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EXTRACTION AND IDENTIFICATION OF VOLATILE BIOACTIVE COMPOUNDS OF *ASPERGILLUS NIGER* AND ITS EFFICACY AGAINST INSECT PEST, *DYSDERCUS KOENIGII* (HETEROPTERA : PYRRHOCORIDAE)

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ABSTRACT : *Dysdercus koenigii* (red cotton bug) is a notorious cotton pest. It is distributed in cotton growing region of India. The aim of the study was to extract and identify the bioactive compounds of *Aspergillus niger* and its implementation in control of red cotton bug; *Dysdercus koenigii*. Bioactive compounds frequently referred as secondary metabolites were analysed by using gas chromatography-mass spectroscopy (GC-MS). Methanolic extract of these secondary metabolites were assessed against adult *Dysdercus koenigii*. A sum of thirty five bioactive compounds were identified in the extract of *Aspergillus niger*. Analyses of Total Ion chromatogram confirmed the presence of Cyclopropaneoctyl-, Hexadecane 1-chloro-, Nonylcyclohexane, 1, 9-decadiene, 4,4,7,7-Tetramethyl-, 1- tetradecene, Nonane, 3,7-dimethyl-, 1-hexyl-1-nitrocyclohexane, Cyclohexane, (1-methylethyl)-, Cyclopropane, 1-methyl-1-(2-methylpropyl)-, 1- Heptadecene, Pentadecane, 2- propenoic acid, dodecyl ester, Propanoic acid, 3-(2,3,6-trimethyl-1,4-dioxaspiro[4,4]non-7-, Tetradecyltrifluoroacetate, Acetamide, N-(2-piperidin-4-ylethyl)-, 5-Methyl-hexahydro-pyrano[3,2-b]pyridin-6-one, 8-Octadecanone etc. The Fourier transform infrared spectrophotometer analysis of *Aspergillus niger* affirmed the existence of C-H bond stretch in aromatic rings, C=C group, R^1—R^3 = alkyl and/or aryl group, C-Nstretch in aromatic nitro compounds, aliphatic flouro compound, methylene C-H assym. Survival analysis and total hemocyte count showed significant differences in comparison to the control. Based on the findings, we can say that the secondary metabolites secreted by fungi could be used as the effective control agent and provide basis for further studies in Insect Pest Management.

Key words : *Aspergillus niger*, gas chromatography-mass spectroscopy, secondary metabolites, *Dysdercus koenigii,* Fourier transform infrared spectrophotometer.

INTRODUCTION

A fungus is the member of the eukaryotic group of organisms comprising microorganisms like yeasts, molds as well as mushrooms. During its active cell growth, it produces primary metabolites like amino acids, proteins, carbohydrates, vitamins, acetone, ethanol, etc. and secondary metabolites like antibiotics, toxins, alkaloids, fatty acids, ketones, alcohols, etc. (Devi *et al*, 2009). However, secondary metabolites play no role in the primary metabolism of fungi. Rather they help other biological phenomena in their hosts such as providing defense against predators, diseases, parasites; bio-control of pathogens and for communication between micro-organisms and their ambience. Studies on the fungal biodiversity reveal that nearly 1.5 million fungal species exist on earth. Out of them, only 5% are known yet (Hawksworth, 2001). Soil fungi are a considerably good source of highly active secondary metabolites, which possess an interesting aspect of the complex fungal development (Bok and Keller, 2004). Identification and evaluation of biological activities of a number of new bioactive metabolites have been done (Gao *et al*, 2010). The species diversity in the fungi group makes it an interesting topic for the researchers to unravel the different unidentified aspects of fungal secondary metabolites.

Aspergillus spp. are pervasive and cosmopolitan moulds that cause both allergic as well as invasive syndromes. It has been used in the biotechnological organization for the manufacture of extracellular as well as intracellular enzymes and organic acids (Perrone *et al*, 2007; Mogensen *et al*, 2010). The synthesis of volatile bioactive compounds or secondary metabolites in *A. niger* is guided by the cascade of genes (Yu and Keller, 2005; Calvo *et al*, 2002), which is further correlated with the arrest of growth and onset of reproduction. The volatile bioactive compounds are usually determined and identified by gas chromatography–mass spectrometry (GC–MS) as it provides the effective separation ability and highly

sensitive performance. These compounds belong to numerous structure classes, such as hydrocarbons, esters, ethers, alcohols, ketones, lactones and glycol ethers (Korpi *et al*, 2009; Schnu¨ rer *et al*, 1999).

Dysdercus koenigii (red cotton bug) is a notorious cotton pest. It is distributed in cotton growing region of India (David and Ananthakrishna, 2004; Sahayaraj and Ilayaraja, 2008). It is polyphagous in nature too; feeds on different host plants like lady's finger, sambhal, hollyhock etc. Nymphs and adults feed on developing or mature cotton seeds (Sprenkel, 2000). In recent years, synthetic insecticides have been used frequently to control this pest. However, rapid dispersal of nymphs and adults makes insecticide partially ineffective. Failure of insecticide and focus on lesser use of chemical insecticide in agriculture practices, propel the researchers to develop alternative ways to control the insect pests.

Implementation of microbes in insect pest management (IPM) is a sustainable method. Organisms used as microbial control agents belong to several taxa namely bacteria, viruses, fungi, nematodes and protozoa (Halouane *et al*, 2013). Amongst all, more than 700 species of fungi are used against arthropods and insects (Khan *et al*, 2012). Despite the broad range of entomopathogenic fungi, there are fewer mycoinsecticides available against *Dysdercus koenigii* (Faria and Wraight, 2007). Till date, meagre work has been reported regarding the importance of *Aspergillus* sp. in insects; especially *A. niger* against *D. koenigii*. Therefore, the present study was designed to screen the bioactive compounds from *A. niger* and evaluation of its effect against notorious insect pest, *D. koenigii*.

MATERIALS AND METHODS

Collection and culture of *Aspergillus niger*

Aspergillus niger (MTCC no. 9652) was obtained from M.T.C.C. Chandigarh, India. Stock cultures of the isolates were stored at -20°C. *A. niger* culture was maintained on Sabouraud dextrose agar medium according to Kumari *et al* (2015). Fungal cultures were incubated at 25 ± 2ºC for 14 days in dark. Stock cultures were stored on agar slants at 4°C until further use.

Extraction of the bioactive compounds

For the extraction of the volatile compounds from the fungal culture, three to five plugs of 6mm diameter were cut from one or several colonies using a cork drill as follows: one in the center of the colony, one at the rim of a colony far away from the other colonies. This was done in replicates to provide variability to the collection of volatile compounds. The plugs pieces were transferred to a 1.5ml auto-sampler screw cap vial containing 500 µl of the solvent mixture [methanol-dichloromethane-ethyl acetate (1:2:3) and 1% (v/v) formic acid] (Garcia *et al*, 2012). The vials containing plugs were sonicated for 60 min to extract volatile compounds. The extract was further transferred to the clean glass vial using a Pasteur pipette. The filtrate was dispersed to dryness at 40ºC on a rotary evaporator–shaker. The sample was then reconstituted in 100µl of methanol, which was filtered through 0.22 µm Randisc PVDF SF syringe filter. It was stored at 4ºC till further analysis by GC-MS (Imad *et al*, 2015a). The experiment was done in three replicates.

GC-MS analysis

The volatile bioactive compounds were scrutinized for their chemical constituents using GCMS – QP2010 Ultra, SHIMADZU (Shimadzu Corp 74707). The separation of bioactive compounds was achieved by Rxi-5Sil MS column (30 m×0.25 mm ID, 0.25 ìmdf film thickness, RESTEK, USA). The oven temperature was programmed at 60ºC and injection temperature was 260ºC. Helium flow was maintained at 1.21 ml/min (split ratio-10.0). Solvent cut time was 7.00 minute.

The eluate from GC column was proceed directly into the MS. Ionization voltage was set at 70 eV while ion source and interface temperature was 230°C and 270°C, respectively. Scan range (m/z) was 40 to 650 amu. The similarity search for the unknown compound identified by the GC-MS was done based on the database of National Institute of Standards and Technology (NIST) 14 library.

Fourier transform infrared spectrophotometer (FTIR)

Fourier transform infrared spectrophotometer based study was performed for the detection of the different functional groups present in the methanolic extract of the fungus. The Fourier transform infrared spectra were recorded on the Shimadzu, IR Affinity 1, Japan (FT-IR) spectrometer in range 4000-400 cm^{-1}. The FT-IR measures bonds vibrations within the functional groups of a compound and creates a spectrum that can be used in the identification of metabolite in the sample (Bobby *et al,* 2012).

Insect maintenance

Dysdercus koenigii

Dysdercus koenigii was collected from Okra field in the Banaras Hindu University campus and agricultural fields abounding Varanasi city. Insect colony was maintained in BOD incubator and fed on the water-soaked cotton seeds under photoperiod of 16L: 8D at 25 ± 2ºC and relative humidity 70% - 80% (Kumari *et al*, 2015).

Insect bioassay and collection of hemolymph

The extracted bioactive compounds were applied topically on the adult *D. koenigii* and observed for six consecutive days for survival analysis by Kaplan Meier using Graph Pad Prism 5 software. Hemolymph was collected 24 hours after the treatment of the insects with the extracted filtrate.

Hemolymph from adult male and female *D. koenigii* was collected in a micro-centrifuge tube containing few crystals of phenyl-thiourea with the help of micro-capillary through amputated legs and antenna (Venugopal and Kumar, 2000).

For THC, hemolymph was diluted with Tauber-Yeager's fluid (Tauber and Yeager, 1935) and then counted in four corners of WBC chamber (1 mm^2) of the improved Neubauer hemocytometer. The number of circulating hemocytes per cubic millimeter (mm^3) was calculated (Jones, 1962).

$$= \frac{Hemocyte\ in\ four\ squares\ \left(1\,mm^2\right) \times Dilution \times Depth\ factor\ of\ chamber}{No.\ of\ squares\ \ counted}$$

Statistical analysis

Data were presented as mean ± SEM and analyzed by student t-test and significant levels were set at $p < 0.05$ using IBM SPSS Statistics for Windows (Version 16.0. Armonk, NY: IBM Corp.). Survival analysis was done by Kaplan Meier survival analysis by Graph Pad Prism 5 software.

RESULTS AND DISCUSSION

In the present study, mixture of methanol-dichloromethane-ethyl acetate (1:2:3) containing 1% (v/v) formic acid was used as solvent for extraction of fungal secondary metabolites. This was further analyzed by GC-MS. The database of National Institute Standard and Technology (NIST 14) was applied for interpreting the GC-MS chromatogram. The similarity search for the spectrum of the unknown components was done in the NIST library. The total ion chromatogram (TIC) of the different bioactive compounds is represented in Fig. 1. The active principle compounds with their retention time (Rt), molecular formula, molecular weight and mass ion fragmentation are represented in Table 1. A sum of thirty five bioactive compounds were identified in the methanolic extract of *Aspergillus niger*. The component pertaining to different retention time and m/z ratio are represented below.

The First set up of peak were Cyclopropaneoctyl- (0.29%), Hexadecane 1-chloro- (0.07%), Nonylcyclohexane (0.08%), 1,9-decadiene,4,4,7,7-Tetramethyl- (0.11%), 1- tetradecene (0.33%), Nonane,3,7-dimethyl- (0.14%), 1-hexyl-1-nitrocyclohexane (0.13%), Cyclohexane,(1-methylethyl)- (0.06%), Cyclopropane,1-methyl-1-(2-methylpropyl)- (0.10%), 1- Heptadecene (0.79%), Pentadecane (0.14%), 2- propenoic acid, dodecyl ester (0.48%), Propanoic acid,3-(2,3,6-trimethyl-1,4-dioxaspiro[4,4]non-7- (0.22%), Tetradecyltrifluoroacetate (1.18%), Acetamide,N-(2-piperidin-4-ylethyl)- (1.48%), 5-Methyl-hexahydro-pyrano[3,2-b]pyridin-6-one (43.72%), 8-Octadecanone (0.31%), Piperidine,2,3-dimethyl- (0.43%), hexadecanoicacid,methyl ester (0.39%), 1,6-Heptadiene, 2-methyl-6-phenyl- (0.45%), 1-Nonadecene (0.77%), 1-octadecanol (0.27%), 1-(4-Ethylpiperazin-1-yl)ethanone (0.69%), 9-Heptadecene-4,6-diyn-8-ol (0.37%), 1,2-Benzenedicarboxylic acid (0.67%), 2-Methyltetracosane (0.23%), 2-(3,7-dimethyl-octa-2,6-dienylideneamino)-4,8-dimethyl-nona-3,7-dienenitrile (1.09%), L-Valine, N-(N-acetyl-L-valyl)- (2.38%), 1H-Indole-3-ethanamine,5-methoxy DIPT (17.89%), N-Ethyl-N-(1-methyl-2-phenylethyl)acetamide (1.55%), Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22E)- (1.43%), l-Glutamic acid, N-(N-acetyl-l-valyl)-,dibutyl ester (1.79%), Cholest-1-eno[2,1-a]naphthalene,3',4'-dihydro- (0.57%), Ergosta-5,8,22-trien-3-ol, (3.beta.,22E)- (18.70%) and Propafenone (0.73%).

The FT-IR spectrum of the dry methanolic extract of *A. niger* revealed the distribution of functional group within the organic fractions (Fig. 2). The IR spectra were recorded in the region 4000- 400 cm^{-1} (Table 2). The spectrum indicated the presence of bands at 3385.21 cm^{-1}, which correspond to intermolecular and intramolecular hydrogen bonding. The aliphatic C-H stretch was observed at 2925.95$cm^{-1}$and 2858.5cm^{-1}. The peaks at 1646.95 and 1552 shows the amide I and amide II bond, representing protein content. The band at 1743 showed C=O bond of ester and carbonyl group. The 1376.27 cm^{-1} band could be assigned as aromatic nitro groups. The peaks at 1155.8, 1239.2 pertain to tertiary amine (C-N stretch). The peaks at 1036.12 and 1076.6 cm^{-1} could be due to the presence of C-F stretch in aliphatic flouro compounds. The peak at 693 cm^{-1} could be assigned to the aromatic groups. Our results showed similarity with the findings of Imad *et al* (2015b).

Since growth of fungus under different culture conditions may influence the production of metabolites, the use of different growth medium and environmental conditions enhance the chemical diversity of fungi (Mayer

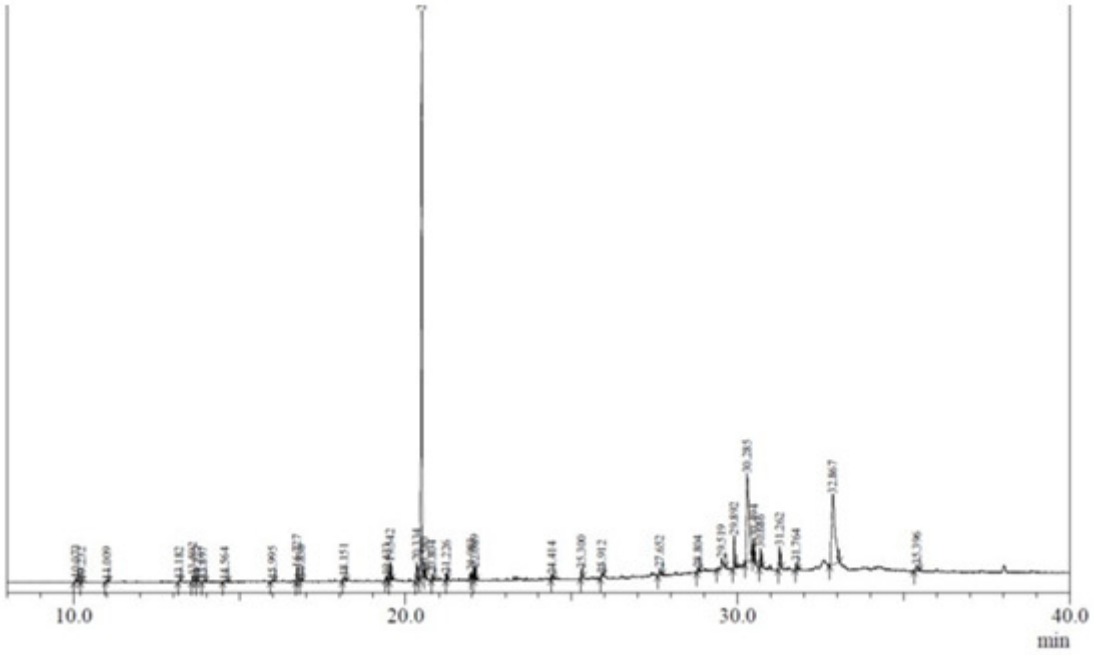

Fig. 1 : GC chromatogram showing the bioactive compounds from culture of *Aspergillus niger* (MTCC 9652).

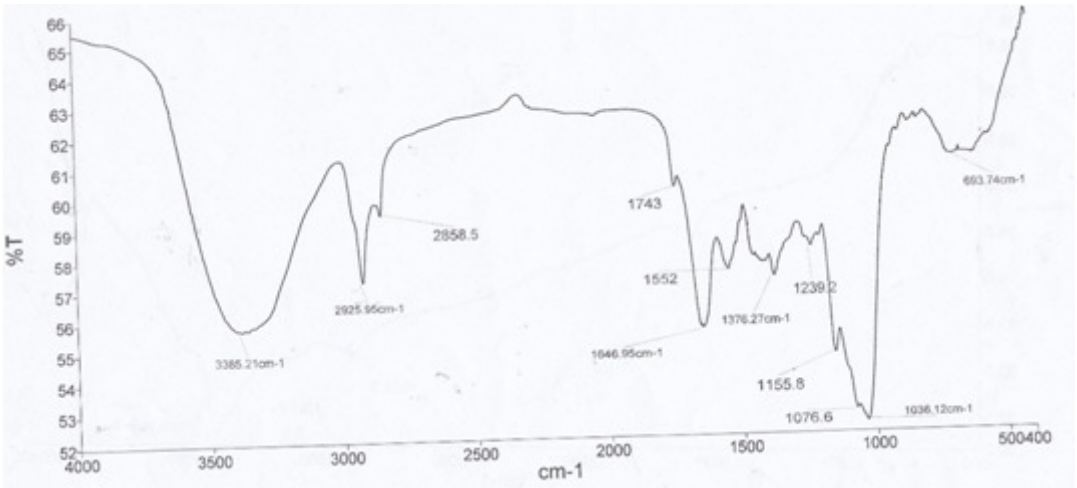

Fig. 2 : Fourier-transform infrared spectroscopy peak values of *A. niger* (MTCC 9652).

and Hamann, 2005; Tayung, *et al*, 2007; Kiran *et al*, 2009; Meenupriya and Thangaraj, 2010, Al-Jassani *et al*, 2016; Kadhim *et al*, 2016; Shareef *et al*, 2016). The present study on bioactive secondary metabolites revealed that *Aspergillus niger* serves as a source of metabolites. These metabolites could be utilized as medicine and in agriculture (Baker, 2006). Nevertheless, in agriculture *A. niger*, as entomopathogenic fungi against various insect pests, is already in practice. However, its application in biocontrol of the notorious insect pests, *D. koenigii* might be an alternative to the synthetic chemical insecticides. Many fungi are found to be parasites on plants, vertebrates and invertebrates including insects. A few of them causes serious diseases in human beings and are proved to be pathogenic against insects. The condition might become fatal if left untreated (Brakhage, 2005).The efficacy of *A. niger* had been observed in control of various insects like in mosquitoes. Lethal effects of *A. niger* has been studied in mosquito vector of filarial, malaria and dengue (Singh and Prakash, 2012).

The fungus caused mortality in the insect pests by modulating the hemolymph thereby affecting the immune system of the host. In our study, the extracted metabolites were applied topically on the abdomen of adult insects and observed regularly for six consecutive days for their mortality analysis. It was observed that the mortality percentage increased with the regular interval in comparison to the control group (Fig. 3). The mortality percentage in the treated group was 80% on sixth days after the treatment whereas in the in the control group it was 23.33%. The differences in mortality between control

Table 1 : Bioactive chemical compounds identified in methanolic extract of *Aspergillus niger* (MTCC no. 9652).

Peak	Name	RT	Formula	Mol.Mass	Exact mol. mass	MS fragment ions
1.	Cyclopropane,octyl	10.073	$C_{11}H_{22}$	154	154.2893	27,41,55,70,84,98,112,126,154
2.	Hexadecane, 1-chloro-	10.232	$C_{16}H_{33}Cl$	260	260.8816	41,43,57,71,91
3.	Nonylcyclohexane	11.009	C_9H_{18}	126	126.2367	27,41,55,67,83,84,98,125,210
4.	1,9-decadiene, 4, 4, 7, 7-tetramethyl-	13.182	$C_{14}H_{26}$	194	194.3526	41,55,69,83,97,111,123, 137,153
5.	1- tetradecene	13.592	$C_{14}H_{28}$	196	196.368	14,27,41,43,57,70,83,97,98,112,126,140,154,168,194
6.	Nonane, 3,7-dimethyl-	13.727	$C_{11}H_{24}$	156	156.3049	27,41,43,57,71,85,98,113,127,141,156
7.	1-hexyl-1-nitrocyclohexane	13.897	$C_{12}H_{22}NO_2$	213	213.3007	39,41,55,69,83,97,111, 125,167,168
8.	Cyclohexane, (1-methylethyl)-	14.564	C_9H_{18}	126	126.2367	27,41,55,67,83,84,111,126
9.	Cyclopropane, 1-methyl-1-(2-methylpropyl)-	15.995	$C_{17}H_{34}$	238	238.4471	41,55,69,83,97,111,125,180,182,238
10.	1- Heptadecene	16.727	$C_{17}H_{34}$	238	238.4471	14,27,41,43,57,70,83,97,111
11.	Pentadecane	16.838	$C_{15}H_{32}$	212	212.4101	41,43,57,71,85,99,113,127,141,155,168,183,212
12.	2- propenoic acid, dodecyl ester	18.151	$C_{15}H_{28}O_2$	240	240.3687	41,55,56,73,97,83,111,127
13.	Propanoic acid, 3-(2,3,6-trimethyl -1,4-dioxaspiro[4,4]non-7	19.433	$C_{14}H_{24}O_4$	256	256.3166	25,27,27,40,55,56,69,81,97,105,125,127,153,169,183 212,227,256
14.	Tetradecyltrifluoroacetate	19.542	$C_{16}H_{29}F_3O_2$	310	310.3812	15,27,41,55,69,83, 97,111,125,140,154,168,194,223,240,292
15.	Acetamide, N-(2-piperidin-4-ylethyl)-	20.334	$C_9H_{18}N_2O$	170	170.2378	33,41,43,56,72,82,85,98,113,127,140,155,170
16.	5-Methyl-hexahydro-pyrano[3,2-b]pyridin-6-one	20.489	$C_9H_{15}NO_2$	169	169.2062	26,27,41,42,57,71,84,98,112,127,141,169
17.	8-Octadecanone	20.580	$C_{18}H_{36}O$	268	268.4683	14,27,41,43,57,71,85,99,124,127,143,155,169,184,197, 211,225,268
18.	Piperidine, 2,3-dimethyl-	20.804	$C_7H_{15}N$	113	113.195	24,27,28,42,56,70,84,98,112
19.	Hexadecanoic acid methyl ester	21.226	$C_{17}H_{34}O_2$	270	270.4369	27,41,57,74,87,101,115,129,143,157,171,185,199,213, 227,239,270
20.	1,6-Heptadiene,2-methyl-6-phenyl-	21.983	$C_{14}H_{18}$	186	186.2902	27,41,51,69,77,91,103,118,129,143,158,171,186
21.	1-Nonadecene	22.089	$C_{19}H_{38}$	266	266.4997	27,41,43,67,69,83,97,111,125,126,140,154,168,182,196 210,224,238,266
22.	1-Octadecanol	24.414	$C_{18}H_{38}O$	270	270.4839	39,41,55,69,83,97,111,125,139,154,168,224,253
23.	1-(4-Ethylpiperazin-1-yl)ethanone	25.300	$C_8H_{16}N_2O$	156	156.2115	14,27,42,56,72,84,99,113,127,141,156
24.	9-Heptadecene-4,6-diyn-8-ol, (Z)-	25.912	$C_{17}H_{26}O$	246	246.3796	27,41,55,67,77,91,105,119,133,147,161,175,189,203 216,333,347,356,390
25.	1,2-Benzenedicarboxylic acid, bis(6-methylheptyl) ester	27.652	$C_{24}H_{38}O_4$	390	390.5326	41,57,71,84,104,113,132,149,167,176,261,279,280,306 333,347,356,390

Table 1 continued...

Table 1 continued...

26.	2-Methyltetracosane	28.804	$C_{27}H_{56}$	380	380.7257	27,41,43,57,71,85,99,113,127,141,155,169,183,197,211, 211,225,239,253,267,281,295,309,337,365
27.	2-(3,7-dimethyl-octa-2,6-dienylideneamino) -4,8-dimethyl-nona-3,7-dienenitrile)	29.519	$C_{21}H_{32}N_2$	312	312.4805	27,41,55,69,81,93,107,121,136,145,161,175,189,216,243, 297,312
28.	L-Valine, N-(N-acetyl-L-valyl)-, butyl ester	29.892	$C_{13}H_{19}NO$	205	205.2853	14,27,41,57,72,85,100,114,127,142,159,174,213,229,241, 263,272,299,314
29.	5-methoxy DIPT	30.285	$C_{17}H_{26}N_2O$	274	274.3858	41,56,72,89,103,114,130,145,160,174,188,216,243,257,274
30.	N-Ethyl-N-(1-methyl-2-phenylethyl)acetamide	30.494	$C_{13}H_{19}NO$	205	205.2853	38,43,56,72,91,103,114,134,148,162,205
31.	Ergosta-5,7,9(11),22-tetraen-3-ol, (3.beta.,22E)-	30.686	$C_{28}H_{42}O$	394	394.6221	41,55,69,81,95,109,125,141,155,181,195,209,211, 225,251,252,277,291,305, 333,348,361,376,379,394
32.	l-Glutamic acid, N-(N-acetyl-l-valyl)-, dibutyl ester	31.262	$C_{20}H_{36}N_2O_6$	400	400.4704	39,41,57,72,84,100,114,130,142,158,170,186,204,213,230,241 241,260,272,288,299,315,327,341,358,385,400
33.	Cholest-1-eno[2,1-a] naphthalene, 3',4'-dihydro-	31.764	$C_{35}H_{52}$	472	472.7801	27,41,43,57,81,93,105,123,134,146,159,173,187,208,227,241 257,273,283,301,311,325,341,355,367,381,396,414,424,443 457,472
34.	Ergosta-5,8,22-trien-3-ol, (3.beta.,22E)-	32.867	$C_{28}H_{44}O$	396	396.6377	41,55,69,81,93,105,119,128,143,157,171,183,199,211,237, 253,271,337, 363,364,378,396
35.	Propafenone	35.396	$C_{21}H_{27}NO_3$	341	341.4231	38,43,65,72,91,98,121,135,147,165,178,207,227,297,312,340

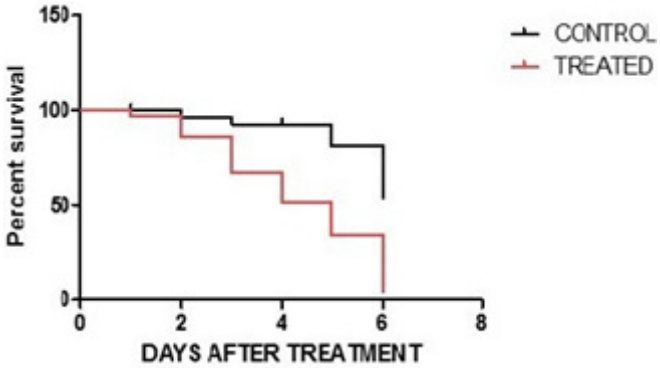

Fig. 3 : Survival analysis of *Dysdercus koenigii* after application of secondary metabolite extract of *Aspergillus niger* (MTCC 9652).

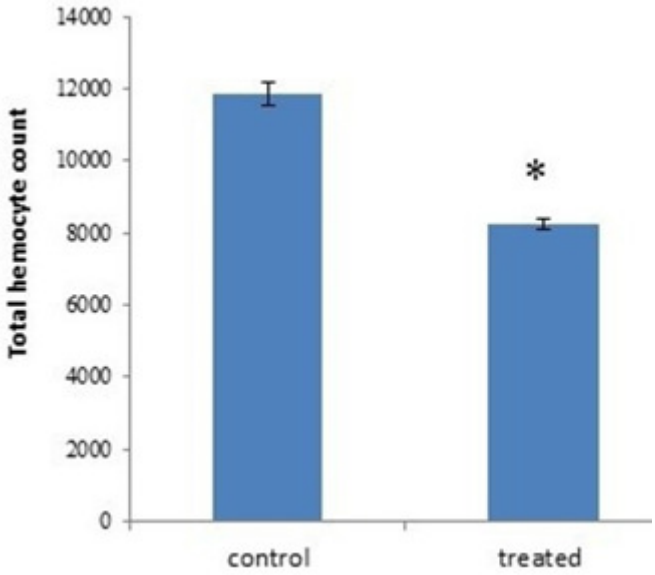

Fig. 4 : Effect of secondary metabolite extracted from *A. niger* (MTCC 9652) on the total hemocyte count of adult *D. koenigii*. Data were represented as mean ± SEM and analysed by unpaired t-test.

and treated group might be due to the presence of bioactive compounds in the *A. niger* extract. Other factors such as alteration in the number of circulating hemocytes may kill the insects. Hemocyte is a primary barrier against any invading pathogens. It provides the cellular response against invaders. Our results revealed the decrease in total hemocyte count i.e. 8236 ± 142.04 after 24 h of the post-treatment in comparison to the control group i.e. 11864.67 ± 328.17 (Fig. 4). The deteriorating condition of the circulating hemocytes after treatment compromises the insect innate defense system, which might prove to be fatal for the host. Our results revealed that enhanced mortality of the insect pest could be due to decreased defense mechanism due to a fall in the number of the circulating hemocytes. As the defense mechanism of the insect was compromised, we are getting an effective result in controlling the insect pests.

Table 2 : Fourier-transform infrared spectroscopy peak values of *Aspergillus niger* (MTCC no. 9652).

S. No.	Peak (Wave number cm^{-1})	Intensity	Bond	Functional group assignment	Group frequency
1.	693.74	74.17	C-H	Aromatic rings	900-690
2.	1036.12	70.48	C-F stretch	Aliphatic flouro compound	1150 -1000
3.	1076.6	70.37	C-F stretch	Aliphatic flouro compound	1150 -1000
4.	1155.8	71.25	C-H	Tertiary amine, C-N stretch	1207-1150
5.	1239.2	73.14	C-H	Tertiary amine, C-N stretch	1207-1150
6.	1376.27	72.55	-	Aromatic nitro compounds	1390-1310
7.	1552	72.18	-	Amide group	1585–1490
8.	1646.95	71.28	-	Amide group	1724–1585
9.	1743	73.17	C=O	Ester carbonyl bond	1724–1585
10.	2858.5	72.59	-	Methylene-CH. asym	2860-2840
11.	2925.95	70.78	-	Methylene-CH. asym	2935-2915
12.	3385.21	71.04	O-H	Normal polymeric O-H stretch	3400-3200

Nevertheless, there are various questions haunting in the mind of researchers; how the secondary metabolites could be effectively used against insect pest in the field without hampering the other beneficial insects and non-target species? What are the mechanisms regulating the synthesis and secretion of secondary metabolites in the fungus culture on a broad scale either intracellularly or extracellularly? These questions remain to be answered to understand the actual phenomena. Moreover, the other underlying mechanism should be traversed while going through the mechanism of action against the insect immune system, like invading cuticle etc.

CONCLUSION

Altogether, thirty-five bioactive chemical compounds have been identified as secondary metabolites from the methanolic extract of *Aspergillus niger* by GC-MS technique. The study related to products of *Aspergillus niger* forms a basic and crucial platform for further pharmacological investigation for the development of a new potential control agent for insect pest management.

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