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Various secondary metabolites of endophytic fungi induced by a newly modified medium and their insecticidal activities

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Abstract. *Sari JMP, Herlinda S, Elfita, Suwandi S. 2025. Various secondary metabolites of endophytic fungi induced by a newly modified medium and their insecticidal activities. Biodiversitas 26: 1881-1890.* This research aimed to identify and increase the variation of secondary metabolites of *Penicillium citrinum*, *Beauveria bassiana*, and *Metarhizium anisopliae* from South Sumatra and to determine their insecticidal activities. The fungi were grown on a Glucose Yeast Extract Broth (GYB) medium enriched with cricket powder and a Potato Dextrose Broth (PDB) medium. Secondary metabolite compounds from peaks in the LC-MS chromatograms of the crude extract of *P. citrinum* cultured in GYB medium were guanidine acetate, 8-deoxygartanin, abafungin, and 2-amino-5-carboethoxy-4-hydroxypyrimidine. Isoquinoline and leucic acid were detected in *P. citrinum* extracts of PDB culture. *B. bassiana* could produce psoralidin and L-(+)-lysine from GYB culture and adenine, guanidine acetate, ethyl carbazate, aceglutamide, and L-(+)-lysine from PDB culture. *M. anisopliae* could synthesize cytosine, adenine, n-aminoguanidine, azanidazole, phenol, methyl carbazate, and antiarol from GYB medium and 3-(Dimethylamino)-1-propanethiol and 2-(7H-Pyrrolo[2,3-d] pyrimidin-4-yl)-1H-isoindole-1,3(2H)-dione from PDB medium. *P. citrinum* extract from GYB culture caused the highest larval mortality (98%) of *Spodoptera frugiperda.* Nevertheless, the mortality was not significantly different from that induced by *B. bassiana* (96.67%) and *M. anisopliae* (97.33%) from GYB culture. Thus, the fungi cultured in the GYB medium have the potential to synthesize more secondary metabolite compounds, and they have high insecticidal activities, inducing high larval mortality.

Keywords: *Beauveria bassiana*, GYB, *Metarhizium anisopliae*, PDB, *Penicillium citrinum*

INTRODUCTION

Endophytic fungi can inhabit plant tissue both intracellularly and intercellularly, establishing a mutually beneficial symbiotic relationship with their host plants (Lira et al. 2020). The endophytic fungi reside within host plant tissues, enhancing the host's growth and protecting against insect pest attacks (Kinyungu et al. 2023). For example, *Beauveria bassiana* (Bals.-Criv.) Vuill. can successfully colonize tomato plants through foliar application, achieving 100% colonization in two weeks and inhibiting *Bemisia tabaci* (Gennadius, 1889) (Wei et al. 2020). *B. bassiana* can also colonize maize seedlings via seed inoculations, enhance seedling growth, and adversely affect larval growth (Herlinda et al. 2020; Sari et al. 2023a). *Metarhizium anisopliae* (Metschn.) Sorokīn successfully colonized *Citrus ×limon* (L.) Osbeck plants and increased their seedling height and leaf number (Bamisile et al. 2020) as well as the fungus can promote maize plant growth and cause mortality (100%) on *Spodoptera frugiperda* J.E.Smith, 1797 larvae (Ramos et al. 2020). Endophytic *Penicillium citrinum* Thom possesses potential as an entomopathogen of *S. frugiperda* (Herlinda et al. 2021).

Fungal blastospores can produce secondary metabolites in vitro, in broth, or on a solid medium (Mancillas-Paredes et al. 2019). Endophytic fungi can exterminate insect pests due to the toxicity of their secondary metabolites. They have synthesized numerous chemically significant compounds, such as *B. bassiana* secretes adenine, toxic secondary metabolites for insects (Yin et al. 2024); bassiacridin and beauvericin are also toxic to insects (Safavi 2012). *Metarhizium anisopliae* synthesizes destruxin, which is also toxic to insects (Borisade et al. 2016). *Penicillium citrinum* produces guanidine acetate, which functions as a biocidal agent (Drozdov and Kotov 2021). The endophytic fungi additionally excrete secondary metabolites in the plants they colonize (Jaber and Ownley 2018). Secondary metabolites can elicit deterrent or antifeedant and antibiosis effects in insect pest larvae (Russo et al. 2021). Our previous study identified three species of endophytic fungi, *P. citrinum*, *B. bassiana*, and *M. anisopliae*, from corn (*Zea mays* L.) and red chili (*Capsicum annuum* L.) in South Sumatra, Indonesia, and they possess the potential as entomopathogens (Herlinda et al. 2021). Nevertheless, their secondary metabolites have not been examined.

The quality, diversity, and quantity of secondary metabolites synthesized by endophytic fungi can be substantially influenced by changes in external environments and their nutritional conditions (Isah et al. 2018). In investigating natural product laboratories, the variation and optimization of growth conditions are necessary to acquire a novel and diverse compound of interest (Singh et al. 2017). Solid or liquid media can induce the production of diverse fungal secondary metabolites (Isah et al. 2018). The methodical modification of cultural growth conditions is referred to as the one-strain, many-compounds approach (OSMAC) (Armin et al. 2021). The liquid medium typically employed for the production of fungal secondary metabolites is a standard medium, such as Potato Dextrose Broth (PDB) (Nasution et al. 2024). Nonetheless, alterations in the liquid medium of endophytic fungal cultures must be conducted to achieve greater diversity and increased quantities of desired secondary metabolites. In an academic environment with moderate results, a diverse array of media will be employed; therefore, only economical, readily available, and easily prepared media should be created (Serrano et al. 2021). For instance, Glucose Yeast Extract Broth (GYB) is a medium utilized for cultivating *Streptomyces libani* A (Azish et al. 2020) and a genus of Oomycetes, *Saprolegnia* sp. (Pant et al. 2025). Nevertheless, GYB is constrained in its application for culturing endophytic fungi to investigate secondary metabolites. The research aimed to identify and increase the variation of secondary metabolites of three fungal endophytic species, *P. citrinum, B. bassiana*, and *M. anisopliae*, grown on a standard medium (PDB) and a new modified medium (GYB) and to determine their insecticidal activities.

MATERIALS AND METHODS

Preparation and rejuvenation of endophytic fungi

The endophytic fungal isolates originated from our previous research and were preserved in the Entomology Laboratory, Faculty of Agriculture, Universitas Sriwijaya. The isolates used were *P. citrinum* JgTpOi(2) isolate, *B. bassiana* JgSPK isolate, and *M. anisopliae* CaTpPGA isolate. They were molecularly identified and deposited in GenBank. The fungal isolates were obtained from maize and red chili in South Sumatra, Indonesia (Table 1). The isolates were recultured in a modified Sabouraud Dextrose Agar (SDA) enriched with cricket powder (Herlinda et al. 2021) for microscopic and macroscopic characterization.

Cultivation and extraction of endophytic fungi, thin layer chromatography (TLC), and LC-MS/MS test

The fungal colonies from the SDA media were transferred to a standard medium, PDB (Nasution et al. 2024), and a new modified medium, GYB. The new modified GYB was enriched with cricket powder for cultivation and extraction. The PDB media contained 200 g of potato, 20 g of dextrose monohydrate, and 1 L aquades (Habisukan et al. 2021). The modified GYB was prepared by the addition of 20 g of yeast, 20 g of sugar, and 1 L aquades (Azish et al. 2020) enriched with 5 g of cricket powder. The culture was subsequently preserved at room temperature (25°C) and 97% relative humidity for four weeks under static conditions. The color change of the sample indicated the production of a secondary metabolite molecule. Furthermore, 300 mL of PDB and GYB media were each dispensed into individual 1 L bottles. Subsequent to the incubation period, the mycelia were extracted from the bulk culture using cylinder paper. Subsequently, 200 mL of ethyl acetate was added to the growth medium and heated to a boil in the partition (Elfita et al. 2019). The EtOAc extract underwent reduced pressure treatment using a rotary evaporator to produce a concentrated extract. The concentrated extract was employed for thin-layer chromatography (TLC) and LC-MS analysis.

The analysis of chemical compounds of fungal extracts was carried out on silica gel Thin Layer Chromatography (TLC) plates (silica gel GF254, Merck) (Praptiwi et al. 2018). The fungal crude extract was dissolved in ethyl acetate at a concentration of 99.8%. Then, the crude extract was applied to a TLC plate. The spots were visualized under ultraviolet (UV) light at 254 nm, yielding additional insights into the compounds present in the extract. Subsequently, the chromatogram was sprayed with 20% H_2SO_4 and heated on a hotplate at 100°C. The spot patterns and retention factors (Rf) were noted as indicators of the diversity of secondary metabolites in the extracts.

Table 1. Species and isolates of endophytic fungi from South Sumatra, Indonesia used in this research

Location (village, district/city)	Coordinates	Altitude (m)	Isolate	Species	Isolate origin	GenBank Acc. No.
Tanjung Pering, Ogan Ilir	104°38'29.058"E 3°12'47.1132"S	36.0	JaTpOi(2)	*Penicillium citrinum*	Maize	MZ359812
Simpang Padang Karet, Pagar Alam	103°15'30.1788"E 4°1'28.0308"S	797.7	JgSPK	*Beauveria bassiana* (Bals.-Criv.) Vuill.	Maize	MZ356494
Tanjung Payang, Pagar Alam	103°14'28.0644"E 4°2'20.8752"S	689.6	CaTpPga	*Metarhizium anisopliae*	Red chili	MZ242073

Note: Source: Herlinda et al. (2021)

The concentrated extract was employed for LC-MS analysis. A crude extract of endophytic fungi underwent an LC-MS analysis utilizing LCM-SMS QTof and the OASIS HLB SPE (Solid Phase Extraction) technique (Qasim et al. 2020). The crude extract of endophytic fungi was obtained through the EtOAc solvent extraction method at a 1:1 ratio with the culture medium. The OASIS HLB SPE cartridge was first activated using methanol. This procedure conditioned the stationary phase in the cartridge to bind the target compound. The crude extract was introduced to the OASIS HLB cartridge. The target compound adhered to the stationary phase within the cartridge while extraneous contaminants were permitted to elude. The cartridge was rinsed with a 5% methanol solution in water to eliminate undesired polar compounds while preserving the target compound attached to the cartridge. The target compound was eluted from the cartridge using pure methanol, a potent solvent that effectively liberated the target compound from the stationary phase. The methanol fraction containing the target compound was evaporated to dryness, and the compound was reconstituted in methanol for subsequent analysis via LC-MS/MS. Upon concluding the SPE process, the sample was analyzed via LC-MS/MS QTof. Liquid Chromatography (LC) segregated compounds according to their physicochemical characteristics, whereas MS/MS QTof identified and characterized the compounds based on their mass-to-charge ratio (m/z). The results were displayed as a mass spectrum, which identified the compound and illustrated the fragmentation of the generated ions.

Bioassay of insecticidal activities of endophytic fungal crude extract

Concentrated extracts from three fungal species (*P. citrinum*, *B. bassiana*, and *M. anisopliae*) were evaluated for toxicity against the first instar larvae of *S. frugiperda*, with three trials conducted, each comprising 50 larvae per repetition and repeated three times. Mass-rearing of *S. frugiperda* followed a method of Sari et al. (2023b). The crude extract of endophytic fungi was weighed to a maximum of 0.1 g and dissolved in 10 mL of ethyl acetate to yield a stock solution of 10,000 ppm. From the stock solution, 100 µL was aliquoted into a microcentrifuge tube and subsequently centrifuged for 15 minutes at a velocity of 150,000 rpm. The solvent was evaporated for 24 hours, after which 10 mL of distilled water was added to the extract to achieve a concentration of 100 ppm. Next, to serve as a control, 10 mL of distilled water was combined with 10 mL of ethyl acetate and subsequently evaporated over 24 hours. Then, 1 mL of the resultant solution was subsequently uniformly applied to corn leaves (2 × 5 cm²). The treated leaves were positioned in a cup lined with damp sterile filter paper, and 50 first instar larvae of *S. frugiperda* were put into the cup containing the corn leaves for their diet. After ingesting the fungal-treated and control leaves for 1 × 24 hours, the larvae were provided with fresh, untreated leaves. The first-instar larvae that proceeded to the second instar were transferred to a separate cup to avoid cannibalistic behavior and provided with fresh corn leaves for their diet (Lestari et al. 2022). The number of dead larvae was recorded daily.

Data analysis

Differences in larval mortality for each treatment were assessed using an analysis of variance. The honestly significant difference (Tukey's test) was utilized to ascertain the differences among the isolates at a significance of 5%. Time and mortality data were subjected to probit analysis. Compounds in crude extracts of the fungi were assessed using TLC and LC-MS/MS analysis.

RESULTS AND DISCUSSION

Secondary metabolites of endophytic fungal crude extract

Three endophytic fungal species used in the present research were *P. citrinum*, *B. bassiana*, and *M. anisopliae*, and their macroscopic and microscopic characteristics were presented in Figure 1. They could produce significantly different wet ($P<0.01$) and dry ($P<0.01$) weights of the colony (Table 2). *M. anisopliae* produced the highest wet and dry weights of the colony, significantly different from those of *B. bassiana* and *M. anisopliae*. The endophytic fungi were grown on the GYB medium, yielding heavier colonies compared to those of the PDB medium.

The TLC analysis of endophytic fungal extracts indicated that the spot patterns from fungi cultured in GYB medium exhibited more variation and were darker in color compared to those cultured in PDB medium (Figure 2). The variations in spot patterns were related to the presence of secondary metabolite compounds in each fungal extract without reagent (Figure 2.A). After spraying with sulfate acid reagent, the spot patterns showed brown to purple for the presence of terpenoid/steroid groups (Figure 2.B). The spot patterns suggested black to dark blue for the presence of phenol/tannin groups with FeCl3 20% reagent (Figure 2.C). The spots displayed yellow to dark orange for the presence of flavonoid/saponin groups with sodium hydroxide reagent (Figure 2.D).

The variations in spot patterns for each endophytic fungal extract displayed different retention factor (Rf) values (0.75-12.40) (Table 3). They related to the presence of different secondary metabolite compounds in each fungal extract. The Rf values of 0.75-2.75 indicated highly polar compounds, but the Rf values of 4.50-12.40 reflected non-polar compounds. The similar Rf value among the fungal isolates represented that the fungal compounds had similar characteristics. The highest RF value (12.40) was found on *B. bassiana* (JgSPK Isolate) cultured in a PDB medium, and this indicated that the compounds from the fungus could move easily in the mobile phase.

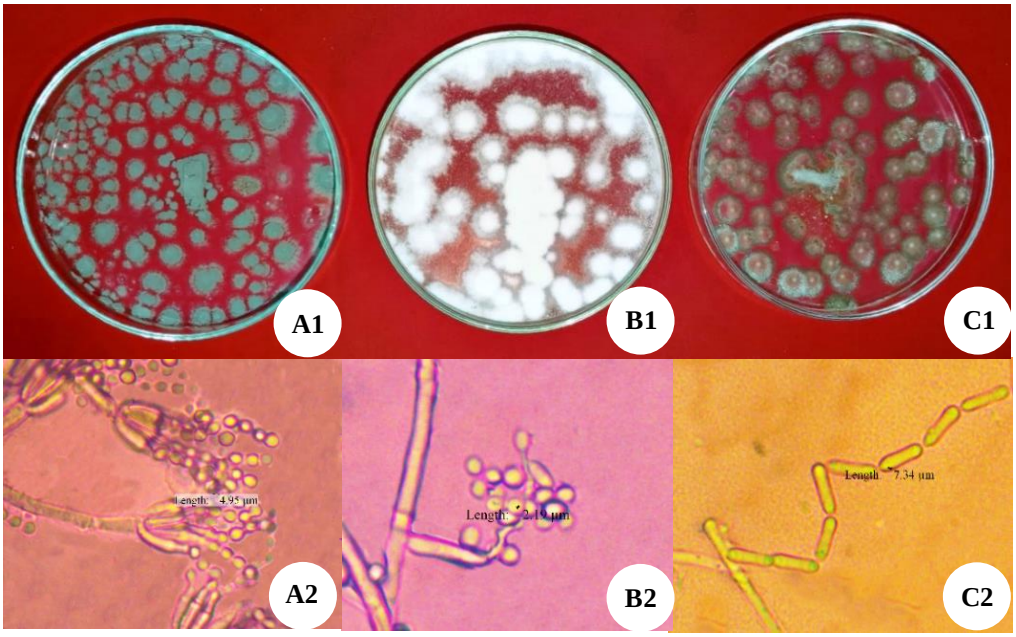

Figure 1. Fungal macroscopic (above) and microscopic (below) characteristics: *Penicillium citrinum* colony (A1) and conidia (A2), *Beauveria bassiana* colony (B1) and conidia (B2), and *Metarhizium anisopliae* colony (C1) and conidia (C2)

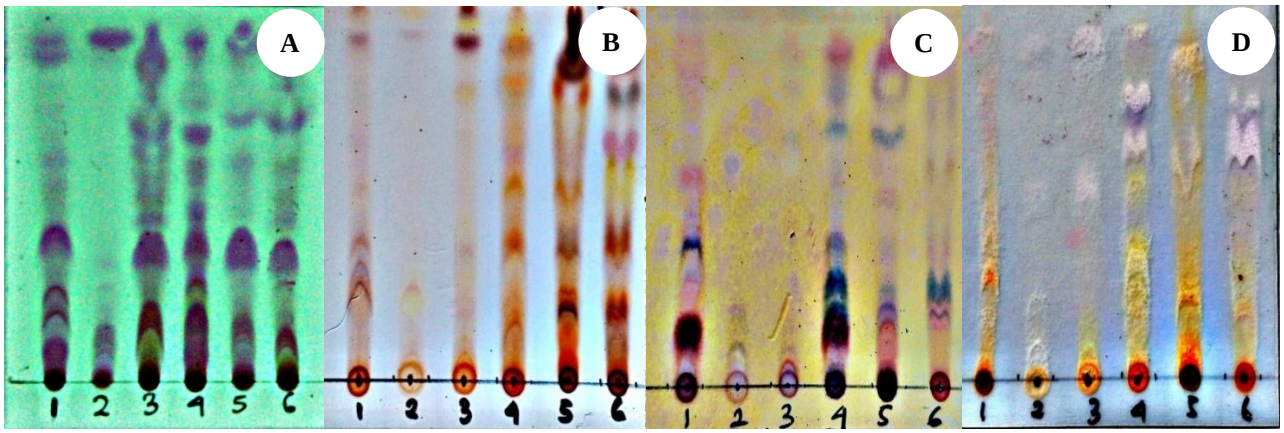

Figure 2. Thin Layer Chromatography (TLC) of endophytic fungi without reagent: A. terpenoids/steroids with sulfate acid reagent; B. phenols/tannins with $FeCl_3$ 20% reagent; C. and flavonoids/saponins with sodium hydroxide reagent; D. Number (below view): endophytic fungi cultured in PDB media: 1. *Penicillium citrinum*, 2. *Beauveria bassiana*, and 3. *Metarhizium anisopliae*; endophytic fungi cultured in GYB media: 4. *Penicillium citrinum*, 5. *Beauveria bassiana*, and 6. *Metarhizium anisopliae*

Table 2. Production of filtered culture after four weeks of cultivation

Media and fungal isolates	Fungal species	Average filter results (mL)	Wet weight of fungal colony (g)	Dry weight of fungal colony (g)
GYB Media				
JgTpOi(2)	*Penicillium citrinum*	207.00	11.16ab	0.94b
JgSPK	*Beauveria bassiana*	202.00	10.56b	1.06ab
CaTpPga	*Metarhizium anisopliae*	204.40	15.25a	1.58a
PDB Media				
JgTpOi(2)	*Penicillium citrinum*	211.60	8.79b	0.88b
JgSPK	*Beauveria bassiana*	209.80	10.02b	0.92b
CaTpPga	*Metarhizium anisopliae*	229.00	12.88ab	1.51a
F value		0.24^{ns}	4.76^{*}	6.26^{*}
P value		0.94	3.66×10^{-3}	7.56×10^{-4}
HSD value		-	4.61	0.55

Note: ns: not significant; *: significant difference, values in columns followed by the same letter are not significant differences at $P < 0.05$ according to the Tukey HSD test

Table 3. Detection of the presence of terpenoids/steroids, phenols/tannins, and flavonoids/saponins in a crude extract of endophytic fungi

Fungal culture media	Fungal species	TLC Retention factor (Rf) (mm)	Biochemical test					
			Steroids	Terpenoids	Tannins	Phenols	Flavonoids	Saponins
GYB	*Penicillium citrinum*	2.25	+	-		+	+	-
		3.25	+	-	+	-	+	-
		2.00	+	-	+	-	-	-
		3.00	-	-	-	-	+	-
		3.00	+	+	-	-	-	-
		3.25	+	-	+	-	-	-
		2.75	+	-	+	-	-	-
		2.75	+	-	+	-	-	-
		3.25	-	-	-	-	-	-
	Beauveria bassiana	3.00	+	-		+	+	-
		1.50	+	-	+	-	-	-
		5.50	+	-	-	-	-	-
		2.75	+	-	+	-	-	-
		1.50	+	-	+	+	+	-
		4.00	+	-	-	+	+	-
	Metarhizium anisopliae	1.25	+	-	-		-	-
		4.50	+	-	-	+	-	-
		2.75	+	-	-	-	-	-
		2.50	+	+	-	-	-	-
		3.00	-	-	-	-	-	-
		4.75	+	-	-	-	-	-
		1.00	+	-	+	-	-	-
		0.75	+	-	-	+	+	-
PDB	*Penicillium citrinum*	1.25	+	-	-	-	-	-
		1.25	+	+	-	-	-	-
		3.75	-	-	-	+	-	-
		2.50	+	-	+	-	+	-
		2.75	+	+	+	+	-	-
		3.50	+	-	+	-	+	-
	Beauveria bassiana	12.40	+	-	-	-	-	-
		3.50	+	-	-	+	-	-
		1.50	+	-	+	-	-	-
	Metarhizium anisopliae	6.50	+	-	-	-	+	-
		2.75	+	-	-	-	-	-
		1.00	-	-	-	-	-	-
		5.25	+	+	-	-	-	-
		4.50	-	-	-	-	-	-
		3.50	+	-	-	+	-	-

Note: + showed the presence of secondary metabolite compounds, and - showed the absence of secondary metabolite compounds

The compound content of crude extract from *P. citrinum* cultured in GYB and PDB media was analyzed using LC-MS/MS. Chromatogram of the fungal extracts of *P. citrinum* from GYB culture showed a major peak and three minor peaks, but the extracts from PDB culture showed a major peak and a minor peak (Figure 3). LC-MS/MS analysis identified key compounds of the crude extract of *P. citrinum* cultured in GYB medium were guanidine acetate, 8-deoxygartanin, abafungin, and 2-amino-5-carboethoxy-4-hydroxypyrimidine. However, the compounds from the crude extract of *P. citrinum* cultured in PDB medium were only isoquinoline and leucic acid (Table 4). The chromatogram of the fungal extracts of *B. bassiana* cultured in GYB medium showed a major peak and a minor peak, but the extracts from PDB medium showed a major peak and four minor peaks (Figure 4). The compounds of crude extract from *B. bassiana* cultured in GYB were Psoralidin and L-(+)-Lysine, and the compounds from PDB culture were such as adenine, guanidine acetate, ethyl carbazate, aceglutamide, and L-(+)-Lysine (Table 5). Compounds from peaks in the LCMS/MS Chromatograms of crude extract of *M. anisopliae* cultured in GYB medium were cytosine, adenine, n-aminoguanidine, azanidazole, phenol, methyl carbazate, and antiarol and in PDB medium were 3-(Dimethylamino)-1-propanethiol and 2-(7H-Pyrrolo[2,3-d] pyrimidin-4-yl)-1H-isoindole-1,3(2H)-dione (Figure 5 and Table 6). The LC-MS/MS analysis indicated that endophytic fungi cultured in GYB medium produced a greater diversity of secondary metabolite compounds compared to those cultured in PDB medium.

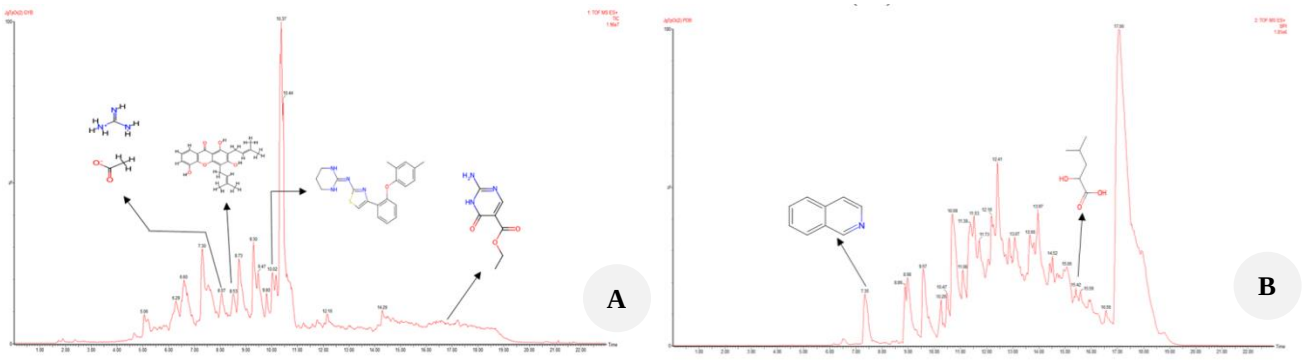

Figure 3. Compounds of crude extract from *Penicillium citrinum* cultured in GYB medium (A) and PDB medium (B)

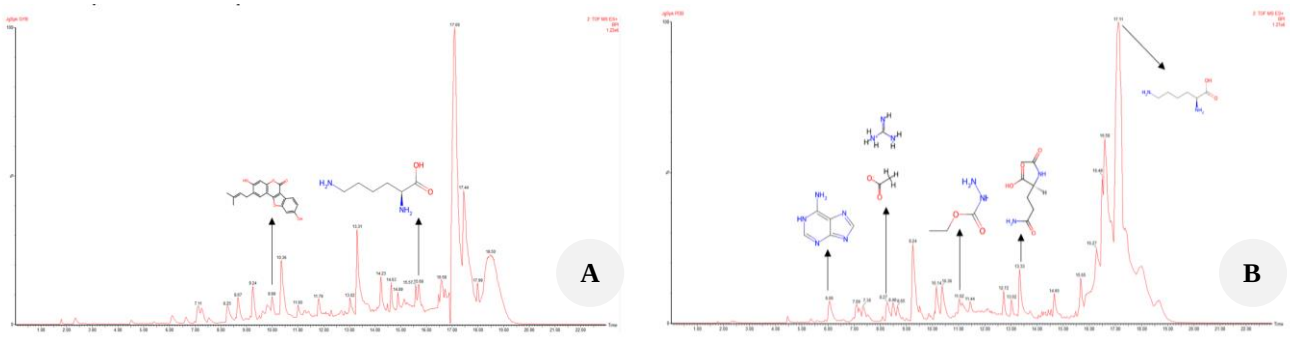

Figure 4. Compounds of crude extract from *Beauveria bassiana* cultured in GYB medium (A) and PDB medium (B)

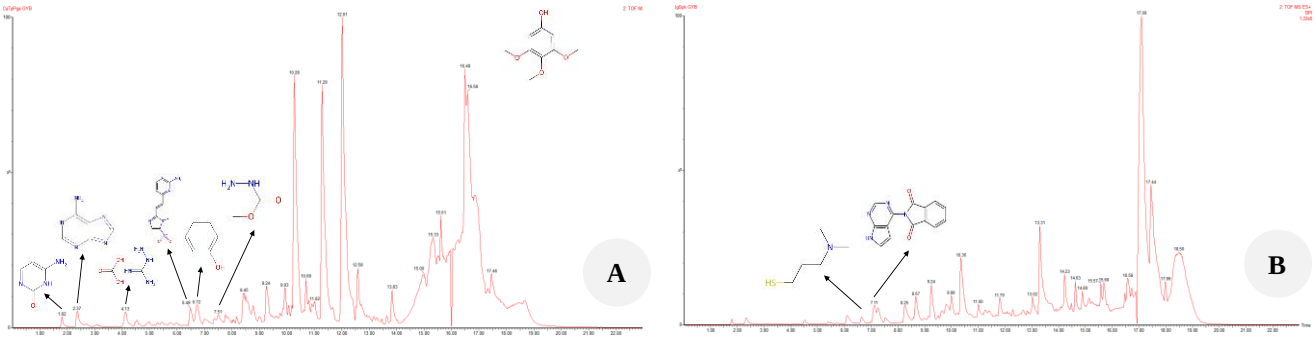

Figure 5. Compounds of crude extract from *Metarhizium anisopliae* cultured in GYB medium (A) and PDB medium (B)

Table 4. Compounds from peaks in the LC-MS/MS Chromatograms of the crude extract of *Penicillium citrinum* cultured in GYB and PDB medium

Fungal culture media	Compound	Molecular formula	Molecular weight (m/z) $[M + H]^+$	Retention time (min)
GYB	Guanidine acetate	$C_3H_{10}N_3O_2$	120.0773	8.07
	8-deoxygartanin	$C_{23}H_{25}O_5$	381.1702	8.73
	Abafungin	$C_{21}H_{23}N_4OS$	379.1593	10.02
	2-amino-5-carboethoxy-4-hydroxypyrimidine	$C_7H_{10}N_3O_3$	184.0722	16.65
PDB	Isoquinoline	C_9H_8N	130.0657	7.35
	Leucic acid	$C_6H_{13}O_3$	133.0865	15.42

Table 5. Compounds from peaks in the LC-MS/MS Chromatograms of the crude extract of *Beauveria bassiana* cultured in GYB and PDB medium

Fungal culture media	Compound	Molecular formula	Molecular weight (m/z) $[M + H]^+$	Retention time (min)
GYB	Psoralidin	$C_{20}H_{17}O_5$	337.1076	9.99
	L-(+)-Lysine	$C_6H_{15}N_2O_2$	147.1134	15.68
PDB	Adenine	$C_5H_6N_5$	136.0623	6.06
	Guanidine acetate	$C_3H_{10}N_3O_2$	120.0773	8.27
	Ethyl Carbazate	C3H9N2O2	105.0664	11.02
	Aceglutamide	$C_7H_{13}N_2O_4$	189.0875	13.33
	L-(+)-Lysine	$C_6H_{15}N_2O_2$	147.1134	17.11

Table 6. Compounds from peaks in the LC-MS/MS Chromatograms of the crude extract of *Metarhizium anisopliae* cultured in GYB and PDB medium

Fungal culture media	Compound	Molecular formula	Molecular weight (m/z) $[M + H]^+$	Retention time (min)
GYB	cytosine	$C_4H_6N_3O$	112.0511	1.82
	Adenine	$C_5H_6N_5$	136.0623	2.37
	N-aminoguanidine	$C_2H_9N_4O_3$	137.0675	4.13
	Azanidazole	$C_{10}H_{11}N_6O_2$	247.0943	6.49
	Phenol	C_6H_7O	95.0497	6.72
	Methyl carbazate	$C_2H_7N_2O_2$	91.0508	7.51
	Antiarol	$C_9H_{13}O_4$	185.0814	16.49
PDB	3-(Dimethylamino)-1-propanethiol	$C_5H_{14}NS$	120.0847	6.54
	2-(7H-Pyrrolo[2,3-d] pyrimidin-4-yl)-1H-isoindole-1,3(2H)-dione	$C_{14}H_9N_4O_2$	265.0726	7.42

Table 7. Larval cumulative mortality caused by a crude extract of endophytic fungi cultured in GYB and PDB Media

Media and fungal species	Larval cumulative mortality (%)								
	2nd day	4th day	6th day	8th day	10th day	12th day	14th day	16th day	18th day
Aquades (control)	0.00	0.67b	2.00b	2.00b	2.00b	2.00b	2.00d	2.00c	2.00d
GYB									
Penicillium citrinum	4.00	20.00a	38.67a	51.33a	62.00a	70.00a	82.00abc	89.33ab	98.00a
Beauveria bassiana	5.33	18.67a	36.67a	54.67a	68.00a	78.00a	88.67ab	96.67a	96.67a
Metarhizium anisopliae	2.00	21.33a	33.33a	44.00a	62.00a	80.67a	93.33a	97.33a	97.33a
PDB									
Penicillium citrinum	4.00	18.00a	31.33a	42.67a	53.33a	62.00a	72.67bc	82.67ab	84.00bc
Beauveria bassiana	8.00	18.67a	36.00a	52.00a	70.00a	80.67a	89.33ab	93.33ab	93.33ab
Metarhizium anisopliae	4.67	19.33a	36.67a	47.33a	54.00a	60.67a	63.33c	72.67b	74.67c
F-value	1.59^{ns}	72.30^{*}	11.60^{*}	15.62^{*}	31.31^{*}	47.31^{*}	65.77^{*}	41.87^{*}	82.06^{*}
P-value	0.22	7.94×10^{-04}	11.23×10^{-04}	1.77×10^{-05}	2.45×10^{-07}	16.80×10^{-08}	18.80×10^{-09}	37.40×10^{-08}	42.30×10^{-10}
HSD value	-	15.03	16.40	17.66	15.10	14.26	14.09	19.75	14.53

Note: ns: not significant, *: significant difference; values in columns followed by the same letter are not significant differences at $P < 0.05$ according to the Tukey HSD test

Insecticidal activities of endophytic fungal crude extract

The fungal crude extracts containing the secondary metabolite compounds were dripped on the corn leaves for the diet of the test larvae. The findings indicated that the cumulative mortality of larvae induced by a crude extract of endophytic fungi cultured in GYB medium was significantly different ($P<0.001$) from that in PDB medium (Table 7). However, the lethal time (LT_{50} and LT_{95}) of larvae after consuming a crude extract of endophytic fungi cultured in GYB was not significantly different from that in PDB medium (Table 8). The lethal time of larvae after consuming a crude extract of endophytic fungi was significantly different from that of the control (untreated fungal extract). At the last observation (18th day after fungal application), the highest larval cumulative mortality occurred on *P. citrinum* extract from GYB culture (98%). It was not significantly different from those induced by *B. bassiana* (96.67%) and *M. anisopliae* (97.33%) extracts from GYB culture and *B. bassiana* (93.33%) extract from PDB culture. Three species of fungi (*P. citrinum*, *B. bassiana*, and *M. anisopliae*) exhibited significant insecticidal activity; however, their toxicity was affected by the fungal culture medium. In the current research, the GYB fungal culture exhibited greater toxicity than the PDB fungal culture.

Table 8. Lethal time (LT_{50} and LT_{95}) of larvae after consuming a crude extract of endophytic fungi cultured in GYB and PDB Media

Media and fungal species	LT_{50} (days)			LT_{95} (days)		
	Lethal time	**Lower**	**Upper**	**Lethal time**	**Lower**	**Upper**
Aquades (control)	20.14±0.02a	20.58a	21.52a	25.57±0.02a	24.06a	27.43a
GYB						
Penicillium citrinum	4.19±0.13b	5.22b	5.23b	9.74±0.13b	8.76b	10.96b
Beauveria bassiana	4.52±0.12b	4.69b	5.10b	9.63±0.12b	8.64b	10.85b
Metarhizium anisopliae	6.37±0.13b	4.10b	5.61b	10.22±0.13b	9.22b	11.45b
PDB						
Penicillium citrinum	4.09±0.18b	5.07b	7.41b	10.39±0.18b	9.45b	11.55b
Beauveria bassiana	4.67±0.12b	4.31b	5.95b	9.84±0.12b	8.83b	11.08b
Metarhizium anisopliae	6.52±0.13b	4.35b	4.82b	10.10±0.13b	9.16b	11.25b
F-value	101.70*	89.85*	113.8*	100.20*	89.55*	111.60*
P-value	9.88×10^{-11}	2.29×10^{-10}	4.58×10^{-11}	1.09×10^{-10}	2.34×10^{-10}	5.23×10^{-11}
HSD value	2.82	2.91	2.76	2.84	2.91	2.81

Note: *: significant difference; values in columns followed by the same letter are not significant difference at $P < 0.05$ according to the Tukey HSD test

Discussion

The current investigation revealed that the crude extracts of *P. citrinum* from GYB culture could produce the secondary metabolites, namely guanidine acetate, 8-deoxygartanin, abafungin, and 2-amino-5-carboethoxy-4-hydroxypyrimidine. The guanidine acetate is known as biocidal (Drozdov and Kotov 2021). The presence of guanidine acetate in the *P. citrinum* extracts may significantly increase mortality rates (98%) in *S. frugiperda* larvae in this study. Other secondary metabolites derived from *P. citrinum* extracts may also enhance insecticidal activities. For instance, 8-deoxygartanin is characterized as cytotoxic (Masullo et al. 2022). The compound of 2-amino-5-carboethoxy-4-hydroxypyrimidine is known as the enzyme inhibition (Chandrappa et al. 2021). Enzyme inhibitors may disrupt metabolic processes in insects, potentially leading to stunted growth, reduced reproduction, or increased mortality. Isoquinoline and leucic acid were detected in the crude extracts of *P. citrinum* from PDB culture. Isoquinoline was recognized for its inhibitory effects (Karanja et al. 2022) and could inhibit the movement of second-stage larvae of *Toxocara canis* (Werner, 1782) Stiles, 1905 (Satou et al. 2002). Isoquinoline had a nematicidal activity (Shang et al. 2019). The presence of isoquinoline in this study may also influence the neuromuscular systems of *S. frugiperda*, depending on coordinated muscle movement. However, leucic acid was recognized as a supplement that inhibits obesity-related inflammation in humans (Lee et al. 2024). The identification of leucic acid in the crude extract of *P. citrinum* grown from PDB culture may augment overall bioactivity. Synergistic interactions among several substances may result in an enhanced inhibitory impact on larval insects.

The crude extracts of *B. bassiana* from GYB culture could produce secondary metabolites, such as psoralidin and L-(+)-lysine. Psoralidin exhibits cytotoxic characteristics, similar to L-(+)-Lysine, which can disrupt cellular functions (Costa and Silva 2022). They could potentially impair essential processes in the target insects. The secondary metabolites, adenine, guanidine acetate, ethyl carbazate, aceglutamide, and L-(+)-lysine were detected in the crude extracts of *B. bassiana* from PDB culture. High concentrations of adenine have toxic effects (Yin et al. 2024). Guanidine acetate is recognized for its cytotoxic effects (Liang et al. 2021). Aceglutamide is associated with cognitive enhancement and stimulating effects on the central nervous system (Zhang et al. 2015). L-(+)-Lysine was reported as cytotoxic (Gorzkiewicz et al. 2020). These chemical compounds may interact synergistically to enhance the efficacy of *B. bassiana* in insect pest control, thereby increasing its metabolic flexibility and adaptability to diverse biological environments. Nonetheless, the exact function of these chemicals in biological control will be elucidated through additional and future research.

The LC-MS investigation of the crude extract from *M. anisopliae* cultivated in a GYB medium identified cytosine, adenine, N-aminoguanidine, azanidazole, phenol, methyl carbazate, and antiarol. Cytosine plays a crucial role in gene regulation (Ngo et al. 2016). Nonetheless, irregularities in adenine metabolism or elevated levels of adenine in some situations may result in hazardous consequences (Yin et al. 2024). N-aminoguanidine is recognized as a potential inhibitor of ribonucleotide reductase (RR) (Ma et al. 2020). Ribonucleotide reductase is an essential enzyme in nucleotide metabolism, as it facilitates the conversion of ribonucleotides into deoxyribonucleotides, which are essential for DNA synthesis and repair (Lundin et al. 2015). N-aminoguanidine's presence in the crude extract of *M. anisopliae* suggests that it may function as an RR inhibitor of insect larvae, *S. frugiperda*, which can lead to a decrease in deoxyribonucleotides and then affect insect metabolism. Azanidazole is recognized for its antimicrobial properties (Shivhare and Vinode 2023). Phenolics are acknowledged for their antimicrobial properties (Ergüden 2021). Methyl carbazate is a commonly studied enzyme inhibitor (Bhat et al. 2024). Antiarol is recognized for its antibacterial properties (Deryabin and Tolmacheva 2015). The constituents discovered in the crude extract of *M. anisopliae* grown in GYB medium indicated that the extract possesses chemical agents that may demonstrate antibacterial activities and toxicity against *S. frugiperda*. However, further research is needed to verify these findings. The crude extracts of *M. anisopliae* from PDB culture could produce 3-(Dimethylamino)-1-propanethiol

and 2-(7H-pyrrolo[2,3-d] pyrimidin-4-yl)-1H-isoindole-1,3(2H)-dione. The 3-(Dimethylamino)-1-propanethiol is a chemical reagent (Laroussi 2022). The 3-(Dimethylamino)-1-propanethiol is an organic compound with a thiol functional group and a dimethylamino group (Kutsumura et al. 2020). Compounds with a thiol group have been studied for alteration in insect behavior and the existence of this secondary metabolite may also influence insect behavior, which can be utilized in pest management strategies (Oliveira et al. 2022).

Finally, these research findings indicate that the endophytic fungi (*P. citrinum*, *B. bassiana*, and *M. anisopliae*) cultured in GYB medium have the potential to synthesize more secondary metabolite compounds, and the metabolite compounds have high insecticidal activities, inducing high larval mortality. *Penicillium citrinum* can produce metabolite compounds, such as guanidine acetate and 8-deoxygartanin. The metabolite compounds, such as psoralidin and L-(+)-lysine, can be extracted from *B. bassiana*. *Metarhizium anisopliae* can produce metabolite compounds, such as N-aminoguanidine and methyl carbazate. The secondary metabolite compounds of the endophytic fungi are potential active compounds of new insecticides.

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