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Colonization of the entomopathogenic fungus *Beauveria bassiana* (Bals.)Vuill. on rice and its impact on nymph mortality and fecundity of brown planthopper (*Nilaparvata lugens* Stål)

Kolonisasi cendawan entomopatogen *Beauveria bassiana* (Bals.) pada tanaman padi dan pengaruhnya terhadap mortalitas nimfa dan keperidian wereng batang coklat (*Nilaparvata lugens* Stål)

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ABSTRACT

Brown planthopper (BPH), *Nilaparvata lugens* Stål, is a significant pest widely recognised for its detrimental impact on rice production. Biological agents, such as the entomopathogenic fungus *Beauveria bassiana* (Bals.) Vuill., can effectively control this pest. *B. bassiana,* known for its endophytic abilities, colonises various plant tissues. This research aims to assess *B. bassiana*'s colonosation capacity on rice plants and its impact on nymph mortality and BPH fecundity. Four isolates of *B. bassiana* (BbWS, Pb211, Td312, and BbJg) were used, each with conidial density of 108 conidia/ml. The application menthod involved soaking rice seeds in the fungus for 24 hours. The results showed that all *B. bassiana* isolates could successfully establish as endophytes within rice plants, colonising all tissue parts, with leaves exhibiting the highest fungal colonisation at 58%. Seed soaking with *B. bassiana* reduced eggs hatching by 23.88% and increased nymph mortality by 40%. The presence of *B. bassiana* in rice tissue also influenced BPH adults fecundity. Notably, the BbWS isolate demonstrated the most significant effectiveness in elevating nymph mortality and reducing BPH fecundity.

Key words: *Beauveria bassiana*, endophytic, entomopathogen, fecundity, mortality, *Nilaparvata lugens*

ABSTRAK

Wereng batang coklat (*Nilaparvata lugens* Stål) sering disebut juga WBC merupakan hama penting yang menyebabkan rendahnya produksi tanaman padi. Pengendalian hama ini dapat dilakukan dengan pemanfaatan agens hayati salah satunya cendawan *Beauveria bassiana* (Bals.) Vuill. Tujuan penelitian untuk mengukur kemampuan kolonisaasi berbagai isolat cendawan *B. bassiana* pada tanaman padi dan pengaruhnya terhadap mortalitas nimfa dan keperidian WBC. Penelitian disusun dalam rancangan acak lengkap (RAL) dengan 5 perlakuan dan 5 ulangan. Perlakuan terdiri atas empat isolat *B. bassiana*, yaitu BbWS, Pb211, Td312, BbJg, dan kontrol. Konsentrasi *B. bassiana* yang digunakan adalah 10^8 konidia/ml. Data yang didapat diolah dengan menggunakan sidik ragam dan dilanjutkan dengan uji LSD taraf nyata 5%. Hasil penelitian menunjukkan bahwa semua isolat cendawan *B. bassiana* mampu mengkolonisasi semua bagian tanaman padi dan tingkat kolonisasi tertinggi terdapat pada daun, yaitu 58%. Kolonisasi *B. bassiana* dipengaruhi oleh jenis isolat. Keberadaan cendawan *B. bassiana* di dalam jaringan tanaman padi dapat menurunkan persentase telur WBC yang menetas sebesar 62,8%, dan meningkatkan mortalitas nimfa 46%. Keberadaan

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cendawan *B. bassiana* di dalam jaringan padi juga mempengaruhi keperidian imago WBC. Isolat BbWS merupakan isolat yang paling efektif dalam meningkatkan mortalitas nimfa dan menurunkan keperidian WBC.

Kata kunci: *Beauveria bassiana*, endofit, entomopatogen, keperidian, mortalitas, *Nilaparvata lugens*

INTRODUCTION

Brown planthopper (BPH) or *Nilaparvata lugens* Stål (Hemiptera: Delphacidae) is a major pest of rice plants, inflicting damage across all growth stages by feeding on sap from plant cells, ultimately leading to desiccation. Furthermore, its impact extends through the transmission of three detrimental viruses: rice dwarf virus, rice grassy stunt virus type 1, and type 2. Previous studies have confirmed that severe infestations can cause hopperburn and result in crop failure (Baehaki 2011; Harini et al. 2013; Syahrawati et al. 2019).

Various technologies have been identified to control BPH, including trap lamps (Afrizal 2021), crop rotation with non-host plants (Kritani 1979), resistant varieties (Ikeda & Vanghan 2004), and synthetic insecticides (Trisnaningsih 2016). However, the inappropriate use of synthetic insecticides leads to adverse outcomes, such as pest resistance and resurgence.

An environmentally friendly pest control method that avoids pesticide resistance is the utilisation of biological agents such as the fungus *B. bassiana* (Koswanudin 2014). *B. bassiana*, an entomopathogenic fungus with broad host range, has been employed as a biological control agent against various insect pests, including *Crocidolomia pavonana* (Fabricius) (Trizelia & Nurdin 2010), *Spodoptera exigua* Hubner (Razak et al. 2016), *Spodoptera litura* Fabricius (Trizelia et al. 2016), *Nezara viridula* (Linnaeus) (Siahaan et al. 2021), *Eurydema pulchrum* (Westwood) (Trizelia et al. 2019) and *N. lugens* (Hendra et al. 2022a).

In addition to its role as an entomopathogen, *B. bassiana* exhibits endophytic behavior in various plant species, colonising plant tissues. Its presence within plants adversely affects insects, particularly plant pests, thereby enhancing plant resistance against pest attacks (Vega 2008; Jia et al. 2013; Trizelia et al. 2020; Flawerina 2021). Guesmi-Jouini et al. (2014) observed that the *B. bassiana* strain RSB effectively colonises broccoli leaves, suppressing the thrips population, *Frankliniella occidentalis* (Pergande) while enhancing plant resistance to pests. According to Batool et al. (2020), *B. bassiana* can thrive endophytically in corn plants.

The presence of the fungus in corn plants can lead to an 85% reduction in *Ostrinia furnacalis* (Guenee) larvae, decreasing both the number and length of larval tunnels. Larval mortality is attributed to secondary metabolites such as proline and polyphenol oxidase present in corn plants, which are toxic to larvae. Additionally, Trizelia et al. (2020) reported that *B. bassiana*, when living endophytically in chili plants, effectively suppressed the *Myzus persicae* (Sulzer) population. Shaalan et al. (2021) noted a detrimental impact on *Aphis gossypii* Glover population following the application of this fungus to cucumber plants via seed soaking. The induction of plant resistance occurs due to changes in the morphological and physiological plant characteristics caused by endophytic fungus, resulting in the production of various toxic compounds, antifeedants, and secondary metabolites (Gao et al. 2010; McCormick et al. 2016). Furthermore, *B. bassiana* has been shown to enhance secondary metabolite contents, such as salicylic acid, and oxalic acid, while reducing primary metabolites like sucrose in plant stems (Hendra 2022).

Previous studi by Hendra et al. (2022b) reported on the colonisation ability of *B. bassiana* in rice plants and its influence on the oviposition preference of adult BPH. It was found that the number of eggs laid by adult BPH and the percentage of hatching were lower on rice plants treated with *B. bassiana*. However, its impact on nymph mortality and the fecundity of BPH remains unreported. Therefore, this research aims to explore the colonisation ability of various isolates of the entomopathogenic fungus *B. bassiana* on rice plants, specifically focusing on its effect on nymph mortality and the fecundity of BPH.

MATERIALS AND METHODS

Preparation of *B. bassiana*

B. bassiana isolate used in this study originated from the Biological Control Laboratory, Department of Plant Pests and Diseases, Andalas University, as shown in Table 1. The isolate was cultured on sabouraud dextrose agar plus yeast extract (SDAY) medium and incubated for 21 days. A suspension was prepared by combining 10 ml of sterile distilled water and 0.01% Tween 80. Subsequently, conidia were collected using a soft brush and transferred into a reaction tube, followed by homogenisation using a vortex. The concentration of fungal conidia utilised stood at 10^8 conidia/ml, with density determined under a binocular microscope using a haemocytometer.

Rearing of BPH

Brown planthoppers are reared using the IR 42 rice variety 7–15 days after planting. Nymphs and adults of brown planthoppers used as the test insects were collected from rice fields in Kuranji District, Padang City, West Sumatra, using an aspirator. Brown planthoppers from the field are then placed into a plastic jar with a diameter of 27.5 cm and a height of 27 cm. This jar already contains vegetative-phase rice plants as a food source and a place for egg-laying. Every 2 days, the food plants are replaced new ones. The rearing of BPH continued until the 3rd generation.

Colonization test of *B. bassiana* on rice plants

Before being treated with *B. bassiana*, the rice seeds are soaked in 70% alcohol for one minute, followed by three rinsed in sterile distilled water, each for one minute. The rice seeds were air-dried for approximately 30 minutes in a laminar flow and then soaked in the *B. bassiana* suspension for 24 hours. The concentration of *B. bassiana* conidia used is 108 conidia/ml. The treated seeds were then ready for sowing, which was carried out in trays measuring 30 cm × 21 cm × 5 cm filled with a mixture of soil and compost (2:1). Fifteen-day-old rice seedlings were transplanted into plastic cups (upper diameter = 15 cm, base = 10 cm, height = 12 cm), containing a mixture of soil and compost (2:1). The colonisation of *B. bassiana* on rice plants was tested using a specific oatmeal agar (OMA) medium and observed at 30 and 60 days after inoculation (DAI). The research was arranged in a completely randomised design (CRD) with 5 treatments and 5 replications, requiring 25 samples for each treatment. Furthermore, one rice plant was taken from a replication of each treatment. Plant parts are sterilised using 70% alcohol, followed by 3% NaOCl, washed three times with sterile distilled water for 1 minute, and air-dried in a laminar flow hood. After drying, plant parts were cultured on OMA medium in petri dishes and incubated for 10 days. The parts of the rice plant colonised by *B. bassiana* were confirmed by the presence of hyphae or mycelia growing on the tips of the root, stem, and leaf tissues. The percentage of *B. bassiana* colonisation was calculated using the formula:

$$\mathrm{PK} = \frac{\sum n}{N} \times 100\%, \text{ where}$$

PK: percentage of colonization; ∑n: number of plant parts colonised by *B. bassiana*; N: total number of plant parts observed.

Effect of *B. bassiana* on nymph mortality and adult fecundity of BPH

Rice seeds were immersed in *B. bassiana* suspension for 24 hours at a concentration of 10^8 conidia/ml. Subsequently, treated seeds were sown in trays containing a soil and manure mixture in a 2:1 ratio. This was followed by the transplanting of 15-day-old rice seedlings (10 stems) into plastic containers with a diameter of 15 cm and height of 12 cm, filled with a soil and compost mixture in a 2:1 ratio as the planting medium. BPH infestation was conducted one day after transplanting the test

Table 1. Sources of *Beauveria bassiana* fungus isolates

Isolate code	Origin	Location
BbWS	*Leptocorisa oratorius*	Duku (Padang pariaman), West Sumatra
Pb211	Chili stem endophytes	Parabek (Agam), West Sumatra
Td312	Wheat stem endophytes	Koto Laweh (Tanah Datar), West Sumatra
BbJg	Corn stem endophytes	Limau Manis (Padang), West Sumatra

plants. A total of five gravid adult were introduced into each plastic container planted with rice. After 24 hours, the gravid adult were removed, and the rice was maintained for 16 days until the appearance of the first instar nymphs. On the 17th day, the rice plants were dissected to count non-hatching egg, and 10 first-instar nymphs were transferred to new containers, which were observed daily until reaching adulthood. The number of adult successfully developed depended on the initial number of nymphs. Observation of adult fecundity was conducted by pairing one male obtained from the breeding results and one female adult. Both adult were placed in the same container, each containing one rice plant, which was replaced daily until female adult died. The stem of the rice plant was dissected every day to observe the daily egg laying by the BPH adult. The number of female adult depended on the number of nymphs that successfully developed.

Data analysis

The data obtained from observations were analysed using analysis of variance (ANOVA) to determine the treatment effect, followed by the least significant difference (LSD) test at a significance level of 5% using Statistix 8 software.

RESULTS

Colonisation of *B. bassiana* on rice plants

The colonisation test showed that all tested *B. bassiana* isolates could live endophytically on rice plants, colonising the root, stem, and leaf tissues. No presence of *B. bassiana* was not found in any control plants. At 30 days after inoculation (DAI), Pb211 and BbWS isolates exhibited higher colonisation abilities compared to the other two isolates. Table 2 illustrates a trend where colonisation ability tends to decrease with the increasing age of the plants.

Table 2 shows that the colonisation of the fungus *B. bassiana* was higher in leaves compared to stems and roots at 30 and 60 DAI, respectively. The percentage of colonisation by the entomopathogenic fungus *B. bassiana* on rice plants was lowest in the roots. Isolate BbWS displayed the highest average colonisation percentage in all plant tissues, while BbJg exhibited the lowest ability at 7.5% (Figure 1).

Effect of *B. bassiana* on nymph mortality and Fecundity of BPH

Percentage of hatched eggs. The number of eggs laid by a single female adult of BPH within 24 hours after infestation on rice plants treated with *B. bassiana* through seed soaking was lower than the control. BPH adults laid around 24.82–37.22% fewer eggs. Application of *B. bassiana* on rice plants significantly reduced the the percentage of hatched eggs. In the control, hatched eggs reached 82.5%, whereas in the fungal treatment, it ranged from 62.8–76.7%, showing a significant reduction of 7.03–23.87%. Table 3 indicates that isolate BbWS was the most effective in reducing the number of laid and hatched BPH eggs, and their percentage.

Table 2. Percentage of *Beauveria bassiana* colonization on rice plants

Plant age (DAI)	Treatment	*B. bassiana* colonization percentage (%)*		
		Root ± SD	Stem ± SD	Leaf ± SD
30	BbWS	20.0 ± 1.25 ab	48.0 ± 1.80 a	53.3 ± 1.59 a
	Pb211	21.0 ± 1.16 a	32.0 ± 1.55 a	58.0 ± 1.62 a
	Td312	12.0 ± 0.74 ab	13.0 ± 0.72 b	26.0 ± 1.11 b
	BbJg	6.0 ± 0.62 bc	14.6 ± 0.74 b	29.3 ± 1.06 b
	Control	0.0 ± 0.00 c	0.0 ± 0.00 c	0.0 ± 0.00 c
60	BbWS	0.0 ± 0.00 a	0.0 ± 0.00 a	0.5 ± 0.46 ab
	Pb211	0.0 ± 0.00 a	0.0 ± 0.00 a	0.2 ± 0.35 abc
	Td312	0.2 ± 0.35 a	0.1 ± 0.26 a	0.6 ± 0.49 a
	BbJg	0.1 ± 0.26 a	0.0 ± 0.00 a	0.1 ± 0.26 bc
	Control	0.0 ± 0.00 a	0.0 ± 0.00 a	0.0 ± 0.00 c

*Values in the same column followed by the same letter are not significantly different based on the LSD test at a 5% level.

Nymph mortality of BPH. Application of *B. bassiana* on rice plants through seed soaking increased the mortality of BPH nymphs and decreased the percentage of adult formation. All *B. bassiana* isolates increased the mortality of BPH nymphs. Isolate BbWS exhibited the highest result at 50%, while isolate Pb211 showed lower nymph mortality of only 42% ($P < 0.05$), as shown in Table 4.

Table 4 shows that the percentage of adult formation from BPH nymphs feeding on *B. bassiana*-inoculated rice plants through seed soaking was lower compared to the control. High nymph mortality significantly affected the percentage of formed BPH adult, showing 70% in the control and 46–56% in the *B. bassiana* treatment. This indicated a decrease of approximately 20–34.28% in the percentage of adults formed from BPH nymphs. Isolate BbWS proved the most effective in reducing the percentage of formed adult.

BPH fecundity. This research showed that *B. bassiana* inoculation into rice plants through seed soaking reduced the fecundity of BPH adults. In the control, one BPH adult could lay a total of 125.6 eggs, while treatment with isolate BbWS resulted in 92.8 eggs (Figure 2).

Daily egg laying by BPH adult. *B. bassiana* inoculation through seed soaking affected the daily egg-laying quantity by BPH adults. One BPH adult laid the highest daily number of eggs, approximately 10,3 eggs, while 15,8 eggs were obtained in the control. Figure 3 illustrates that the lowest daily egg laying by BPH occurred in rice plants inoculated with *B. bassiana* isolate BbWS. In the control, egg laying by female adults began

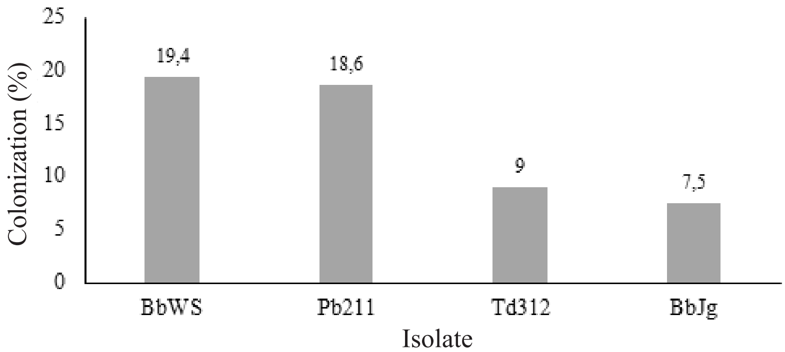

Figure 1. Average colonization ability of four *Beauveria bassiana* isolates on rice plant tissues.

Table 3. Percentage of hatched eggs 24 hours after BPH adult infestation

Treatment	Laid eggs ± SD	Hatched eggs ± SD	Percentage of hatched eggs (%)
BbWS	17.2 ± 3.1 c	10.8 ± 0.8 c	62.8 b
Pb211	20.6 ± 1.5 a	13.8 ± 1.3 b	67.0 ab
Td312	18.0 ± 1.4 bc	13.8 ± 2.9 b	76.7 ab
BbJg	20.4 ± 2.3 b	14.6 ± 0.9 b	71.6 ab
Control	27.4 ± 1.9 a	22.6 ± 2.3 a	82.5 a

*Values in the same column followed by the same letter are not significantly different based on the LSD test at a 5% level.

Table 4. BPH nymph mortality on rice plants inoculated with *B. bassiana* via seed soaking

Treatment	Nymph mortality (%) ± SD	Percentage of adult formed (%) ± SD
BbWS	50.0 ± 0.0 a	46.0 ± 0.0 c
Pb211	42.0 ± 4.5 b	52.0 ± 8.4 bc
Td312	44.0 ± 5.5 ab	56.0 ± 5.5 b
BbJg	46.0 ± 0.0 ab	54.0 ± 5.5 bc
Control	30.0 ± 8.8 c	70.0 ± 8.9 a

*Values in the same column followed by the same letter are not significantly different based on the LSD test at a 5% level.

on the second day after pairing, while in rice plants treated with *B. bassiana*, it occurred three days after pairing. The treatment slowed down the egg-laying process and reduced the number of eggs laid.

DISCUSSION

B. bassiana, an entomopathogenic fungus, demonstrates the ability to exist endophytically in plants without inducing disease symptoms. In this study, all tested isolates of *B. bassiana* successfully colonised root, stem, and leaf tissues, indicating a systemic nature where the fungus could grow and translocate within all plant tissues. The distribution of the fungus within rice plant tissues was non-uniform, attributed to passive transmission facilitated by water through the plant's transpiration process. According to Akbar et al. (2022), *B. bassiana* may infiltrate seeds during imbibition and diffuse into seedlings via seed soaking.

The colonisation capacity of *B. bassiana* on rice plants varied based on the isolate and plant part, with the most pronounced impact observed in treatments involving isolate BbWS at 30 DAI. Leaf colonisation by *B. bassiana* was higher than on stems and roots, exhibiting a declining trend as the plant matured. Zheng et al. (2021) reported that the ability of *B. bassiana* to colonise plant tissues was influenced by the fungal strain. Akutse et al. (2013) highlighted that *B. bassiana* colonization of plants was affected by the host plant, specific plant part, method of application, and plant age (Posada & Vega 2005). Parsa et al. (2013) reported that

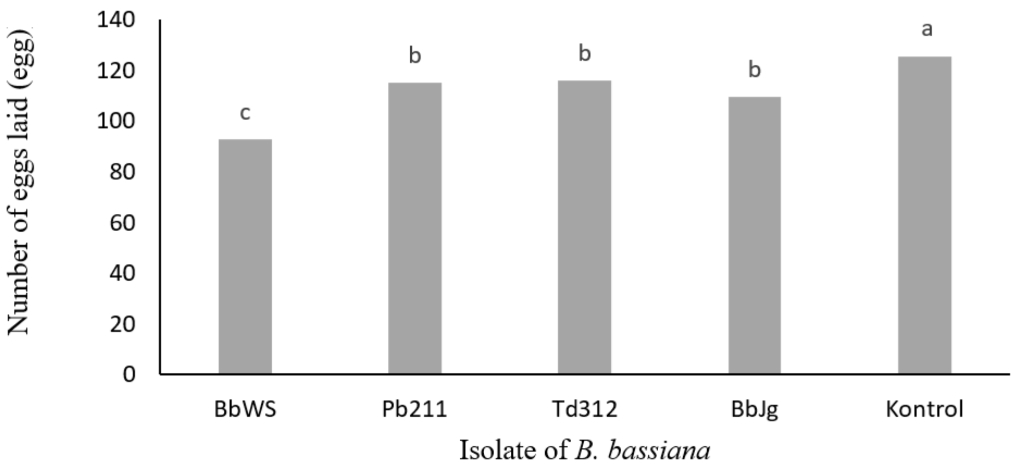

Figure 2. Average number of eggs laid by one BPH female adult during its lifetime.

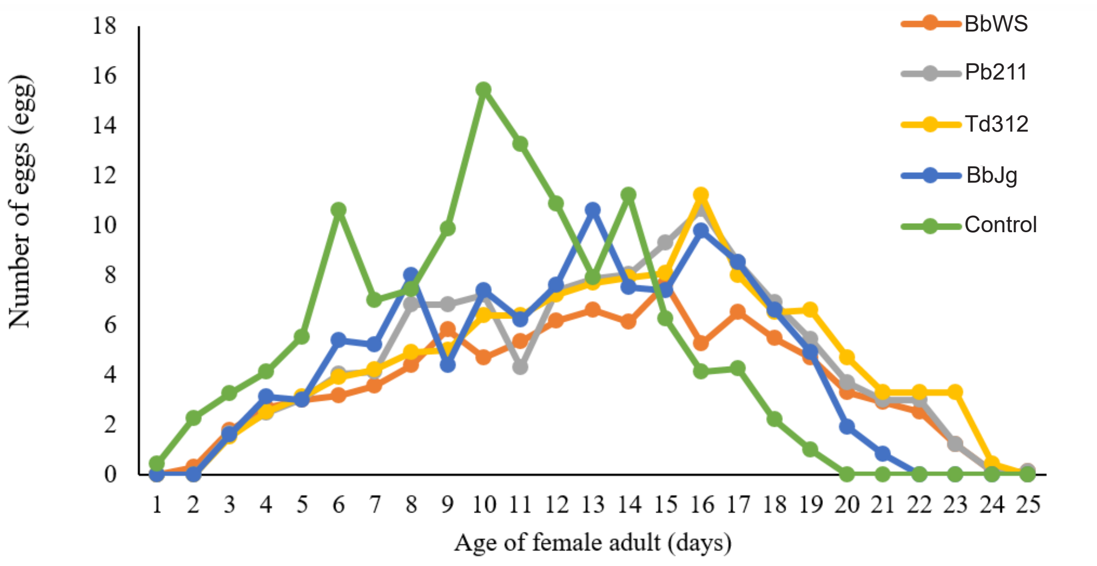

Figure 3. Average number of eggs laid by one BPH female adult.

the establishment of this fungus as an endophyte in plant tissues was affected by multiple factors, including the physiology of the fungus, the plant itself, and differences in fungal isolate strains (Artunes et al. 2008). Based on the study by Jia et al. (2013), *B. bassiana* colonisation through soil drenching on rice plants sustained for only 15 DAI on leaf tissues. The percentage of *B. bassiana* colonisation decreased with increasing plant age, potentially due to competition with the host plant or the faster growth of plants compared to the development rate of *B. bassiana*.

The number of eggs laid and the hatching percentage of BPH significantly decreased on rice plants inoculated with *B. bassiana.* This phenomenon occurred because the fungus growing within plant tissues produced repellent compounds, affecting the attraction of BPH to lay eggs and the hatching rate. Moreover, this was evident as adults did not correlate with plants previously inoculated with *B. bassiana*. Budiprakoso (2010) reported that rice plants inoculated with *Nigrospora* sp. endophyte influenced BPH preferences, resulting in fewer landing on fungus-treated rice plants after 24 and 48 hours of infestation. BPH landings on rice plants indirectly influenced the quantity of eggs laid. According to Mawan et al. (2013), rice seeds treated with *Nigrospora* sp. reduced the number of eggs laid by BPH and decreased the hatching percentage.

BPH nymphs reared on rice plants treated with *B. bassiana* seed soaking exhibited lower mortality compared to direct spraying. Abdullah et al. (2020) noted that *B. bassiana* applied through seed soaking induced mortality in *Nephotettix virescens* (Distant). This treatment also reduced BPH adult emergence, impacting the quantity of eggs laid, laying time, and the fecundity. Furthermore, the pre-oviposition period and the age of females when laying eggs for the first time can affect reproductive success and organism fitness (Stearns 1992).

The mechanisms of endophytic fungi in protecting plants can occur due to changes in chemical nutrition, qualitatively and quantitatively, such as variation in carbohydrate and nitrogen content, including phytosterol composition (Schulz & Boyle 2005). Inoculation of *B. bassiana* affected 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 (Vlot et al. 2009). Hendra (2022) reported that *B. bassiana* inoculation via seed soaking increased the content of salicylic acid and oxalic acid while decreasing sucrose content in rice plant stems. Salicylic acid (Zhou et al. 2009), sucrose, and oxalic acid (Rahmini 2012) played important roles in BPH survival. Salicylic acid defends against leafhoppers by producing antibiotics or repellents capable of affecting their infestation patterns on tomato plants (Moran & Thompson 2001). Raad (2016) reported that *Arabidopsis thaliana* plants colonised by *B. bassiana* strains FRh2 and BG11 could activate the salicylic acid and jasmonic acid signaling pathways. Oxalic acid functions as an inhibitor of fluid sucking by BPH in phloem, while sucrose acts as a feeding stimulant. Soluble oxalic acid is toxic to insects, and the high level absorbed into the herbivore digestive system can cause death (Korth et al. 2006). According to Yoshihara et al. (1980), oxalic acid is an pivotal in controlling the rice plant resistance to BPH.

The ability of BbWS isolate exhibited greater efficacy compared to other *B. bassiana* isolates in increasing BPH mortality and reducing fecundity. The BbWS isolate, obtained from the stink bug in the same order as BPH (Hemiptera) or from *Leptocorisa oratorius* (Fabricius) in the same order as the source isolate used, was noted for its efficacy. Flawerina (2021) reported that the BbWS isolate displayed the highest virulence against eggs and instar 2 nymphs of *Bemisia tabaci* (Gennadius). Isolates *B. bassiana* originating from the same order as the test insect showed higher virulence compared to isolates from plants (endophytes).

CONCLUSION

This research showed the successful colonization of all parts of rice plants by the fungus *B. bassiana*, with the highest colonisation rate observed in the leaves. The colonisation ability of *B. bassiana* in rice plants was influenced by the origin of the isolate, with the highest result obtained in plants treated with the BbWS isolate. The presence of this fungus in rice plant tissues led to a reduction in the hatching percentage of BPH eggs by 7.03–23.87% and an increase in BPH nymphs mortality. Seed soaking also impacted the fecundity of BPH adults, where BbWS proved to be the most effective isolate in increasing nymph mortality and reducing BPH adult fecundity.

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