

Ability of commercial *Beauveria bassiana* to suppress the brown planthopper attack by inducing resistance into different rice variety

ANTONI LISWANDI, MY SYAHRAWATI*, HASMIANDY HAMID, ARNETI, JAMES RINALDI

Department of Plant Protection, Faculty of Agriculture, Universitas Andalas. Jl. Unand, Limau Manis, Padang 25163, West Sumatra, Indonesia.

Tel./fax.: +62-751-72701, *email: mysyahrawati@agr.unand.ac.id

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Abstract. Liswandi A, Syahrawati M, Hamid H, Arneti, Rinaldi J. 2023. Ability of commercial *Beauveria bassiana* to suppress the brown planthopper attack by inducing resistance into different rice variety. *Biodiversitas* 24: 6704-6710. *Beauveria bassiana* (Bb) is one of the biological agents for controlling the Brown Planthopper (BPH), *Nilaparvata lugens*, which is generally applied directly to BPH. Nowadays, it is also used to induce resistance in rice plants; this ability has never been tested on commercial *B. bassiana*. The research aimed to study the effect of different rice varieties and induced resistance of commercial Bb on BPH and rice plants. The study used a Factorial Completely Randomized Design (FCRD). The first factor was the difference in rice varieties (IR 42, Cisokan, Batang Piaman), and the second factor was the soaking of the seeds and roots by commercial Bb (with commercial Bb, without commercial Bb). The results showed no interaction between rice variety and commercial Bb against BPH and rice plants. Commercial Bb reduced the attack intensity of BPH (13.31%); meanwhile, different rice varieties influenced BPH mortality, colonization, and plant growth. The Cisokan variety was better at suppressing the BPH population (45%) and suitable for colonization by commercial Bb. The IR 42 variety was more responsive than other varieties in accepting the presence of commercial Bb (16%).

Keywords: Colonization, *Nilaparvata lugens*, resistance induction, rice variety, soaking

Abbreviations: Bb: *Beauveria bassiana*, BPH: brown planthopper

INTRODUCTION

Brown Planthopper (BPH), *Nilaparvata lugens* Stal, Hemiptera: Delphacidae is a monophagous insect that is harmful to most rice-producing countries in Asia, including Indonesia (Syahrawati et al. 2019; Iamba and Dono 2021). This is because BPH saps the fluid produced by rice plants, which causes them to wilt, stunt, turn yellow, and eventually burn (Bao and Zhang 2019). In addition, BPH is a vector for viruses that cause grass dwarf diseases (Suprihanto et al. 2015). BPH attacks in Indonesia fluctuated with a decreasing trend during 2018-2022 (21,781.8 ha, 10,317.1 ha, 20,425 ha, 9,399.7 ha, and 6,068.21 ha, respectively). Meanwhile, in West Sumatra Province, it also fluctuated with an increasing trend (440.45 ha, 628.75 ha, 1,103.56 ha, 281.85 ha, and 284.56 ha, respectively) (Suwarman et al. 2023). It is estimated that it will continue to increase in 2024.

Intensive and excessive application of insecticides has encouraged the emergence of BPH resistance (Datta and Banik 2021; Iamba and Dono 2021; Utari et al. 2023). Various environmentally friendly controls have been recommended to reduce the BPH population, such as ecological engineering (Horgan et al. 2016) and the use of natural enemies such as predators (Syahrawati et al. 2015; Nasral et al. 2020; Syahrawati et al. 2021a; Syahrawati et al. 2021b; Siregar et al. 2023; Utari et al. 2023), parasitoids (Minarni et al. 2018; Abdilah and Susilo 2020), and insect pathogens, such as *Beauveria bassiana* (Hendra et al. 2022;

Kurniawati et al. 2021; Mascarin and Jaronski 2016; Trizelia et al. 2023).

Beauveria bassiana is an insect pathogen widely studied and declared safe and effective as a biocontrol agent. It can control plant pests directly, such as *Helicoverpa armigera* (Qayyum et al. 2015) and *Rhynchophorus ferrugineus* (Ahmed and Freed 2021). *Beauveria bassiana* can suppress *Spodoptera litura* larvae up to 95% (Trizelia et al. 2017; Afandhi et al. 2020) and *Nezara viridula* up to 66.67% (Permadi et al. 2019), reaching 100% 5 days after application (Siahaan et al. 2021). *Beauveria bassiana* reduced 48% of *Lycorma delicatula* nymphs formed after 14 days of application (Hajek et al. 2023). *Beauveria bassiana* can also control BPH, with mortality reaching 85-95% at a density of 10^8 - 10^9 conidia (Ihsan et al. 2023). Nowadays, it can colonize plant tissues endophytically (Afiyanti et al. 2019; Barra-Bucarei et al. 2020; Faddilah et al. 2022), which can induce the plant resistance to the pests. Inducing the plant resistance by *B. bassiana* was reported in rice plants against BPH by Hendra (2021), the number of eggs laid had been fewer, the percentage of egg hatching had decreased, and the stadia length became longer.

Furthermore, the presence of *B. bassiana* in rice plants has changed the biochemical composition of plants, such as increasing salicylic acid and oxalic acid and reducing sucrose (Hendra 2022). Salicylic acid effectively increases plant resistance to disturbances from insect pests (Schweiger et al. 2014) and pathogens (Rivas-Franco et al.

2020). Oxalic acid is a toxic compound to insects, while sucrose can be a stimulant to increase insect appetite (Hendra 2022). Currently, *B. bassiana* is widely packaged and produced as a commercial biopesticide to control various plant pests (Dannon et al. 2020; Sopialena et al. 2022; Chavez et al. 2023). However, there have been no reports regarding the ability of commercial *B. bassiana* in Indonesia to control BPH through resistance induction. This research aimed to determine the ability of one commercial *B. bassiana* to induce resistance of different rice varieties to control BPH populations when applied through soaking seeds and roots.

MATERIALS AND METHODS

This research was carried out from April to July 2022 at the Insect Bioecology Laboratory, Plant Protection Department, Faculty of Agriculture, Universitas Andalas, Indonesia. There were two stages of testing: the effectiveness of a commercial *B. bassiana* against BPH and the inducing resistance into different rice varieties using a commercial *B. bassiana*.

Providing of BPH

The BPH used was the 18th generation from the collection of Insect Bioecology laboratory, the initial generation of which was collected from rice fields in Pauh District, Padang City, West Sumatra, Indonesia. BPH was taken to the laboratory to be reared in the IR-42 variety in rearing boxes measuring 40x60x60 cm. A total of 50g of rice seeds were soaked in a seedling box (30x20x8 cm) using distilled water for 24 hours, air-dried for 60 minutes, then sprinkled evenly in the seedling box containing a mixture of water, soil, and manure to a height of 2 cm. BPH was infested on the rice seedlings 7 days after sowing (Figure 1).

Testing the effectiveness of the commercial *B. bassiana* against BPH

The study was arranged in a completely randomized design (CRD) by application of commercial *B. bassiana* (Natural BVR) 1.5 g/L directly to BPH with a conidia density of 10¹⁰ conidia/mL. Control treatment was prepared using only distilled water; each had 10 replications.

A total of 20 plastic buckets (15x10x12 cm) were prepared with planting media consisting of soil and manure with a volume ratio of 3:1. The mixture of soil and manure was autoclaved for 1 hour at 121°C and then left for 24 hours. In each bucket, 10 seedlings of the IR-42 variety were planted, and 10 individual 3rd instar nymphs were transported. One day after infestation, a commercial *B. bassiana* suspension was sprayed to wet the nymphs in each bucket. Then, observations were made every day until 10 days after the infestation. The effectiveness of commercial *B. bassiana* was determined by calculating BPH mortality using the following formula (Hadi et al. 2023):

$$\text{Mortality} = \frac{\text{Number of dead BPH}}{\text{Number of infested BPH}} \times 100 \quad (1)$$

If mortality in control was between 5-20%, data correction was carried out using the Abbot formula (Campbell and Miller 2017):

$$\text{Mortality} = \frac{\text{mortality in treatment} - \text{mortality in control}}{100 - \text{mortality in control}} \times 100 \quad (2)$$

Inducing resistance on different rice varieties with commercial *B. bassiana*

This study used a Factorial Completely Randomized Design (CRD) consisting of two factors: rice variety differences (IR 42, Cisokan, Batang Piaman) and seed and root soaking (with commercial *B. bassiana*, without *B. bassiana*). Description of rice varieties used are as follows (Table 1).

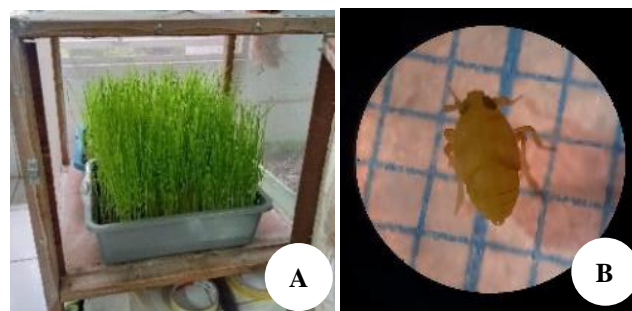


Figure 1. Preparation of tested BPH: A. IR 42 for rearing BPH, B. 3rd instar BPH nymphs used for testing

Table 1. Characteristics of rice varieties of IR-42, Cisokan, and Batang Piaman that induced resistance using commercial *Beauveria bassiana* during the study

Description	IR-42	Cisokan	Batang Piaman
Original parent	IR2042/CR94-13	PB36/Pelita I-1	IR25393-57/RD203// IR27316-96///SPLR7735/SPLR2792
Age (day)	135-145	110-120	100-117
Height (cm)	90-105	90-100	105-117
Tiller	20-25	20-25	14-19
Amylose levels (%)	27	26	28
Protein content	8.9	6.9	10.4
Glycemic index (%)	58	34	71

Primary Source: (Suprihatno et al. 2015)

The commercial *B. bassiana* concentration was 1.5 g/L with 10^{10} conidia/mL (as recommended). Seed soaking was carried out in a combination of 0.15 g commercial *B. bassiana* and 100 mL distilled water. In comparison, root soaking was carried out in a combination of 0.375 g commercial *B. bassiana* and 250 mL distilled water (Figure 2).

A total of 20 g of seeds from each variety was soaked in 70% alcohol for one minute. The seeds were then washed using distilled water thrice for one minute and air-dried for 60 minutes at room temperature. Then, the seeds were soaked using a commercial *B. bassiana* suspension for 24 hours in an Erlenmeyer glass with a volume of 250 mL. Seeds treated are air-dried for 60 minutes, then sown in a seedbed (30x21x5 cm) according to the treatment.

The seedlings 14 days after sowing were removed from the seedbed and then dipped in distilled water to remove the remaining soil attached to the roots. After air-drying for 2 minutes, the seeds were soaked using a commercial *B. bassiana* suspension for 15 minutes. Next, six seedlings were planted into the plastic buckets provided according to the treatment. Three days after planting, eight 3rd instar BPH nymphs were infested into a plastic bucket, covered with a cylindrical mica cage (d = 24 cm and t = 80 cm) at the top, covered with gauze.

Variables Observed

Mortality of BPH (%)

BPH mortality was observed 7 days after BPH infestation, equal to 24 days after seed soaking or 10 days after root soaking. The mortality of BPH was determined by using the formula (1). If mortality in control was between 5-20%, data correction was done using the formula (2).

Attack intensity (%)

Attack intensity was observed 7 days after BPH infestation, equal to 24 days after seed soaking or 10 days after root soaking. The attack intensity is calculated using the formula (Supartha et al. 2021):

$$I = \frac{\sum(n_i \times v_i)}{Z \times N} \times 100\%$$

Note: I = attack intensity, n_i = number of rice plants attacked in i score, v_i = scale for each part of the plant

observed in i score, Z = highest score, N = number of rice plants observed.

Observation of the attack intensity of BPH uses the scoring presented in Table 2.

Assessment of stem colonization by the commercial *B. bassiana*

The ability of the commercial *B. bassiana* to colonize rice stems was observed 24 days after seed soaking or 7 days after root soaking. The rice stems not infested with BPH are used for colonization assessment. Stems are cut into small pieces with a size of ± 1 cm. The pieces were sterilized with 70% alcohol for 1 minute, 3% NaOCL for 1 minute, and distilled water three times. A total of 5 pieces of rice stalks were placed into a petri dish containing Oat Meal Agar (OMA) media and repeated five times. After ten days, observations were made for mycelium or conidia at the tips of the stem tissue, indicating the presence of *B. bassiana*. The percentage of *B. bassiana* colonization was calculated based on the formula (Hendra 2021):

$$C = \frac{\sum n}{N} \times 100\% \quad (4)$$

Where: C: Percentage of colonization, n: Number of stem pieces colonized by *B. bassiana*, N: Number of all pieces observed.

Table 2. Scoring for determination of BPH attack level (Lakshmi et al. 2021)

Score	Symptoms
0	No BPH was found in the rice plants
1	The first leaves turn yellow, but the plant has not wilted yet
3	The first and second leaves on most plants turn yellow
5	Leaves turn yellow, growth is stunted or wilted, and almost half of the plants die.
7	Over half of the plants died, and the living plants looked stunted
9	Rice plants died



Figure 2. Preparation of the suspension of commercial *B. bassiana*: (A). Commercial *B. bassiana* with the trademark Natural BVR (B). Weighing as recommended, (C). Commercial *B. bassiana* suspension (1.5 g/L)

Plant growth observations

These observations were made on plant height, number of leaves, and number of tillers 21 days after BPH infestation/ 38 days after seed soaking/ 24 days after root soaking.

Data analysis

Data on BPH mortality to test the effectiveness of commercial Bb was processed using one-way ANOVA (analysis of variance). Data on BPH mortality, attack intensity, stem colonization, and plant growth on several rice varieties induced by commercial Bb was processed using two-way ANOVA (analysis of variance). All data were tested further with LSD at the 5% level using Stat 8 software.

RESULTS AND DISCUSSION

Effectiveness of the commercial *B. bassiana* against BPH

Based on the statistics presented in Table 3, it observed a significant difference between the treatment using commercial *B. bassiana* and the control in reducing the BPH population 10 days after the infestation ($P = 0.0001$). The mortality rate of BPH using commercial *B. bassiana* was $78.89\% \pm 3.50$.

Induced resistance on different rice varieties with commercial *B. bassiana*

BPH Mortality and Attack Intensity

The study showed no interaction between the factors of different rice varieties applied with *B. bassiana* on BPH mortality and attack intensity ($P_{\text{mortality}}=0.5822$, $P_{\text{attack}}=0.9597$). BPH mortality ranged from 12.5-45.0%. Applying commercial *B. bassiana* through soaking seeds and roots did not affect BPH mortality directly ($P=0.2268$), but different rice varieties influenced BPH mortality ($P=0.0004$). The highest mortality was found in the Cisokan (45%), and the lowest was found in the IR 42 (13.75%). Differences in rice varieties did not influence BPH attacks ($P = 0.1407$); however, the application of commercial *B. bassiana*, through seed and root soaking, reduced BPH attacks ($P = 0.0016$) (Table 4).

Stem colonization by the commercial *B. bassiana*

The results showed no interaction between differences in rice varieties and the colonization ability of commercial *B. bassiana* ($P=0.0647$). Commercial *B. bassiana* could colonize rice plant tissue in the range of 4-16% 24 days after seed soaking or 10 days after root soaking. The highest colonization was found in IR 42 stems applied with *B. bassiana* (16%). In contrast, *B. bassiana* colonization was only found in the three rice varieties with soaking seeds and roots (Table 4). Commercial *B. bassiana* was found growing on IR42 and Cisokan but has yet to be seen growing on the tissue of the Batang Piaman (Figure 3).

Plant growth observations

The observations of plant height, tillers and leaves number showed no interaction between the differences in rice varieties and the application of commercial *B. bassiana* ($P_{\text{height}}=0.9018$, $P_{\text{tillers}}=0.8182$, $P_{\text{leaves}}=0.3917$). Applying commercial *B. bassiana* through soaking seeds and roots did not affect the height of rice plants, but Batang Piaman was generally taller than the other two varieties. Differences in varieties affected the tillers' number. The highest number of rice tillers was found in the Cisokan variety (5.30 tillers). Meanwhile, the application of commercial *B. bassiana* did not affect the tillers number in each variety. The observations of leaves number showed no interaction between the difference in rice varieties and the commercial application factor of *B. bassiana* on the number of leaves (Table 5).

Table 3. Effectiveness of the commercial *B. bassiana* against BPH 10 days after the infestation

Treatment	Mortality of BPH (%) \pm SD		
<i>B. bassiana</i>	78.89	\pm	3.50 ^a
Control	0.00	\pm	0.00 ^b

Note: Numbers in the same column followed by the same letters are not significantly different based on the LSD test at the 5% significance level. The mortality number has been corrected using the Abbott formula

Table 4. BPH mortality, attack intensity and colonization in different rice varieties after induction with commercial *B. bassiana* (Bb) (7 days after BPH infestation/ 24 days after seed soaking/ 10 days after root soaking)

Variety	BPH Mortality (%) \pm SD			Attack intensity (%) \pm SD			Colonization (%) \pm SD		
	With Bb (%)	Without Bb (%)	Average	With Bb (%)	Without Bb (%)	Average	With Bb (%)	Without Bb (%)	Average
IR 42	15.0 \pm 1.6	13.0 \pm 2.0	13.8 ^c	15.5 \pm 2.9	28.9 \pm 2.9	22.2 ^a	16.0 \pm 2.0	0 \pm 0	8.0 ^a
Cisokan	45.0 \pm 0.7	45.0 \pm 1.0	45.0 ^a	15.4 \pm 2.9	28.9 \pm 2.9	22.2 ^a	4.0 \pm 2.0	0 \pm 0	2.0 ^{ab}
Batang Piaman	35.0 \pm 0.8	23.0 \pm 2.0	28.8 ^b	8.9 \pm 2.3	20.0 \pm 3.5	14.4 ^a	0 \pm 0	0 \pm 0	0 ^b
Average	31.7 ^A	26.7 ^A	(-)	13.3 ^B	25.9 ^A	(-)	6.7 ^A	0 ^B	(-)

Note: Numbers in the same column/row followed by the same letters are not significantly different based on the LSD test at the 5% significance level. The mortality number has been corrected using the Abbott formula. The (-) indicates there is no interaction between the factors tested



Figure 6. Colonization of commercial *B. bassiana* in different rice varieties (24 days after seed soaking/ 10 days after root soaking): a. IR42, b. Cisokan, c. Batang Piaman

Table 5. Plant height, tiller and leaves number of different rice varieties after seeds and roots soaking with commercial *B. bassiana* (Bb) (38 days after seed soaking/ 24 days after root soaking)

Variety	Height (cm) \pm SD			Tiller number \pm SD			Leaves number \pm SD		
	With Bb (cm)	Without Bb (cm)	Average	With Bb	Without Bb	Average	With Bb	Without Bb	Average
IR 42	18.9 \pm 1.5	19.4 \pm 1.5	19.2 ^b	4.0 \pm 0.5	4.0 \pm 0.6	4.2 ^b	16.0 \pm 1.9	15.0 \pm 1.4	15.5 ^a
Cisokan	20.3 \pm 1.6	21.6 \pm 2.0	21.0 ^b	5.0 \pm 0.5	5.0 \pm 0.8	5.3 ^a	17.0 \pm 1.2	16.0 \pm 1.4	16.8 ^a
Batang Piaman	26.1 \pm 2.2	27.3 \pm 2.9	26.7 ^a	4.0 \pm 0.6	4.0 \pm 0.6	4.0 ^b	14.0 \pm 1.4	14.0 \pm 1.5	14.0 ^b
Average	21.7 ^A	22.8 ^A	(-)	4.6 ^A	4.4 ^A	(-)	15.6 ^A	15.3 ^A	(-)

Note: Numbers in the same column followed by the same letters are not significantly different based on the LSD test at the 5% significance level. The (-) indicates there is no interaction between the factors tested

Discussion

Based on the results, it is known that commercial *B. bassiana* applied directly to BPH is classified as effective because it can cause up to 78.89% mortality; more than half of the insects tested died. So far, *B. bassiana* has been reported to be able to cause mortality of insects (Bugti et al. 2018). Nelly et al. (2023) reported that *B. bassiana* could cause mortality in 70% of *S. frugiperda* larvae after 14 days of application. The higher the density of *B. bassiana* conidia, the higher the insect mortality. Ortiz-Urquiza and Keyhani (2013) reported that most pathogenic fungi infect insects through the epidermis and then multiply in the hemolymph.

Studies on inducing resistance to different rice varieties using commercial *B. bassiana* applied by soaking the seeds and roots have varying results. In general, no interaction exists between rice variety and commercial *B. bassiana* on all variables observed. However, different rice varieties influenced BPH mortality, Bb colonization, and rice plant growth, while commercial *B. bassiana* influenced the intensity of BPH attacks and *B. bassiana* colonization. The highest BPH mortality was found in the Cisokan rice variety (Table 4). This result indicated that Cisokan has specific characteristics that suppress the presence of BPH. Meanwhile, although it did not affect BPH mortality, commercial *B. bassiana* reduced the intensity of BPH attacks (13.31%).

BPH prefers rice variety to feed because of its high nutritional content and low secondary metabolite compound levels. According to Afroz et al. (2021), the anti-herbivore effect on plants increases when the protein content decreases. The secondary metabolite compounds

produced can help plants change the physiology and behavior of insects. These compounds can be toxic to insects or unpreferred by insects, so insects avoid taking nutrients from the plant. Furthermore, interactions between endophytic fungi and plants can influence insect behavior (Wei et al. 2020). The interaction between endophytic fungi and plants includes an increase in secondary metabolite compounds. Secondary metabolites produced by endophytic fungi are toxic, and plants induced by *B. bassiana* had high emissions of volatile compounds. Therefore, induced by *B. bassiana* protects plants against attacks by herbivorous insects (Chebet et al. 2021).

The colonization ability of commercial *B. bassiana* showed different effects on each variety. The highest colonization was found in the IR 42 rice variety (16%), but the Batang Piaman variety could not be colonized by it yet. Besides that, the intensity of BPH attacks was low in Batang Piaman. Fitri (2018) reported that the preference for BPH in Batang Piaman was lower than in IR 42, partly because the Batang Piaman stem structure is more rigid than IR 42. The successful colonization of plant tissue by endophytic fungi is influenced by several factors, including the hardness or softness of the plant's morphological structure, genotype (Hardoim et al. 2015), age, and inoculum density (Bamisile et al. 2018). Endophyte colonization is specific to certain organs and tissues due to selective pressure. The specificity in colonization protects plant tissues from disturbances (Mengistu 2020).

Hendra (2021) reported that the colonization ability of *B. bassiana* was influenced by the isolate, inoculation time, plant age, and plant tissue. Colonization ability will decrease when the age of the rice plant increases. Another

factor that influences the colonization ability is the duration of the soaking process. *Beauveria bassiana* conidia will enter the seed during imbibition and diffuse through the seed coat. The conidia that enter will develop and spread through the vesicular tissue, namely the xylem (Landa et al. 2013). Increasing the soaking duration will increase the colonization ability of *B. bassiana* and plant growth (Jaber and Enkerli 2016). The form of *B. bassiana* formulation also influences its colonization ability. Commercial formulations of *B. bassiana* are directly associated with BPH mortality. So, its colonizing ability will differ from *B. Bassiana* from an endophytic fungus in plant tissue.

Applying commercial *B. bassiana* by soaking the seeds and roots did not affect the growth of rice plants, as represented by height, number of leaves, and number of tillers. The Batang Piaman was taller than Cisokan and IR 42 but had fewer leaves. Cisokan had a more significant number of tillers than the other two varieties. This condition has become a characteristic of each variety. Besides that, the result is in line with Mandasari et al. (2015), who stated that there was no effect of *B. bassiana* application on the growth of soybean plants. On the other hand, several studies report that *B. bassiana* can stimulate the growth of plants such as grapes (Rondot and Reineke 2019) and *Phaseolus vulgaris* (Afandhi et al. 2019). Interactions between *B. bassiana* and plants generally vary from antagonism to mutualism depending on the availability of nutrients (Tall and Meyling 2018).

Generally, the commercial *B. bassiana* applied directly was still effective in increasing BPH mortality, reaching 78.89%. There was no interaction between different rice varieties with commercial *B. bassiana* against BPH and rice plants. Inducing rice plant resistance with commercial *B. bassiana* by soaking seeds and roots did not affect BPH mortality and plant growth but reduce the intensity of attacks (13.31%). The Cisokan variety was better at suppressing the BPH population (45%) and suitable for colonization by commercial Bb. The IR 42 variety was more responsive than other varieties in accepting the presence of commercial Bb (16%). Commercial *B. bassiana* can be used by farmers to control BPH directly or inducing resistance by soaking seeds and roots. Direct application can increase BPH mortality, and induction of resistance can reduce the attack intensity of BPH, especially in the Cisokan variety.

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