**Schizophyllum commune** induced genotoxic and cytotoxic effects in *Spodoptera litura*

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In search for ecofriendly alternatives to chemical insecticides the present study was conducted to assess the insecticidal potential of an endophytic fungus *Schizophyllum commune* and its mechanism of toxicity by studying genotoxic and cytotoxic effects as well as repair potential using *Spodoptera litura* (Fabricius) as a model. Different endophytic fungi were isolated and tested for their insecticidal potential against *S. litura*. Among the tested endophytic fungi maximum mortality against *S. litura* was exhibited by *S. commune* isolated from *Aloe vera*. Extended development, reduced adult emergence was observed in larvae fed on diet supplemented with fungal extract. In addition to it the fungus also has propensity to increase oxidative stress which leads to significantly higher DNA damage. The significantly lower frequency of living haemocytes and increased frequency of apoptotic and necrotic cells was also observed in larvae treated with fungal extract. The extent of recovery of damage caused by fungus was found to be very low indicating long term effect of treatment. Phytochemical analysis revealed the presence of various phenolics, terpenoids and protein in fungal extract. Biosafety analysis indicated the non toxic nature of extract. This is the first report showing the insecticidal potential of *S. commune* and the genotoxic and cytotoxic effects associated with it.

Excessive use of insecticides over the years has resulted in the development of insecticide resistant populations of insects. Additionally, these insecticides have many hazardous effects on environment as well as on human health¹. Thus, there is a need to reduce reliance on chemical insecticides and look for other alternative ecofriendly strategies for the control of insect pests. Biological control of insect pests using microbial insecticides is highly beneficial due to its low cost, specificity and safety to ecosystem². Among the various microorganisms fungal endophytes have been known to impart resistance to insect pests. Fungal endophytes are organisms that grow internally in plant tissues without causing any deleterious effect to host plant³. These endophytes produce various bioactive secondary metabolites like alkaloids, flavonoids, phenolic acids and others which have been documented to be responsible for securing their hosts from unfavorable conditions and providing protection from infectious agents⁴. Adverse effects of fungal endophytes on biological parameters of insects have been reported in a number of studies⁵⁻⁶. These have been reported to induce mortality, prolong development period, reduce adult emergence as well as cause various morphological deformities in insects⁷⁻¹¹.

Though a number of studies attributing the effect of fungal bio-pesticides to inhibition of various enzymes and interference with physiological processes are available¹²⁻¹³ the effect at cellular and genetic level has not been explored. The level of DNA damage after treatment with fungal metabolites would be related to the ability of a pest to survive and reproduce. Impaired activity of antioxidant defense and DNA repair contribute to the DNA damage by free radicals. Previously the effect of radiations on the genetic material for the purpose of pest management has been evaluated on *Plodia interpunctella* (Hubner)¹⁴, *Carcelio sikkimensis* (Heller)¹⁵, *Plutella xylostella* (Linnaeus)¹⁶, *Liriomyza trifolii* (Burgess)¹⁷, and *Spodoptera litura* (Fabricius)¹⁸ but no studies are available which show the impact of microbial bio-pesticides up to the DNA level. In the present study genotoxicity of fungal metabolites was assessed by using comet assay. Among the various techniques of genotoxicity testing, the use of comet assay or Single cell gel electrophoresis (SCGE) assay is considered as a simple, quick and inexpensive

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method. It allows the detection of single and double strand breaks, oxidative base damage and DNA cross linking\(^{19}\) as well as assesses the repair potential. So, in the present study attempts have been made to analyze the oxidative stress, cytotoxicity and genotoxicity as well as repair of damage by fungal extract using \textit{S. litura} as a model.

\textit{S. litura} is one of the economically important pest with wide host range of more than 180 plant species\(^{20}\). It has been reported to cause huge crop losses to a number of agriculturally important crops like cabbage, soybeans, cotton, and many other vegetables\(^{21}\). Chemical control is the main strategy being adopted by the farmers which has resulted in development of resistant populations of insects to a large number of insecticides\(^{22}\).

Keeping this in view the present investigation has been undertaken to isolate and screen endophytic fungi for their insecticidal potential against \textit{S. litura}. Oxidative stress, cytotoxicity and genotoxicity induced by ethyl acetate fungal extract were also assessed. Further recovery of all the parameters was ascertained. Biosafety analysis of the selected isolate was also determined.

**Results**

**Isolation and Screening results.** Fifteen different fungi were isolated from different plants and screened for their insecticidal activity. Maximum mortality exhibiting A4 culture (Table 1) was selected for further studies.

**Identification of fungus.** Fungus was identified on morphological and molecular basis. The fungus was rapid growing and produced white cottony colonies on Potato Dextrose Agar (PDA). Microscopic observations revealed branched and septate hyphae. For molecular identification the DNA of the selected fungus was isolated and amplified using ITS1-5.8S-ITS2 region. The length of the amplified sequence was found to be 616 bp. The sequence was deposited into GeneBank under accession number: MF680077. The sequence similarity of obtained sequence was matched with other available databases from NCBI database using BLAST and a phylogenetic tree was constructed using MEGA7 program (Fig. 1). The strain was found to show 100% similarity with different letters a, b, c within the column are significantly different (Tukey's test, \(p < 0.05\)).

| Cultures          | Larval mortality (%) (Mean ± S.E) |
|-------------------|-----------------------------------|
| Control           | 13.33 ± 4.22a                     |
| T1(Tulsi leaf)    | 23.33 ± 6.15a                     |
| T2(Tulsi leaf)    | 46.66 ± 6.66a                     |
| T3(Tulsi leaf)    | 30.00 ± 4.47a                     |
| T4(Tulsi leaf)    | 16.66 ± 6.15a                     |
| A1(Aloevera leaf) | 20.00 ± 7.30a                     |
| A4(Alovera leaf)  | 63.33 ± 9.54                      |
| AL(Almaltas leaf) | 30.00 ± 6.83                      |
| AS(Almaltas stem) | 26.67 ± 8.43                      |
| F value           | 5.357**                           |

Table 1. Screening of fungi isolated from different plants for insecticidal potential against \textit{S. litura}. Values are Mean ± S.E. Significance ascribed as *\(p < 0.05\) and **\(p < 0.01\) (One way ANOVA). The values followed by different letters a, b, c within the column are significantly different (Tukey's test, \(p < 0.05\)).

![Figure 1. Phylogenetic tree showing the position of A4 on the basis of ITS1-5.8 rDNA-ITS2 gene sequence analysis. The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. The analysis involved 14 nucleotide sequences. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 521 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.](image-url)
increased but the significant increase was recorded at 2000 for the concentration of ethyl acetate extract with respect to control (F = 2.870, p < 0.05) and signify the effect of concentration. The highest concentration resulted in 7.333 times higher mortality than control. The percentage of adult emergence (%)

Table 4. Influence of ethyl acetate extract of S. commune on percentage of adult emergence of S. litura. The values are mean ± standard error. Significance ascribed as *p < 0.05 and **p < 0.01 (One way ANOVA). Different letters a, b within the column are significantly different (Tukey’s test, p ≤ 0.05) and signify the effect of concentration.

Table 5. Effect of ethyl acetate extract of S. commune on lipid peroxidation. Table 5 revealed the effect of treatment with ethyl acetate extract of S. commune on lipid peroxidation as indicated by malondialdehyde
Table 5. MDA content in haemolymph of S. litura after treatment with ethyl acetate extract of S. commune. EG = Exposed group. The values represented as mean ± standard error. * Ascribes the significant difference between exposed group and control group (t-test, p < 0.05). Different letters a, b, c between the columns are significantly different (Tukey’s test, p ≤ 0.05) and signify the effect of duration.

| Parameter | Group          | Exposure Time (hrs) |
|-----------|----------------|---------------------|
|           | Control        | 24                  |
|           |                | 48                  |
|           |                | 72                  |
|           |                | 96                  |
| MDA Content | EG (LC50)  | 5.65 ± 0.06*        |
|           |                | 5.83 ± 0.11**       |
|           |                | 5.79 ± 0.07*        |
|           |                | 6.04 ± 0.09**       |

Table 6. Living cells, Apoptotic cells and Necrotic cells percentage in haemolymph of S. litura after treatment with ethyl acetate extract of S. commune. EG = Exposed group. The values represented as mean ± standard error. * Ascribes the significant difference between exposed group and control group (t-test, p < 0.05). Different letters a, b, c between the columns are significantly different (Tukey’s test, p ≤ 0.05) and signify the effect of duration.

(MDA) content in haemolymph of S. litura at different hours of exposure. A non-significant (p > 0.05) change in MDA content at 24 hr exposure group was observed while a significant (p < 0.05) increase in MDA content was observed in 48 hr, 72 hr and 96 hr exposure groups with respect to control. The effect of duration of exposure was also found to be significant (p < 0.05).

Effect of ethyl acetate extract of S. commune on cell viability. The data summarized in Table 6 showed living cells, apoptotic cells and necrotic cells percentage obtained with Acridine Orange/Ethidium Bromide (AO/EB) double staining of haemocytes of S. litura exposed to fungal extract amended diet at different time intervals. A significant (p < 0.05) decrease in living cells percentage while a significant (p < 0.05) increase in apoptotic cells and necrotic cells percentage was observed in larvae exposed to fungal extract as compared to their respective controls (t-test) at each time interval. Further to determine the effect of duration of exposure, ANOVA followed by post hoc Tukey’s test was carried out. It was found that the values of living cells percentage for all exposed groups increased significantly (p < 0.05) up to 72 hours and then decreased. Although, the overall effect of time duration was found to be significant (p < 0.05) in case of apoptotic cells percentage, however, a corresponding increase in percentage of apoptotic cells was not found with increase in time duration. Highest apoptotic cells percentage was observed after a 24 hr of exposure. In case of necrotic cells percentage, a time dependent significant (p < 0.05) increase in induction of necrosis was observed. The images of living cells, apoptotic cells and necrotic cells obtained after AO/EB double staining of haemocytes of S. litura are shown in Fig. 2.

Effect of ethyl acetate extract of S. commune on DNA damage. Table 7 revealed the genotoxic effect of fungal extract following treatment with ethyl acetate extract of S. commune at different hours of exposure using comet assay. The parameters used to assess genotoxicity were Percent Tail DNA and Olive Tail Moment (OTM). The nuclei with a clear tail like extension were observed after treatment indicating that the cells of insect were damaged and DNA strands were also broken. A typical rounded nuclei was observed in control while maximum length of tail was formed after treatment at 96 hrs of exposure. A significant (p < 0.05) increase in Percent Tail DNA and OTM values was observed in exposed groups as compared to their respective controls at all time intervals. The effect of duration of exposure was also found to be significant (p < 0.05). At 72 hour of exposure the values decreased slightly but again at 96 hour of exposure the values of Percent Tail DNA and OTM were found to be increased.

Recovery of DNA damage. Recovery of DNA damage, MDA content and cell viability was assessed in both recovery groups (R1 and R2) and compared with their respective exposure time groups. The results have been summarized in Fig. 3(a–f).

S. litura larvae exposed to fungal extract for 24 hr and 48 hr did not show any significant (p > 0.05) change in MDA content in recovery groups as compared to their respective exposure time group. However 72 hr exposure time group showed a significant (p < 0.05) decrease in MDA content in recovery groups as compared to its exposure time group, and 96 hr exposure time group showed a significant (p < 0.05) increase in MDA content in both recovery groups with respect to its exposure time group. So it is clearly indicated that the level of lipid peroxidation as indicated by value of MDA content did not get lowered after feeding larvae to control diet (Fig. 3a).

Figure 3(b–d) summarize the results of Living Cells Percentage, Apoptotic Cells Percentage and Necrotic Cells percentage in recovery groups of each time exposure as compared to their respective exposure time group.
24 hr exposure time group the value of living cells percentage was found to be increase significantly \((p < 0.05)\) and the value of apoptotic cells percentage was decreased significantly \((p < 0.05)\) and the value of necrotic cells increased but non-significantly \((p > 0.05)\). Perusal of the Fig. 3(b–d) clearly indicated that for the three parameters of cell viability testing, no recovery was observed. Living cell percentage was found to be low in recovery groups while the necrotic cells percentage was found to be significantly \((p < 0.05)\) high in recovery groups. So in overall apoptosis and necrosis was not getting lowered after giving control diet to larvae which were fed with fungal extract amended diet.

Figure 3(e–f) reveal the results of recovery of DNA damage. The values showed variable trend in the recovery groups for both the parameters. The values of %Tail DNA and OTM increased significantly \((p < 0.05)\) after 1 day of recovery and then decreased after 2days of recovery for 24 hr exposure time group. however for 48 hr and 72 hr exposure time groups the values of %Tail DNA and OTM significantly \((p < 0.05)\) decreased in both recovery groups as compared to their respective exposure time groups. For 96 hr exposure time group the values of both parameters did not found to be differ significantly \((p > 0.05)\) in recovery groups as compared to exposure time group.

Phytochemical detection results. Phytochemicals tests revealed the presence of glycosides, proteins, flavanoids, triterpenoids and phenolics in the ethyl acetate extract of *S. commune*. Folin-Ciocalteu assay determined the Total Phenolic Content (TPC) which was equivalent to 870 \(\mu g/ml\) of gallic acid. Ultra-high performance liquid chromatography (UHPLC) analysis confirmed the presence of gallic acid, catechin, chlorogenic acid, epicatechin, caffeic acid, coumaric acid, rutin, quercetin and kaempferol phenolic compounds in ethyl acetate extract of fungus *S. commune* (Fig. 4).

Effect of ethyl acetate extract of *S. commune* on Chinese Hamster Ovary (CHO) cell lines. To find out the effect of fungal extract on mammals MTT assay on CHO cell lines was carried out. The data is summarized in Fig. 5 which revealed the effect of treatment with different concentrations of *S. commune* fungal extract on Relative cell viability. Relative cell viability of control was found to be 100% and after treatment with highest concentration of fungal extract it was found to be 81.82% which is very much higher as compared to doxorubicin (DOX) a cytotoxic drug treated cells. So *S. commune* fungal extract is proved to be safe for mammalian cell lines showing negligible effect on CHO cell lines.

| Parameters | Groups | Exposure Time (hrs) |
|------------|--------|---------------------|
| Percent Tail DNA | Control | 5.54 ± 0.38a |
| | EG(LC50) | 10.02 ± 0.51* |
| OTM | Control | 4.01 ± 0.46a |
| | EG(LC50) | 6.55 ± 0.58** |

Table 7. Percent Tail DNA and OTM in haemocytes of *S. litura* after treatment with ethyl acetate extract of *S. commune*. EG = Exposed group. The values represented as mean ± standard error. * Ascribes the significant difference between exposed group and control group \((t-test, p \leq 0.05)\). Different letters a, b, c between the columns are significantly different \((Tukey's test, p \leq 0.05)\) and signify the effect of duration.

To find out the effect of fungal extract on mammals MTT assay on CHO cell lines was carried out. The data is summarized in Fig. 5 which revealed the effect of treatment with different concentrations of *S. commune* fungal extract on Relative cell viability. Relative cell viability of control was found to be 100% and after treatment with highest concentration of fungal extract it was found to be 81.82% which is very much higher as compared to doxorubicin (DOX) a cytotoxic drug treated cells. So *S. commune* fungal extract is proved to be safe for mammalian cell lines showing negligible effect on CHO cell lines.

![Typical morphological changes of haemocytes induced by ethyl acetate extract of *S. commune*, stained with AO/EB.](image)
Discussion

Within the last two decades endophytes have attracted greater attention due to their capabilities to protect their host from pest and pathogens. Studies have concluded that plants inoculated with endophytic fungi showed resistance against insect pests as compared to non inoculated plants. Prior inoculation of tomato plants with an endophytic fungi *Acremonium strictum* was found to decrease the performance of the pest cotton bollworm (*Helicoverpa armigera*) (Hubner)\(^{23}\). Similarly inoculation of squash plants with an endophytic strain of *Fusarium oxysporum* is reported to provide protection from *Aphis gossypii* (Glover)\(^{24}\). Retarded development was also observed in insects fed on endophytes inoculated plants\(^{25}\). Endophytic fungi *Paecilomyces* sp. and *Cladosporium* sp. isolated from leaves of *Nerium oleander* were evaluated as biocontrol agents against bean weevil...
Acanthoscelides obtectus (Say)\textsuperscript{30}. On a wide variety of crops, fungal endophytes have been reported to deter feeding, oviposition and performance of stem boring, sap sucking, chewing and leaf mining insects\textsuperscript{37}. On the other hand endophytic fungi are also used as a potential candidate for systemic delivery of biopesticides to the host plant without direct manipulation of plant genome and can improve anti-insect activity of the plant. Zhao et al.\textsuperscript{38} used recombinant fungus to control sap sucking pest. Qi et al.\textsuperscript{28} developed a pest management strategy to control sap sucking insect using recombinant endophytic fungi Chetomium globosum expressing Pinellia ternate agglutinin.

In the present study endophytic fungi were isolated from various plants and their insecticidal activity was evaluated against S. litura. Maximum mortality was evinced by culture A4 identified as Schizophyllum commune. S. commune is basidiomycetes belonging to the schizophyllaceae family of order agaricales. It is a cosmopolitan species and wood rot basidiomycetes\textsuperscript{30}. It is also popular as an edible mushroom in some regions eg. Peninsular Malaysia\textsuperscript{44} and Mexico\textsuperscript{52}. It has been found to be endophyte of Musa spp., Tectona grandis and Panax ginseng etc.\textsuperscript{33-35}.

Various bioactivities like antibacterial\textsuperscript{36} and antifungal\textsuperscript{37,38} have been reported from of S. commune. It has been reported to produce some volatile organic compounds (VOCs) having nematicidal and nematostatic effects\textsuperscript{39}. Though various activities shown by S. commune were reported earlier but insecticidal properties of this fungus have not been described yet. So to the best of our knowledge this is the first report indicating its insecticidal potential.

It was observed that the artificial diet supplemented with different concentrations of ethyl acetate extract of S. commune exerted negative effect on larval survival. All concentrations showed significantly higher mortality than control. The fungal extract was found to extend the development period and reduce the emergence of S. litura adults. Recently few studies have documented the insecticidal activity of endophytic fungi viz Alternaria sp., Cladosporium sp. and Nigrospora sp. on S. litura\textsuperscript{40,41}. Some endophytic fungi Aspergillus flavus, Nigrospora sp. and Comomyrtium sp. showed adverse effects on life cycle of Aphis gossypii (Glover)\textsuperscript{42}. S. commune has been reported to produce secondary metabolites like phthalic acid and triterpenoid saponin\textsuperscript{1,36}. There are some reports which showed insecticidal activity of phthalic acid derivatives. Phthalic acid diamides derivatives have been reported to show insecticidal activities against Pseudoaletia separate (Walker), Plutella xylostella (Linnaeus.), Mythimna separate (Walker), Spodoptera exigua (Hubner)\textsuperscript{44,45}. Triterpene saponin was also been found to exert negative impact on Acyrthosiphon pism (Harris) and on some other insects\textsuperscript{46,47}. In the present study phytochemical screening of extract showed the presence of phenolics. Previous literature has evinced the insecticidal activity of few phenolic compounds like gallic acid, rutin, quercetin, chlorogenic acid etc\textsuperscript{48,49}. So the high amount of phenolics present in the extract could also be responsible for insecticidal activity.

To ascertain the cause of high mortality and abnormal development of S. litura after treatment with fungal extract further observations at cellular and genetic levels were made. The DNA damage and repair as well as cell viability and induced oxidative stress in the exposed group were studied. MDA content in fungal extract further observations at cellular and genetic levels were made. The DNA damage and repair as well as cell viability and induced oxidative stress in the exposed group were studied. MDA content in fungal extract further observations at cellular and genetic levels were made. The DNA damage and repair as well as cell viability and induced oxidative stress in the exposed group were studied. MDA content in fungal extract further observations at cellular and genetic levels were made. The DNA damage and repair as well as cell viability and induced oxidative stress in the exposed group were studied.

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Aphis gossypii sp., Aphis gossypii gossypii (Glover)\textsuperscript{43}. S. commune has been reported to produce secondary metabolites like phthalic acid and triterpenoid saponin\textsuperscript{1,36}. There are some reports which showed insecticidal activity of phthalic acid derivatives. Phthalic acid diamides derivatives have been reported to show insecticidal activities against Pseudoaletia separate (Walker), Plutella xylostella (Linnaeus.), Mythimna separate (Walker), Spodoptera exigua (Hubner)\textsuperscript{44,45}. Triterpene saponin was also been found to exert negative impact on Acyrthosiphon pism (Harris) and on some other insects\textsuperscript{46,47}. In the present study phytochemical screening of extract showed the presence of phenolics. Previous literature has evinced the insecticidal activity of few phenolic compounds like gallic acid, rutin, quercetin, chlorogenic acid etc\textsuperscript{48,49}. So the high amount of phenolics present in the extract could also be responsible for insecticidal activity.

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DNA damage in context of biocontrol:\(^\text{18}^\text{,}^\text{61}\). Shetty et al.\(^\text{65}\) evaluated the gamma radiation induced DNA damage in \textit{Aedes aegypti} using comet assay. All these studies used insect as a model to check toxicity of pollutants and other toxic chemicals.

In addition to DNA damage DNA repair was also studied in the exposure groups after feeding the control diet for 1 day and 2 days. Other parameters viz oxidative stress and cytotoxicity was also measured in repair groups and compared with their respective exposure time groups. It is clear from the results that the lipid peroxidation profile and cytotoxicity was also not decline in recovery groups as well as the DNA damage have not showed any repair in the all recovery groups. Low level of DNA damage was partially repaired but high DNA damage was not repaired. DNA damage and repair studies are more prominent in vertebrates; invertebrates are less explored in context of this. Recently one study examined the susceptibility to oxidative stress, DNA Damage, and repair in four invertebrates by using lymphocytes from Atlantic cod (\textit{Gadus morhua}) as a reference. Results indicated that invertebrate's cells were more susceptible to oxidative stress induced DNA damage as compared to lymphocytes of \textit{Gadus morhua} as well as no recovery observed in any species of invertebrates\(^\text{63}\). Yun et al.\(^\text{66}\) studied the electron beam induced DNA damage and repair in \textit{S. litura} and reported that low dose of electron beam radiation resulted repair in damaged DNA but high dose of electron beam irradiation induced non-repairable DNA damage. The reason behind this might be a DNA repair capacity and cell proliferation rate. In invertebrates repair system is slight less efficient than vertebrates and very less difference in repair capacity leads to 10 to 20 fold more susceptibility to genotoxicants. On the other hand if cell proliferation rate is slow then the damaged cells were less frequently eliminated which might be a reason behind more damage and less repair.

At the end MTT assay was carried out on CHO cell lines. The results showed negligible toxicity of fungal extract on CHO cell lines which indicated its safety for mammals. Earlier cytotoxicity study by MTT assay was carried out on \textit{Spodoptera litura} SL-1 cells to check the toxicity of destruxin A and it was observed that destruxin A was highly toxic for SL-1 cells\(^\text{64}\).

Conclusion

In the light of above discussion it has been concluded that an endophytic fungus \textit{Schizopyllum commune} exhibited insecticidal activity due to its long term cytotoxic and genotoxic effects against \textit{S. litura}. The study suggests the potential of \textit{S. commune} as a biocontrol agent. Further there is need to conduct field studies to evaluates its efficacy under natural conditions.

Materials and Methods

Insect rearing. Larvae of \textit{S. litura} were collected from the cauliflower fields around Amritsar (Punjab) India. The collected insect population was reared in laboratory under controlled temperature (25 ± 2°C) and humidity (60–70%) conditions\(^\text{81}\).

Isolation of fungi. Endophytic fungi were isolated from leaves and stems of healthy plants (Tulsi, Aloevera and Amaltas) collected from Amritsar (India). Collected samples were thoroughly washed with running tap water and sterilized sequentially with 70% ethanol for 2 min, 5% sodium hypochlorite solution for 5 min and then rinsed with sterile distilled water. Surface sterilization was confirmed by plating the water obtained after last wash. Each sterilized sample was cut into small pieces and placed on water agar plates supplemented with ampicillin (200 mg/ml) to suppress the bacterial growth. Plates were incubated at 30°C till the emergence of hyphae. After emergence of hyphae, the hyphae tips were picked and cultured on Potato Dextrose Agar (PDA) plates. The culture was purified and maintained on PDA and stored at 4°C\(^\text{60}\).

Production of fungal extract. The production was carried out in 50 ml malt extract (malt extract = 20 g/l, dextrose = 20 g/l, peptone = 1 g/l, pH = 5.5) broth in 250 ml Erlenmeyer flask by inoculating one plug (1 cm square) taken from the periphery of an actively growing culture. The flasks were incubated at 30°C and 250 rpm for 10 days. After 10 days extraction was carried out twice using ethyl acetate at 120 rpm and 40°C. The extracts were concentrated by using rotavapor and dissolved in 1 ml Dimethyl sulfoxide (DMSO) and stored at 4°C.

Screening of fungi. Fifteen endophytic fungi were isolated and their insecticidal potential was screened. To screen the insecticidal potential of all isolated fungi, an artificial diet as recommended by Koul et al.\(^\text{66}\) with slight modifications was supplemented with ethyl acetate extract of all isolated fungal strains at a concentration of 500µg/ml in 0.5% Dimethyl sulfoxide (DMSO). The second instar larvae (6 days old) were fed on control (0.5% DMSO) as well as on extract supplemented diet. The experiment was replicated six times with five larvae per replication. The larvae were observed daily. Among the all tested cultures of fungi, A4 induced maximum mortality. So A4 culture was selected for further studies.

Identification of fungus. The active culture A4 exhibiting maximum insecticidal activity was identified on morphological and molecular basis. Slide culturing was done for morphological characterization using standard taxonomic keys\(^\text{60}\). For Molecular identification, DNA was isolated and amplification of the region covering ITS1-5.8S- rDNA- ITS2 was carried out by using forward ITS1 and reverse ITS4 primer. Purification and sequencing of DNA amplicons was performed at first base sequencing (Malaysia). The sequence similarity was matched with other available databases retrieved from NCBI using BLAST\(^\text{67}\).

Phylogenetic Analysis. Alignment of retrieved sequences was done using ClustalW program. Phylogeny tree was constructed using Phylogeny in MEGA 7 program with 1000 bootstrap. Evolutionary history was inferred using neighbor-joining method. Evolutionary distance was computed using kimura-2-parameter model.
Bioassay studies. To evaluate the effect of A4 fungal extract on different biological parameters of S. litura different concentrations (125, 250, 500, 1000 and 2000 µg/ml) of fungal extract were made from stock solution having concentration 470 mg/ml in 0.5% DMSO and added in artificial diet. The Second instar larvae (6 days old) were reared on fungal extract amended diets as well as with control diet (0.5% DMSO) at controlled temperature 25 ± 2°C and relative humidity 70 ± 5%. The experiment was replicated three times. For each treatment and control there are 10 larvae per replication. Each larva was put in separate container (4 × 6 cm) and the diet was changed daily. All these parameters were assessed using haemolymph of 12 days old larvae. For this haemolymph was collected from 10 larvae fed with same concentration and then it was pooled. The effect of fungal extract has been recorded on all above mentioned parameters after different time intervals (24 hr, 48 hr, 72 hr and 96 hr) using the following protocols: (a,b,c)

(a) Lipid peroxidation or malondialdehyde (MDA) content analysis:
For lipid peroxidation estimation, collected pooled haemolymph was mixed with PBS (Potassium Phosphate buffer pH 7.0) containing 0.01% phenylthiourea. Haemocytes were removed from haemolymph by centrifuging it for 20 min at 1000 g, at 4°C and supernatant was used for MDA content determination...

(b) Cell viability assay:
For this acridine orange/ethidium bromide (AO/EB) double staining was used. Collected pooled haemolymph was stained with dye mixer (Acridine orange 100 µg/ml Sigma Chemical Co. and Ethidium bromide 100 µg/ml Sigma Chemical Co.). Finally stained cells were analyzed under fluorescent microscope at 400 × magnification. Viable cells emit green fluorescence and necrotic cells emit red fluorescence Er et al...37.

(c) Comet assay:
The DNA damage was quantified by alkaline single cell gel electrophoresis according to Youssef et al.39 and Yun et al.40 with slight modifications. For this collected pooled haemolymph (from 12 days old larvae) was mixed with phosphate buffered saline (PBS) [Sodium chloride (NaCl) 8 g/l, Potassium chloride (KCl) 0.20 g/l, sodium dihydrogen phosphate 11.50 g/l, potassium dihydrogen orthophosphate 0.20 g/l, double distilled water, pH 7.4] containing 0.01% Phenylthiourea (PTU) and layered on microscopic slides precoated with Normal Melting Point Agarose (NMPA 1% in PBS) after mixing with Low Melting Point Agarose (LMPA 0.75% in PBS), LMPA and haemolymph mixed slides were covered with cover slips and placed on ice. After solidification of agarose cover slips were removed and slides were placed in lysing buffer [2.5 M NaCl, 100 mM Ethylene diamine tetra acetic acid (EDTA), 0.25 M Tris aminomethane, 0.25 M Sodium hydroxide (NaOH), 1% triton X-100, 10% Dimethyl sulfoxide (DMSO), double distilled water, pH 10.0] for 2 hrs at 4°C. After lysing slides were put in an electrophoresis buffer (1 mM EDTA, 300 mM NaOH, double distilled water, pH 13) for 20 mins to unwind DNA and then electrophoresis was carried out for 20 min at 25 V and 300 mA. At the end neutralization was done for 15 min by using neutralization buffer (0.4 M Tris amino methane, double distilled water pH 7.5). The slides were dried overnight and then stained with ethidium bromide (20 µg/ml) and analysed under a Nikon fluorescent microscope. DNA damage was assessed by using Casplab software.

Recovery. After giving treatment of fungal extract at each time interval recovery of DNA damage was checked after 1 day and 2 days by feeding treated larvae to control diet. Each exposure time (ET) has two recovery groups. i.e R1 = Recovery after 1 day, R2 = Recovery after 2 days. The experiment was replicated three times. For each treatment there are 20 larvae per replication. After treatment at particular time duration these 20 larvae were fed with control diet, out of these 10 larvae were used to study recovery after 1 day and 10 were used to study recovery after 2 days. Each larva was put in separate container (4 × 6 cm) and the diet was changed daily. The recovery of DNA damage was assessed by comet assay. Before checking recovery of DNA damage of each group, their lipid peroxidation profile and cell viability was also checked.

Phytochemicals analysis. Detection of various phytochemicals viz phenolic, saponins, tannins, steroids, triterpenoids, glycosides, proteins was carried out by following the methodology of et al...39–72. Total phenolic content (TPC) was determined by using Folin-Ciocalteu assay according to Hossain...73. For identification of phenolic compounds High performance liquid chromatography (HPLC) analyses were performed with Ultra-high performance liquid chromatography (UHPLC) (Shimadzu) system consisting of Nexera model. The mobile phase composed of 0.1 acetic acid and methanol. Flow rate was adjusted to 1 ml/min. The injection volume was 10 µl and the column was maintained at 40°C and 2500 psi. The separation was achieved by a reversed-phase chromatographic using a C18 column (150 mm length and 4.6 mm diameter) having particle size of 5 µm. HPLC chromatograms were detected using a photo diode array detector (Dionex UVD 340U UV/Vis) at 280 nm wavelengths according to absorption maxima of analyzed compounds. Detection of phenolic compounds was carried out by comparison...
with standards. Each compound was identified by its retention time and by spiking with standards under the same conditions.12

Safety evaluation of *S. commune* fungal extract by MTT assay on Chinese hamster ovary (CHO) cell lines. The *in vitro* cytotoxicity of *S. commune* fungal extract was assessed by using CHO cell lines by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay. CHO cell line was procured from the National Centre for Cell Science, Pune, India. Cells were grown in Dulbecco’s modified Eagle’s medium supplemented with streptomycin (100 U ml⁻¹), gentamycin (100μg/ml⁻¹), amphotericin B (0.25μg/ml⁻¹) and 10% fetal bovine serum (Hydema) in a CO₂ incubator (5% CO₂, 90% Relative Humidity) at 37°C. 5 × 10⁴ cells were added in each well of 96 well plate and incubated at 37°C, 5% CO₂ for 4 h. The cells were treated with different concentration of *S. commune* fungal extract (1000, 500, 250, 125, 62.5μg/ml) for 48 h, washed and 100μl of fresh medium with 20μl MTT solution (5 mg ml⁻¹) was added in each well. The cells were incubated at 37°C, 5% CO₂, for 4 h. After incubation the medium was removed and formazan product was dissolved in 100μl of DMSO (Dimethyl sulfoxide) and shaken for 10 min. The optical density was measured at 550 nm by microplate reader. Percentage of cell growth was calculated by using formula:

\[
\text{Percentage cell viability} = \left( \frac{\text{OD of treated cells}}{\text{OD of control}} \right) \times 100
\]

Statistical analysis. To study the effect of duration, recovery and to compare different concentrations while screening and bioassay studies one way analysis of variance (ANOVA) with Tukey’s test was performed and to study the effect of treatment student’s *t*-test was applied

Availability of data and materials. All data generated or analyzed during this study are included in this published article and its additional files.

Ethical approval and consent to participate. This article does not contain any studies involving human participants or animals performed by any of the authors.

References
1. Aktar, W., Sengupta, D. & Chowdhury, A. Impact of pesticides use in agriculture: their benefits and hazards. *Interdiscip Toxicol*. 2(1), 1–2 (2009).
2. Castillo, M. A., Moya, P., Hernandez, E. & Primo-Yufera, E. Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to entomopathogenic fungi and their extracts. *Biol control*. 19(3), 274–282 (2000).
3. Wu, H., Yang, H., You, X. & Li, Y. Isolation and characterization of saponin-producing fungal endophytes from *Aralia elata* in Northeast China. *Int J of Mol Sci*. 13(12), 16255–16266 (2012).
4. Knight, V. et al. Diversifying microbial natural products for drug discovery. *Appl Microbiol Biotechnol*. 62(3–6), 446–458 (2003).
5. Shi, Y., Zhang, X. & Lou, K. Isolation, characterization, and insecticidal activity of an endophyte of drunken horse grass, *Achnatherum inebrians*. *J of Insect Sci*. 13(1), 151–162 (2013).
6. Namasiyawat, S. K., Sekar, S. & Bharani, R. A. Pesticidal activity of endophytic fungal metabolites against major groundnut defoliator *Spodoptera littoralis* (Fab.) (*Lepidoptera: Noctuidae*). *J Biopest*. 86, 577–587 (2004).
7. Shi, Y., Zhang, X. & Lou, K. Isolation, characterization, and insecticidal activity of an endophyte of drunken horse grass, *Achnatherum inebrians*. *J of Insect Sci*. 13(1), 151–162 (2013).
8. Namasiyawat, S. K., Sekar, S. & Bharani, R. A. Pesticidal activity of endophytic fungal metabolites against major groundnut defoliator *Spodoptera littoralis* (Fab.) (*Lepidoptera: Noctuidae*). *J Biopest*. 86, 577–587 (2004).
9. Bensaci, O. A., Daoud, H., Lombarkia, N. & Rouabah, K. Formulation of the endophytic fungus *Cladosporium oxysporum* subsp. *luteola* as a new biocontrol tool against the black bean aphid (*Aphis fabae* Scop.). *J Plant Prot Res*. 55(1), 80–87 (2015).
10. Kaur, H. P., Singh, B., Kaur, A. & Kaur, S. Antifeedent and toxic activity of endophytic *Alternaria alternata* against tobacco caterpillar *Spodoptera littoralis*. *J Pest Sci*. 86(3), 543–550 (2013).
11. Singh, B., Kaur, T., Kaur, S., Manhas, R. K. & Kaur, A. Insecticidal potential of an endophytic *Cladosporium velox* against *Spodoptera littoralis* mediated through inhibition of alpha glycosidases. *Pestic Biochem Physiol*. 131, 46–52 (2016).
12. Abraham, S., Basukriadi, A., Pawiroharsono, S. & Sjamsuridzal, W. Insecticidal activity of ethyl acetate extracts from *Achnatherum inebrians* (*Cyperaceae*) against major coniferous forest pests in Indonesia. *Mycoecology*. 43(2), 137–149 (2015).
13. Imamura, T. et al. Effect of “soft-electron” (low-energy electron) treatment on three stored-product insect pests. *J of Stored Prod Res*. 40(2), 169–177 (2004).
14. Todoroki, S. et al. Assessment of electron beam-induced DNA damage in larvae of chestnut weevil, *Curculio sikkimensis* (Heller) (*Coleoptera: Curculionidae*) using comet assay. *Radiat Phys Chem*. 75(2), 292–296 (2006).
15. Koo, H. N. et al. Effect of electron beam irradiation on developmental stages of *Plutella xylostella* (Lepidoptera: *Plutellidae*). *Asia Pac Entomol*. 14(3), 243–247 (2011).
16. Koo, H. N., Yun, S. H., Yoon, C. & Kim, G. H. Electron beam irradiation induces abnormal development and the stabilization of p53 protein of American serpentine leafminer, *Liriomyza trifoli* (Burgess). *Radiat Phys Chem*. 81(1), 86–92 (2012).
17. Yun, S. H., Lee, S. W., Koo, H. N. & Kim, G. H. Assessment of electron beam-induced abnormal development and DNA damage in *Spodoptera littoralis* (E) (*Lepidoptera: Noctuidae*). *Radiat Phys Chem*. 96, 44–49 (2014).
18. Yousef, H. A., Afly, A., Hasan, H. M. & Meguid, A. A. DNA damage in hemocytes of *Schistocerca gregaria* (Orthoptera: Acrididae) exposed to contaminated food with cadmium and lead. *Natural Science*. 2(2), 290 (2010).
19. Rhamphithilax, J., Krishnappa, K. & Elumalai, K. Effect of plant oil formulations against armyworm, *Spodoptera littoralis* (Fab.). *J Insect Prot Res*. Int J Curr Life Sci. 2(1), 1–4 (2012).
20. Khan, R. R., Ahmed, S. & Nisar, M. Mortality responses of *Spodoptera littoralis* (Fab.) (*Lepidoptera: Noctuidae*) against some conventional and new chemistry insecticides under laboratory conditions. *Pakistan Entomologists*. 33(2), 147–150 (2011).
21. Kramhi, K. R. et al. Insecticide resistance in five major insect pests of cotton in India. *Crop Protection*. 21(6), 449–460 (2002).
22. Jallow, M. F., Dugassa-Gobena, D. & Vidal, S. Indirect interaction between an unspecialized endophytic fungus and a polyphagous moth. *Basic Appl Ecol*. 5(2), 183–191 (2004).
24. Martinuzz, A., Schouten, A., Menjivar, R. D. & Sikora, R. A. Effectiveness of systemic resistance toward Apis gossypii (Hom., Aphididae) as induced by combined applications of the endophytes Fusarium oxysporum Fo162 and Rhi zobium elli G12. Biol Control. 62(3), 206–212 (2012).

25. Muvea, A. M. et al. Colonization of onions by endophytic fungi and their impacts on the biology of Thrips tabaci. PLoS ONE. 9(9), 1–7 (2014).

26. Benací, O. A., Lombarka, N. & Lah, D. E. 5–10 Initial evaluation of endophytic fungi, isolated from Nerium oleander L. for their biocontrol action against the bruchid Acanthoscelides obtectus (Say) (Coleoptera: Bruchidae) in Algeria. Endophytes for plant protection: the state of the art. p. 162 (2013).

27. Kamberk, D. N. New paradigms in exploration of microbial endophytes in insect pest management. J. Farm Sci. 29(4), 420–435 (2016).

28. Zhao, X., Qi, G., Zhang, X., Lan, N. & Ma, X. Controlling sap-sucking insect pests with recombinant endophytes expressing plant lectin. Nature preceedings. 21, 1–30 (2010).

29. Qi, G., Lan, N., Ma, X., Yu, Z. & Zhao, X. Controlling Myzus persicae with recombinant endophytic fungi Chaetomium globosum expressing Pinnelia ternata agglutinin. Journal of appl microbiol. 110(5), 1314–1322 (2011).

30. Takemoto, S., Nakamura, H., Imamura, Y. & Shimane, T. Schizophyllum commune as a ubiquitous plant parasite. Jap Jpn Agr Res Q: 44(4), 357–364 (2010).

31. Lee, S. S., Chang, Y. S., Mohd, N. & Rashid, N. Common edible mushrooms of Orang Asli communities in Peninsular Malaysia. Institut Penyelidikan Perhataan Malaysia (2006).

32. Olivo-Aranda, F. & Herrera, T. The species of Schizopyllum in México, their ecological distribution and ethnomycological importance. Rev. Mex. Mic. 10, 21–32 (1994).

33. Assunção, M. M., Cavalcanti, M. D. & Menezes, M. Schizophyllum commune isolated from banana leaves (Musa spp.), as endophytic fungi, in Pernambuco, Brazil. Agrotropica. 22(2), 67–70 (2010).

34. Chareprasert, S. et al. Endophytic fungi of tea leaves Tectona grandis L. and rain tree leaves Samaan saman Merr. World J. Microbial Biotechnol. 22(5), 481–486 (2006).

35. Zhai, X. et al. A stable beneficial symbiotic relationship between endophytic fungus Schizophyllum commune and host plant Panax ginseng. bioRes. 175885 (2017).

36. Joel, E. L. & Bhimba, B. V. A secondary metabolite with antibacterial activity produced by mangrove foliar fungus Schizophyllum commune. International J of Chem. Environ & Bio Sci. 1(1), 2320–4087 (2013).

37. Teoh, Y. P., Don, M. M. & Ujang, S. Production of Biomass by Schizophyllum commune and Its Antifungal Activity towards Rubberwood-Degrading Fungi. Sains Malays. 46(1), 123–128 (2017).

38. Schalchli, H. et al. Antifungal activity of volatile metabolites emitted by mycelial cultures of saprophytic fungi. Chem. Ecol. 27(6), 503–513 (2011).

39. Pimenta, L., Ferreira, M. A., Pedroso, M. P. & Campos, V. P. Wood-associated fungi produce volatile organic compounds toxic to root-knot nematode. Scientia Agricola. 74(4), 303–310 (2017).

40. Singh, B. et al. Acetyloleimolestine inhibitory potential and insecticidal activity of an endophytic Alternaria sp. from Ricinus communis. Appl Biochem Biotechnol. 168(5), 991–1002 (2012).

41. Thakur, A., Kaur, S., Kaur, A. & Singh, V. Detrimental effects of endophytic fungus Nigrospora sp. on survival and development of Spodoptera litura. Biocontrol Sci Techn. 22(2), 151–161 (2012).

42. Thakur, A., Singh, V., Kaur, A. & Kaur, S. Insecticidal potential of an endophytic fungus, Cladosporium uredinicola, against Spodoptera litura. Phytoparasitica. 41(4), 373–382 (2013).

43. Hernawati, H., Wiyono, S. & Santoso, S. Leaf endophytic fungi of chil (Capsicum annuum) and its role in the protection against Aphis gossypii (Homoptera: Aphididae). Biodiversitas. 21(4) (2011).

44. Zhou, S. et al. Design, Synthesis, Structure-Activity Relationship and Insecticidal Activities of Trifluoromethyl-Containing Sulfilimyl and Sulfoximinyl Phthalic Acid Diamide Structure. Chin. J. Chem. 32(7), 567–572 (2014).

45. Yan, T. et al. Synthesis and Insecticidal Activities of Novel Phthalic Acid Diamides. Chin. J. Chem. 30(7), 1445–1452 (2012).

46. De Geyer, E., Lambert, E., Geelen, D. & Smaghe, G. Novel advances with plant saponins as natural insecticides to control pest insects. Pest Technol. 1(2), 96–105 (2007).

47. De Geyer, E., Smaghe, G., Rabbe, Y. & Geelen, D. Triterpene saponins of Quillaja saponaria show strong aphidical and deterrrent activity against the pea aphid Acyrthosiphon pisum. Pest Manag. Sci. 68(2), 164–169 (2012).

48. Marques, T. R. et al. Muliginia emarginata DC. bagasse acetone extract: Phenolic compounds and their effect on Spodoptera frugiperda (JE Smith) (Lepidoptera: Noctuidae). Chin J Agr Res. 76(1), 55–61 (2016).

49. Bouzouina, M. M. et al. Effects of Citrus sinensis crude phenolic extract on the larval development of Phylocnistis citrella stain (lepiderpota: Gracillaridiae). Biol J Agric Sci. 18, 334–341 (2012).

50. Buyukguzel, E. et al. Effect of boric acid on glutantiam enzyme activity, lipid peroxidation, and ultrastructure of midgut and fat body of Galleria mellonella. Cell Biol. Toxicol. 29(2), 117–129 (2013).

51. Huang, Y. et al. Structure and expression of glutathione S-transferase genes from the midgut of the common cutworm, Spodoptera litura (Noctuidae) and their response to xenobiotic compounds and bacteria. J Insect Physiol. 57(7), 1033–1044 (2011).

52. Tayarani-Najaran, Z. et al. Cytotoxic and apoptogenic properties of three isolated diterpenoids from Salvia chassarasia through bioassay-guided fractionation. Food Chem Toxicol. 57, 346–351 (2013).

53. Curcic, M. G. et al. Antiproliferative and proapoptotic activities of methanolic extracts from Ligustrum vulgare L. as an individual treatment and in combination with palladium complex. Int J Mol Sci. 13(2), 2521–2534 (2012).

54. Yoo, J. et al. Assessment of the cytotoxic and apoptotic effects of chaetominin in a human leukemia cell line. Biomol Ther. 24(2), 147 (2016).

55. Pumiputavon, K. et al. Cell cycle arrest and apoptosis induction by methanolic leaves extracts of four Annonaceae plants. BMC Complement Altern Med. 17(1), 294 (2017).

56. Fernandez, D. M. et al. Antioxidant potential, in vitro cytotoxicity and apoptotic effect induced by crude organic extract of Anthracophyllum lateritium against RD sarcoma cells. BMC Complement Altern Med. 15(1), 398–407 (2015).

57. Sr., A., Uçkan, E., Rivers, D. R. & S. O. Epigallocatechin gallate and epicatechin gallate as antiinflammatory and antiangiogenic agents for human astrocytoma cells. J Neurooncol. 130(3), 225–236 (2011).

58. Siddique, H. R. et al. Validation of Drosophila melanogaster as an in vivo model for genotoxicity assessment using modified alkaline Comet assay. Mutagenesis. 20(4), 285–290 (2005).

59. Sharma, A., Shukla, A. K., Mishra, M. & Chowdhuri, D. K. Validation and application of Drosophila melanogaster as an in vivo model for the detection of double strand breaks by neutral Comet assay. Mutat Res Genet Toxicol Environ Mutagen. 721(2), 142–146 (2012).

60. Augustyniak, M. et al. Zinc-induced DNA damage and the distribution of metals in the brain of grasshoppers by the comet assay and micro-PiXe. Comp Biochem Physiol C Toxicol Pharmacol. 144(3), 242–251 (2006).

61. Packiam, S. M., Emmanuel, C., Baskar, K. & Ignacimuthu, S. Feeding Deterrent and Genotoxicity analysis of a novel Pytopesticide by using Comet Assay against Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae). Braz. arch. biol. technol. 58(4), 487–493 (2015).

62. Shetty, V. et al. Evaluation of gamma radiation-induced DNA damage in Aedes aegypti using the comet assay. Toxicol Ind Health. 33(12), 930–937 (2017).
63. Sahlmann, A. et al. Baseline and oxidative DNA damage in marine invertebrates. *Toxicol. Environ. Health Part A.* **80**(16–18), 807–819 (2017).
64. Meng, X. *et al.* Toxicity and differential protein analysis following destruxin A treatment of *Spodoptera litura* (Lepidoptera: Noctuidae) SL-1 cells. *Toxicol.* **58**(4), 327–333 (2011).
65. Koul, O. *et al.* Bioefficacy of crude extracts of *Aglia* species (Meliaceae) and some active fractions against lepidopteran larvae. *J of Appl Entomol.* **121**(1–5), 245–248 (1997).
66. Larone, D. H. Medically important fungi: a guide to identification. *MBio.* 203 (1993).
67. Sharma, M. *et al.* Molecular characterization of multiple xylanase producing thermophilic/thermotolerant fungi isolated from composting materials. *J Appl Microbiol.* **46**(5), 526–535 (2008).
68. Jain, S. K. & Levine, S. N. Elevated lipid peroxidation and vitamin E-quinone levels in heart ventricles of streptozotocin-treated diabetic rats. *Free Radic Biol Med.* **18**(2), 337–341 (1995).
69. Yadav, M., Chatterji, S., Gupta, S. K. & Watal, G. Preliminary phytochemical screening of six medicinal plants used in traditional medicine. *Int J of Pharm Pharm Sci.* **6**(5), 539–542 (2014).
70. Sofowara, A. Medicinal plants and traditional medicine in Africa. *Spectrum Books Ltd., Ibadan: Nigeria.* 289–300 (1993).
71. Harborne, J. B. Phytochemical methods: A guide to modern techniques of plant analysis. *2nd ed. London: Chapman and Hall.* 54–84 (1998).
72. Kokate, K. C. Practical pharmacognosy. 4th ed. *Delhi: Vallabh Prakashan,* p.218 (1997).
73. Hossain, M. A. *et al.* Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown *Thymus vulgaris.* *Asian Pac J Trop Biomed.* **3**(9), 705–710 (2013).

**Acknowledgements**

The grant received from UGC (University Grant Commission), New Delhi, India under SAP (Special Assistance Program) scheme is thankfully acknowledged. This study was funded by UGC (University Grant Commission) – SAP (Special Assistance Program) grant. Grant number-F.4-4/2016/DRS-I (SAP-II).

**Author Contributions**

Pooja Chadha, Amarjeet Kaur and Sanehdeep Kaur designed the study and analyzed the content. Mandeep Kaur performed the experiments except Biosafety studies and wrote the manuscript. Arun Kumar and Ramandeep Kaur performed the Biosafety assay and analyzed the content related to it. Rajvir Kaur helps in fungus identification as well as in isolation. All authors read and approved the final manuscripts.

**Additional Information**

**Competing Interests:** The authors declare no competing interests.

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