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Identification and Production of Beauvericin by *Fusarium subglutinans*and *F. sacchari* from Sugarcane

AeshahMhana Mohammed[1],
https://orcid.org/0000-0002-8001-4175

Laith Khalil Tawfeeq Al-Ani [2,3*]
https://orcid.org/0000-0001-5138-0224

[1]University of Baghdad,, College of Education for pure science (Ibn al-Haytham), Department of Chemistry, Baghdad, Iraq; [2]University of Baghdad, College of Agriculture Engineering Science, Department of Plant Protection, Baghdad, Iraq; [3]Universiti Sains Malaysia, School of Biology Science, Minden, Pulau Pinang, Malaysia.

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*Correspondence: cmv_virus2002@yahoo.com (L.K.T.A.A.).

HIGHLIGHTS

- Isolation two species of pathogens *Fusarium* from sugarcane.
- Identification the difference in morphological characterization between two species of Fusarium.
- Determination the ability of pathogens *Fusarium* to produce mycotoxin Beauvericin.
- Determination of the toxicity Beauvericin on the brine shrimp.

Abstract: *Fusarium* is producing several important mycotoxins including beauvericin (BEA). Two species of *Fusarium* viz. *F. subglutinans* and *F. sacchari* cause the Pokkahboeng disease of sugarcane. The studies on the occurrence and toxicity of BEA are scarce. Therefore, this study aimed to identify the isolates of *Fusarium* and detect their ability to produce BEA. The toxicity of BEA was also tested on brine shrimp *Artemia salina*. Many isolates of *Fusarium* were isolated from the infected plants of sugarcane in Malaysia. We identified the species of *Fusarium* according to their morphological characteristics. The capability of *Fusarium* isolates for producing the BEA was estimated by using a thin layer chromatography. The toxicity bioassay of BEA was conducted on the brine shrimp larvae. The results were identified on *F. subglutinans* and *F. sacchari* in 55 isolates of *Fusarium*. All isolates demonstrated the ability to produce BEA. Interestingly, BEA exhibited variation in toxicity between low toxic to very higher toxicity 100%. *F. subglutinans* and *F. sacchari* were able to produce BEA and possibly BEA may be causing toxicity in the host tissue and may be acting as a potential pathogenicity factor. Therefore, we consider BEA as an interesting factor in determining the virulence of fusarium isolate.

Keyword: *Fusarium*; *F. sacchari;F. subglutinans;* Beauvericin; Brine shrimp; mycotoxin.

INTRODUCTION

Sugarcane is one of the top ten food crops in the world and an important economic crop in many tropical countries [1]. Sugarcane is infested with many plant pathogens including fungi, bacteria, and viruses as well as, insect pests. The genus *Fusarium* is one of the most devastating plant pathogens producing several mycotoxins [2]. Two species of *Fusarium* including *F. subglutinans* and *F. sacchari*are well reported to cause Pokkahboeng disease of sugarcane. *Fusarium* species are able to produce numerous phytotoxins and mycotoxins that could be causing major diseases in humans, animals, and plants [3-5]. Mycotoxins were produced by *Fusarium* species and other fungi comprising moniliformin, fusaproliferin, fusarins, butenolides, beauvericin, enniatins, and fusaric acid, etc [6-10]. One of interesting mycotoxins is a beauvericin affecting for health of animals and human due to this toxin is more common contaminant for grains after infection with *Fusarium* [10-11].

Beauvericin (BEA) is a bioactive cyclodepsipeptide that contains three of N-methyl-L-phenylalanyl and D-a-hydroxy-isovaleryl [12]. It was first isolated from entomopathogenic fungi *Beauveriabassiana*[13-14]. However, the first detected BEA of *Fusarium* species was by Gupta and coauthors [15]. It is limiting data on the occurrence and toxicity of BEA compared with other mycotoxins. BEA was considered as insecticidal and phytotoxic that exhibited several biological properties [15], antibacterial [16], as well as, showing cytotoxicity towards cell lines from invertebrates, animals and human [17]. The methods for the detection of mycotoxins include gas chromatography (GC), GC-MS, enzyme-linked immunosorbent assay (ELISA), high performance liquid chromatography (HPLC), matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF/TOF), liquid chromatography-tandem mass spectrometry (LC-MS/MS), and tin-layer chromatography (TLC) [18-21]. TLC is using a qualitative screening method that assayed many mycotoxins [22].

Several methods have been used for determining the toxicity of mycotoxins by bioassay test. Bioassay could test on organisms including microorganisms, insects, aquatic animals, insects, plants, tissue culture, and organ. There are three bioassays systems including brine shrimp, zebrafish, and chicken embryo known to be able to detect more than 10 types of mycotoxins [23]. The popular choice for the bioassay test is brine shrimp according to the high sensitivity to mycotoxin [24-25]. Therefore, the study aims to identify the *Fusarium* isolates and investigate their ability for producing BEA. Then, the biotoxicity of BEA was determined using brine shrimp (*Artemiasalina*).

MATERIALS AND METHODS

Culture of *Fusarium* isolates from sugarcane

A total of 55 isolates for *Fusarium* spp. were reculturedfrom the *Fusarium* culture collection unit, School of Biology Science, University Sains Malaysia, Penang, Malaysia. These isolates were taxonomically identified in a previous study [26] by using the *Fusarium* laboratory manual for Leslie and Summerell [2].

Identification of the *F. subglutinans* and *F. saccharif*rom sugarcane

Morphological characteristics were used to identify *F. subglutinans* and *F. sacchari* isolates to reconfirm their identity from the stock culture. The morphological descriptions were based on Booth [27], Gerlach and Nirenberg [28], Nelson and coauthors [29], Burgess and coauthors [30], Leslie and Summerell [2], and Wijayawardeneand coauthors [31]. To study microscopic characteristics, the isolates cultured onto CLA (Carnation Leaf Agar) for 7 to 10 days [32]. Morphological features observed for identification of *Fusarium* species:

A. Macroconidia: Presence or absence, overall shape, shapes of apical and basal cell and the number of septa.
B. Microconidia: Presence or absence, shape, and the number of septa.
C. Mesoconidia: Presence or absence.
D. Conidiophores: Presence or absence, monophialides, and polyphialides.
E. Chlamydospores: Presence or absence, mode of formation, and cell wall.

All the characteristics were observed using a light microscope (Olympus model BX-50F4) and photographed using a camera (JVC model KY-F55BE) with an image Analyzer-single image stereogram (SIS) program. For macroscopic characteristics, PDA (Potato Dextrose Agar) was used for observation of

culture appearances, such as the texture of the colony, colony colour, pigmentations, and growth pattern. Mycelial disc of 6 mm diameter was plated onto the fresh PDA plate and the growth rates were recorded after 3 days of incubation. The culture appearance of each isolate on PDA was visually assessed after the mycelia were fully grown. The determination of colony colour and pigmentation was based on the colour description in the Methuen handbook of colour chart [33].

Cultivation of fungal isolates for mycotoxin screening

The *Fusarium* isolates were transferred to PDA plates and incubated for 7 to 10 days. Corn grits were used as a culture medium to analyses the presence of mycotoxin as it enhances the mycotoxin production. 15 g of corn grits with 45% of moisture was autoclaved in 250 ml Erlenmeyer flask. Spore suspension (approx. $1x10^6$ spores) of 1-2 week old culture was prepared to inoculate on the autoclaved corn grit. The inoculated corn grit and control (uninoculated corn girt) were incubated in dark at room temperature for 28 days. Control was treated the same except inoculants was substituted with substituted with sterilized double distilled water.

Extraction of Beauvericin (BEA)

BEA was extracted from 28 days old inoculated corn grit following the procedure of Logriecoand coauthors [34]. 15 g of corn grits were extracted overnight with 75 ml of acetonitrile, methanol, and water (16:3:1) and grounded in a warring blender for 5 min. The crude extracted was filtered through Whatman no.4 filter paper that was defatted twice with 25 ml of n-heptane. The bottom layer was evaporated to near dryness at 80°C by a rotary evaporator (Buchi 461, Switzerland). The residue was dissolved in 50 ml of a mixture of methanol and water with 1:1 proportion. Extraction was carried out twice using 25 ml of dichloromethane, which was evaporated and re-dissolved in 50 ml mixture of methanol and water with 1:1 proportion. Extraction was carried out twice using 25 ml of dichloromethane. BEA in the dichloromethane was evaporated and re-dissolved in 1 ml of methanol prior to the detection of BEA through TLC. The extracted mycotoxins were stored in a refrigerator.

Detection of Beauvericin (BEA)

The detection of BEA was carried out by using thin layer chromatography (TLC). TLC is a sensitive method for mixture analysis by separating the compound in the mixture. About 5 to 10 µl of extract were spotted onto a 20 x 20 cm silica TLC plates (Pre-coated with silica gel 60 F254; E. Merck AG, Darmstadt, Germany) along with the standard of BEA at 200 ppm from Sigma, USA. Plates developed in the solvent system according to Song and coauthors [35] in a mixture of acetic acid, methanol, water (100:5:1). The plate was air-dried and subsequently, the spot on the TLC plate detected by iodine vapour. Retention factor (R_f) values for the standard and samples were measured. The R_f values were calculated according to Touchstone [36] and Fessenden and coauthors [37]:

$$R_f = \frac{\text{Distance travelled by the compound (Y)}}{\text{Distance travelled by the compound (X)}} \quad (1)$$

Bioassay test on brine shrimp, *Artemia salina*

Brine shrimp medium (BSM) described by Panigrahi and Dallin [38] with slight modification was used. This was prepared by using 30 g sodium chloride; 0.3 g calcium chloride dehydrate; 1.6 g magnesium chloride hexahydrate; 0.5 g magnesium sulphate heptahydrate; 0.8 g potassium chloride; and 6.0 g glycine. The BSM was autoclaved at 121°C for 15 min stored in a brown colored Scott bottle.

Toxicity of each detectable BEA to brine shrimp larvae (*Artemia salina* L.) was determined. Dried *A. salina* eggs were hatched in BSM prepared for 24 hours at 27°C in a small water tank provided with the aeration from the motor. 30 mature larvae were selected and transferred into 24-well cell culture plates by exposing it with 5 µl of mycotoxin extract and sterile BSM was added until 500 µl. The test performed in triplicate for BEA extracted against BSM and methanol as a control. Numbers of dead larvae were counted in each dish after 24 hours of incubation at 27°C. Surviving larvae were killed by freezing at -20°C for 12 hours.

Statistical analysis

The toxicity of BEA from *F. sacchari* and *F. subglutinans* on adult were studied on *A. salina* (Brine shrimp medium). One treatment was carried after 24 hours, data were subjected to analysis of variance test (95% confidence level) using independent samples T-test mean values are presented. The mortality was recorded in the control afterward, for LC_{50}, data were analyzed by analysis of variance T-test (Spss 20.0 version).

RESULTS

Identification of *F. subglutinans* and *F. sacchari*

A total of 55 different isolates grew from *Fusarium* Laboratory consisting of *F. sacchari* and *F. subglutinans* which was the causal agent of Pokkahboeng disease in sugarcane. Observation of pigmentation, macroscopic and microscopic characteristics were done after cultured on PDA and CLA. This media culture is mainly used for identification purposes as recommended by Leslie and coauthors [2].

The colony morphology of *F. sacchari* showed abundant mycelia growth after 7 days of incubation on PDA (Figure 1-A). In addition, the pigmentation produced in various colour from pale violet, pink, and peach (Figure 1-B). The observation on CLA, macroconidia usually had 3-septation with slightly falcate and thin-walled (Figure 2 A). The apical cells curved while the basal cells were poorly developed. Microconidia with oval shape together with 1-septate mesoconidia (Figure 2, (A) and (B)) and simple polyphialides were observed on the prepared slide (Figure 3) Mesoconidia and microconidia in situ were present on CLA (Figure 4, (A) and (B)). Chlamydospores and microconidia chains were absent.

For *F. subglutinans*, the mycelia were abundantly growing on PDA whereas the pigmentations ranging from pale violet to deep violet (Figure 5). From in situ observation on CLA, macroconidia were sparsely formed, slender, and thin-walled with 3- or 4- septa (Figure 6 A). Curved apical cells and poorly developed basal foot-shaped cells were recorded. Oval shaped microconidia with 0-septate (Figure 6 B and 7 A) were found from agar plates attached to monophialides or polyphialides (Figure 7). No chlamydospores or microconidia chains were observing in CLA.

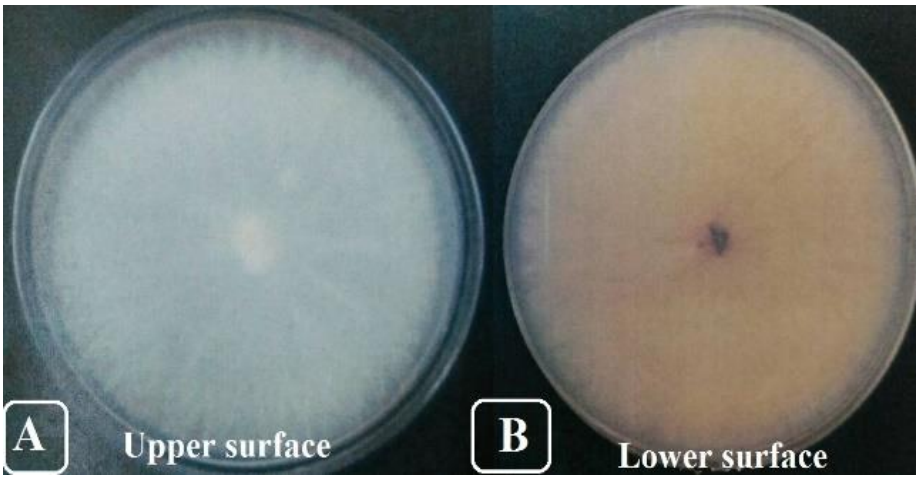

Figure 1. Colony appearance and pigmentation of *Fusariumsacchari* on PDA. A) Upper surface, B) lower surface.

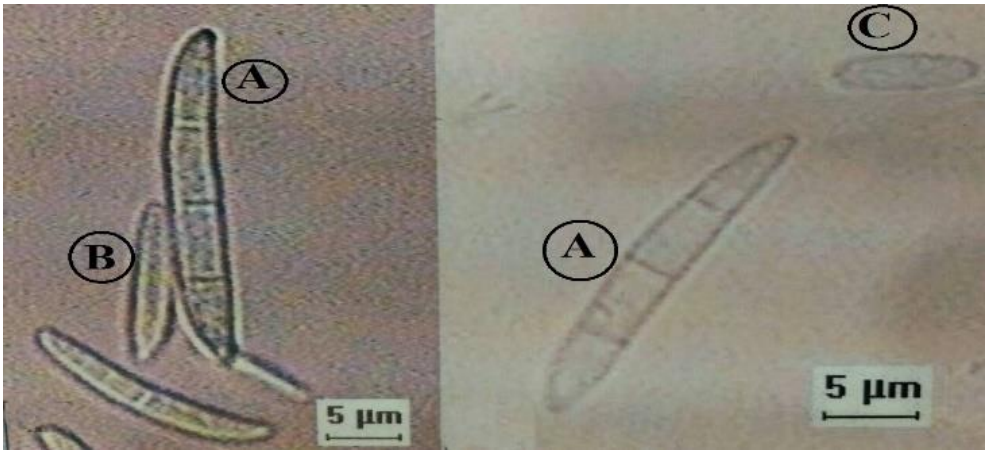

Figure 2. Microscopic characteristics of *Fusariumsacchari*, A) Macroconidia, B) Mesoconidia, C) Microconidia.

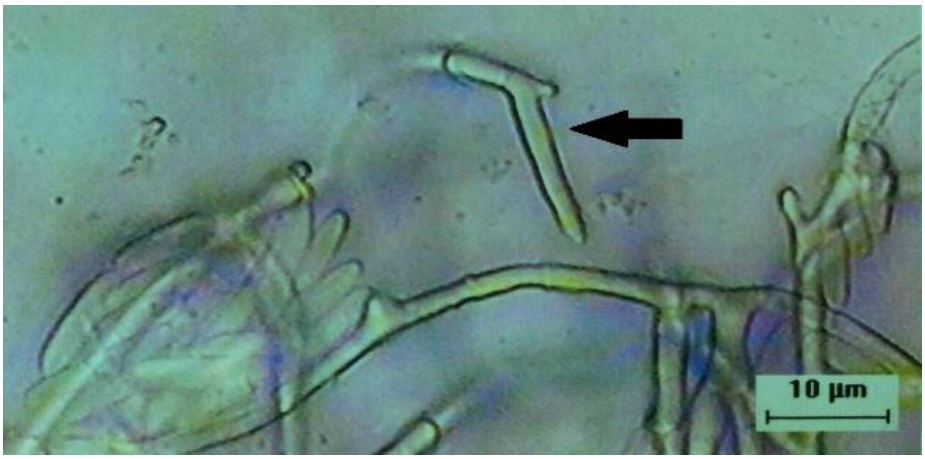

Figure 3. Simple polyphialidicconidiophores of *Fusariumsacchari* aerial mycelium

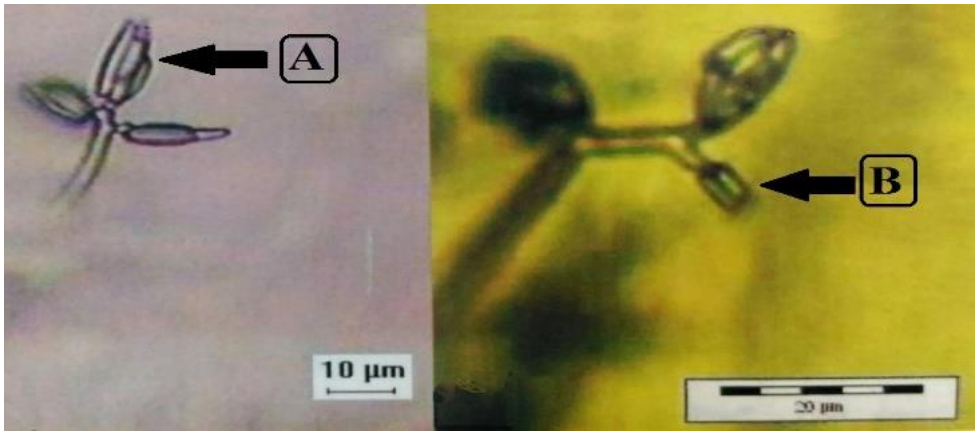

Figure 4. Polyphialides of Fusarium sacchari on CLA. A) Mesoconidia, B) Microconidia

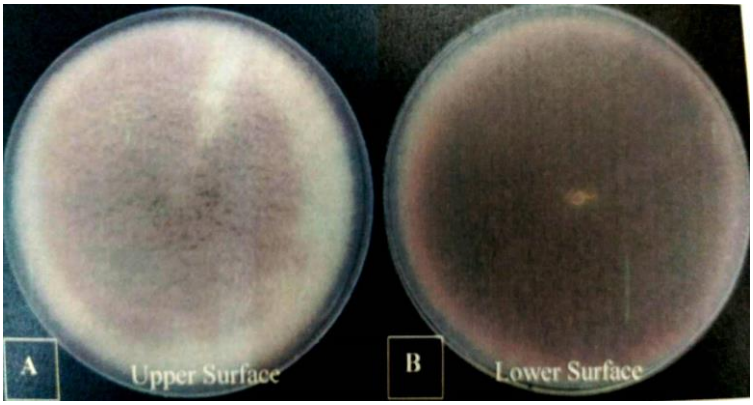

Figure 5. Colony appearance and pigmentation of *Fusariumsubglutinans* on PDA. A) Upper surface. B) Lower Surface.

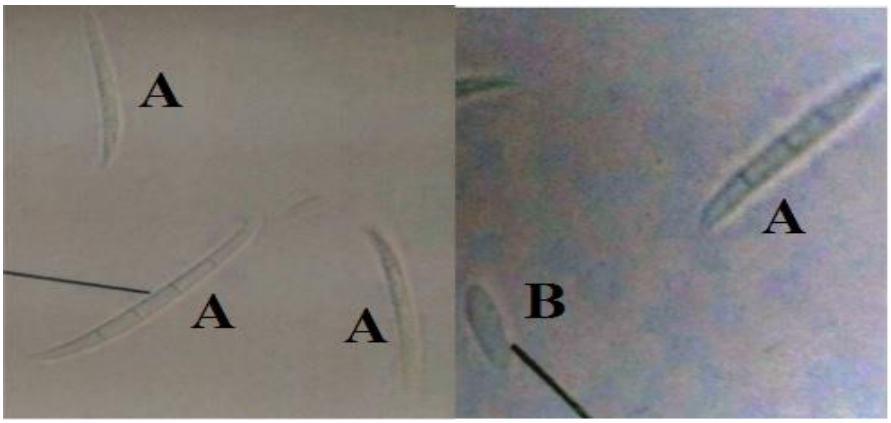

Figure 6. Microscopic characteristics of *Fusariumsubglutinans*, A) Macroconidia, B) Microconidia.

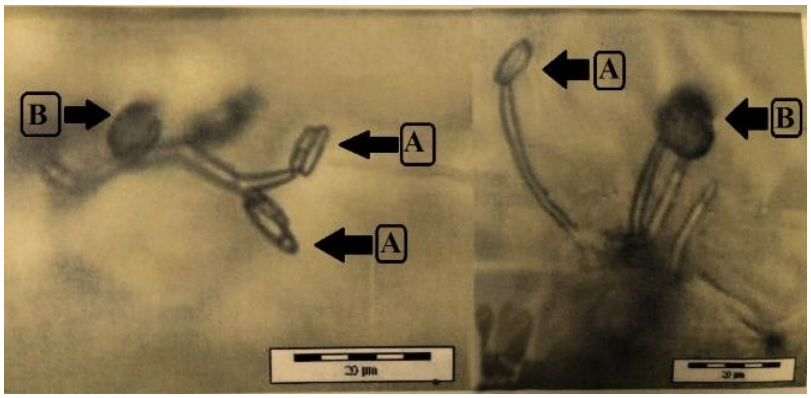

Figure 7. Polyphialides of *Fusariumsubglutinans* on CLA. A) Microconidia, B) False head.

Detection of BEA by TLC

BEA was extracted after 28 days of the inoculation process with *F. sacchari* and *F. subglutinans* spore suspension on corn grit cultures. The results of screening were based on the colour spot and comparison of the retention factor (R_f) values of the samples together with the standard of BEA at a concentration of 200 ppm and further visualized under iodine vapour as shown in the Figure (8).

From Table 1, data showed that all the tested isolates of *F. sacchari* (46) and *F. subglutinans* (9) having the capable to produce BEA with the TLC method. The visualisedcolour spot of the standard with aid of iodine vapour was brown in colour and the R_f values ranging from 0.93 till 0.97 (Mean value = 0.95). Samples extracted lies almost at the same position with BEA standard colour spots were compared to verify the presence of BEA. Briefly, all isolates of *F. sacchari* and *F. subglutinans* showed R_f value in reference to standard BEA. While the colour spots of all isolates appeared resemblance in terms with BEA standard but it is different in the colour intensity. The intensity of colour spots for all isolates was darker compared to the BEA standard exhibited brownish in colour.

Table 1. Beauvericindetection of *Fusarium sacchari* and *Fusarium subglutinans* strains isolated from Pokkahboeng disease of sugarcane.

N.	***Fusarium* species**	**Isolates**	**Location**	**BEA**
1.	*F. sacchari*	K3243U	Gula Padang Terap (GPT), Kedah.	+
2.		K3247U	Gula Padang Terap (GPT), Kedah.	+
3.		K3249U	Gula Padang Terap (GPT), Kedah.	+
4.		K3251U	Gula Padang Terap (GPT), Kedah.	+
5.		K3252U	Gula Padang Terap (GPT), Kedah.	+
6.		K3256U	Gula Padang Terap (GPT), Kedah.	+
7.		K3257U	Gula Padang Terap (GPT), Kedah.	+
8.		K3259U	Gula Padang Terap (GPT), Kedah.	+
9.		K3260U	Gula Padang Terap (GPT), Kedah.	+
10.		K3261U	Gula Padang Terap (GPT), Kedah.	+
11.		K3266U	Gula Padang Terap (GPT), Kedah.	+
12.		K3268U	Gula Padang Terap (GPT), Kedah.	+
13.		K3269U	Gula Padang Terap (GPT), Kedah.	+
14.		K3271U	Gula Padang Terap (GPT), Kedah.	+
15.		K3272U	FeldaChunping, Perlis	+
16.		K3273U	FeldaChunping, Perlis	+
17.		K3275U	FeldaChunping, Perlis	+
18		K3277U	FeldaChunping, Perlis	+
19.		K3282U	FeldaChunping, Perlis	+
20.		K3283U	FeldaChunping, Perlis	+
21.		K3284U	FeldaChunping, Perlis	+
22.		K3285U	FeldaChunping, Perlis	+
23.		K3287U	FeldaChunping, Perlis	+
24.		K3288U	FeldaChunping, Perlis	+
25.		K3290U	FeldaChunping, Perlis	+
26.		K3291U	FeldaChunping, Perlis	+
27.		K3296U	FeldaChunping, Perlis	+
28.		K3303U	FeldaChunping, Perlis	+
29.		K3304U	FeldaChunping, Perlis	+
30.		K3305U	FeldaChunping, Perlis	+
31.		K3306U	FeldaChunping, Perlis	+
32.		K3307U	Jelutong, Penang.	+
33.		K3309U	Kupang, Kedah.	+
34.		K3311U	Kupang, Kedah.	+
35.		K3312U	Kupang, Kedah.	+
36.		D3325U	Rantau Panjang, Kelantan.	+
37.		D3326U	Rantau Panjang, Kelantan.	+
38.		D3327U	Rantau Panjang, Kelantan.	+
39.		T3332U	Sri Langkap, Terengganu.	+
40.		T3334U	Setiu, Terengganu.	+
41.		C3338U	KampungAwah, Pahang.	+
42.		C3339U	KampungAwah, Pahang.	+
43.		K3350U	Baling, Kedah.	+
44.		K3352U	Baling, Kedah.	+
45.		K3354U	Alor Star, Kedah.	+
46.		J3357U	Rengit, Johor Bahru.	+
47.	*F. subglutinans*	K3258U	Gula Padang Terap (GPT), Kedah.	+
48.		K3267U	Gula Padang Terap (GPT), Kedah.	+
49.		K3270U	Gula Padang Terap (GPT), Kedah.	+
50.		K3293U	FeldaChunping, Perlis	+
51.		K3295U	FeldaChunping, Perlis	+
52.		K3308U	Kupang, Kedah.	+
53.		K3324U	Rantau Panjang, Kelantan.	+
54.		K3349U	Kuantan, Pahang.	+
55.		K3443U	Cameron Highlands, Pahang.	+
56.	Control			-

+ = detected

- = not detected

Control = non-inoculated corn grit cultures

Brine shrimp bioassay

The extracts containing BEA were used to evaluate the toxicity of BEA towards *A. salina* (Brine shrimp larvae). Two species of the *Fusarium* in section Liseolais known as *F. sacchari* and *F. subglutinans* exhibited a mortality rate up to 100%. Yet each of the isolates of *F. sacchari* and *F. subglutinans* was observed to show different mortality rate on *A. salina* larvae. The results tabulated in Table 2 with the toxicity level indication [39]. All the toxicity levels exhibited varying results ranging from slightly toxic to highly toxic. The calculated t (1.86) was larger than tabulated $t_{0.05}$ (1.65). In conclusion, the toxicity of BEA extracts from *F. subglutinans* was more in comparison to the toxicity of BEA extracts from *F. sacchari*.

BEA toxicity towards brine shrimp larvae was highly variable. In *F. sacchari* alone, the mortality rates of brine shrimp towards extracts containing BEA was ranging from 15.56% to 100% as shown in Table 2. The distribution in scale from slightly toxic to very toxic was 5 isolates in the slightly toxic group, 20 isolates in the toxic group, and 21 isolates in the very toxic group. Among those in a very toxic group, 9 isolates showed a 100% mortality rate on brine shrimp larvae.

While BEA extracted from corn grits inoculated with *F. subglutinans* had a mortality rate in between 66.67-100%. The K3324U isolate of *F. subglutinans* showed 100% mortality for brine shrimp larvae. Narrow variations in extracts containing BEA extracted from *F. subglutinans* were more consistent in the BEA production.

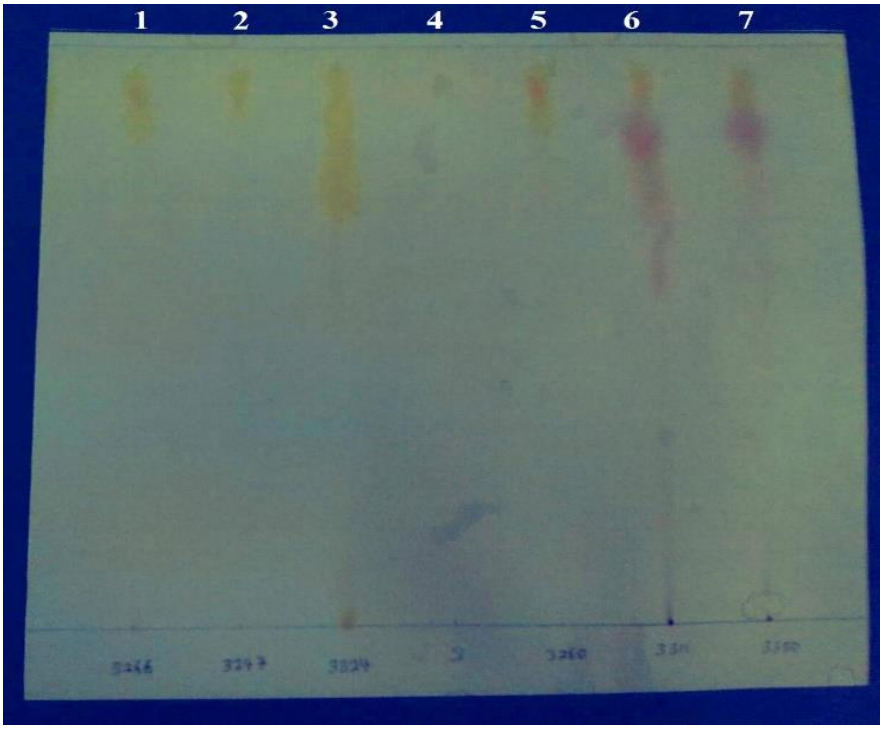

Figure 8. Thin layer chromatograms of Beauvericin developed in acetic acid; methanol: water (100:5:1) solvent system. Brown colour spots on silica gel sheet of Beauvericin standard and samples after visualization under iodine vapour. (Lane 1: K3266U, Lane 2: K3247U; Lane 3: K3324U, Lane 4: standard Beauvericin, Lane 5: K3260U, Lane 6: K3311U and Lane 7: K3350U.

Table 2. Beauvericindetection of *Fusarium sacchari* and *Fusarium subglutinans* strains isolated from Pokkahboeng disease of sugarcane.

N.	*Fusarium* species	Isolates	*A. salina* toxicity mortality (%)	Toxicity
1.	*F. sacchari*	K3243U	92.22 ± 8.39	VT
2.		K3247U	21.11 ± 11.70	ST
3.		K3249U	70.00 ± 17.32	T
4.		K3251U	100	VT
5.		K3252U	96.67 ± 3.34	VT
6.		K3256U	75.56 ± 12.62	T
7.		K3257U	95.56 ± 5.09	VT
8.		K3259U	100	VT
9.		K3260U	54.44 ± 9.62	T
10.		K3261U	74.45 ± 3.85	T
11.		K3266U	56.67 ± 8.82	T
12.		K3268U	75.56 ± 8.39	T
13.		K3269U	100	VT
14.		K3271U	91.11 ± 3.85	VT
15.		K3272U	94.45 ± 3.85	VT
16.		K3273U	15.56 ± 7.70	ST
17.		K3275U	61.11 ± 10.71	T
18		K3277U	97.78 ± 1.92	VT
19.		K3282U	43.33 ± 5.77	ST
20.		K3283U	41.11 ± 11.70	ST
21.		K3284U	68.89 ± 5.09	T
22.		K3285U	64.44 ± 29.88	T
23.		K3287U	85.56 ± 5.09	T
24.		K3288U	64.44 ± 8.39	T
25.		K3290U	96.67 ± 3.34	VT
26.		K3291U	76.67 ± 23.34	T
27.		K3296U	92.22 ± 6.94	VT
28.		K3303U	100	VT
29.		K3304U	74.44 ± 24.57	T
30.		K3305U	73.34 ± 11.55	T
31.		K3306U	90.00 ± 8.82	VT
32.		K3307U	95.55 ± 3.85	VT
33.		K3309U	100	VT
34.		K3311U	85.56 ± 1.93	T
35.		K3312U	65.55 ± 27.96	T
36.		D3325U	90.00 ± 6.67	VT
37.		D3326U	100	VT
38.		D3327U	100	VT
39.		T3332U	100	VT
40.		T3334U	82.22 ± 22.19	T
41.		C3338U	73.33 ± 29.63	T
42.		C3339U	88.89 ± 19.42	T
43.		K3350U	96.67 ± 3.34	VT
44.		K3352U	100	VT
45.		K3354U	84.44 ± 7.70	T
46.		J3357U	45.56 ± 8.39	ST
47.	*F. subglutinans*	K3258U	81.11 ± 5.09	T
48.		K3267U	88.89 ± 7.70	T
49.		K3270U	96.67 ± 3.34	VT
50.		K3293U	66.67 ± 31.80	T
51.		K3295U	68.89 ± 16.44	T
52.		K3308U	92.22 ± 6.94	VT
53.		K3324U	100	VT
54.		K3349U	84.44 ± 1.93	T
55.		K3443U	95.55 ± 3.85	VT
56.	Control		0	NT

NT = non-toxic (0-9% mortality
ST = slightly toxic (10-49% mortality)
T = toxic (50-89% mortality)
VT = high toxic (90-100% mortality)

DISCUSSION

The media PDA and CLA were used instead of others as these media allowed pigmentation of *Fusarium* and the production of conidia for identification purposes. Fisher and coauthors [32] reported that CLA is a natural substrate medium that promoted sporulation rather than mycelia growth. Microconidia chains, false heads, polyphialides, monophialides, chlamydospores, and sporodochia can be seen *in situ* if these structures are present. The results showed no difference between *F. sacchari*and *F. subglutinans*in producing microconidia, and shape of macroconidia. Morphological characteristics are possible to discriminate into the two species based on the presence of mesoconidia. The distinguishable trait in producing of mesoconidia showed the ability of *F. sacchari* in producing a higher number of mesoconidia compared with *F. subglutinans* in situ on CLA media.

Interestingly, the results showed the ability of *F. sacchari* and *F. subglutinans* to produce BEA by using the TLC method. At detectable levels on the TLC method after visualization under iodine vapour. They assumed the mycotoxin profile of *F. sacchari* similar to those of *F. subglutinans*[2]. Therefore, this assumption was well supported in this study. BEA is a nonpolar cyclodepsipeptide compound as it gives high Rf value due to weak interaction with the polar adsorbent on the TLC plate [40]. The R_f value 0.95 detected in the experiment of BEA was in agreement with previous studies with minor changes in R_f value due to the diverse developing solvent system. The solvent system made up of methanol, water, and formic acid (30:45:25) showed the Rf value of BEA = 0.90 [41]. While, the BEA R_f value under the solvent system of 1-butanol, water, and acetic acid (12:5:3) was 0.92 [40]. The results showed slight dissimilarity in BEA R_f value compared with the previous studies due to the different solvent systems. The difference in the R_f value might be due to several factors including the amount of material spotted, temperature, solvent system, and chemical nature of absorbent. The condition of temperature, the equilibrium between liquids and vapour in tank, sample impurities are the key to a high range of R_f value [42-44]. In addition, the results showed a difference in intensity of colour spots between BEA that extracted from all isolates and BEA standards. The darker brown colour denoted a higher concentration for BEA which was more than 200 ppm contained in the sample extraction [45].

However, the crude extracts for all *F. sacchari* isolates in this study tested positive for the production of BEA. Moretti and coauthors [25] detected the ability of *F. sacchari* to produce BEA. Petrovic and coauthors [46] mentioned about *F. sacchari* produced BEA and a plant pathogen for sugarcane and other crops. *F. subglutinans* is considered the producer for BEA. This study showed the ability of all tropical isolates of *F. subglutinans* for producing BEA. Moretti and coauthors [24], Moretti and caouthors [47], and Reyes-Velázquez and coauthors [48] found some isolates of *F. subglutinans* of different geographic areas could not produce BEA. Several *Fusarium* sp. produced a BEA toxin including *F. verticillioides*, *F. oxysporum*, *F. poae*¸ *F. redolens*, *F. proliferatum*, and *F. subglutinans* [20,35,48-51].

On the other hand, the result of a biotoxicity assay for the biological activity could provide the detection of either known or unknown mycotoxin in foodstuffs and useful for verification about the toxin presence after screened through chemical means. Hamill and coauthors [52] further confirmed brine shrimp as a sensitive organism towards BEA, so it was selected as the targeted organism in this bioassay test for this study. The study was focus on the mortality rate of brine shrimp towards BEA that calculated to determine the toxicity of BEA. Bioassay test confirmed by many certain studies including Logriecoand coauthors[53], Moretti and caouthors[24], and Moretti and coauthors[25], that calculated the mortality rate of brine shrimp to resolve the toxicity of mycotoxins. Indeed, BEA of *F. sacchari* and *F. subglutinans* showed a toxicity for the brine shrimp in the different levels among the same species. Two species of *Fusarium* in this study is shown three levels of toxicity. Butt and Goettel[54] reported the role of fungal toxins causing death for the host tissue by the combination of colonizing of fungal with nutrient depletion and the action of toxins from fungal. The toxicity BEA levels of *F. sacchari* included 46% high toxic, 43% toxic, and 11% slightly toxic of isolates. As well, the BEA toxicity of *F. subglutinans* appeared two groups included 44% high toxicity, and 56% toxic of isolates. The difference in the production BEA among the same species of *Fusarium* could occur in the wild population of *Fusarium* spp. because it may have happened a mutation. The change in production of mycotoxins resulted from the effect of a mutation on plant pathogens [55].The mutation in the *Fusarium* isolates may occur as a result of several factors; (1) the interaction between *Fusarium* and the host plant such as influence of plant defences,(2) the blend in mating occurred among avirulence and high virulence (56),(3) the type of nutrient, the preservation conditions, and the length of preservation time.

CONCLUSION

The difference in toxicity for the brine shrimp reflected the difference in the capability of *Fusarium* isolates to cause the pathogenicity. This study manifests the capability of both *F. sacchari* and *F. subglutinans* to produce effective BEA. Therefore, BEA may play a big role in the occurrence of the infection for the host plant by causing the toxicity for the host tissues and can be a potential pathogenicity factor.

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