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Research Article

Effectiveness of Secondary Metabolites from Entomopathogenic Fungi for Control *Nilaparvata lugens* Stål. in the Laboratory Scale

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ABSTRACT

Nilaparvata lugens Stål. is an essential pest in rice plants. This pest attack can reduce crop yields and even crop failure. This research was conducted to obtain secondary metabolites that are effective in controlling brown planthopper (BPH). A randomized block design was used to test the effectiveness of secondary metabolites against BPH. The treatments tested were secondary metabolites produced by eight isolates of fungi consist of three concentrations: 5, 10, and 15%. Water and imidacloprid insecticide were used as control. The eight isolates were: J11 (*Aspergillus* sp.), J22 (*Lecanicillium saksenae*.), J34 (*Myrothecium* sp.), J35 (*Beauveria* sp.), J41 (*Fusarium* sp.), J56 (*Fusarium* sp), J60 (*Simplicillium* sp.), and J65 (*Curvularia* sp.). Each treatment was repeated three times. The variables observed were mortality and time of death of BPH. Data were analyzed using the F test and followed by a DMRT if significant differences existed. The results showed that the secondary metabolites of the *Lecanicillium saksenae*. *Myrothecium* sp., and *Simplicillium* sp. fungi effectively controlled BPH pests by 80–100% within 3.22–5.47 days. The fungus *L. saksenae*, *Myrothecium* sp., and *Simplicillium* sp. contain insecticidal compounds, clogging the insect spiraculum, antifeedant, repellant, and antimicrobial.

Keywords: controlling; entomopathogenic fungi; *Nilaparvata lugens*; secondary metabolites

INTRODUCTION

The brown planthopper (*Nilaparvata lugens* Stål.) is a major rice pest threatening rice production in Indonesia. Rashid *et al.* (2016) reported that in the last decade, there was an explosion in the brown planthopper (BPH) population throughout Asia, which resulted in large yield losses. In Thailand, there was a continuous BPH population explosion for ten consecutive growing seasons from 2008 to 2012 and caused a loss of US $ 52 million or the equivalent of approximately 173,000 tonnes. This pest also caused an estimated loss of 1,000,000 tonnes in Vietnam in 2007 and resulted in the government canceling rice exports. According to Bhatt and Tiwari (2015), in Southeast and East Asia, BPH caused yield losses of 30–50 percent. In Indonesia, BPH from October 2016 to August 2017 caused damage to rice crops covering an area of 63,075 hectares and resulted in 20,152 hectares of rice crops experiencing crop failure (Julianto, 2017). The percentage of BPH attacks ranges from 51.6–94.1% in Padang, Indonesia (Syahrawati, 2019).

The frequency of BPH attacks in developing Asian countries continues to increase; this is due to the unwise use of synthetic chemical insecticides, so that natural enemies are killed (Khan *et al.*, 2018; Minarni *et al.*, 2018; Zhu *et al.*, 2018). BPH pests have high genetic plasticity, the use of the same insecticides and continuously can cause BPH resistance to these insecticides (Surahmat *et al.*, 2016; X. Zhang *et al.,* 2016; Y. Zhang *et al.*, 2017; Minarni *et al.*, 2018; Wu *et al.*, 2018; Tian *et al.*, 2019). These problems need to be addressed immediately so that there is no explosion of BPH pests.

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The use of natural enemies of BPH is a safe control technique for the environment. One such natural enemy is the entomopathogenic fungus. Entomopathogenic fungi are fungi that can infect and kill insects (Litwin *et al*., 2020). Entomopathogenic fungi that have been widely researched and known to be effective in controlling BPH are *Beauveria bassiana* (Suryadi *et al*., 2018; Sumikarsih *et al*., 2019; Atta *et al*., 2020), *Metarhizium* sp. (Chinniah *et al*., 2016; Atta *et al*., 2020), and *Lecanicillium lecanii* (Atta *et al*., 2020). However, in its implementation in the field, the use of entomopathogenic fungi to control BPH pests still has many weaknesses. After application in the field, entomopathogenic fungi are exposed to various abiotic stresses, such as temperature (Saldarriaga Ausique *et al*., 2017; Zaman *et al*., 2020), humidity (Hsia *et al*., 2014; Rai *et al*., 2014; Zaman *et al*., 2020), ultraviolet (UV) radiation (Kaiser *et al*., 2018), and edaphic factors (soil) (Klingen *et al*., 2015; Niu *et al*., 2019).

The entomopathogenic fungus Hypocreales produces a variety of secondary metabolites. This group of fungi has a very high genome rank and is predicted to have a number of gene clusters that produce unique secondary metabolites. Secondary metabolites have very diverse roles in insect pathogenicity as virulence factors by modulating various interactions between fungi and insect hosts. In addition, secondary metabolites also protect the host carcass from attack by other microbes, play a role in intra and inter-species communication, and reduce biotic stress (L. Zhang *et al*., 2020).

Secondary metabolites are genetic properties inherent in an organism, which are usually used for the adaptation of fungi to their environment (Hautbergue *et al*., 2018). The secondary metabolites of entomopathogenic fungi generally have a small molecular size, usually <2,000 MW (molecule weight). The overall production of certain secondary metabolites can be significantly altered by optimizing growth conditions, such as nutrition, temperature, humidity (Mishra *et al*., 2015; Zaman *et al*., 2020), and UV radiation (Kaiser *et al*., 2018, Herlinda *et al*., 2019). Some entomopathogenic fungi can kill the host even more rapidly by secreting some mycotoxins (such as beauvericin, cyclodepsipeptide, destruxin, and desmethyldestruxin) in the early stages of infestation (Wang *et al*., 2018).

Based on the previous description, it is necessary to research entomopathogenic fungi' secondary metabolites' effectiveness in BPH control. Results of the literature search show the use of entomopathogenic fungal secondary metabolites to BPH control has not been reported.

MATERIALS AND METHODS

Propagation of Entomopathogenic Fungi

The entomopathogenic fungi used in this study resulted from the exploration of entomopathogenic fungi that infected BPH in the Banyumas Regency. These fungi in the laboratory could infect BPH > 70% (Minarni *et al*., 2020). The fungus isolates were J11 (*Aspergillus* sp.), J22 (*Lecanicillium saksenae*.), J34 (*Myrothecium* sp.), J35 (*Beauveria* sp.), J41 (*Fusarium* sp.), J56 (*Fusarium* sp.), J60 (*Simplicillium* sp.)), and J65 (*Curvularia* sp.). The fungi were grown on PDA (*Potato Dextrose Agar*) for 14 days at 27°C (Donzelli & Krasnoff, 2016).

Extraction of Secondary Metabolites of Entomopathogenic Fungi

Fungal secondary metabolites are produced by multiplying the entomopathogenic fungi in PDB (*Potato Dextro Broth*). Propagation was carried out using five 1 cm diameter fungi cultures and was incubated in a 250 ml Erlenmeyer at room temperature and shaken at 200 rpm for ten days (Kim *et al*., 2013). Fungi culture was separated between fungal mycelium and its supernatant using a 5,000× g speed centrifuge (Hitachi himac CR 7) for 10 minutes at 4°C. The supernatant was then filtered with Whatman No 1 filter paper. The supernatant was grown on PDA (*Potato Dextro Agar*) to ensure no more mycelium was carried (Bandani *et al*., 2000).

The extraction results were analyzed using GC-MS Shimadzu Type QP-2010 SE, with an SH-Rxi-5Sil MS column, 30 m long, 0.25 mm inside diameter, with an initial column temperature operating conditions of 80°C and a final temperature of 300°C, an injector temperature of 128°C, detector temperature 280°C, Helium carrier gas, ionizing type EI (Electron Impact), the volume of the sample injected was 0.1 µL. Compound identification was carried out computer-aided by Wiley 229, NIST 12, and NIST 62 Library software. Compound analysis using GC-MS will obtain the active ingredient content of the tested fungus.

Testing of Secondary Metabolites to BPH Mortality

Testing of secondary metabolites to BPH mortality was performed using an experimental method with a randomized block design. The treatments tested were secondary metabolites produced by 8 fungal isolates, namely J11 (*Aspergillus* sp.), J22 (*Lecanicillium saksenae*.), J34 (*Myrothecium* sp.), J35 (*Beauveria* sp.), J41 (*Fusarium* sp.), J56 (*Fusarium* sp), J60 (*Simplicillium* sp.), and J65 (*Curvularia* sp with three concentration of 5, 10, and 15%. Water and the imidacloprid insecticide (active ingredients 350 g/l) were used as control. Each treatment was repeated 3 times. Each experimental unit used 10 third instar nymphs of BPH. The rice plant used was 21 days after seedlings in a plastic cylinder with a diameter of 5 cm and a height of 20 cm with a leotard cloth. Suspension of secondary metabolites was prepared according to the concentration tested.

The application has been made in contact by spraying the suspension of secondary metabolites, aqua dest, and imidacloprid insecticides on 10 individual third instar nymphs. The distance between the nozzle of the sprayer and the BPH was about 5 cm, the number of sprays was three times. Then the BPH nymphs were transferred into plastic cages containing rice plants. Rice plants were placed in the screenhouse. The experiment was repeated three times; observations were made every 24 hours for seven days. Observed variables: nymph mortality and time of death. BPH's time of death was calculated based on the formula from Susilo *et al*. (1993):

$$W = \frac{\sum(\frac{a}{n} \times b)}{\sum \frac{a}{n}}$$

Description:
W = Time of death of BPH
a = Number of BPH died on the day of infection
b = day when the dead BPH
n = number of dead BPH at each treatment

Data Analysis

Analysis of secondary metabolites from fungi was done using GCMS Shimadzu Type QP-2010 SE. Data of mortality and time of BPH death were analyzed using ANOVA, and whether real differences were further tested by the Duncan test of 95% accuracy. The analysis of the secondary metabolites content of fungi was carried out on isolates which caused 80% mortality in BPH.

RESULTS AND DISCUSSION

Three fungal isolates were found to be effective in controlling BPH pests in the laboratory, with a mortality of 80–100 percent (Table 1). The fungus isolates were J22 (*Lecanicillium saksenae*), J34 (*Myrothecium* sp.), and J60 (*Simplicillium* sp). Secondary metabolites that have been produced by the three fungi at a concentration of 5 % caused the death of BPH of 80.00; 86.67; and 83,33% in the time of 4.74; 5.33; and 5.47 days. At a concentration of 10%, it can cause death to brown planthopper of 90.00; 96.67; and 90.00% within 4.69; 5.33; and 4.43 days, while at a concentration of 15%, resulting in 86.67; 100; and 90% deaths within 4.54, 3.22; and 4.01 days. The high mortality of BPH due to the treatment of secondary metabolites of *Lecanicillium saksenae*, *Myrothecium* sp., and *Simplicillium* sp. was suspected because the three fungi produced toxic compounds. The BPH exposed to secondary metabolites of the entomopathogenic fungi showed inactivity, decreased feeding activity, then died drying, and was not overgrown with fungal hyphae.

The secondary metabolites of the three fungi were then analyzed for their chemical content using GCMS. The results of GCMS analysis of the three fungi are presented in Tables 2, 3, and 4. The literature search results show that the fungus *Lecanicillium saksenae*, *Myrothecium* sp., and *Simplicillium* sp. contain insecticidal compounds. *L. saksenae* fungus produces secondary metabolites methyl ester of ricinoleic acid and selinane. *Myrothecium* sp. produces four secondary metabolites, namely (2.4,4,4,16,16-D6)-3. alpha.,17.beta.-dihydroxy-5.beta.-androstane; (+)-nepetalactone; Alloaroma dendrenoxid-(1) and 2-(4-Bromobenzylidene) cyclohexanone. Meanwhile, the fungus *Simplicillium* sp. produces secondary metabolites phenylalanine, N, N-bis (trimethylsilyl)-trimethylsilyl ester; papaverine; and octadecanoic acid trimethylsilyl ester.

Based on Table 5, it's known that the more types of secondary metabolites that are insecticidal produced by entomopathogenic fungi, the higher mortality of BPH. *Myrothecium* sp. produced four toxic compounds, while the *Simplicillium* sp. and *L. saksenae* fungi produced three and two toxic compounds. The biological activity of each secondary metabolite produced by each fungus can also be seen in Table 5.

Table 1. Mortality (percent) and time of death (days) of brown planthopper at 1 to 7 days after application of secondary metabolites of entomopathogenic fungi

Treatment	Mortality of brown planthopper (percent)														Time of death (days)
	1 dat		2 dat		3 dat		4 dat		5 dat		6 dat		7 dat		
K0	0.00	a	0.00	a	0.00	a	0.00	a	0.00	a	0.00	a	3.33	a	7.00 d
M1K1	3.33	ab	26.67	ijk	30.00	gh	30.00	efgh	46.67	fgh	53.33	ef	66.67	ef	5.13 cd
M2K1	0.00	a	6.67	abc	13.33	bcd	36.67	ghij	53.33	hi	56.67	f	80.00	gh	4.74 bc
M3K1	0.00	a	10.00	bcd	30.00	gh	40.00	hij	33.33	de	43.33	cde	53.33	cd	4.82 bc
M4K1	0.00	a	10.00	bcd	13.33	bcd	20.00	bcde	63.33	jk	73.33	hi	86.67	hi	5.33 cd
M5K1	0.00	a	13.33	cde	16.67	cde	23.33	cdef	33.33	de	46.67	def	56.67	cd	4.18 bc
M6K1	6.67	b	10.00	bcd	13.33	bcd	13.33	bc	23.33	bc	33.33	bc	53.33	cd	4.88 bc
M7K1	0.00	a	16.67	def	20.00	def	46.67	jkl	56.67	ij	70.00	g	83.33	hi	5.47 cd
M8K1	0.00	a	0.00	a	16.67	cde	30.00	efgh	33.33	de	46.67	def	70.00	f	5.59 cd
M1K2	6.67	b	30.00	jkl	30.00	gh	33.33	fghi	26.67	bcd	36.67	bcd	56.67	cd	4.82 bc
M2K2	0.00	a	6.67	abc	10.00	bc	10.00	ab	66.67	kl	70.00	g	90.00	ij	4.69 bc
M3K2	0.00	a	23.33	ghi	33.33	h	43.33	ijk	40.00	ef	43.33	cde	56.67	cd	4.56 bc
M4K2	0.00	a	13.33	cde	13.33	bcd	16.67	bcd	66.67	kl	76.67	hi	96.67	jk	5.33 cd
M5K2	3.33	ab	16.67	def	20.00	def	26.67	defg	33.33	de	46.67	def	60.00	de	5.59 cd
M6K2	0.00	a	10.00	bcd	10.00	bc	16.67	bcd	30.00	cd	36.67	bcd	66.67	ef	5.00 bc
M7K2	0.00	a	23.33	ghi	53.33	j	56.67	kl	66.67	kl	76.67	hi	90.00	ij	4.43 bc
M8K2	0.00	a	3.33	ab	6.67	ab	16.67	bcd	20.00	b	26.67	b	40.00	b	4.48 bc
M1K3	0.00	a	33.33	kl	33.33	h	36.67	ghij	43.33	fg	53.33	ef	66.67	ef	4.35 bc
M2K3	0.00	a	16.67	def	23.33	efg	33.33	fghi	70.00	kl	73.33	hi	86.67	hi	4.54 bc
M3K3	0.00	a	30.00	jkl	33.33	h	46.67	jkl	50.00	ghi	53.33	ef	66.67	ef	5.12 cd
M4K3	0.00	a	20.00	fgh	26.67	fgh	40.00	hij	73.33	l	80.00	hi	100.00	k	3.22 b
M5K3	0.00	a	20.00	fgh	26.67	fgh	30.00	efgh	40.00	ef	46.67	def	66.67	ef	5.04 cd
M6K3	0.00	a	13.33	cde	20.00	def	36.67	ghij	40.00	ef	46.67	def	73.33	fg	4.85 bc
M7K3	0.00	a	36.67	l	43.33	i	53.33	kl	73.33	l	83.33	i	90.00	ij	4.01 bc
M8K3	0.00	a	6.67	abc	10.00	bc	13.33	bc	23.33	bc	33.33	bc	50.00	c	5.81 cd
K4	90.00	c	100.00	m	100.00	k	100.00	l	100.00	m	100.00	j	100.00	k	1.14 a

Note: Numbers followed by the same letters indicate no significant difference in the DMRT test with 95% accuracy. M1 (*Aspergillus* sp.), M2 (*Lecanicillium saksenae*), M3 (*Beauveria* sp.), M4 (*Myrothecium* sp.), M5 (*Fusarium* sp.), M6 (*Fusarium* sp.), M7 (*Simplicillium* sp.), M8 (*Curvularia* sp.), K0 (water), K1 (5% Concentration), K2 (10% concentration), K3 (15% concentration), K4 (imidacloprid), dat (days after treatment)

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Table 2. Test results of secondary metabolite compounds of *Lecanicillium saksenae* using GC-MS Shimadzu Type QP-2010 SE

Peak	Real Time	Area%	Height%	A/H	Compound
1	3.955	27756	6432	4.02	Ergost-5-ene-3.25-diol. (3.beta.)- (CAS)
2	4.255	22980	5698	3.56	4.8.8-trimethyl-3-oxa-bicyclo(5.4.0)undec-1.7-ene
3	5.08	29211	5144	3.22	Dihydro am-toxin 1
4	5.36	29125	6104	3.82	4-(4-[4-methyl-3-[(pyridin-2-ylmethyl)-sulfamoyl]-phenyl]-phthalazin-1-ylamino)-benzoic acid methyl ester
5	6.304	25197	5468	3.42	{[4-(tert-Butyldimethylsiloxy)-2.6-dimethylphenyl](2-ethylhex-2-ynyloxy)methoxy]methylene} pentacarbonylchromium(0)
6	7.418	22359	7102	4.44	12-Desoxyphorbol 13-isobutyrate
7	7.504	36502	6338	3.97	4-Acetoxy-3-methylbut-2-enoic acid. methyl ester
8	9.455	23624	3236	2.02	2-(3-Methylphenoxy)octahydro-1H-1.3.2-benzodiazaphosphole 2-oxide
9	10.349	32818	7695	4.81	Sandaracopimar-7.15-dien-6-one
10	10.46	25901	7826	4.9	Methyl ester of ricinoleic acid
11	11.239	31024	5414	3.39	3HO-16:1 ME TMS
12	11.835	42908	5016	3.14	9H-Purine-9-butanoic acid. .alpha...beta.-bis(acetyloxy)-6-amino-. methyl ester. [R-(R*.R*)]-(CAS)
13	12.015	39800	8725	5.46	(14.beta..20R)9.19-Cyclo-6.7-epoxylanostan-3-ol. acetate
14	12.136	22146	12983	8.12	1-pyrazineacetamide. n-(2-cyano-4.5-dimethoxyphenyl)hexahydro-4-(2-hydroxyethyl)-
15	12.196	31206	12357	7.73	syn-4.4'-Dimethylidene-2.2'-bi(tricyclo[3.3.0.0(3.7)]octylidene)
16	12.255	29985	13809	8.65	4-[2-(1R*.2S*)-(2-Hydroxycyclohexylmethyl)allyl]tetrahydro-2H-pyran-4-ol
17	12.294	24948	12937	8.09	(S)-3-Methylazepin-2-one
18	12.355	35891	10152	6.35	Zomepirac
19	12.38	44986	11025	6.9	Selinane
20	12.53	30981	6378	3.99	N-Acetyl-O-benzylelmerrillicine

Table 3. Test results of secondary metabolite compounds of *Myrothecium* sp. using GC-MS Shimadzu Type QP-2010 SE

Peak	R. Time	Area%	Height%	A/H	Compound
1	3.028	19263	6314	3.05	Unknown name
2	3.97	25340	4348	5.83	5H-Cyclopropa[3.4]benz[1.2-e]azulen-5-one. 9.9a-bis(acetyloxy)- 3-[(acetyloxy)methyl]-1.1a.1b.4.4a. 7a.7b.8.9.9a-decahydro-7b-hyd
3	4.223	23235	5325	4.36	(m-Methoxymesityl)mesitylacetic acid
4	5.971	23733	5726	4.14	5.12-Naphthacenedione. 7-(acetyloxy)-8-ethyl-7.8.9.10-tetrahydro -1.4.6.8.11-pentahydroxy- (CAS)
5	6.965	26035	6321	4.12	3-Propylglutaric acid
6	8.076	22476	4392	5.12	2-(4-Bromobenzylidene)cyclohexanone
7	8.905	41561	6593	6.3	Bis(4-chlorophenyl)methanone oxime
8	9.025	25338	6952	3.64	1-(1'-Acetoxyethyl)-2-chloro-3.4-dimethoxybenzene
9	9.316	28466	5882	4.84	Trimethyl tridecane-1.5.13-tricarboxylate
10	10.546	52671	7218	7.3	Tricarbonyl-[1-acetyl-1.2-diazepino]-iron
11	10.69	22018	6107	3.61	1-Pentatriacontanol (CAS)
12	10.769	21274	5756	3.7	5.5'-[(Z)-but-2-enylidenedithio]-bis[3-methyl-1.3.4-thiadiazole-2(3H)-thione]
13	11.123	24205	7819	3.1	Tridecan.1.13-dibromo
14	11.275	19309	3164	6.1	2.3.22.beta.-Trihydroxy-24.29-dinor-1.3.5(10).7-friedelatetraen-6.21-dione-23-al
15	12.015	21574	4757	4.54	Thiazole-5-carboximidamide. 2-allylamino-4-amino-N'-cyano-
16	12.325	22636	5351	4.23	Alloaromadendrenoxid-(1)
17	12.6	33192	9235	3.59	(+)-nepetalactone
18	12.64	22573	12141	1.86	Eicosane. 1.20-dibromo- (CAS)
19	12.71	19852	5641	3.52	2.4-Imidazolidinedione. 1.3-diethyl-5-phenyl-5-[3-[(trimethylsilyl)oxy]phenyl]- (CAS)
20	12.794	21223	6509	3.26	(2.4,4,4,16,16-D6)-3.alpha.,17.beta.-dihydroxy-5.beta.-androstane

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Table 4. Test results of secondary metabolite compounds of *Simplicillium* sp. using GC-MS Shimadzu Type QP-2010 SE

Peak	Real-Time	Area%	Height%	A/H	Compound
1	3.19	32249	5659	5.69	2-tert-Butyl-4-(dimethylaminomethyl)-6-(a-methylbenzyl)phenol
2	3.384	46574	7989	5.83	Phenylalanine. N.N-bis(trimethylsilyl)-. trimethylsilyl ester
3	4.895	27676	5276	5.25	Papaverin
4	5.935	20966	4934	4.25	Ethyl 2-phenylthio-3-phenylpropanoate
5	6.272	25800	6816	3.79	Octadecanoic acid. trimethylsilyl ester (CAS)
6	6.435	29793	5519	5.4	1-{1-[4-(ethylamino)-6-(1-piperidinyl)-1.3.5-triazin-2-yl]-5-methyl- 1h-1.2.3-triazol-4-yl}ethanone
7	7.72	29917	6684	4.48	N-(p-Bromophenyl)selenoacetamide
8	7.833	28147	7079	3.98	(4S.4aS.8aS)-1.2.3.4.4a.5.8.8a-Octahydro-3.3.4-trideuterio-4-[(trideuterio)methyl]-4.8a-dimethylnaphthalen-4a-ol
9	10.866	26955	4942	5.45	Ergost-9(11)-ene-3.6.20-triol. 3.6-diacetate. (3.beta..5.alpha.. 6.alpha..20R)- (CAS)
10	11.31	22544	5531	4.08	1.4-Dimethylcyclododeca-5.11-diene-1.4-diol
11	11.594	27372	9567	2.86	1.2.4-Triazaspiro[4.5]decane-3-thione. 2-(3-methylbutyl)-
12	11.745	28404	5261	5.4	Methyl cativate
13	12.028	41134	7924	5.19	O o'-biphenol. 4.4'.6.6'-tetra-t-butyl-
14	12.11	31510	6755	4.66	(4aR*.4bR*.6aR*.8R*.10aR*.10bS*.12aS*)-8-Methoxy-10b.12a-dimethylhexadecahydrobenzo[a]phenanthren-4(1H)-one
15	12.24	25452	7669	3.32	1-(3'.4'-diethoxybenzoyl)-6.7-diisopropyl-3.4-dihydroisoquinoline
16	12.416	28081	9201	3.05	Cholest-5-en-3-ol. 4.4-dimethyl-. (3.beta.)- (CAS)
17	12.45	40081	7580	5.29	Methanesulfonic acid. 2-(3-hydroxy-4.4.10.13.14-pentamethyl-2.3.4.5.6.7.10.11.12.13.14.15.16.17-tetradecahydro-1H-cyclopenta[a]
18	12.545	29944	6271	4.77	Dihydroxyquinalbarbitone 2.3-
19	12.665	22806	6651	3.43	N-(2-cyano-ethyl)-n-methyl-acetamide
20	12.985	21034	4181	5.03	4-Methyl-heptadecanoic Acid

Table 5. Some of the results of research on the secondary metabolite of *Lecanicillium saksenae*, *Myrothecium* sp., and *Simplicillium* sp. and its biological activities

Fungi	Compound	Biological activity	References
Lecanicillium saksenae	Methyl ester of ricinoleic acid	Clogging the spiraculum of insects	Celestino (2016)
		Larvicidal	Sogan *et al.* (2018)
	Selinane	Antifeedant	Wada *et al.* (1970)
		Insecticidal	Sosa *et al.* (2017)
Myrothecium sp.	(2.4,4,4,16,16-D6)-3.alpha.,17.beta.-dihydroxy-5.beta.-androstane	Insecticidal	Surahmaida & Umarudin (2019)
	(+)-nepetalactone	Repellent	Birkett *et al.* (2011); Sengupta *et al.*(2018); Reichert *et al.* (2019)
	Alloaromadendrenoxid-(1)	Insecticidal	Hamada *et al.* (2018)
	2-(4-Bromobenzylidene) cyclohexanone	Antifungal, mosquito deterrent, and larvicidal activity	Tabanca *et al.* (2013)
Simplicillium sp.	Phenylalanine. N.N-bis (trimethylsilyl)-. trimethylsilyl ester	Insecticidal	Romeh (2009)
	Papaverin	Insecticidal	Huddart & Saad (1980) Shimizu *et al.* (2000)
	Octadecanoic acid. trimethylsilyl ester (CAS)	Antimicrobial	Abubakar & Majinda (2016)

The toxin production will differ depending on fungal isolates, culture composition, and pH so that the culture extracts or filtrates from different fungi are thought to contain secondary metabolites or compounds that have different insecticide activity (Sánchez-Pérez *et al.*, 2016). The type and concentration of a compound can vary according to fungal isolates, the composition of the culture medium, and the conditions of propagation (Valencia *et al.*, 2011; Safavi, 2013).

Secondary metabolites of entomopathogenic fungi have the following characteristics: small molecular weight, high stability, not easily damaged, and penetrate the barrier. These characteristics significantly affect the effectiveness of entomopathogenic fungal applications in BPH. Secondary metabolites of *L. saksenae*), *Myrothecium* sp., and *Simplicillium* sp. caused BPH death by 80–100% in the laboratory. These results indicate that the secondary metabolites of entomopathogenic fungi have the potential to be further investigated in the field. If it shows good results, it can be used as an alternative to controlling BPH pests and as a substitute for synthetic chemical insecticides.

CONCLUSION

The results showed that the secondary metabolites of the *Lecanicillium saksenae*. *Myrothecium* sp., and *Simplicillium* sp. fungi effectively controlled BHP pests by 80–100 percent within 3.22–5.47 days. The fungus *L. saksenae*, *Myrothecium* sp., and *Simplicillium* sp. contain insecticidal compounds, clogging the spiraculum insect, antifeedant, repellant, and antimicrobial. *L. saksenae* fungus produces secondary metabolites methyl ester of ricinoleic acid and selinane. *Myrothecium* sp. produces four secondary metabolites, namely (2.4,4,4,16,16-D6)-3.alpha.,17.beta.-dihydroxy-5.beta.-androstane; (+)-nepetalactone; Alloaroma dendrenoxid-(1) and 2-(4-Bromobenzylidene) cyclo hexanone. Meanwhile, the fungus *Simplicillium* sp. produces secondary metabolites phenylalanine, N, N-bis (trimethylsilyl)-trimethylsilyl ester; papaverine; and octadecanoic acid trimethylsilyl ester.

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