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Evaluation of Insecticidal Activity of *Fusarium venenatum* Metabolites Againstsf-21 Cell Lines

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Abstract: The crops world wide are damaged economically because of insect infestation. Chemical insecticides are used for their control but their use comes with certain issues like development of resistant populations, environmental and health hazards. Hence research on alternatives for chemical pesticides has gained importance. Plant and microbial compounds are effectively assessed for their insecticidal activities as they are compounds of natural origin.In this context, insecticidal activity of methanol and ethyl acetate extracts of *Fusarium venenatum* against Sf-21 insect cell line has been studied. The crude extracts obtained from the fungus were found to show noticeable cytotoxicity in Sf-21 cells. Among them maximum cytotoxicity activity was observed with methanol extracts with IC_{50}value being 125µg/mL compared to that of ethyl acetate extract (IC_{50} value250µg/ml).
Key words: *Fusarium venenatum*, Methanol and ethyl acetate extracts. Insecticidal activity; SF-21cell lines.

Introduction

Microorganisms are an abundant resource of various primary and secondary metabolites that are well known for their enormous use in pharmaceutical and agricultural industries. Fungi are a fascinating group of microorganisms that are a repository of innumerable secondary metabolites[1]with diverse functions that include antibacterial activity, anti-fungal activity, antitumor activity, plant growth promoters, immunosuppressive activity, pigments, herbicidal activity and insecticidal activity to mention a few.

Secondary metabolites play an important role in the control of certain plant pathogens. These are used to control the phytopathogenic fungi and bacteria[2]. *Trichoderma harzianum* produces an antifungal agent, alkyl-pyrone which is active against wide range of fungi and bacteria[3]. *Pisolithusarhizus* produce compounds, hydroxy benzyl formic acid and R-(-)-p-hydroxymendelic acid which are active against *Truncatella hartigii*[4]. Most of the *Trichoderma* sp. producesgliotoxin active against root pathogenic*Rhizoctoniasolani*[5].

These fungal metabolites also display insecticidal activity and various formulations of microbial pesticides are commercially available. Formulations of *Bacillus thuringiensis*, *Trichoderma*sps, *Metarhizium*sps, *Beauveria*sps, *Verticillium*sps and *Baculovirus* are used against agriculture pestsnamely *Helicoverpa*&*Spodoptera*

In view of the potential shown by fungal secondary metabolites, the present study has been undertaken to evaluate the insecticidal activity of the crude extracts of *Fusarium venenatum* against SF-21 cell lines.

Materials and Methods

Fungal strain

*Fusarium venenatum*was obtained from Fungal biodiversity centre, Netherland in lyophilized form and the fungi was activated in oats meal medium. An activated fungus was maintained on the oats meal agar slant as monosporic culture at 4°C.

Cultivation and extraction of secondary metabolites

Fusarium venenatum was cultivated in designed medium that included Jaggery water, date extract, KH_2PO_4, K_2HPO_4, $MgSO_4$. The inoculated medium was incubated at 28°C for 10 days. After incubation, the broth was centrifuged at 10000 rpm for 10 min. The supernatant was collected and extracted with ethyl acetate (1:1) and Methanol (1:1) separately and concentrated using rotary evaporator. The dried extract was scraped and resuspended in DMSO.

Cytotoxic activity against Sf-21 (*Spodoptera frugiperda*-21) cell line

Sf-21 (*Spodoptera frugiperda*-21 cell lines) derived from the primary explants ofpupal tissues of *Spodoptera frugiperda*, were obtained from National Centre for Cell Sciences (NCCS), Pune, India. The culture was routinely maintained at 27°C using Grace's TNM-FH medium supplemented with 10% FCS and 1% penicillin–streptomycin solution.

Cells were collected six days after sub culturing and diluted with fresh medium to a density of 7.5 x 10^4cells/ml. Each well of a 96-well microtiter culture plate was loaded with 100 µL of cell solution containing 2 µL of the fungal metabolite, prepared in DMSO. The final concentration of fungal metabolites was 3.95, 7.8, 15.6, 31.2, 62.5, 125, 250µg/ml. Each concentration tested consisted of four replicates and the test was repeated two times after 72 hours of exposure[6]. The test medium was replaced with 20 µL of 2 mg/ml MTT followed by overnight staining at 27 ºC.The staining solution was carefully removed and DMSO (150 µL/well) was added to solubilize the purple formazan crystals produced within the cell. The absorbance of each well was measured at 540 nm using a microplate reader[7].The cell growth was expressed as percentage of absorbance ratio (absorbance in wells with fungal metabolites to control well without fungal metabolites).The inhibition concentration (IC_{50}) was calculated using the formula-

$$\% \, Cytotoxicity = \frac{(Abs_{Control} - Abs_{Test})}{Abs_{Control}} \times 100$$

Results

In the recent past, research is focused towards the identification and development of new insecticides of natural origin as the chemical substances used currently are associated with certain problems related to health and environmental instabilities. Moreover, the use of certain insecticides has been reduced by law in many countries and hence the search for new compounds has become the prime focus[8-11].

The answer was provided in terms of microbial pesticides that undermine the above mentioned disadvantagesof synthetic pesticides by being the natural products or their derivatives. The fungal species produce diverse number of natural compounds that differ in their chemical structure, biological activityand mechanism of action, specificity and environmental impact[12]. Fungal strains belonging to entomopathogenic fungi are one group that are known to effect insects and can be extensively used as biocontrol agents against economically destructive insects and pests in various parts of the world.

Table 1: Effect of Methanol extract against SF 21 cell lines

Concentration(µg)	Absorbance	Cytotoxicity %	Cell viability %
3.95	1.110	9.89	98.84
7.8	0.907	15.05	80.76
15.6	0.821	20.57	73.10
31.2	0.783	23.51	69.72
62.5	0.629	37.4	56.01
125	0.569	42.84	50.66
250	0.481	51.12	42.83

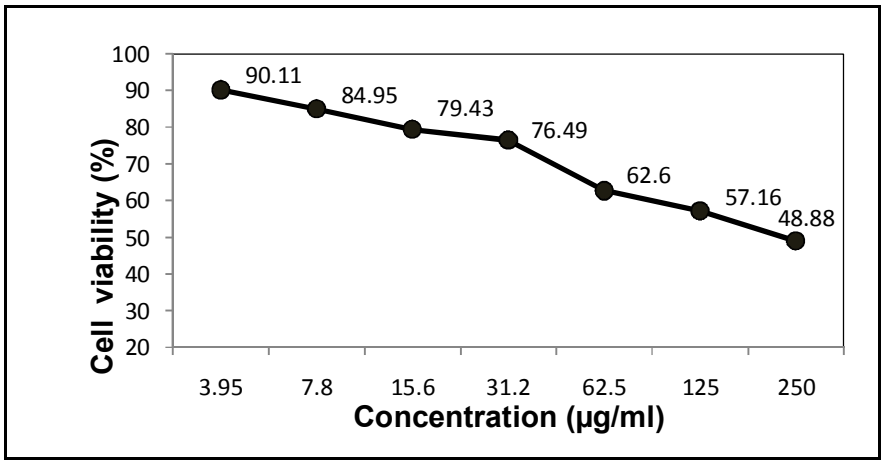

Figure 1. Cytotoxicity of methanol extracts on SF 21 cell lines

Table 2: Cytotoxicity of ethylacetate extracts against Sf 21 cell lines

Concentration(µg)	Absorbance	Cytotoxicity %	Cell viability %
3.95	1.012	9.89	90.11
7.8	0.954	15.05	84.95
15.6	0.892	20.57	79.43
31.2	0.859	23.51	76.49
62.5	0.703	37.4	62.60
125	0.642	42.84	57.16
250	0.549	51.12	48.88

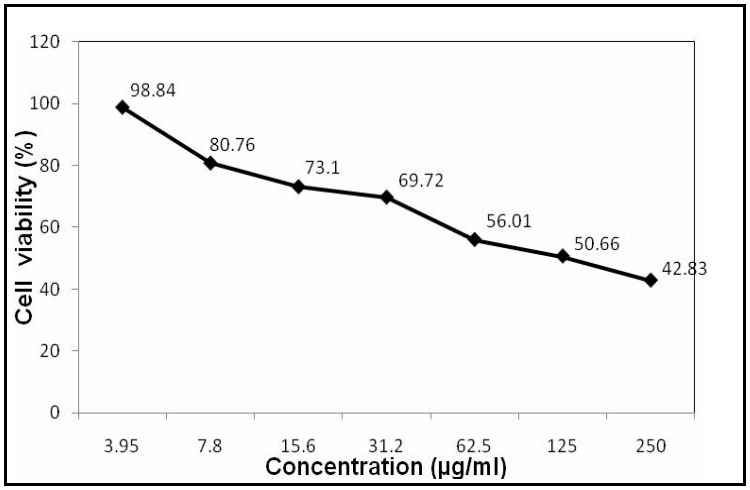

Figure 2. Cytotoxicity of methanol extracts on SF 21 cell lines

Discussion

In the present study the fungi *Fusarium venenatum* was used for the production of secondary metabolites and was assessed their insecticidal potential. The secondary metabolites were extracted using two solvents namely ethyl acetate and methanol separately. Thesecrude extracts were checked for their insecticidal activity against Sf-21 insect cell lines.The different concentrations of the extracts wereused (methanol and ethyl acetate) and the cytotoxicity values are provided in Table 1 and Table 2 along with the cell viability percentages which were calculated from the absorbance values and these effect of the extracts on the cell lines are provided inFigure 1 and 2.

It has been observed that the cytotoxicity effect was dose dependent in both the extracts. As the concentration of the extract increased, there is an increased level of cytotoxicity as is evident in the tables provided above which indicates that the growth and viability of the cells is influenced by the metabolites. The

IC50 value was found to be 125 µg/ml (methanol extract) and 250 µg/ml (ethyl acetate extract). Though cytotoxicity in cell lines was noticed the study showed that a slightly higher concentration of the metabolites was required for insecticidal activity.

Microscopic examination of the cells (Fig 3) revealed distortion in the shape and structure of the cells followed by the reduction in the number of cells in both the extracts,there by clearly indicating the role of metabolites on cell viability.

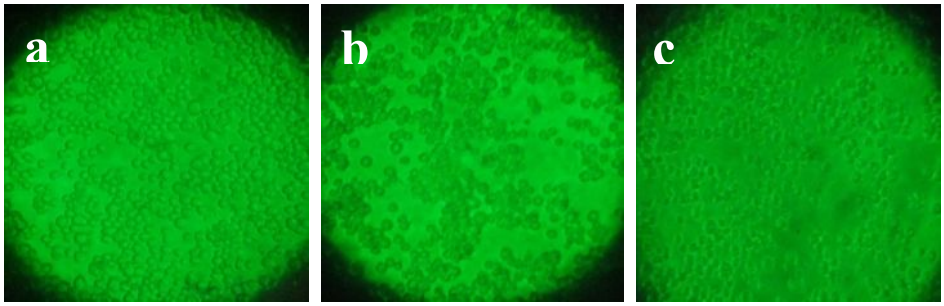

Figure3.Effect of the extracts on Sf 21 cell lines a) Control b) Methanol extract c) Ethyl acetateextract

Certain other fungi have been reported to produce insecticidal metabolites include *Alternaria, Chaetomium, Paecilonyces, Verticillium, Cephalosprium, Botrytis, Myrothecium, E, Gliocladium, Trichoderma, Rhizopus, Trichothecium, Lasidipldia, Sterigomatocystis, Amanita, Tricholoma* and *Clitocybe*[13-16]

Previous study carried out by Prakash and Namashivayam(2014)[17]reported the presence of various compounds in the extracts of *Fusariumvenenatum* identified by GC MS analysis. These compounds identified would have displayed the bioactivity and further studies with regard to purifying the extract would throw light on the molecules involved in insecticidal activity. Additional studies with respect to optimising the media and growth conditions would help in producing the metabolites in large quantities with potential bioactivites.

Conclusion

The present study involved the use of *Fusariumvenenatum* to extract the secondary metabolites using methanol and ethyl acetate and evaluate its insecticidal activity against Sf 21 cell lines. It was observed that these extracts displayed noticeable cytotoxicity against the insect cell lines with IC_{50} values being 125 µg/mL (methanol extract) and 250µg/mL (ethyl acetate extract). This study reveals the potential of *Fusarium venenatum* as synthesizer of secondary metabolites with significant bioactivities. The advantage of using this fungus is that the biomass can be used as a source of mycoprotein and the culture filtrate can be used to extract certain pharmacologically significant compounds.

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