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Toxicity of *Fusarium solani* Strains on Brine Shrimp (*Artemia salina*)

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Abstract: Discovery of anticancer drugs that must kill or disable tumor cells in the presence of normal cells without undue toxicity is an extraordinary challenge. Cytotoxicity of plant or fungal materials is considered as the presence of antitumor compounds. Brine shrimp lethality for larvae (nauplii) is used as prescreening test for the antitumor compounds. In this study, culture filtrates of eight strains of *Fusarium solani* isolated from seeds of various crops were tested for the toxic effect on brine shrimp. Five of the strains (TS, S-29, B-17, C-10, W-5) showed highest toxic effect and three of the strains (SR, T-9, L-25) showed low toxic activity on brine shrimp. Toxic activity reduced when culture filtrates were diluted. However, *F. solani* strains TS, B-17, SR, T-9 and L-25 caused more than 30% mortality at 1:10 dilution. Toxic activity was slightly reduced when the filtrates were neutralized with sodium hydroxide indicating possible role of pH of culture filtrate on toxicity. Lyophilized filtrates of these strains showed less activity as compared to un-lyophilized filtrates. *n*-Hexane soluble fraction was obtained only in three strains which showed mild toxicity whereas chloroform soluble fraction was obtained in negligible quantity and could not further be proceeded. Toxic effect of these strains showed variation from strain to strain. Compounds from *F. solani* could be exploited for the development of toxic compounds.

Key words: *Fusarium solani* strains; Toxicity; Brine shrimp; Compounds

镰刀菌 *Fusarium solani* 菌株对卤虫 *Artemia salina* 的毒性

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摘要：寻找能杀伤肿瘤细胞而对正常细胞无毒的抗癌药物极具挑战性。具有细胞毒性的植物或者真菌可能含有抗肿瘤的化合物。卤虫无节幼体的致死性可作为筛选抗肿瘤化合物的试验。本研究运用从不同农作物种子分离的 8 株镰刀菌（*Fusarium solani*）培养滤液来测试卤虫的细胞毒性效果。结果表明，5 株菌株（TS、S-29、B-17、C-10 和 W-5）对卤虫显示高毒性；3 株菌株（SR、T-9 和 L-25）显示低毒性，且毒性随着培养滤液的稀释而减弱。5 株菌株（TS、B-17、SR、T-9 和 L-25）按照 1∶10 稀释能导致 30％以上的死亡率。NaOH 中和后的滤液毒性略微降低，表明培养滤液的 pH 值可能影响毒性。这些菌株冻干的滤液相对于未冻干的滤液毒性较低。只在 3 株温和毒性的菌株中得到正己烷可溶萃取物；氯仿可溶萃取物的量极微而不能作进一步处理。各菌株的毒性效果各不相同。从镰刀菌（*F. solani*）分离的化合物可开发为毒性化合物。

关键词：镰刀菌菌株；毒性；卤虫；化合物
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The fight against cancer has not been successful so far, particularly in the development of therapies for growing tumors. It is an extraordinary challenge to find drug for the effective treatment of the cancer. The drug must kill or disable tumor cells in the presence of normal cells of extremely similar type without undue toxicity (Suffness, 1985). Toxicity of plant or microbial material is considered as the presence of antitumor compounds.

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Brine shrimp bioassay is very simple, rapid and inexpensive method and has successfully been used as prescreening of bioactive compounds having antitumor activity (McLaughlin et al, 1993). This test has been established as a safe, practical and economical method for the determination of the bioactivity of synthetic compounds (Almeida et al, 2002), mycotoxins of fungal pathogens (Schmidt et al, 1995), marine products (Ara et al, 1999) as well as higher plant products (Nino et al, 2006, Stefanello et al, 2006;). National Cancer Institute (NCI), USA has found a significant co-relation between the brine shrimp assay and *in vitro* growth inhibition of human solid tumor cell lines (Silva et al, 2007). Antibiotics produced by fungi, are used currently in chemotherapy against bacterial and fungal infection, besides antibiotics production, fungi have potential role of producing medicinally important antitumor agents (Arora, 2003; Wainwright, 1992).

Fusarium solani is one of the most ubiquitous soil fungus and a destructive plant pathogen of hundreds of hosts, causing chiefly root and fruit rots (Domsch et al, 1980). *F. solani* is known to produce naphthoquinone compounds (Baker et al, 1990) including fusarubin, anhydrofusarubin, javanicin, norjavanicin, methyl ether-fusarubin, marticin, isomarticin, bostrycoidia, ethyl ether-fusarubin, solaniol, nectriafusarubin and dihydro-fusarubin lactone (James & Robert 1983; Kurobane et al, 1980; Tatum et al, 1985). Some naphthaquinones have antimicrobial, toxic, insecticidal and antitumor properties (Ammar et al, 1979). In our previous study, *F. solani* strains showed nematicidal activity against *Meloidogyne javanica* (Hameed et al, 2001) and antimicrobial activity against some Gram positive and negative bacteria and root rot strain of *F. solani* (Qureshi et al, 2003). The present report describes the toxicity of culture filtrates of some strains of *F. solani* on brine shrimp.

1 Materials and Methods

1.1 *Fusarium solani* strains

Eight strains of *Fusarium solani* used in this study were isolated from seeds of *Sorghum bicolor* (S-29), *Brassica compestris* (B-17), *Capsicum annuum* (C-10), *Triticum aestivum* (W-5), *Helianthus annuus* (SR), *Lycopersicon esculentum* (TS), *L. esculentum* (T-9) and *Lens culinaris* (L-25). Isolated strains of fungi were grown on Czapek's Dox broth for 15 days at 28 °C. After 15 days each strain was filtered over Whatman No. 1 filter paper. The culture filtrates were divided into three set: i) culture filtrates, ii) lyophilized filtrate, and iii) soluble fractions of *n*-hexane and chloroform.

1.1.1 Culture filtrates The pH of the filtrates was acidic. Therefore this set was further divided into two sets, a) standard: used as such (1:0) and further diluted in artificial sea water as 1:10, 1:100, 1:1000, and b) the pH of the filtrates was adjusted to 7.0 by using NaOH after making a dilution of 1:0, 1:10, 1:100 and 1:1000 in artificial sea water.

1.1.2 Lyophilized filtrate Culture filtrates were lyophilized on Freez Dryer (Eyela FD-1). Dilutions of 0.002, 0.02, 0.2, 2, and 20 mg/mL of lyophilized powder were made in artificial sea water

1.1.3 Hexane and chloroform soluble fractions Culture filtrates were extracted with hexane and chloroform in separating funnel. The solvents were concentrated on rotary vacuum evaporator to obtain a gummy mass of fraction. A series of dilutions of 0.001, 0.01, 0.1 and 1 mg/mL were made of each fraction in their respective solvents.

1.2 Brine shrimp lethality test

Brine shrimp lethality test for larvae nauplii was used to determine the toxicity of culture filtrates of *F. solani* (McLaughlin et al, 1993).

1.2.1 Sample preparation Two millilitres from each dilution of culture filtrate, lyophilized filtrate or fraction were transferred in sample vials, used for bioassay. In case of fraction, each vial was left for 48 hours to evaporate organic solvent before adding the brine shrimp larvae along with 2 mL artificial sea water.

1.2.2 Hatching of shrimp Brine shrimp eggs (Carolina Biological Supply Company, Burlington, NC, USA) were hatched in a shallow rectangular container (60 x 30 cm) filled one fourth with artificial sea water (prepared with sea salt and distilled water). A plastic divider with holes was placed in the container to make two unequal compartments. The eggs were sprinkled into the larger compartment which was darkened, while the smaller compartment was illuminated. After 48 hours, the phototropic nauplii were collected from the illuminated side.

1.2.3 Bioassay Ten shrimps were transferred in previously prepared sample vial. Survivors were counted under the stereomicroscope after 24 hours and the percent death at each dose and control was determined.

1.2.4 LC50 Determination LC50 was determined from the 24 hours count using the probit analysis method described by Finney (1971). The dilutions of culture

filtrate were transformed as 1:0 =1, 1:10 = 0.1, 1:100 = 0.01 and 1:1000 = 0.001 to calculate the LC50 value.

2 Results and Discussion

Fusarium species occur throughout the world in a variety of climate and on many plant species as epiphytes, parasites or pathogen (Park et al, 1999). Many species are known to produce a wide array of mycotoxins, having toxic effect on various cell lines and on brine shrimp (Cetin & Bullerman, 2005; Gutleb et al, 2002; Hartl & Humpf, 2000). In the present study, culture filtrates of eight different strains of seed-borne *F. solani* (TS, S-29, B-17, SR, C-10, T-9, L-25, W-5) and their diluted fractions were tested for toxicity. Of these, undiluted filtrates of TS (LC50 =0.06), B-17 (LC50 =0.06), C-10 (LC50 =0.12), W-5 (LC50 =0.12) and S-29 (LC50 =0.14) caused greater toxic effect on brine shrimp. The toxic effect of all *F. solani* strains was found to decline when diluted. However, *F. solani* strains TS, B-17, SR, T-9 and L-25 caused more than 30% mortality at 1:10 dilution (Tab. 1). Pathogenicity of *Fusarium solani* on seedlings of different plants and their antimicrobial activity has been reported (Qureshi et al, 2003). There are also reports that *Fusarium* species, parasitize the eggs of root knot nematodes *Meloidogyne* (Tabreiz & Hussain, 1986) and reduced gall formation by nematode on host plants (Parveen et al, 1993). In some cases neutralization of culture filtrates slightly reduced their toxicity. However, greater toxicity in neutralized culture filtrates was observed in *F. solani* strains TS (LC50 = 0.10), W-5 (LC50 = 0.20), SR (LC50 = 0.27) and B-17 (LC50 =0.36). There are reports that the culture filtrates of *F. oxysporum* and *F. solani* have adverse effects on hatching and mortality of *M. incognita*, which attributed to factors like low pH and toxins produced by the fungal pathogens during their initial growth period (Mani, 1983). In this study, lyophilized culture filtrate of *F. solani* strain TS caused maximum death (46.6%) at 20 mg/mL, while other strains showed less activity (Tab. 2). *n*–Hexane fractions were obtained only in three strains and found unable to cause significant toxic effect at 1 mg/mL (Tab. 3). Chloroform soluble fractions were obtained in very small quantities, hence further studies could not proceeded. In this study, *F. solani* strains showed variation in their toxic effect on brine shrimp.This kind of variation has also been reported by Hameed et al (2001) while studying the nematicidal activity of *F. solani* strains against root knot nematode, *Meloidogyne javanica*. Lyophilized filtrates of these strains showed less activity as compared to un-lyophilized filtrates. *n*-Hexane soluble fraction was obtained only in three strains which showed mild toxicity. Antimicrobial naphthoquinones are widely distributed in plants, fungi and some animals (Riffel et al, 2002) are also reported from *F. solani* (Ammar et al, 1979; Baker et al, 1990). Besides naphthoquinones, *F. solani* is also known to produce diterpene and paclitaxel (Chakravarthi et al, 2008), and has been used alone or in combination with other chemotherapeutic agents for the treatment of variety of cancers, as well as AIDS-related Kaposi sarcoma (Brown, 2003; Croom, 1995). Production of chemotherapeutic and antitumor agents could be possible by the industrial fermentation of potential strain of *F. solani*.

Tab. 1 Effect of culture filtrates of *Fusarium solani* strains on Brine shrimp

Fusarium solani strains	% Death after 24 hours (mg/mL)				LC50
	0.001	0.01	0.1	1.0	
(TS) Standard	3.3	3.3	63.3	96.6	0.06
Neutralized	0	10	30	96.6	0.10
(S-29) Standard	0	3.3	13.3	96.6	0.14
Neutralized	0	3.3	13.3	83.3	0.36
(B-17) Standard	6.6	20	46.6	96.6	0.06
Neutralized	3.8	6.6	6.6	30	>1
(SR) Standard	6.6	26.6	36.6	60	0.36
Neutralized	0	6.6	50	60	0.27
(C-10) Standard	0	10	23.3	96.6	0.12
Neutralized	0	3.3	16.6	33.3	>1
(T-9) Standard	6.6	20	30	63.3	0.76
Neutralized	0	6.6	10	53.3	0.56
(L-25) Standard	6.6	20	30	63	0.76
Neutralized	0	6.6	10	53	0.56
(W-5) Standard	0	16.6	23.3	96.6	0.12
Neutralized	0	6.6	23.3	86.6	0.20

Means of 3 replicates.

Tab. 2 Effect of lyophilized culture filtrates of *Fusarium solani* strains on Brine shrimp

Fusarium solani strains	% Death after 24 hours (mg/mL)					LC50 (mg/mL)
	0.002	0.02	0.2	2	20	
TS	3.3	10	26.6	33.3	46.6	>20
T-9	0	10	20	26.6	33.3	>20
W-5	6.6	13.3	20	23.3	30	>20
L-25	0	3.3	13.3	23.3	33.3	>20
SR	3.3	6.6	13.3	23.3	33.3	>20
S-29	0	3.3	10	16.6	23.3	>20
B-17	0	3.3	13.3	20	30	>20
C-10	3.3	10	13.3	26.6	33.3	>20

Means of 3 replicates.

Tab. 3 Effect of *n*-Hexane soluble fraction of *Fusarium solani* strains on Brine shrimp

Fusarium solani strains	% Death after 24 hours (mg/mL)				LC 50 (mg/mL)
	0.001	0.01	0.1	1.0	
S-29	0	5.0	6.6	20	>1
B-17	0	3.3	6.6	16.6	>1
L-25	5	6.6	13.3	20	>1

Means of 3 replicates.

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昆明动物研究所 4 项科技成果荣获 2008 年度云南省科学技术奖

2009 年 6 月 16 日,云南省委、省政府在昆明连云宾馆礼堂召开“2008 年度云南省科学技术奖颁奖大会”，云南省委和省级国家机关有关部委办厅局有关领导和在滇的“两院”院士及有关人民团体、高等院校、科研院所、部分大型企事业单位、中央驻滇有关单位、昆明市人民政府相关领导和相关人员参加了颁奖大会，荣获 2008 年度云南省科学技术奖所有类别一等奖和二等奖的成果主要完成人员也应邀参加了颁奖大会。昆明动物所获奖的赖仞研究员、郑永唐研究员和牛昱宇等主要完成人员出席了此次颁奖大会。

中科院昆明动物所有 4 项科技成果荣获 2008 年度云南省科学技术奖。他们是:

1 由赖仞研究员主持，张云、李建许、张亚平、郑永唐、徐学清、杨海龙和李文辉参加研究的“**两栖动物活性多肽的结构与功能研究**”荣获自然科学类一等奖;

2 由郑永唐研究员主持，杨柳萌、王睿睿、张高红、王云华、欧阳东云和王媛媛参加研究的“**抗 HIV 药物研发关键技术建立及其应用**”荣获科技进步类一等奖;

3 由季维智研究员主持，牛昱宇、杨世华、李天晴、王淑芬、和协超、唐向辉、谢云华、陈永昌和纪少珲参加研究的“**灵长类核移植与干细胞的研究**”荣获自然科学类二等奖;

4 由云南省疾病预防控制中心主持，昆明动物所郑永唐研究员参加研究的“**云南省 HIV 毒种库建设及分子流行病学研究**” 荣获科技进步类三等奖。