stink bug*' by farmers.

During the second crop, 2022, the pesticide residue analysis for Pronto (Imidacloprid) and Renova (Thiamethoxam) was examined. Three separate trials were conducted as follows:

2.4.1. Number of Repeat Application of Pesticides/Cut-Off Point

Two separate trials were carried out to determine the number of times: (1) Renova (Thiamethoxam) at 100 g/ac and (2) Pronto (Imidacloprid) at 40 g/ac can be applied against paddy bugs without exceeding the MRLs 0.01 mg/kg for Pronto (Imidacloprid 70 WP) and Renova (Thiamethoxam) as listed on the EU restricted list of insecticides. The treatments were applied on a variety GRDB 16 in Field #21 of the Seed Production Plot at RRS, Burma. The plot size per treatment was 5 m × 12 m with 4 replicates of samples collected for the pesticide residue analysis at harvest. The details for the two experiments were as follows:

2.4.1.1. Experiment No.1–Repeat Application Using Thiamethoxam (Renova)

Renova was applied at 100 g per acre for each treatment on days 67, 72, 77, 82, 87, 92, 97, 102, and 107 DAS. Applications on treated plots were repeated every five (5) DAS (Table II).

TABLE II: Details of the Treatment Schedule and Application Frequency-Repeat Applications

Days after sown (DAS)	No. of times treatment applied
Untreated control	×0
67	×1
67 + 72	×2
67 + 72 + 77	×3
67 + 72 + 77 + 82	×4
67 + 72 + 77 + 82 + 87	×5
67 + 72 + 77 + 82 + 87 + 92	×6
67 + 72 + 77 + 82 + 87 + 92 + 97	×7
67 + 72 + 77 + 82 + 87 + 92 + 97 + 102	×8
67 + 72 + 77 + 82 + 87 + 92 + 97 + 102 + 107	×9

TABLE III: Details of the Treatment Schedule and Application Frequency-Cut Off Point

Days after sown (DAS)	No. of times treatment applied
Untreated control	×0
67	×1
72	×1
77	×1
82	×1
87	×1
92	×1
97	×1
102	×1
107	×1
112	×1

2.4.1.2. Experiment No. 2–Repeat Application Using Imidacloprid (Pronto)

Pronto was applied at 40 g per acre for each treatment on days 67, 72, 77, 82, 87, 92, 97, 102, and 107 DAS. Applications on treated plots were repeated every five (5) DAS (Table II).

2.4.2. Identification of Growth and Suitable Cut-Off Points

In the third trial (Cut-off Point), Pronto (Imidacloprid) was applied only once at the recommended rate of 40 g/ac from 67 Days After Sown (DAS) and continued at an interval of 5 days until harvest at 112 DAS (Table III) to determine at what growth stage of the rice plant should be the cut-off point for applying this product.

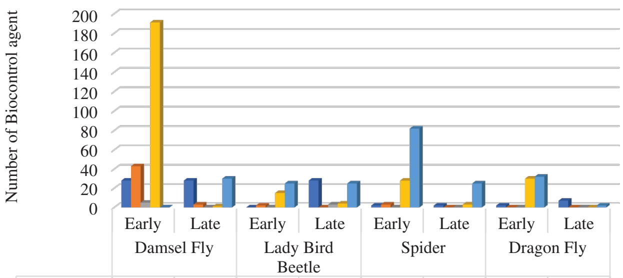
After all treatments were applied at the different growth stages and timings/intervals; samples were harvested at maturity, dried, carefully weighed to 1.0 kg, labelled, packaged in separate bags as per each treatment and taken to Pesticides and Toxic Chemical Control Board (PTCCB) Laboratory at Mon Repos for pesticide residue analysis of the paddy grains. The pesticide residues analysis was determined in the paddy and shelled grains (cargo rice) samples using, a QuEChERS (quick, easy, cheap, effective, rugged, and safe) based protocol coupled with liquid chromatography-tandem mass spectrometry (LC–MS/MS) following the protocols as described by [4]. Based on the results of the analysis, 5 samples that recorded high MRLs and low MRLs were randomly selected. The paddy grains of these samples were shelled and then re-analyzed by the PTCCB laboratory for the pesticide residue detected in the cargo rice grains to ascertain if there was any difference in pesticide residues between paddy and cargo rice for the same treatment.

3. Results

3.1. Identification and Development of Suitable Biological Control Agents

The survey for biological control agents, especially the predators of the paddy bugs, throughout the regions, was monitored twice in the second crop of 2022. It revealed relatively low populations of damsel flies, lady bird beetles, spiders, and dragon flies (Fig. 1). Damsel flies were recorded in almost all the monitoring zones within Regions #2, #3, #4, #5, and #6, with populations ranging from 5 to 191. The population of the lady bird beetles was extremely low, ranging from 2 to 25, with none being recorded in Regions #2 and #4. The presence of spiders was also observed in all Regions, except Region No. 4. Likewise, a relatively low population of dragon flies was recorded in Regions #2, #4, and #5 (Fig. 1).

In addition, isolated cases of an egg parasitoid, *Telenomus podisi* and adult parasitoids, Beskia aelops, two known principal natural enemies of the paddy bug (Oebalus poecilus), were recorded within the rice growing regions of Guyana at extremely low levels.



	Damsel Fly Early	Damsel Fly Late	Lady Bird Beetle Early	Lady Bird Beetle Late	Spider Early	Spider Late	Dragon Fly Early	Dragon Fly Late
Region # 2	28	28	0	28	2	2	2	7
Region # 3	43	3	2	0	3	0	0	0
Region # 4	5	0	0	3	0	0	0	0
Region # 5	191	1	15	4	28	3	30	0
Region # 6	0	30	25	25	82	25	32	2

Fig. 1. Number of bio-agents recorded during the second season, 2022.

3.2. Screening Advanced Germplasm for Tolerance/Resistance

The advanced yield trial entries screened were monitored for the number of bugs present during the growing season, and paddy bug damage was determined at maturity.

The incidence of paddy bugs (*Oebalus poecilus*) was higher during the second crop of 2022, and the bugs invaded all the entries (Table IV). The highest number of bugs was recorded from G-14-10 with 107 and FG-18-222 with 158, while the lowest numbers were from G18-05 with 49 and FG-15-35 with 87 for the first and second crops in 2022, respectively. In terms of damage, FG-18-222 recorded the highest per cent for both crops in 2022, while the lowest damage was recorded from FG-15-35 with 2.13 and G17-109 with 3.93 for the first and second crops in 2022, respectively. These entries (FG-15-35, G17-109)

TABLE IV: Number of Paddy Bugs and Mean Paddy Bug Damage for Entries in Advanced Yield Trial

Treatment	Number of bugs recorded		Mean paddy bug damage (%)	
	1st Crop	2nd Crop	1st Crop	2nd Crop
G-14-10	107	99	4.10^{BC}	6.82^{BC}
FG-15-35	97	87	2.13^{C}	4.83^{C}
G17-109	78	89	9.16^{AB}	3.93^{C}
G18-05	49	92	7.95^{ABC}	15.16^{B}
G18-110	78	98	5.30^{ABC}	9.93^{BC}
G18-124	109	106	4.77^{BC}	6.96^{BC}
UKM-RC-2	106	128	5.05^{BC}	11.23^{BC}
UKM-RC-8	101	145	5.94^{BC}	10.33^{BC}
FG-17-28	82	102	5.94^{BC}	5.76^{BC}
FG-17-94	87	93	8.02^{ABC}	6.06^{BC}
FG-18-222	99	158	22.00^{A}	28.63^{A}
G19-07	87	105	21.76^{A}	11.50^{BC}
			CV = 2.08	CV = 2.08
			SE = 3.01	SE = 4.94

Note: SE = Standard Error of a Mean, CV = coefficient of variation. Mean values in columns followed by same superscript letter(s) are not differ significantly at 95% confidence interval according to Fisher's Least Significant Difference (LSD) procedure.

appeared to be the least preferred entries for the PB due to the high presence and relatively low percentage of grain damage recorded (Table IV). The highest damage recorded from FG-18-222 corresponded with a high incidence of bugs during both crops. Although G-14-10 had a high incidence of bugs during both crops, the damage recorded as 4.10 and 6.82 were significantly different (0.05) from FG-18-222 for both crops in 2022 (Table IV). All entries were susceptible to paddy bug lighting and feeding.

3.3. *Evaluation of Bio-Pesticides (Boom) and New Synthetic Molecules (Mal-Shon) With Higher Maximum Residue Limits (MRLs)*

During the second crop 2022, Mal-Shon (Malathion) which has an 8.0 mg/kg MRLs as listed on the European Union (EU) restricted database was evaluated against the paddy bug. Treatments were applied after monitoring was done and the paddy bug population observed to be above the threshold limits (Table V). Also, the number of paddy bugs were monitored and recorded after 24 hours after the various treatment application recorded (Table V). The data recorded before and after treatment application showed number of paddy bugs ranging from 2 to 6, while the monitoring after 24 hours of treatment application only the untreated control recorded paddy bug population of 4. All the treatments with Malathion recorded 0 paddy bugs after treatment included the Pronto check (Table V).

Table VI shows that the control recorded the highest damage percent (8.4%) which was significantly different ($P \leq 0.05$) from all the other treatments. The lowest damage was 2.8%, which was recorded from Pronto @ 40 g/ac, treatment 4. The different rates of Malathion were not significantly different from each other, but treatments 2 (Mal-Shon @ 12 ml/ac) and 3 (Mal-Shon @ 18 ml/ac.) were comparable with treatment 4 (Pronto @ 40 g/ac). As the rates of the Malathion increased, higher levels of percent grain damage reduction were observed with the reductions ranging from 33.49% to 57.01% over the untreated control (Fig. 2). The Pronto check (67.82%) recorded the highest percent reduction in grain damage percent over the untreated control (Fig. 2).

Similarly, Boom, *Bio-insecticide* (Pyrethrins 7.0%, Soyabean oil 3.0%, Clove oil 2.6%, Peppermint oil 2.6%, Citronella oil 2.2%, Cinnamon oil 0.5%, Cedar oil 0.5%, Thyme oil 0.2%) were evaluated against the paddy bugs during second crop 2022. The paddy bugs population recorded before treatment application ranged between 6 to 10 (Table VII); while the paddy bugs population after treatment application ranged between 0 and 4. All treatment recorded 0 paddy bugs after treatment application except treatment with Boom @ 162 ml/ac which recorded 4 bugs (Table VII).

Paddy bug damage in the control, treatment 0 with no insecticide was the highest with 4.9%, while the lowest damage was from Pronto, treatment 4 with 1.5% (Table VIII). Control, treatment 0 was significantly different (0.05) from all treatments with Boom and the Pronto check (treatments 1 to 4). The different levels of Boom were not significantly different from each other; however, treatments 2 (Boom @ 201 ml/ac) and 3 (Boom 243 ml/ac)

TABLE V: Number of Paddy Bugs BEFORE and AFTER Exposure to the Different Treatments of Malathion and the Two Checks Under Field Conditions-Second Crop 2022

Treatments	No of bugs before application of treatments	No of bugs after application of treatments
T_0–Control (no insecticide)	6	4
T_1–Malathion @ 09, ml/ac	6	0
T_2–Malathion @ 12, ml/ac	6	0
T_3–Malathion @ 18, ml/ac	2	0
T_4–Pronto @ 40, g/ac	5	0

TABLE VI: Mean Percent Paddy Bug Damage for the Different Treatments of Malathion and the Two Checks Evaluated Under Field Conditions-Second Crop, 2022

Trt.	Treatment	Rates/ac	*Mean % grain damage, 2022 Autumn crop
T_0	Untreated control	–	[1]8.42^{A}
T_1	Mal-Shon	9 ml	5.60^{B}
T_2	Mal-Shon	12 ml	4.16^{BC}
T_3	Mal-Shon	18 ml	3.62^{BC}
T_4	Pronto (Check)	40 g	2.71^{C}
	Grand mean		**4.90**
	SEM		**0.88**
	CD (P = 0.05)		**2.03**
	CV (%)		**22.03**

Note: * = Average of four replications; [1] = Average from five samples per each replications. Mean values in columns followed by same superscript letter(s) are not differ significantly at 95% confidence interval according to Fisher's Least Significant Difference (LSD) procedure.

were comparable with treatment 4 (Pronto check @ 40 g/ac) (Table VIII).

Additionally, Boom demonstrated a reduction in the percentage grain damage ranging from 40.08% to 47.98% when compared to the untreated control (Fig. 3).

Boom treated plots showed higher grain yields than the Pronto treated plots (Table IX).

3.4. Pesticide Residue Analysis for Imidacloprid (Pronto) and Thiamethoxam (Renova)

Paddy samples from each treatment carefully harvested, dry, packaged, weighed in 1.0 kg and sent to the PTCCB laboratory in Mon Repos for residual analyses of Imidaclorpid and Thiamethoxam. Figs. 4 to 6 represent the concentration levels detected from samples harvested from Experiments 1, 2, and 3, respectively.

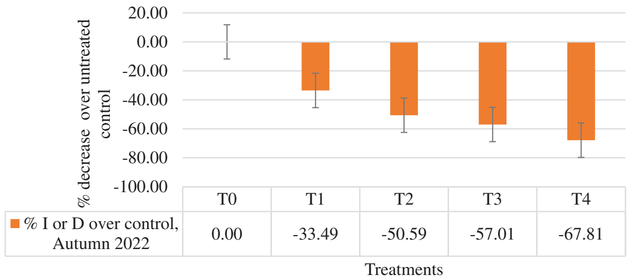


Fig. 2. Increase (I) or decrease (D) of % PB damage over the untreated control during autumn crop, 2022.

TABLE VII: Number of Paddy Bugs BEFORE and AFTER Exposure to the Different Treatments of Boom and the Two Checks Under Field Conditions-Second Crop, 2022

Treatments	No of bugs before application of treatments	No of bugs after application of treatments
T_0–Control (no insecticide)	8	0
T_1–Boom @ 162, ml/ac	7	4
T_2–Boom @ 201, ml/ac	6	0
T_3–Boom @ 243, ml/ac	6	0
T_4–Pronto @ 40, g/ac	10	0

3.4.1. Experiment 1

In this trial where the repeat application was done using Thiamethoxam (Renova) the results indicated the application of only one treatment of Thiamethoxam at 67 DAS showed MRLs of 0.00613 mg/kg which falls below the 0.01 mg/kg as stated in the EU restricted list of chemicals. Additionally, it was observed from the analysis that all the other repeat application at 5 days interval from 72 DAS until 107 DAS showed higher MRLs than 0.01 mg/kg (Fig. 4).

3.4.2. Experiment 2

In this trial that examined the Repeat application of Pronto (Imidacloprid 70 WP) the results showed that the application of Pronto (Imidacloprid 70 WP) once at 67 DAS and twice (at 67 DAS and 72 DAS) pesticide residue limits of 0.00534 and 0.00651 mg/kg, respectively were recorded. This was found to be below the MRLs for Pronto (Imidacloprid 70 WP) as listed as 0.01 mg/kg on the EU restricted list of insecticides. All other repeat applications showed higher MRLs than 0.01 mg/kg (Fig. 5).

3.4.3. Experiment 3

The results from the cut-off points trial indicated that treatment at 67 DAS pesticide residue from paddy sample detected Imidacloprid levels 0.000417 mg/kg and at 72 DAS as 0.000316 mg/kg. While at 77 DAS and 82 DAS, higher levels of Imidacloprid residue levels of 0.0187 and 0.0336 mg/kg, respectively were detected which was above the MRLs for Pronto (Imidacloprid 70 WP) as listed on the EU restricted list of insecticides. Thereafter, treatments from 77 DAS to 112 DAS showed MRLs levels below 0.01 mg/kg (Fig. 6).

3.5. Comparison of MRLs Quantity Detected (mg/kg) for Paddy Grains Versus Shelled Rice (Cargo Rice) Grains 2022

During the second crop 2022, the samples that were analyzed for pesticide residues in Experiment 1 to 3 that recorded higher and lower concentration of residues of Thiamethoxam and Imidacloprid in paddy samples analyzed were randomly selected and shelled to give cargo rice and re-analyzed to assess the quantity of pesticide residues

TABLE VIII: Mean Paddy Bug Damage (%) for the Different Treatments of Boom and the Two Checks Evaluated Under Field Conditions-Second Crop, 2022

Trt.	Treatment	Rates/ac.	*Mean % grain damage, 2022 Autumn crop
T_0	Untreated control	–	[1]4.94^{A}
T_1	Boom	162 ml	2.79^{BC}
T_2	Boom	201 ml	2.96^{B}
T_3	Boom	243 ml	2.57^{BC}
T_4	Pronto (Check)	40 g	1.54^{C}
	Grand mean		**2.96**
	SEM		**0.57**
	CD (P = 0.05)		**1.32**
	CV (%)		**23.63**

Note: * = Average of four replications; [1] = Average from five samples per each replication. Means values in columns followed by same superscript letter(s) do not differ significantly at 95% confidence interval according to Fisher's Least Significant Difference (LSD) procedure.

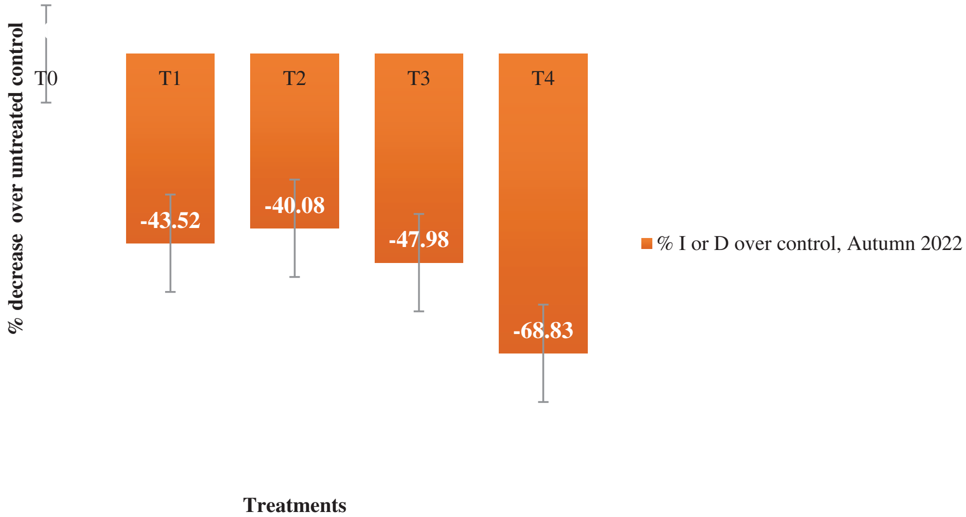


Fig. 3. Increase (I) or decrease (D) of % PB damage over the untreated control during the autumn crop, 2022.

(mg/kg) for paddy grains versus shelled rice (cargo rice). The result of this analysis is presented in Table X.

In this analysis Thiamethoxam demonstrated a 0.0628% reduction in quantity of pesticide residue detected between paddy versus cargo rice. This accounts for an 86.62% reduction in the pesticide residues. Likewise, Imidacloprid expresses a 0.0769% and 0.09853% reduction in quantity of pesticide residue detected between paddy versus cargo rice, respectively when sample 19 and 20 were analyzed. This accounts for 84.41% and 85.68% reduction in quantity of pesticide residue detected between paddy versus cargo rice (Table X). However, when the lower concentrations of pesticide residues of samples of Imidacloprid (sample 22 and 29, respectively) were analyzed a mere 0.000084 increase and 0.000076 decrease in pesticide residues of Imidacloprid was detected between paddy grains and cargo rice grains. This accounts for a 26% increase and 15.97% decrease in pesticide residue concentrations detected (Table X).

TABLE IX: Mean Yield (kg/ha) for the Different Treatments of Boom and the Two Checks Evaluated Under Field Conditions-Second Crop 2022

Treatments	*Mean yield (kg/ha) 2nd crop
T_0–Control (no insecticide)	3528.06
T_1–Boom @ 162, ml/ac	3011.01
T_2–Boom @ 201, ml/ac	3531.47
T_3–Boom @ 243, ml/ac	3945.74
T_4–Pronto @ 40, g/ac	2492.25
	CV = 18.02

Note: * = Average of four replications.

Based on the results of these pesticide residue trials (Experiment 1 to 3) it can be determined that the Active Ingredient (A.I.)-Imidacloprid and Thiamethoxam, each can only be applied once in a rice growing season for paddy bug control no later than 67 DAS. Additionally, these 2 AI along with the other recommended alternatives insecticides (Table X) that have similar MRLs should be used on a *strict rotation* basis for managing paddy bugs, prevent grain damages caused by the PB and at the same time protect Guyana rice export markets. Further, based on the results of the comparison of MRLs quantity detected (mg/kg) for Paddy grains versus Shelled Rice (Cargo rice) grains 2022 it can determine that greater than 84.41% reduction in the detected pesticide residues for the A.I. Imidacloprid and Thiamethoxam can be achieved when paddy grains with higher MRLs are shelled (cargo rice) (Table X).

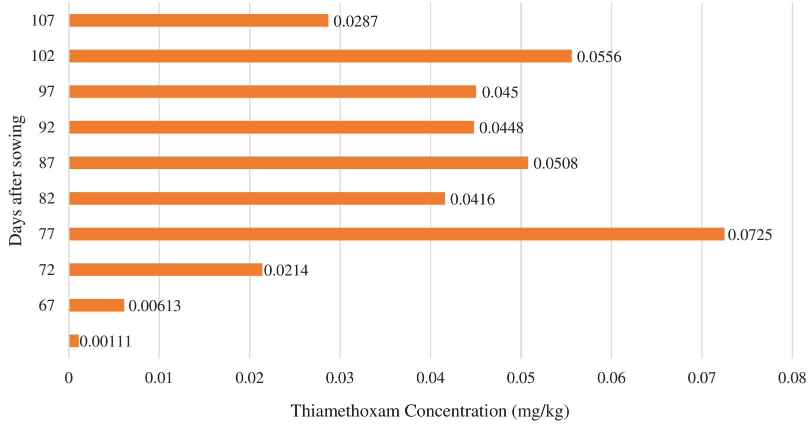


Fig. 4. Concentration detected from paddy treated repeatedly with Thiamethoxam (Renova).

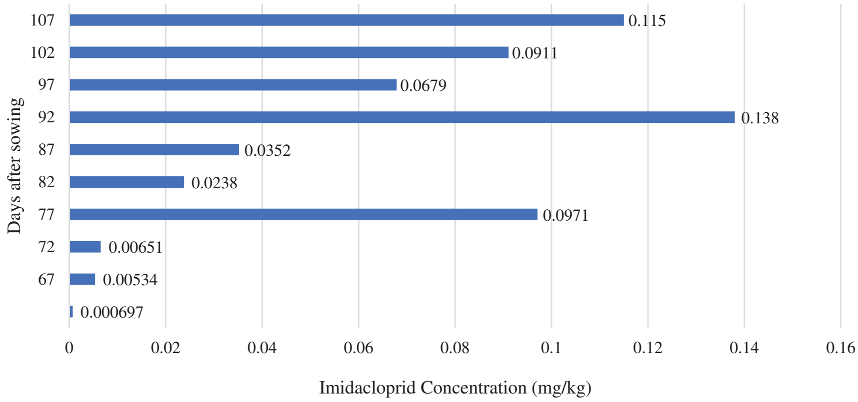


Fig. 5. Concentration detected from paddy treated repeatedly with Imidacloprid (Pronto).

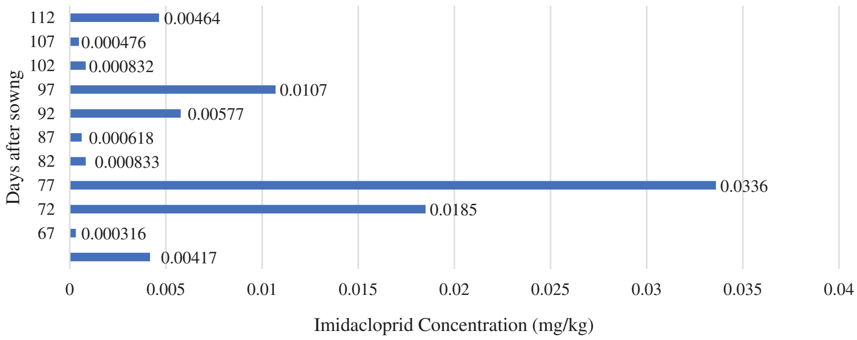


Fig. 6. Concentration detected from paddy treated Imidacloprid (Pronto) at various cut-off points.

4. Discussion

4.1. *Identification and Development of Suitable Biological Control Agents*

Nearly 40% of the world's potential food production is lost each year due to pests. Overreliance on chemical pesticides has led to increased resistance among several species, making them even harder to manage [12]. The advent of chemical solutions such as pesticides revolutionized plant protection techniques has brought about environmental and health concerns. Biological controls offer a middle-ground, leveraging natural predators and bio-pesticides to combat agricultural threats [13]. Based on the forgoing this research project identifies suitable biological control agents that are present within the rice ecosystem in Guyana, with the view of developing them to use in the management of the paddy bug. Results from the survey and monitoring exercise throughout the rice industry in Guyana found the presence of predators such as damsel fly, lady bird beetle, spider, and dragon fly at relatively low populations with in almost all the monitoring zones in Regions #2, #3, #4, #5 and #6. Of these predators to the paddy bugs damsel flies were recorded in with the highest population in Region #5 (191), followed by Region #6 (82). Also, isolated cases of parasitoids, *Telenomus podisi* and *Beskia aelops*, were found at extremely low levels within the rice-growing regions of Guyana. Similar findings were reported by [14] regarding the presence of the parasitoids, *Telenomus podisi* and *Beskia aelops* within the rice industry in Guyana. Also, [9] conducted a review of natural enemies of stink bugs in the US, and reported that that the eggs, nymphal and adult stages are the most attacked by parasitoids like *Telenomus podisi* and *Beskia aelops* and various types of predators.

TABLE X: Results of the Comparison of MRLs Quantity Detected (mg/kg) for Paddy Grains Versus Shelled Rice (Cargo Rice) Grains, 2022

Sample id	A.I.	(Quantity detected (mg/kg)		Actual increase or decrease in quantity detected (mg/kg): Paddy *vs.* Cargo rice grains (%)	Per cent increase or decrease in quantity detected (mg/kg): Paddy *vs.* Cargo rice grains (%)
		PDDDY grains	Cargo rice grains		
4	Thiamethoxam	0.0725	0.00970	0.0628	−86.62
19	Imidacloprid	0.0911	0.01420	0.0769	−84.41
20	Imidacloprid	0.115	0.01647	0.09853	−85.68
22	Imidacloprid	0.000316	0.0004	−0.000084	26.58
29	Imidacloprid	0.000476	0.00040	0.000076	−15.97

4.2. Screening of Advanced Germplasm to Identify Tolerance/Resistance

Results from this study reveal that entry, FG-18-222 recorded the highest percent of damage which corresponded with high incidence of bugs during both crops 2022, while the lowest damage was recorded from FG-15-35, G17-109 and G-14-10 (2.13%, 3.93%, and 4.10%, respectively) for the first and second crops 2022. These entries (FG-15-35, G17-109, G-14-10) appeared to be the least preferred entries for the PB due to the high presence and relatively low percentage grain damage recorded. The damage recorded in entries viz. FG-15-35, G17-109, G-14-10 were significantly different (0.05) from FG-18-222 for both crops in 2022. However, all entries were found to be susceptible to paddy bug lighting and feeding. Reference [10] conduct similar research and report like findings reported as observed in this present study, where host-plant resistance varied from one variety to another. In the feeding preference and performance study of different cultivated and non-cultivated rice varieties the researchers reported that the choice tests showed that the rice varieties *Cheniere* has been the least preferred by *O. pugnax* for feeding and while *Kaybonnet* was the most preferred for feeding, respectively. Reference [15] also revealed that host–plant resistance study is a very good method of combating pest, and it is perhaps the easiest, most economical, and effective means of controlling insect pests as there is no special technology which has to be adopted by farmers. The expenses to the farmer are also limited because he only has to buy the seeds and there are no environmental hazards involved. The researcher also states that this method of pest control is quite compatible with other methods of control.

4.3. Evaluation New Bio-Pesticides and Synthetic Molecules With Higher Maximum Residue Limits (MRLs)

Many countries globally have experienced nonconformance in the levels of pesticides residues detected in food crops for local consumption and export. Sometimes this leads to rejections and other forms of embarrassment from the importing countries [7]. Reference [16] reported that the dangers associated with the use of synthetic pesticides necessitated the need for alternative pesticides (biopesticides), which are cheaper, environment friendly, and sustainable. Biopesticides can be sourced from microbes (e.g., metabolites), plants (e.g., from their exudates, essential oil, and extracts from bark, root, and leaves), and nanoparticles of biological origin (e.g., silver and gold nanoparticles). Reference [17] also reported that pesticide residues in food grains and green vegetables are an emerging threat to food security, food safety and environment, as well as countries import and export markets. The impact of pesticide residues can be minimized by taking certain measures such as the rational use of pesticides, promoting organic farming, exploit natural and bio-pesticides, and proper implementation and amendment of pesticide-related laws [18]. In Guyana this scenario is no different, hence this present research was undertaken where Boom, a *Bio-insecticide* (A.I.-Pyrethrins-7.0%, Soyabean oil-3.0%, Clove oil-2.6%, Peppermint oil-2.6%, Citronella Oil-2.2%, Cinnamon oil-0.5%, Cedar oil-0.5%, Thyme oil-0.2%) with MRLs of 3.0 mg/kg on the EU restricted list of insecticides were evaluated against the paddy bugs. The results found and recommend Boom at 162 ml/ac as the most effective rates providing excellent control of the paddy bugs population and demonstrated lower levels of grain damages compared to the untreated control and were not significantly different to the Pronto (Imidacloprid) check (that has an MRLs of 0.01 mg/kg on the EU restricted list of insecticides). Similar findings were reported by [19] when these researchers evaluated three essential oils (*viz*. ginger, garlic, and Mexican marigold) against late and early blight caused by *Phytophthora infestans* and *Alternaria solani*, respectively and found all 3 to have significant potency against the two diseases which was comparable to the synthetic fungicide, (Ridomil Gold). Likewise, [20] highly recommended the use of similar natural pesticides (bio-pesticides) as a pest control alternative to synthetic pesticides because of less impact on food security and environmental sustainability. Also, [21] endorse that these type of biopesticides revived the interest in the use of more environmentally friendly crop protection products or well known as phytochemical pesticides. Moreover, these types of biopesticides do not feature residue problems which are matter of significant concern for consumer and one which this study aims to encourage and explore [21]. Another, field study conducted by [22] on tomato to compared the effect of the application of *Leucaena leucocephala* leaf extracts as an alternative to carbofuran a synthetic nematicide against the control of nematodes and to look at the pesticide residues (MRLs) after application. The results revealed that pesticide residue (MRLs) was significantly ($p = 0.05$) higher in plots treated with Carbofuran compared with standard MRLs for Carbofuran in tomatoes. Also, Crude extracts of *L. leucocephala* were as effective as Carbofuran, while the fraction was however significantly better in producing higher numbers of fruits and reduced nematode population in root and soil of tomato plants which also provide further support to the findings of this present study. The A.I. (Pyrethrins) in the Boom probably responsible for the effect on the paddy bug by leading to the disruption of sodium and potassium ion exchange in insect nerve fibres, which eventually caused immediate paralysis leading to death [7].

In a separate experiment, a synthetic molecule Mal-Shon (Malathion) which has an 8.0 mg/kg MRLs on the EU restricted list of insecticides was evaluated against the paddy bugs. The results for that trial found Mal-Shon at 12–18 ml/ac to be the most effective rate in controlling the paddy bug population and reducing the percent grain damage incidence. Similar results were reported by [23] who evaluated seven insecticides including Malathion 50% EC. The researchers revealed that all the treatments (including Malathion 50% EC treatment) were significantly effective to suppress population of '*gundhi bug'* as compared with control (3.51 bug/hill). As well, [24] found Malathion 57 EC was the most effective chemical in controlling rice bug and give the best yields compared to the control and other treatments.

4.4. Pesticide Residue Analysis for Imidacloprid (Pronto) and Thiamethoxam (Renova)

One of the important issues facing approximately 6.48 billion world populations is food security due to pesticide residue above the MRLs set by importing and exporting countries (PRB. 2005). The widespread use of pesticides for improving agricultural productivity has raised public concern about the possible presence of residues in food [21]. Reference [5] reported that lab test data analysis from a study conducted over 26 provinces in Indonesia showed that 98.58% of total samples of food products do not contain or have residues under the MRLs required to achieve the food safety requirements. The pesticide residues other 1.42% of samples analyzed include residues of Organophosphate, Carbamate, Pyrethroid, Organochlorine, N-Phenylpyrazole, and Neonicotinoid groups of pesticide that were above the acceptable MRLs. These researchers also concluded that considering the effect of pesticide (residue) on human health and to environmental sustainability, efforts to control pesticide use need to be continued. This call is also supported by the research findings of [25]. They reported that pesticide residues in various crops have been found in the environment many times higher than the maximum permissible limit and causing serious health and economic implications. Due to this issue, this present research was focus of looking at addressing some of the issue with MRLs in Guyana by looking into two of the most widely use pesticides A.I. that were used by rice farmers for pest control. Imidacloprid (Pronto) and Thiamethoxam (Renova) are two systemic insecticides, which means that it is taken up by plants and spreads throughout the plant's stems, leaves, fruit, and flowers [2]. These two A.I. are widely used by rice farmers/s for Early, Mid and Late season pest control, especially against the Late season pest-*Oebalus poecilus* (Dallas) commonly called 'Paddy bug' or 'Stink bug' by farmers [1]. In view of this, several experimental studies were executed with different objectives using these two A.I. Results from the pesticide residue analysis for Imidacloprid (Pronto) and Thiamethoxam (Renova) from the Experiment 1 to 3 found that the Active Ingredient (A.I.)-Imidacloprid and Thiamethoxam, each can only be applied once in a rice growing season for paddy bug control no later than 67 DAS to prevent grain damages caused by the PB and at the same time protect Guyana rice export markets since the levels found were below the MRLs of 0.01 mg/kg as listed on the EU database for these two A.I. Reference [26] studied the dissipation rates and half-life values on eggplant after being treated

TABLE XI: List of Alternative Recommended Insecticides to be Used for Paddy Bug Control (GRDB Advisory, 2023)

S. n.	Active ingredient (%)	Common name of insecticides	Rates/ac (ml)	Mode of action	MRLs status on the EU list
1*	Lambda-Cyhalothrin-5% Thiamethoxam-15%	Undersiege	90 ml	Contact + Systemic	0.20 mg/kg 0.01 mg/kg
2	Acetamiprid-15%	Caprid, Super capri	60 ml	Contact + Systemic	0.01 mg/kg
3*	Lambda-Cyhalothrin-5%	Jackpot, Karatex	50–60 ml; 42 ml	Contact	0.20 mg/kg
4	Pyriproxyfen-10%	Advance 10	192 ml	Contact + Systemic	0.01 mg/kg
5*	Malathion-57%	Mal-Shon	12–18 ml		8.0 mg/kg
6*	Bio-insecticide (Pyrethrins-7.0%, Soyabean oil-3.0%, Clove oil-2.6%, Peppermint oil-2.6%, Citronella oil-2.2%, Cinnamon oil-0.5%, Cedar oil-0.5%, Thyme oil-0.2%)	Boom	162 ml	Contact + Repellant	3.0 mg/kg
7*	Cypermethrin	Bestac, Fastac	80 ml	Contact	2.00 mg/kg
8	Thiamethoxam	Renova, Medal, Protector	80–100 ml	Systemic	0.01 mg/kg
9*	Permethrin	Tenguard	60 ml	Contact	0.05 mg/kg
10	Abamectin	Abamectin	100–120 ml	Systemic	0.01 mg/kg
11	Bifenthrin	Binder	80–100 ml	Contact	0.01 mg/kg
12	Triazophus	Triazophus	60 ml	Systemic	0.02 mg/kg

Note: *Those can be used for *repeat/multiple* applications within the rotation schedule, the others with MRLs of 0.01 mg/kg, including the Thiamethoxam, should only be applied once in a rice growing season for paddy bug control before 67 DAS (GRDB Advisory, 2023).

with three synthetic insecticides. They found that the half-life of indoxacarb to be 3.9 days, thiacloprid 11.6 days and methyl parathion 3.4 days on eggplant fruits after treatment application. Thus, agrees with the findings of the present study that the residues of these two pesticides, Imidacloprid (Pronto) and Thiamethoxam (Renova) can gradually eroded over time (67 DAS) to the level below the MRLs of 0.01 mg/kg as listed on the EU database for these two A.I. in rice.

4.5. Comparison of MRLs Quantity Detected (mg/kg) for Paddy Grains Versus Shelled Rice (Cargo Rice) Grains 2022

Further, based on the results of the comparison of MRLs Quantity Detected (mg/kg) for Paddy grains versus Shelled Rice (Cargo rice) grains 2022 it can determine that greater than 84.41% reduction in the detected pesticide residues for the A.I. Imidacloprid and Thiamethoxam can be achieved when paddy grains with higher MRLs are shelled (cargo rice). This finding also concurs with the findings of [26].

5. Conclusion

This present research identifies the presence of essential biological control agents that are predators and parasitoids in nature against the '*Paddy bug*' (*Oebalus poecilus* (Dallas)) in Guyana. Also, found three entries viz. FG-15-35, G17-109, G-14-10 which appeared to be the least preferred entries for the PB due to the high presence and relatively low percentage grain damage recorded. Of which the entry G-14-10 was recently released as a new rice variety in 2023 named GRDB IICA 17 (Zinc Biofortify). Additionally, two suitable alternative pesticides with higher MRLs, *Bio-insecticide*, Boom @ 162 ml/ac with MRLs listed as 3.0 mg/kg and Malathion @ 12–18 ml/ac with MRLs listed as 8.0 mg/kg were tested and found to be effective in controlling the PB and resulted in significant reduction in percent damage as compared to the untreated control. These pesticides are recommended by GRDB (Tables VI,VIII and XI) and form part of the recommended pesticides to be used by farmers in the rotation spray program. Apart from these findings, a series of pesticide residue analysis was conducted and found that systemic Active Ingredient (A.I.) such Thiamethoxam, can only be applied once in a rice growing season for paddy bug control no later than 67 DAS to prevent grain damages caused by the PB and at the same time protect Guyana rice export markets since the levels found were below the MRLs of 0.01 mg/kg as listed on the EU database. Further, the MRLs quantity detected (mg/kg) for Paddy grains versus Shelled Rice (Cargo rice) grains found a >84.41% reduction in the detected pesticide residues for the A.I. This provides a base for exporters to test the paddy samples when received to assess the MRLs of the various restricted pesticides and if found to be above the MRLs of the desired export markets to utilize the option of shelling the paddy and sell as cargo rice instead. This in a way offers another option of trying to safeguard the rice export markets and avoid paddy or cargo rice being rejected. Notwithstanding this, it is important to note that the availability of alternative pest-management tools will be critical to meet the production standards and stiff competition as is expected in these niche rice export markets [13].

6. Recommendation and Future Research Work

To protect Guyana rice export to the EU and other important rice market, the following is recommended:

1. Chemicals reside in grains are a major food safety concern globally. In this this regard GRDB would need to partner with other research institutions that is advanced in developing bio-control methods of insect pests in rice. Also, the biological control agents found within the rice ecosystem need to be protected and enhanced by using more bio-friendly pesticides. Further, a more structural biological control program needs to be researched and developed using the baseline information gather from this present study.
2. Further research needs to focus of identifying germplasms that have more tolerance/resistance to the paddy bug and use in the breeding program to develop resistance/tolerant cultivars.
3. Additional research to be conducted to identify and screen more bio-pesticides which will be less harmful to the bio-agents present withing the rice ecosystem and also steps to be taken to explore the option of developing nano-pesticides and systemic pesticides with higher MRLs that has proven to be effective in managing pest and have less negative impact on none target organisms and the environment.
4. Moreover, application restrictions exist for the products with the Active Ingredient (A.I.)–Imidacloprid. This product should be avoided and use the other available alternatives as recommended by the GRDB (Tables VI,VIII and IX) on a strict rotation schedule [2].

Further, the A.I. with MRLs of 0.01 mg/kg must be applied on a strict rotation schedule with the other recommended A.I. with higher MRLs such as Cypermethrin and Lamba-cyhalothrin, Malathion, Boom (bio-insecticide) and Permethrin that has their MRLs limits listed on the EU restricted list as 2.0, 0.2, 8.0, 3.0, and 0.5 mg/kg, respectively [2].

5. This research also observed gaps and recommended further studies to be conducted to better under the paddy bugs and to develop a comprehensive management strategy that can be utilized to effectively reduce its economic impact on rice cultivation in Guyana. Further studies are recommended in the following areas:

I. Screening of pheromones against paddy bugs,
II. Evaluation of more medicinal plant extracts, bio-pesticides, and commercially available bio-agents,
III. Conduct residue analysis of insecticides-storage degradation,
IV. Study the spatial and temporal distribution of paddy bugs & incidence of emerging insect pests in Guyana,
V. Species identification and distribution,
VI. Life cycle studies,

VII. Threshold studies,
VIII. Host preference studies,
IX. Training and awareness.

All of these will further contribute and lead to the development, promotion and implement an integrated pest management (IPM) program for the management of paddy bug in Guyana.

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Author Contributions

Rajendra Persaud designed and executed the study analyzed the data and drafted the paper. All other authors assist with data analysis and interpretation, literature review and editing support, technical advice, and read, and agreed with the content of the manuscript.

Data Availability

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding authors.

Ethical Standards

The authors declare that the current research did not involve human participants or animals as test materials.

Funding

The authors have no relevant financial or non-financial interests to disclose.

Conflict of Interest

The authors declare that there is no potential conflict of interest to report.

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