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Effect of Rice Seed Treatment with *Beauveria bassiana* on Salicylic Acid Content and Population of Brown Planthopper (*Nilaparvata lugens* Stal.)

Ravindra C. Joshi[1], Yolma Hendra[2*], Trizelia[3], My Syahrawati[3]

[1] Philippine Rice Research Institute, Nueva Ecija, Philippines
[2] Agotechnologi, Faculty of Agriculture, Universitas Islam Riau, Indonesia
[3] Plant Protection, Faculty of Agriculture, Universitas Andalas, Indonesia

*E-mail: hendrayolma@agr.uir.ac.id (Corresponding author)

Abstract : The brown planthopper (BPH), *Nilaparvata lugens* Stal., is a significant pest that adversely affects rice yields. Utilizing *Beauveria bassiana* (Bals.) Vuill. as a biological control agent offers a sustainable solution to combat this pest. This study investigated the impact of *B. bassiana* applied via rice seed treatment for 24 hours on BPH population dynamics and salicylic acid levels in rice stems. The experiment followed a completely randomized design (CRD) with five treatments and five replications, including four *B. bassiana* isolates (BbJg, BbWS, Pb211, Td312) and a control. A concentration of 10^8 conidia/ml was applied uniformly across treatments. Data analysis was performed using ANOVA and LSD at a 5% significance level. Results indicated that *B. bassiana* treatment significantly suppressed BPH populations. The BbWS isolate demonstrated the lowest egg hatch rate (28.39%), outperforming other treatments. While the number of adult BPH remained unaffected, a notable reduction in 4th and 5th instar nymphs was observed. Furthermore, all isolates elevated salicylic acid content in rice stems, peaking at 47.87 ppm, as measured using High-Performance Liquid Chromatography (HPLC). The BbWS isolate emerged as the most effective in curbing BPH and boosting salicylic acid levels. These findings underscore the potential of *B. bassiana* seed treatment as an effective strategy for BPH management and enhancing plant defense mechanisms through increased salicylic acid production..

1. Introduction

One of the important pests of rice (*Oryza sativa* Linnaeus) is the brown planthopper (BPH) or *Nilaparvata lugens* Stal (Hemiptera: Delphacidae). BPH attacks rice at all stages of growth, destroying it by sucking plant cell fluids and as virus vectors. Heavy attacks can cause hopperburn and crop failure[1]. Various efforts have been made to control BPH, one of which is to use superior varieties that are resistant to BPH. The continuous use of resistant varieties can only last for 2-3 seasons because BPH is a pest with high genetic plasticity and is able to quickly adapt to existing varieties. The resistance of rice plants can be quickly broken with the emergence of new biotype BPH [2]. Efforts to control using insecticides are also carried out, but if carried out continuously it can cause environmental pollution and BPH resistance to these insecticides [3]. One of the pest control that has a fairly good prospect is the use of entomopathogenic fungi such as *Beauveria bassiana* [4]

B. bassiana is used as a biological control agent that is very effective in controlling a number of insect species. In addition to acting as an entomopathogenic fungus, *B. bassiana* fungus is an endophytic fungus [5]. Endophytic fungi in plant tissues are able to increase plant resistance by forming various toxic compounds, antifeedants and secondary metabolites which include salicylic acid, jasmonic acid and ethylene which affect insect development [6] Endophytic fungus *B. bassiana* in tomato plants caused the salicylic acid content to be higher and suppressed the development of the *Bemisia tabaci* population [7] .

The Fungus of *B. bassiana* isolate Td312 that colonizes chili plants has a higher salicylic and jasmonic acid content compared to control plants [8]. The content of sucrose and oxalic acid influenced the development of the BPH population [9]. The high sucrose content causes the prolonged development of BPH nymphal stadia to be shorter. Dissolved oxalic acid is toxic to insects, the entry of this acid on the digestion of insects in high levels can lead to death [10]. The rice seeds inoculated by the endophytic fungus *Nigospora sp*. by the seed soaking method, can increase plant resistance to BPH, increase egg mortality with the number of eggs hatched ±10%, prolong the length of nymph development, preoviposition period, oviposition period as well as delayed egg laying time by female adults [11].

Trizelia et al. reported that chili plants inoculated with the endophytic fungus *B. bassiana* are able to suppress the development of the *Myzus persicae* population and increase plant resistance to pests [12]. Similarly, studies on *Helicoverpa armigera* (Hubner) larvae in tomato plants inoculated with the endophytic fungus *Acremonium strictum* showed prolonged egg and larval stadia [13]. Endophytic fungi found in the root system of plants have also been shown to extend the development of *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) nymphs [14].

Despite the promising potential of *B. bassiana* as a biocontrol agent, there is limited research on its specific effects on BPH population dynamics and its ability to induce secondary metabolite production, such as salicylic acid, in rice plants. Furthermore, the effectiveness of different *B. bassiana* isolates in controlling BPH and their impact on plant defense mechanisms remains underexplored. This study aims to address these gaps by evaluating the ability of *B. bassiana* isolates from West Sumatra to influence BPH biology and plant metabolite content, particularly focusing on salicylic acid as a key defense compound.

2. Research Methods

This research was conducted at the Biological Control Laboratory, Insect Bioecology Laboratory and Greenhouse Faculty of Agriculture, Andalas University, Padang from December 2020 to March 2021. The design used in this study was a Completely Randomized Design (CRD), with 5 treatments and 5 replications, 4 isolates of *B. bassiana* and control, soaking using sterile distilled water (Table 1). Data were analyzed using variance, if significantly different, then continued with the 5% LSD test.

Table 1. List of isolates code of *Beauveria bassiana* isolates used in research

Isolates code	Source	Location
BbWS	Leptocorisaoratorius	Duku (Padang pariaman) West Sumatra, Indonesia
Td312	Wheat stems endophyte	Koto Laweh (Tanah Datar), West Sumatra, Indonesia
Pb211	Chili stems endophyte	Parabek (Agam), West Sumatra, Indonesia
BbJg	Corn stems endophyte	Limau Manis (Padang), West Sumatra, Indonesia

2.1 Rearing of test insects

20 pairs of BPH adults collected from rice fields in Kuranji District, Padang City were infested into jars containing rice seeds 14 days after sowing. After 3 days of infestation, all adultss were removed so that the BPH stage was uniform. The BPH used is the third generation pregnant adults.

2.2 Preparation of B. bassiana

The *B. bassiana* fungus isolates used are the collection of the Biological Control Laboratory, Department of Pests and Plant Diseases. The provision of fungi was carried out by rejuvenating the fungus using Sabouraud Dextrose Agar Yeast (SDAY) media and incubated for 21 days.

2.3 B. bassiana suspension making and seed soaking

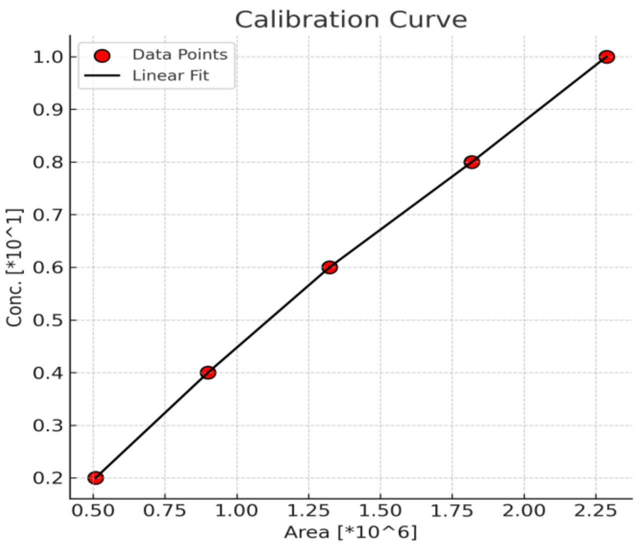
The suspension of the fungus *B. bassiana* is made by adding sterile aquades as much as 10 ml and 3 drops of 80 0.01% Tween solution. The concentration used was 108 conidia/ml. Before being treated rice seeds are first disinfected by soaking the seeds in 70% alcohol, and washed 3 times with sterile aquades for one minute. The seeds that have dried the wind are then soaked using a 20 ml volume *B. bassiana* suspension with a density of 108 conidia/ml for 24 hours in a 250 ml erlenmeyer. Seeds are sown on the upper surface of a tray measuring 30 cm x 21 cm x 5 cm which already contains a mixture of soil and manure in a ratio of 1:1 as much as 2 kg, then moistened with water until the soil condition is macak-macak. Rice seeds that have been aged 21 days after planting (DAP) can be used for experiments. Planting Seedlings using plastic cup containers containing a mixture of soil medium and manure 1:1. The planting medium used is as much as 4/5 of the plastic cups (400 grams). 5 stalks of rice seedlings that have reached the age of 21 day after inoculation are ready to be planted on the media. The water level is always maintained during the test.

2.4 Analysis of salicylic acid content in rice plant stems

2.4.1 Pembuatan kurva standard

The calibration curve was constructed using a quantitative External Standard method with a PDA detector. The analyzed compound had a retention time (RT) of 2.708. The model used for this curve was linear regression, with the method set to ZeroThrough: Not Through.

The calibration curve was developed based on the relationship between the concentration of the standard solution and the peak area generated by the detector. The standard concentrations used in this analysis were 2.000, 4.000, 6.000, 8.000, and 10.000 in units of µg/mL or ppm. The peak area reflects the detector's response to each given standard concentration (Figure 1).



Conc. (Ratio)	Mean Area	Area
2.000	508876.0	508876
4.000	900115.8	900116
6.000	1323422.8	1323423
8.000	1818918.2	1818918
10.000	2287579.7	2287580

Figure 1. Calculation of the standard curve for testing salicylic acid content using the quantitative External Standard method with a PDA detector in HPLC

2.4.2 Sample Preparation and Calculation of Salicylic Acid Concentration (ppm)

Salicylic acid content was analyzed on rice plants' leaves aged 2 weeks after planting (WAP) , by combining 5 plants for each treatment. Salicylic acid extraction was carried out by a modified method Anggraini [15]. A total of 5 grams of rice stalks were crushed with the addition of 10 ml of methanol, the extract was centrifuged at 6,000 rpm for 15 minutes and then the supernatant was taken. Salicylic acid content was analyzed by High Performance Liquid Chromatography (HPLC). The concentration of salicylic acid was measured using a linear distance from the calibration standard containing 0-1.3 mg/50 ml of salicylic acid.

2.5 Effect of B. bassiana Fungus on BPH

Five pregnants female adults were infested into rice seedlings of IR42 variety as many as 3 rice seedlings aged 15 days after inoculation. The bucket was covered with a cylindrical mica cage (d = 24 cm and t = 80 cm) at the top and covered with gauze. The female is released from the cage after 3 days assuming that within that time the female adults has laid a sufficient number of eggs for testing. Observations were made every day by recording the number of first instar nymphs that emerged from each bucket. Plants were then dissected on day 17 to determine the number of unhatched eggs. The process of dissecting rice seedlings and counting the number of BPH eggs was carried out under a stereo microscope. As many as 10 nymphs were taken as samples to see the popuaton of BPH.

2.6 Data analysis

The data obtained are presented in tabular form and the standard definition (SD) is sought. Then the data is analyzed using analysis of variance (STAT 8) and if it is significantly different, it is further tested with a level of 5%

3. Results and discussion

3.1 Salicylic acid content in rice stems

Based on the chromatographic results, it is evident that treatment with *Beauveria bassiana* isolates influences the salicylic acid content in rice plants. The isolate BbWS exhibited the highest peak intensity in the chromatogram, indicating that it is the most effective in enhancing salicylic acid production (Figure 2).

These findings align with the role of salicylic acid as a compound that activates plant defense mechanisms, such as Systemic Acquired Resistance (SAR), which helps plants combat pest infestations like the Brown Planthopper (BPH). Meanwhile, isolates BbJg and Td312 showed moderate peak intensities, suggesting a moderate effect on increasing salicylic acid levels. On the other hand, the isolate Pb211 produced lower peaks, indicating a lesser impact on salicylic acid production. As a comparison, the control (untreated) displayed the lowest peak, reflecting the natural salicylic acid content in rice plants.

The average salicylic acid content across treatments revealed that the BbWS isolate recorded the highest value (47.87 ppm), followed by Td312 (44.70 ppm), Pb211 (44.32 ppm), BbJg (35.21 ppm), and the control (33.79 ppm). These data provide insights into the effectiveness of each isolate in boosting salicylic acid production, which can serve as an indicator of their potential as biological control agents to enhance rice plant resistance against BPH infestations (Table 2).

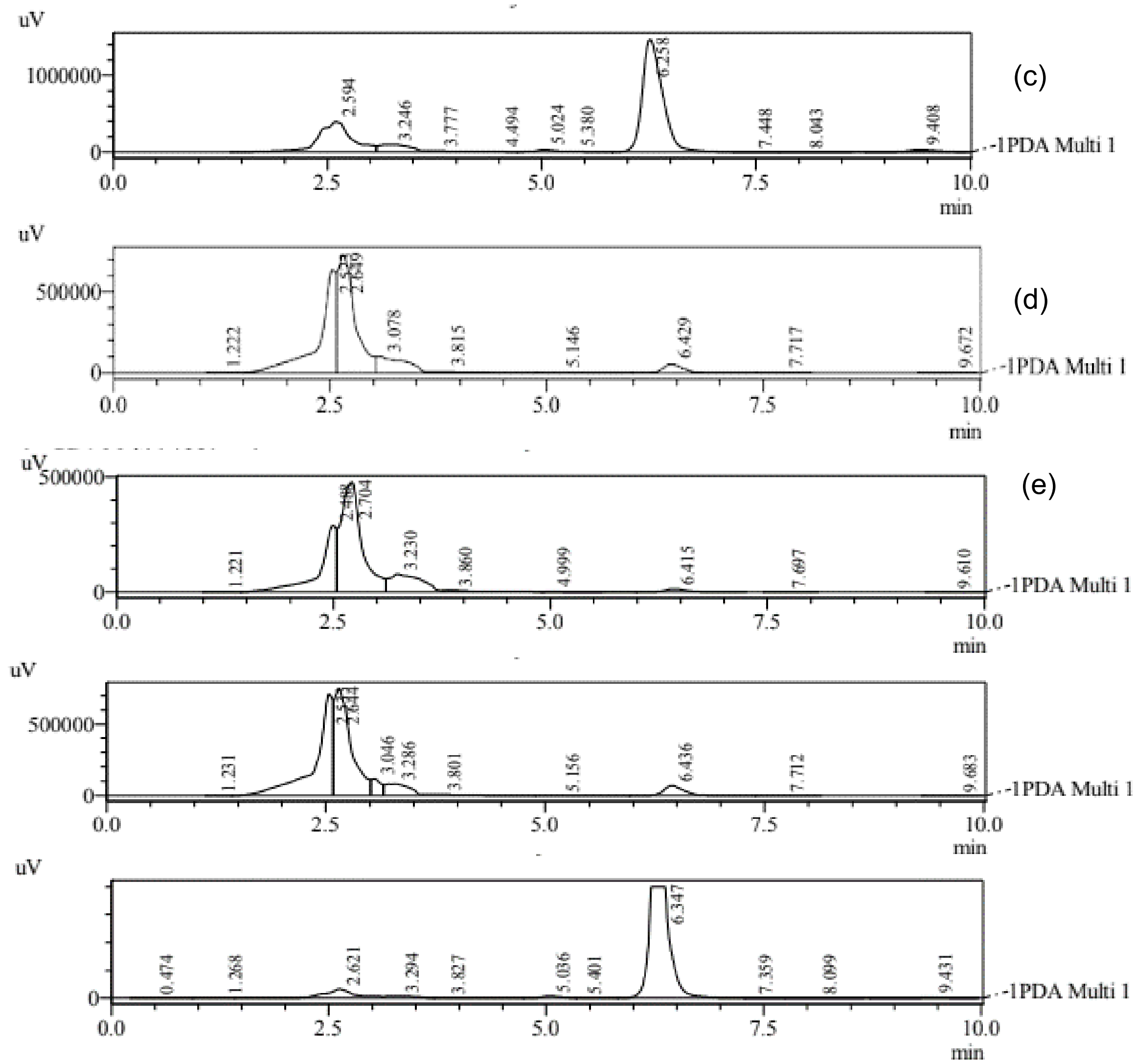


Figure 2. Chromatography of the best treatment isolates, including (a) BbWS, (b) Td312, (c) BbJg, (d) Pb211, and (e) Control.

Table 2. The average salicylic acid content in rice stems with the application of *B. bassiana 2* weeks after planting

Treatments	Salicylic acid content (ppm)
BbWS	47.87 a
Td312	44.70 b
Pb211	44.32 b
BbJg	35.21 c
Kontrol	33.79 d

Numbers followed by different letters in the same column indicate significant differences at the 5% level as determined by the LSD (Least Significant Difference) test.

Salicylic acid (SA) is a phenolic compound that plays a crucial role in plant defense systems. This compound is recognized as one of the plant hormones involved in activating systemic defense mechanisms, such as Systemic Acquired Resistance (SAR), which helps plants combat pathogen and pest attacks [9]. Increased levels of salicylic acid in plant tissues are often associated with the plant's response to biotic stress (such as pest or disease infestations) and abiotic stress (such as drought or extreme temperatures) [6]{7}[9]. The finding that *B. bassiana* can enhance salicylic acid content in rice plants has significant implications for integrated pest management (IPM). The combination of using biological agents like *B. bassiana* and enhancing the plant's natural defense mechanisms through salicylic acid can be an effective and sustainable strategy for controlling pests such as the brown planthopper (BPH). Furthermore, this approach can reduce reliance on chemical pesticides, which often have negative impacts on the environment and human health.

B. bassiana inoculation can affect the physiological and biochemical responses of plants by increasing the production of chemical compounds such as ethylene, chitinase, phytoalexins, alkaloids, jasmonic acid and salicylic acid [18], levels of sucrose and oxalic acid [9]. The content of salicylic acid increased in the treatment with *B. bassiana*. Salicylic acid is effective in increasing plant resistance to pests from piercing-sucking insects and salicylic acid has an important role in plant defense against aphids by producing antibiotics or repellants that can affect aphid infestation patterns on tomato plants [19].

3.2 Effect of *B. bassiana* on BPH

3.2.1 Number of Eggs Laid and Percentage of Eggs Hatch

The results showed that the number of eggs laid by BPH and the number of eggs that hatched were lower in rice plants that were applied to the fungus *B. bassiana* compared to controls. Rice plants applied with BbWS isolate produced the lowest percentage of hatched eggs compared to other isolates (Table 3)

Table 3. Number of eggs laid and percentage of eggs hatched in rice plants treated with *B. bassiana.*

Treatment	Laid eggs of BPH ±SD		Hatched eggs of BPH ±SD		Percentage of eggs that hatch (%)
BbWS	553 ±	3.56 a	157 ±	4.72 a	28.39 a
Td312	553 ±	5.07 a	222 ±	5.94 b	40.14 b
BbJg	572 ±	3.91 ab	227 ±	3.21 b	39.68 b
Pb211	594 ±	3.42 b	261 ±	3.90 b	43.93 b
kontrol	634 ±	7.22 c	340 ±	9.27 c	53.62 c

Numbers followed by different letters in the same column indicate significant differences at the 5% level as determined by the LSD (Least Significant Difference) test.

From the observation, the number of eggs laid by BPH adults and the number that successfully hatched into nymphs treated with the fungus *B. bassiana* was lower than the control. This is presumably because the fungi that develop in plant tissues produce repellent compounds that affect the ability of BPH adults to perch on plants. Budiprakoso reported that plants inoculated with the endophytic fungus *Nigrospora sp* could affect BPH preference, the results showed that fewer BPHs perched when infested after 24 hours and 48 hours [18]. This is supported by research by Mawan that the application of the fungus *Nigrospora sp* in treated seeds treated with eggs laid by BPH was less and the percentage of eggs that hatched was also lower when compared to controls [11].

3.2. Number of Individuals at Each Nymph and Adults Stage BPH

The results showed that rice plants inoculated with the fungus *B. bassiana* affected the number of nymphs living on rice plants. The number of surviving 1-3 instar nymphs in rice plants inoculated with *B. bassiana* was not significantly different from the control. The treatment of the fungus *B. bassiana* significantly affected the survival of 4 and 5 instar BPH nymphs on rice plants (Table 5).

The fungus *B. bassiana* which was applied through soaking rice seeds gave a negative effect on the length of the BPH stadia, and the egg stage until the adults became longer. This is in accordance with the research of Mawan that rice plants treated with the fungus *Nigrospora sp* BPH nymph stadia became longer[11]. The results of Jallow on the larvae of *Helicoverpa armigera* (Hubner) in tomato plants inoculated with the endophytic fungus *Acremonium strictum*, the mortality of the larvae increased[13]. The same effect of the endophytic fungus *Neotyphodium coenophilalum* on the larvae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) on fescue 'KY, resulting in a longer larval stage[23]. This shows that *B. bassiana* has antibiosis properties, namely the ability to produce chemical compounds that can inhibit the growth and development of pests. This property arises because certain endophytic fungi are able to induce plants to produce secondary metabolites such as alkaloids, flavonoids, terpenoids, phenolics, anthobiotics or toxins[24]. Secondary metabolites of *B. bassiana* have the ability to produce substances that act as biological insecticides, one of which is by interfering with the metabolic system of insect pests[25]. The insect control effect is related to the ability of endophytes to produce toxicants[26]

Tabel 5. Number of individuals nymph stage BPH

Treatments	Individuals Nymph and Adults Stage BPH ± SD				
	Instar 1	Instar 2	Instar 3	Intar 4	Instar 5
Control	9.2 ± 0.89 a	9.2 ± 0.84 a	9.0 ± 1.22 a	8.0 ± 0.71 a	7.8 ± 0.45 a
BbWS	9.4 ± 0.55 a	8.0 ± 0.71 a	7.0 ± 1.00 a	6.6 ± 0.55 c	6.4 ± 0.55 b
Pb 211	9.8 ± 0.45 a	8.6 ± 0.55 a	8.0 ± 1.22 a	7.8 ± 1.30 ab	7.4 ± 1.14 a
Td 312	9.6 ± 0.89 a	9.0 ± 1.41 a	7.6 ± 1.52 a	6.8 ± 0.45 bc	6.4 ± 0.55 b
Bbjg	9.6 ± 0.55 a	8.8 ± 1.30 a	8.4 ± 1.40 a	7.6 ± 0.55 abc	7.4 ± 0.55 a

the numbers in the same row followed by the same letters are not significantly different based on the results of the LSD level of 5%.

B. bassiana isolate in BbWS isolate was the best isolate on BPH biological observation parameters. Different strains of isolates are factors that give different responses to pests. in accordance with the statement of Sallem[27] that the effect of fungi that can develop in plant tissues has different responses depending on the fungal isolates used and the host plant. The mechanism of endophytes in increasing plant resistance from pests and herbivores is by producing toxic compounds, compounds that affect insect development processes and decrease appetite Mandyam and Jumpponen and cause qualitative and quantitative changes in plant chemical nutrition, such as changes in carbohydrate and nitrogen content[28], as well as phytosterol composition. Secondary metabolites involved in this defense mechanism usually come from compounds belonging to the alkaloid group. Clay reported the presence of toxic alkaloids produced by leaf endophytes from grasses[29]. This statement is reinforced by Kuldau and Bacon[30] who conducted a study on the effect of endophytic fungi on 45 insect species. From these studies found sufficient evidence for the presence of specific alkaloids such as peramine from the pyrrolopyrozine alkaloid group (often found in grass species) and these compounds act against toxicity in insects.

3.2.3 Percentages of adults emergence

The results showed that rice plants that were applied to *B. bassiana* did not significantly affect the percentage of emerged adults. Of the 10 first instar nymphs that were infested on plants treated with *B. bassiana*, the percentage of adults emergence ranged from 62-70%, not significantly different from the control (Table 4).

Table 4. Percentage of Adults Formed from 10 Iinstar 1 nymphs infested on rice plants

Perlakuan	Perxentages of emerged adults (%)
BbWS	62
Td312	64
Pb211	70
BbJg	70
Kontrol	72

Numbers followed by the same letter indicate no significant difference when tested with the LSD test at a 5% significance level.

The implications of these results indicate that *B. bassiana* does not have a significant effect in suppressing the development of BPH adults, even though it may influence the 4th and 5th instar nymphs. *B. bassiana* can also suppress the development of the brown planthopper (BPH) during the nymphal stages, but it has a lesser impact on the final stage, such as the adult BPH {Formatting Citation}. However, further research is necessary to evaluate the effectiveness of *B. bassiana* on other life stages of the insect or its effects on the developmental duration of BPH and other physiological impacts on BPH adults. Additionally, studies under different environmental conditions are required to gain a more comprehensive understanding. Combining B. bassiana with other control methods may also need to be considered to enhance the overall effectiveness of pest management strategies.

4. Conclusion

The BbWS isolate demonstrated the lowest egg hatch rate (28.39%), outperforming other treatments. While the number of adult BPH remained unaffected, a notable reduction in 4th and 5th instar nymphs was observed. Furthermore, all isolates elevated salicylic acid content in rice stems, peaking at 47.87 ppm. The BbWS isolate emerged as the most effective in curbing BPH and boosting salicylic acid levels. These findings underscore the potential of *B. bassiana* seed treatment as an effective strategy for BPH management and enhancing plant defense mechanisms through increased salicylic acid production..

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