Sains Malaysiana 54(5)(2025): 1281-1290
http://doi.org/10.17576/jsm-2025-5405-07

Effectiveness of Biological Agents to Control *Lepidiota stigma* (Coleoptera: Scarabaeidae) Larvae in Endemic Sugarcane Plantation

(Keberkesanan Agen Biologi untuk Mengawal Larva *Lepidiota stigma* (Coleoptera: Scarabaeidae) di Ladang Tebu Endemik)

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Received: 30 April 2024/Accepted: 14 January 2025

ABSTRACT

The information on the effectiveness of nematode and fungus entomopathogenic is essential for the appropriate management of white grubs *Lepidiota stigma* in endemic sugarcane plantations. Despite the significant benefits, there is still limited information on the management practices of white grubs control particularly in sugarcane plantations with sandy soil. Therefore, this study aimed to explore the effectiveness of *Steinernema* sp., *Beauveria bassiana*, and registered biological agents, as well as farmers' methods in controlling white grubs. The Randomized Block Design was used to set up the experiment. The results showed that the three biological agents had a significantly lower population of white grubs compared to the control (untreated) at one month after treatment (MAT). At 3 and 4 MAT, the population of white grubs were also significantly lower compared to the untreated plots, suggesting that all treatments had suppressed the population in sandy soil. This was supported by the finding of the glasshouse trial which generally showed that the three biological agents were still effective in the soil media for a couple of weeks. Furthermore, this study showed that yields of sugarcane obtained from the application of the three biological agents produced economic benefits.

Keywords: *Beauveria bassiana*; economic benefits; *Saccharum officinarum*; *Steinernema* sp.; white grubs

ABSTRAK

Maklumat mengenai keberkesanan nematod dan kulat entomopatogen dalam mengawal belatung putih *Lepidiota stigma* di ladang tebu endemik masih sangat terhad. Maklumat ini penting untuk pemahaman yang lebih baik tentang amalan pengurusan kawalan grub putih yang sesuai di kawasan tertentu iaitu ladang tebu dengan tanah berpasir. Keberkesanan agen biologi untuk mengawal belatung putih, iaitu *Steinernema* sp., *Beauveria bassiana*, agen biologi berdaftar dan kaedah peladang yang disusun dalam Reka Bentuk Blok Rawak. Keputusan menunjukkan bahawa ketiga-tiga agen biologi mempunyai populasi belatung putih yang jauh lebih rendah berbanding kawalan (tidak dirawat) pada satu bulan selepas rawatan (BSR). Pada 3 dan 4 BSR, populasi grub putih juga jauh lebih rendah daripada plot yang tidak dirawat, menunjukkan bahawa semua rawatan telah menindas populasi di tanah berpasir dengan ketara. Ini disokong oleh penemuan percubaan rumah kaca kajian ini, yang merekodkan bahawa, secara amnya, ketiga-tiga agen biologi masih berkesan dalam media tanah selama beberapa minggu. Selain itu, kajian menunjukkan hasil tebu yang diperoleh daripada penggunaan ketiga-tiga agen biologi tersebut masih memberi manfaat ekonomi.

Kata kunci: *Beauveria bassiana*; belatung putih; faedah ekonomi; *Saccharum officinarum*; *Steinernema* sp.

INTRODUCTION

Efforts to increase sugarcane production are currently facing serious problems. This is because farmers' enthusiasm for growing sugarcane has been hindered by the major invasion of insect pests, which also poses a danger to the industry's ability to grow sustainably (Allsopp, Croft & Fillols 2020; Shang et al. 2023; Widyasari et al. 2022). One of the pests contributing to a significant reduction in sugarcane production is white grubs of *Lepidiota stigma* (Coleoptera: Scarabaeidae) (Allsopp, Croft & Fillols 2020; Gite, Mohite & Rathour 2015; Shang et al. 2023; Widyasari et al. 2022). White grubs of *L. stigma* are an endemic pest in various sugarcane areas, particularly in areas with sandy soil (Subiyakto 2016; Sunarto & Subiyakto 2020). The attack of white grubs on sugarcane has also become a problem in several countries causing a reduction in productivity by more than 50% due to relatively high intensity and a more widespread distribution. Fields are severely impacted and seedlings die due to infestations. Therefore, replanting with different species of plant is essential when all seedlings are lost, leading to high costs.

Currently, farmers are more prone to use chemical pesticides to control attacks of white grub larvae, as the last alternative to Integrated Pest Management (IPM) implementation. Chemical application is challenging, practically unfeasible, expensive, and causes issues, such as environmental contamination through water pollution by pesticides (FitzGibbon, Allsopp & De Barro 1998; Manisegaran, Lakshmi & Srimohanapriya 2011). This shows the need to use non-chemical pest control to minimize the impact of traditional pest control such as environmentally friendly biological control. The use of biological agents for pest control has good potential and prospects, serving as host-specific and harmless to humans, natural enemies, and the environment. A potential alternative for biological control of white grubs on sugarcane plantations is the use of natural entomopathogens. Natural entomopathogens including nematodes, fungi, bacteria, and viruses are important components of soil ecosystems that can effectively control pests, showing potential for future application (Bohara et al. 2018; Chandel et al. 2015; Gabarty et al. 2014; Guo et al. 2013; Mane & Mohite 2015; Sarma et al. 2023).

Several studies have explored the use of *Metarhizium anisopliae* and *Beauveria bassiana* as entomopathogens against white grubs (Tiago, de Oliveira & de Luna Alves Lima 2014; Visalakshi, Bhavani & Rao 2015). A pot experiment showed that the fungus of *M. anisopliae* could cause mortality in white grubs found in sugarcane growing media reaching 62.7%. Meanwhile, the fungus *B. bassiana* caused larvae mortality of 55.18% (Mane & Mohite 2015). Indrayani, Prabowo and Wijayanti (2019) and in the lab tests, Bohara et al. (2018) conducted laboratory experiments and found that *M. anisopliae* caused mortality in sugarcane white grubs by 91.7% and 50%, respectively. Furthermore, laboratory experiments have been carried out on the use of entomopathogenic nematodes as biological agents. The results suggested that entomopathogenic nematodes could be promising biological agents for the control of sugarcane white grubs (Purnomo et al. 2021; Rathour, Mohite & Gite 2015; Supekar & Mohite 2015; Wagiyana, Habriantono & Alfarisy 2021). Despite the numerous studies, there is still limited information on the effectiveness of natural entomopathogens in controlling sugarcane white grubs, particularly in Indonesia with specific soil types and agro-climate.

The information is required to devise appropriate management practices for white grub control in specific sites of sugarcane plantation areas. Therefore, this study aimed to determine the effectiveness of biological agents in controlling white grubs, namely nematode *Steinernema* sp., fungus *B. bassiana*, and a registered entomopathogen. The assessment was also carried out on farmers' methods in a grub endemic area of sugarcane plantations at Kalasan Village, Yogyakarta, Indonesia. Furthermore, yields of sugarcane and the economic benefits of each treatment were recorded.

MATERIALS AND METHODS

The experiment was carried out in the endemic area of white grubs on a sugarcane plantation in Tempelsari Village, Sleman Regency, Yogyakarta, Indonesia (Utami, Muniningsih & Ciptadi 2021), located at 209 m altitude, with coordinates 110°44'31'' EL and -7°74'93'' SL (Figure 1). The climate of Tempelsari Village is warm and humid, with mean annual rainfall of approximately 2,342 mm and temperature of 33 to 36 °C. Soil classification at the experimental site is Regosol according to Subardja et al. (2014). Soil texture at the depth of 0-20 cm is loamy sand (sandy 81-9%, silt 7-21%, and clay 1-4%) (Jayanti, Sudira & Sunarminto 2015).

FIGURE 1. Heavy attack symptoms by white grubs on sugarcane plantations in untreated endemic areas

EXPERIMENTAL DESIGN AND TREATMENTS

The sugarcane variety PS682 (national superior and tolerant variety of white grubs) was planted with a spacing of 100 cm between rows at a plot size of about 2500 m^2. As basic fertilizer, 5 tons/ha of cow dung manure, 500 kg/ha of SP36, and 500 kg/ha ZA were applied to all experimental plots. The treatment effectiveness on white grub control methods in sugarcane plantation was (1) Control (untreated), (2) *Steinernema* sp., (3) *B. bassiana*, (4) a commercially registered biological agent (an unknown strain of *Beauveria* spp.), and (5) farmers' method. The treatments were arranged in a Randomized Block Design and replicated five times.

Steinernema sp. used was a Lembang isolate that originated from vegetable crops in Lembang, West Java, Indonesia. The isolate was applied by spraying approximately 75-100 mL of nematode suspension per clump on the sugarcane roots. The density of nematode concentration used was approximately 1 million Infective Juveniles (IJ) per L of nematode suspension. Meanwhile, *B. bassiana* used was a Bbl strain obtained from the Indonesian Spice and Medicinal Crops Research Institute (Bogor, Indonesia) that has been increased in capacity in the form of formulations. *B. bassiana* with a concentration of 10^9 conidia/mL of water was used by spraying a suspension of 75-100 mL per clump on the sugarcane roots. A commercially registered biological agent was applied following the previous procedures. Farmers' method of white grub control was by using a formula based on experience, consisting of 1 ton/ha of lime, 500 kg/ha of SP36, 500 kg/ha ZA, and 20 kg/ha of the synthetic pesticide (fipronil 50).

The observation on the population of white grubs was carried out by making holes in the sugarcane root zone of inter raw with a size of 1 m × 0.5 m × 0.5 m at 5 points of observation. The observation was recorded before the application of the treatments and carried out every 1-2 months after treatment (MAT). Observations were also performed on the growth of the sugarcane plant, the development of the population of white grubs, and sugarcane production at harvest. The sustainability of the biological agent treatment was observed by sampling the presence of *Steinernema* sp. and *B. bassiana* population in the treatment plots. Samples were taken in the form of soil in each plot and plants remained to observe the presence of *Steinernema* sp. and *B. bassiana* populations in the laboratory. Each replication unit of each treatment was taken as much as 3 kg of soil. Furthermore, the soil sample was divided into 5 parts (as a replication), put into polybags, and given healthy grubs. The grubs were observed for mortality. The living grubs were observed until they became adults.

An additional study in a glasshouse at Bogor was carried out using soils from field trial plots that had been treated with the three biological agents, namely *Steinernema* sp., *B. bassiana*, and registered entomopathogen to confirm the effectiveness of the three treatments. Soil samples from each treatment plot (each of 3 kg) were taken from the field at harvesting time and used as media of white grubs. Each of the treatments was repeated five times and the mortality time (the day after treatment) was observed. Healthy grubs inserted into soil samples from the field were observed for their development. Grubs that died showing symptoms of being attacked by the biological agent were recorded for the length of the period time of infestation until grubs died, while healthy grubs would develop into pupae and imago.

STATISTICAL ANALYSIS

A randomized block design analysis of variance (ANOVA) was carried out. Honest significant difference (HSD) test at $p = 0.05$ was used to separate the mean when ANOVA results showed significant treatment effects (Steel & Torrie 1980). The data concerning the population of white grubs were square root transformed when the spreads (or standard deviation) were proportional to the square root of the mean. When the data conformed to ANOVA assumptions, particularly the homogeneity of variance and normality, there was no transformation. In this study, return above variable costs (RAVC) and marginal benefit-cost ratio (MBCR) (Rifiana 2012) were calculated to determine the potential benefits of biological agent treatments, by the formula:

$$\text{RAVC} = (Y \times P) - \text{TVC}$$

where Y is the yield of sugar cane (ton/ha); P is the price of sugar cane (IDR/ton); and TVC is a total of variable cost (IDR/ha).

$$\text{MBCR} = \frac{\sum_{t=0}^{n} \frac{Bim}{(1+i)^t} - \sum_{t=0}^{n} \frac{Bfm}{(1+i)^t}}{\sum_{t=0}^{n} \frac{Cim}{(1+i)^t} - \sum_{i=0}^{n} \frac{Cfm}{(1+i)^t}}$$

where B_{im} is the benefit of introduced method; B_{fm} is the benefit of farmers' method; C_{im} is the cost of the introduced method; and C_{fm} is the cost of farmers' method. Feasibility criteria: MBCR >1 is feasible; MBCR<1 is not feasible.

RESULTS AND DISCUSSION

FIELD EFFICACY OF BIOLOGICAL CONTROL AGENTS

The information on using biological agents to control white grubs in sugarcane plantations is still limited, as the majority focuses on laboratory experiments. Field tests on white grubs control in sugarcane plantations showed that the three biological control treatments in Figure 2 suppressed the population from 1 MAT, while the farmers' method had no significant effect. This suggested that all three isolates were capable of showing fairly good performance. The population of white grubs before the application of the treatments ranged from 2.0 to 2.8/m^2, as shown in Figure 2. The results showed that at 1 MAT, there was no difference in the population of white grubs between farmers' methods (2.0 grubs/m^2) and all treatments (<1.0 grub/m^2). However, the application of the three biological control treatments significantly decreased the population of white grubs compared to the control treatment.

Previous studies supported the efficacy of these bioagents in laboratory experiments to select the higher pathogenicity of entomopathogenic nematode isolates (*Steinernema* sp.) for controlling white grubs of sugarcane. The results showed that the highest enhancement regarding mortality of white grubs occurred 72 h after treatment and the percentage of mortality was 57% (Indrayani, Prabowo & Wijayanti 2019). Purnomo et al. (2021) found that *Steinernema* sp. was effective in controlling larvae to 100% in laboratory conditions. The ability of *Steinernema* sp. to kill host larvae was attributed to the poison released by *Xenorhabdus* bacteria which were in symbions with *Steinernema* nematodes. Furthermore, in the body of the host cadaver, nematodes would multiply, grow, and develop inside the hemocoel (Ley & Blaxter 2002; Purnomo et al. 2020). Hidayah et al. (2019) determined the effectiveness of *M. anisopliae*, *B. bassiana*, and *Streptomyces* sp. on the mortality of white grubs in the laboratory. The results showed that the most effective biological agent was *M. anisopliae* at 80%, followed by *B. bassiana* and *Streptomyces* sp. at 60% and 30%, respectively. The capability of *B. bassiana* to cause mortality in white grubs was related to beauvericin (toxin) produced by *B. bassiana* (Kučera & Samšiňáková 1968). After invading insect hosts, *B. bassiana* generated a range of secondary metabolites, including beauvericin, bassianin, bassianolide, beauverolides, tenellin, oosporein, and oxalic acid. These toxins aided *B. bassiana* in parasitizing and killing its hosts (Wang et al. 2021).

There was also no difference in the population among the three biological control treatments, as shown in Figure 2. At 3 MAT, the population of white grubs in all treatment plots was significantly lower than in the untreated plots. This suggested that either the three biologicals treatments or the farmers' method had suppression effects on white grub in soil. At the observation 4 MAT, the population of white grubs in the untreated plots (2.5/m^2) was still significantly higher compared to other treatments (0/m^2). However, there was no difference in the population among the four treatments. These results suggested at 4 MAT, the application could suppress the population of white grubs in the soil. When *B. bassiana* talc-based formulations containing 7.8 × 109 conidia/mL were applied at a rate of 1 kg/acre, *Holotrichia serrata* mortality was 62% at 20 days after treatment (Chelvi, Thilagaraj & Kandasamy 2010). In another study in the field, *B. bassiana* (5.0×10^{13} spores/g) caused plant mortality and a reduction in grub population was 25.18% and 54.75%, consecutively (Pandey 2010). Tr*eatment of Steinernema glaseri at 2.5 × 10^9 infective juveniles (IJ)/ha caused the maximum grub (Anomala communis) mortality of 71.33%. But, in the field test, the nematode S. glaseri was the most adept at surviving at the lowest temperature (Sharmila* et al. 2023).

EFFICACY EFFECTIVENESS

The glasshouse study was conducted using soil media taken from the field experimental site at the harvesting time of the cane, which had been treated by *Steinernema* sp., *B. bassiana*, and the commercially registered biological agent. The results showed that the efficacy of the three biological agents was still capable of causing mortality of white grubs at 4 MAT in the field, as shown in Figure 3.

The results showed that soil media from the plot treated with *Steinernema* sp., *B. bassiana*, and registered biological agents, caused mortality of white grubs in 17.8 days (10-32 days), 13.5 days (10 -17 days), and 11 days (7-15 days), respectively (Figure 4). For soil media taken from the untreated (control) and treated with farmers' method plots, there was no death of white grubs. This suggested that the three biological agents persisted in the field soils and were still effective in causing mortality. The registered biological agents cause the death of white grubs faster than *B. bassiana*, and *Steinernema* sp. in the glasshouse condition.

The three agents persisted in the field soils and were still effective in causing the death of white grubs. According to Laznik and Trdan (2015), *Steinernema* sp. was an effective parasite for insects that live in the soil or at certain stages of life in the soil, including white grubs. Although the efficacy of biological agents decreases when larvae enter the third stage or older, there is a need for a combination with other agents or a search for suitable time to increase effectiveness.

Donga, Vega and Klingen (2018) found that *B. bassiana* suspension applied to sugarcane fields would produce a population of *B. bassiana* colonies on the roots and some sugarcane stems. San-Blas, Pembroke and Gowen (2012) stated that another advantage of entomopathogenic nematodes was the presence of virulence properties, killing host insects quickly, and possessing a wide range of hosts. Furthermore, entomopathogenic nematodes are harmless to non-target organisms, serving as scavengers, and can be easily propagated *in vitro.* Alfarizi, Purnomo and Jadmika (2012) conducted laboratory experiment to determine the effects of nematodes combined with the fungus *Metharhizium* sp. The results showed that the treatment could suppress the population of white grubs by approximately 92%.

NUMBER OF PLANTS, LENGTH, AND DIAMETER OF STEM

The results in Table 1 showed that the number of plants that survived in *B. bassiana* treatment was the highest.

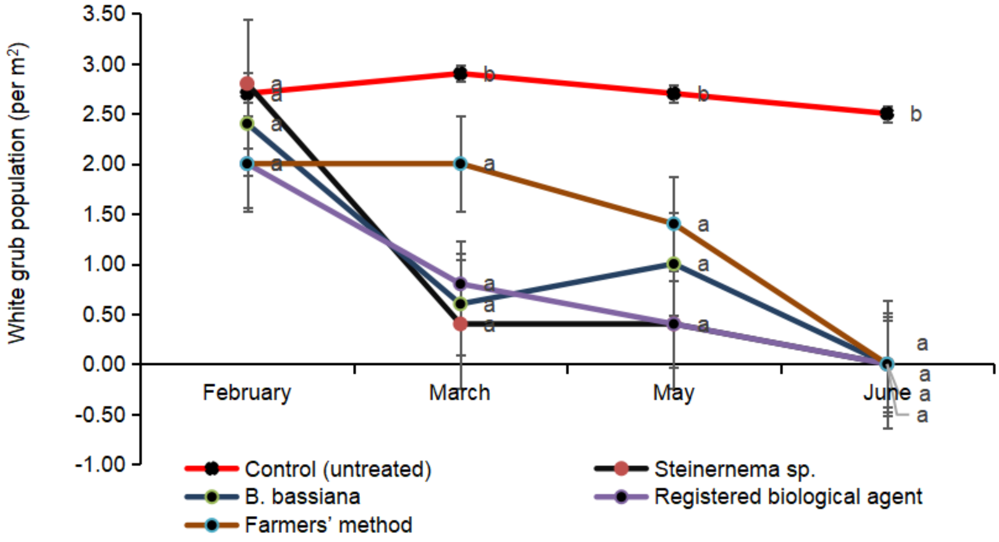

FIGURE 2. The effects of the biological agents on population of white grubs in the field (the same letters in the same month are not significantly different at $p < 0.05$. Error bar represent standard errors. Statistical analysis was carried out on $\sqrt{x} + 1$ transformed data)

FIGURE 3. White grubs which died due to (A) B. bassiana, (B) BVR – registered biological agent, and (C) Steinernema sp. infection

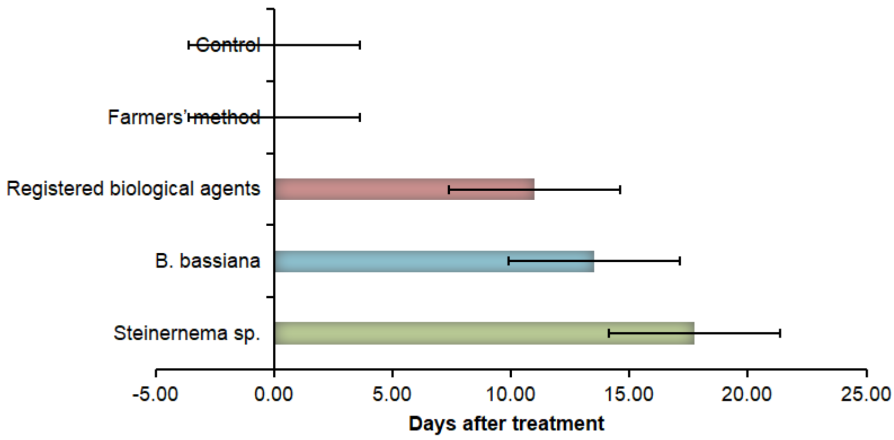

FIGURE 4. Effect of biological agents on the time of death of *L. stigma* (days) (error bar represent the standard errors)

However, there was no significant difference between *Steinernema* sp., farmers' method, and registered biological agent treatments, with variations in the number of plants that grew 2-14 per 10 m row 20 weeks after treatment. For stem length, the treatment of *Steinernema* sp. showed the longest stem compared to the others, with a difference of 173.2 cm, 38.2 cm, 12.4 cm, and 9.4 cm compared to the control, farmers' method, registered biological agent, and *B. bassiana*. Stem diameter of all treatments was not significantly different compared to the control. In *B. bassiana* treatment, the stem diameter was higher than in other treatments. The results also showed that the presence of more white grubs in control treatment affected the growth of sugarcane plantations.

YIELD OF SUGARCANE

For plant growth, there was no difference in the number of sugarcane plants per 10 m row among the four treatments, as shown in Table 1. This suggested that the application of all treatments to sugarcane in the plantation suppressed the infestation of white grubs. As observed for the number of sugarcane plants, stem length in the control plots was significantly lower compared to others. Meanwhile, there was no difference in the diameter of stems among the four other treatments.

Measurements in the field showed that the three biological agent treatments produced sugarcane of 117,419.0 - 146,543.8 kg/ha, as presented in Table 2. The productivity was relatively higher compared to the farmers' method (102,961.3 kg/ha) and the control (29,226.7 kg/ha). Among the three biological agent treatments, the application of *B. bassiana* produced the highest yield, followed by *Steinernema* sp. and the registered biological agents. The yields achieved by the application of the three biological agent treatments (Table 2) were still favorable (Tables 3 & 4). However, the sugarcane was grown in endemic areas of white grubs (Utami, Muningsih & Ciptadi 2021), showing the effectiveness of the three treatments in controlling attacks. This was shown in the achievements in productivity, which significantly suppressed the attacks of white grubs capable of inhibiting growth.

FINANCIAL BENEFITS

In this study, the financial benefits were assessed based on the saving of farm costs and income increase due to the application of the control regarding pests of white grubs (Tables 3 & 4). From the viewpoint of technology saving farm costs (Table 3), the application of *B. bassiana* was most effective in saving the cost of pest control of white grubs. Although the allocation of all components presented in Table 4 showed that farming costs due to the application of *B. bassiana* was higher compared to others. However, the increased cost components were logging and transportation because of the higher productivity due to the low levels of pest infestation. The cost of cultivation in the application of *B. bassiana* method was smaller than *Steinernema* sp. and the registered biological agent methods.

The economic analysis (Table 4) showed that the values of MBCR test of the three biological agent treatments were >1. The results showed that among all biological agent treatments, the application of *B. bassiana* produced the highest financial benefit (MBCR value of 2.76), followed by *Steinernema* sp. and registered biological agent method with MBCR of 1.98 and 1.35, respectively. These results showed that the three biological agent treatments economically were feasible to be applied as alternative methods to control the attack of white grubs in sugarcane plantations. The application of the farmers' method was effective in controlling white grubs but required a higher cost. Moreover, the farmers' method was still based on the chemical insecticide (imidaclopride). Indrayani, Prabowo and Wijayanti (2019) reported that the application of manure and the chemical insecticide imidacloprid was not effective in increasing the mortality of white grubs. This suggested that the application and development of biological agents in controlling white grubs in sugarcane plantations could be attributed to technical, environmental, and economic considerations.

TABLE 1. Number of sugarcane plants, length and diameter of stem under different biological agent treatments in the field 20 weeks after treatments (9 months old)

Treatments	Number of plants per 10 m row	Stem length (cm)	Diameter of stems (cm)
Steinernema sp.	106.00 b	313.20 b	2.73 a
B. bassiana	108.00 b	303.80 b	2.95 a
Registered biological agents	94.00 b	300.80 b	2.60 a
Farmers' method	98.00 b	275.00 b	2.60 a
Control	36.00 a	140.00 a	2.40 a

Numbers within the same column followed by the same letter were not different at $p < 0.05$

TABLE 2. The effects of pest control treatments of white grubs on the productivity of sugarcane

Methods	Productivity on field observation		Correction factor 20% (kg)	Estimated productivity (kg/ha/y)
	Productivity/plot (kg)	Productivity/ha (kg)		
Steinernema sp.	25,409.84	164,998.96	32,999.79	131,999.17
B. bassiana	28,209.68	183,179.74	36,635.95	146,543.79
Registered biological agents	22,603.16	146,773.77	29,354.75	117,419.01
Farmers' method	19,820.04	128,701.56	25,740.31	102,961.25
Control	5,626.13	36,533.33	7,306.67	29,226.66

TABLE 3. The cost of sugarcane farming under pest control treatments of white grubs applied in 1 ha (IDR)

No.	Component of Cost	*Steinernema* sp.	*B. bassiana*	Registered biological agents	Farmers' method
1.	Land rent (IDR/ha/y)	6,000,000	6,000,000	6,000,000	6,000,000
2.	Cultivation costs	21,819,622	20,852,086	21,787,079	18,604,810
	a. Worker wage	8,500,000	7,750,000	8,500,000	8,550,000
	b. Seed	4,800,000	4,800,000	4,800,000	4,800,000
	c. Material	8,225,000	7,975,000	8,225,000	5,025,000
	d. Others	294,622	327,086	262,079	229,810
3.	Logging and transportation	13,041,518	14,478,527	11,448,354	10,172,571
	a. Logging	7,919,950	8,792,628	7,045,141	6,177,675
	b. Transportation	5,121,568	5,685,899	4,403,213	3,994,896
	Sum of 1+2+3	40,861,140	40,861,140	41,330,612	39,235,433
4.	Capital interest	3,903,337	3,959,673	3,708,252	3,173,286
	Total cost of farming	44,764,477	45,290,286	42,943,685	37,950,666

TABLE 4. Analysis of cost margins, increased income, and MBCR improvement under pest control treatments of white grubs applied in 1 ha

Treatments	Cost of farming (IDR)		Cost difference to farmers' method (IDR)		Farming income (IDR)		Income difference to farmers' method (IDR)		MBCR
	White grubs Control	Total	White grubs Control	Total	Gross	Net	Gross	Net	
Steinernema sp.	4,900,000	44,764,477	1,450,000	6,813,810	61,419,213	16,654,736	13,511,345	6,697,535	1.98
B. bassiana	4,150,000	45,290,286	2,200,000	7,339,620	68,186,827	22,896,541	20,278,958	12,939,339	2.76
Registered biological agents	4,400,000	42,943,685	1,950,000	4,993,019	54,635,067	11,691,382	6,727,199	1,734,180	1.35
Farmers' method	6,350,000	37,950,666	-	-	47,907,868	9,957,202	-	-	

CONCLUSIONS

In conclusion, this study showed that the three biological agents, namely *Steinernema* sp., *B. bassiana*, and the registered biological agent, including the farmers' method significantly suppressed the population of white grubs and reduced plant mortality. The results from the glasshouse experiments showed that these three biological agents remained effective in soil medium for several weeks. Additionally, the application of the agents continued to be beneficial in terms of productivity and provided economic benefits, with the MBCR test value >1.

ACKNOWLEDGMENTS

We are grateful to the Indonesian Agency for Agricultural Research and Development for financially supporting this study and to Mr. Endang Sugandi who assisted in observing and collecting data in the field.

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