Inhibitory potential of ethyl acetate extract from mushrooms against root-knot nematode
(*Meloidogyne incognita*)

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Abstract

The present study focused on the nematicidal activities of bio-molecules extracted from mushrooms against *M. incognita*. The experimental results revealed that the highest hitching inhibition and the juvenile mortality were recorded with the ethyl acetate fraction of cell-free culture (CFC) filtrate of *Ganoderma lucidum* followed by *Lentinus edodes*. At 1000 ppm concentration, the bio-active molecules of *G. lucidum* exhibited the maximum inhibition of egg hitching (92.6%) and juvenile mortality (93.2%) of *M. incognita* at 72 hours of incubation. GC – MS analysis of *G. lucidum* revealed the presence of 23 compounds viz., Octadecane, 3-ethyl-5-(2-ethyl butyl), Decane, Undecane, 3,7-dimethyl-, Dihydroartemisin, Benzaldehyde, 3,4-dimethyl-, Heptadecane, 2,6,10,15-tetramethyl- 1,4-Di-tet-butyl-phenol, 1-Propanamine, Pyrrole[1,2-a]pyrazine-1,4-dione, hexahydro-1,2-Benzenedicarboxylic acid, butyl octyl ester, n-Hexadecanoic acid, Dibutyl phthalate, 2,2,4-Trimethyl-3-(3,8,12,16-tetramethylheptadeca-3,7,11,15-tetraenyl)-cyclohexanol, Methyl stearate, Cyclohexane, Octadecanoic acid, Deoxyspergulain, Methyl glycocholate, 3TMS derivative, 1,25-dihydroxyvitamin D3, TMS derivative, Spirost-8-en-11-one, 3-hydroxy-, 1H-Indene, 1-hexadecyl-2,3-dihydro- and Bis(2-Ethylhexyl) phthalate. Among these, 2, 4-Di-tet-butyl-phenol, n-Hexadecanoic acid and Dibutyl phthalate might have been responsible for antinemic activity.

Keywords: *G. lucidum*, biomolecules, cell-free culture filtrate, GC-MS and antinemic activity

Introduction

Root-knot nematodes, *Meloidogyne* spp. constitute one of the major important groups of plant-parasitic nematodes posing a major threat in the cultivation of agricultural and horticultural crops. Root-knot nematodes are sedentary endoparasites and occupy the first position of the top ten plant-parasitic nematodes occurring across the world [11, 2] and their parasitic lifecycle is found to induce feeding sites in the roots of host plants [20]. There are more than 100 species of *Meloidogyne* spp. dispersed worldwide and they parasitize both cultivated and uncultivated plants [13]. The four important *Meloidogyne* species viz., *Meloidogyne incognita*, *M. javanica*, *M. arenaria*, and *M. hapla* are economically important and are responsible for 95% of the infestations in cultivated lands [16, 24]. The most favorable condition for survival and multiplication of nematodes are in tropical climates than in temperate climates [4, 12]. The root-knot nematode is soil-borne and the infection starts with the penetration of juveniles (J2) present in soil and modifies the vascular tissues [10, 15, 18]. Infested plants show reduced growth, discoloration of leaves, and wilting due to uptake nutrient partitioning alterations and limited water uptake due to deformations of conducting vessels [12]. The management of plant-parasitic nematodes by biological methods has been very much realized in recent years [1, 7]. Perusal literature showed that mushroom fungi are important as natural sources of medicines and possess several bioactive compounds viz., antibacterial, antifungal, antioxidant, antiviral, antinemic, and anti-tumor activity [9, 28, 14, 21, 22]. Owing to the current emphasis on the eco-friendly approaches for plant disease management, mushroom fungi can serve as a promising source of antimicrobials against nematodes as evidenced by the antimicrobial and anti-nemic activity of phenolic compounds and water extracts of *Lentinula edodes*, *Boletus edulis*, *Pleurotus ostreatus*, and *Agaricus bisporus* against *M. incognita* [16, 26].
Indeed, the methanolic extract of cell-free culture (CFC) filtrate and mycelium borne bioactive molecules of *Ophiocordyceps sinensis* and *O. neovolkaniana* has been known to possess antimicrobial and anti-nematic activity against *M. incognita* [23]. Therefore, the present investigation was conducted to test the efficacy of ethyl acetate extract of cell-free culture (CFC) filtrate bioactive molecules of *Lentinus edodes*, *Ganoderma lucidum*, and *Schizophyllum commune* against root-knot nematode incited by *M. incognita*.

Materials and Methods

Pure culture of root-knot nematode, *M. incognita*

The egg masses were collected from the infected roots of tomato plants by prudently uprooting the plants and the roots with prominently noticeable galls were washed gently in water and the egg masses were then handpicked under the stereo zoom microscope and allowed to hatch by placing the egg masses in 100 ml beaker containing distilled water and incubated at room temperature. Pure culture of root-knot nematode, *M. incognita* was maintained on tomato in earthen pots. The potting mixture was prepared (1:1:2 red earth, sand, and farmyard manure) and sterilized in an autoclave at 121°C, 15 lbs for 20 min. Then the hatched out second-stage juveniles (J2) of *M. incognita* were inoculated @ 1 J2 / g of soil in the tomato rhizosphere at two weeks after transplanting in the earthen pots and the nematodes were multiplied and maintained in the Nematology glasshouse.

Extraction of Bioactive molecules from mushrooms

Five days old mycelial disc of mushroom fungi viz., *L. edodes*, *G. lucidum*, and *S. commune* measuring 5mm diameter were cut from the margin of the colony and were inoculated in 250 mL conical flasks containing 100 mL of sterilized mushroom complete (Medium) broth in each flask (adjusted to pH 6.0). The flasks were placed in an incubator cum rotary shaker for incubation at 25°C and agitated at 120 rpm 20 d. Later, the culture filtrate was collected and the mycelial mat was separated by filtration through Whatman No.40 filter paper. Further, the culture filtrate was centrifuged at 10,000 rpm for 15 mins and the cell-free culture filtrate was extracted with ethyl acetate solvent (v/v) sequentially. Liquid-liquid extraction was carried out three to four times for the same solvent. The solvent extract was evaporated separately under reduced pressure using a rotary evaporator to obtain the residues. The condensate of the solvent extract was dried and dissolved in methanol (1mg/mL) and filtered with a membrane filter (0.2 μm), stored at 4°C for further studies.

Bioassay of CFC condensates of mushrooms on egg hatching of *M. incognita*

Two ml of ethyl acetate fraction of CFC condensate of mushroom fungi were prepared from the stock solution (w/v) using distilled water at different concentrations (250, 500, 750, and 1000 ppm) and transferred into 5cm Petri plates. One egg mass of *M. incognita* was placed in each petri dish and incubated at room temperature (28±1°C). One egg mass was placed in distilled water as a control. The number of hatched juveniles was counted after 24, 48, and 72 h intervals. The data showed that a gradual increase in mortality

Characterization of Biomolecules produced by *G. lucidum* through Gas chromatography and Mass Spectrometry (GC-MS)

The effective isolate of *G. lucidum* was analyzed for the detection of active bio-molecules responsible for the suppression of root-knot nematode (*M. incognita*) through GC-MS (GC Clarus 500 Perkin Elmer) using a column Elite-5MS (100% Dimethylpolysiloxane), 30 × 0.25 mm × 0.25 μm df equipped with GC Clarus 500 Perkin Elmer. The turbo mass gold- Perkin-Elmer detector was used. The carrier gas flow rate was 1 ml per min, split 10:1, and injected volumes were 3 μl. The column temperature was maintained initially at 110°C at the rate of 10°C/min-No hold followed by an increase up to 280°C at the rate of 5°C/min-9 min (hold). The injector temperature was 250°C and this temperature was held constant for 36 min. The electron impact energy was 70 eV, Julet line temperature was set at 2000°C and the source temperature was set at 200°C. Electron impact (EI) mass scan (m/z) was recorded from the 45-450 aMU range. The GC-MS compounds were identified by comparing the obtained mass spectra with NIST/EPA/NIH Mass Spectral Library.

Statistical Analysis

The design of experiments (CRBD) and statistical analysis was followed as suggested by [8]. Statistical software SPSS was used for the analysis of ANOVA and Duncan’s Multiple Range Test (DMRT) of the data.

Results

Effect of Bioactive compounds of mushrooms on egg hatching of *M. incognita*

As root-knot nematodes in several cases predispose *Fusarium* infection, this experiment was conducted to test the effect of biomolecules of mushrooms against *M. incognita*. The egg mass exposed to various concentrations (250 ppm, 500 ppm, 750 ppm, and 1000 ppm) of mushroom biomolecules and observed at different time intervals viz., 24, 48, and 72 hours. In 24 hours, the ethyl acetate CFC condensate of *G. lucidum* decreased the hatching inhibition by 88.5 percent at 1000 ppm followed by 750 ppm 76.8 percent respectively. In *L. edodes* and *S. commune* the percentage of hatching inhibition was recorded in 1000 ppm concentration at 24 hrs interval which was 80.3 percent and 74.1 percent respectively. The CFC condensate of *G. lucidum* was exhibited more antinemic activity and the highest percentage inhibition was recorded in 72 hrs (92.3 percent) at the concentration of 1000 ppm when compared to other treatments. The least inhibition was observed at 250 ppm when compared with methanol and water control is appended in Table 1 and Figure 1.

Effect of Bioactive compounds of mushrooms on juvenile mortality of *M. incognita*

The CFC condensate of *G. lucidum* showed a significant increase in mortality of J2 of *M. incognita* at different time intervals. The data showed that a gradual increase in mortality
was observed (24 – 51.2, 48- 73.7, and 72 hours- 93.2 percent, respectively) at 1000 ppm followed by 750 ppm. Therefore, *L. edodes* and *S. commune* also increase the juvenile mortality at the concentration of 1000 ppm at different hour intervals appended in Table 2 and Fig 2. The mortality gradually decreased in the treatment of *S. commune* and *L. edodes* at a concentration of 250 ppm and recorded 3.25 percent and 8.75 percent mortality, respectively at 24 hrs. There was no mortality observed in control at different time intervals.

**Detection of compounds present in Cell-free culture filtrate of G. lucidum**

Among the CFC condensate of different mushrooms tested, *G. lucidum* revealed the highest hatch inhibition and juvenile mortality of *M. incognita*. Further CFC filtrate of *G. lucidum* was subjected to GC-MS analysis for compound identification. The results revealed that the presence of 23 compounds viz., Octadecane,3-ethyl-5-(2-ethyl butyl), Decane, Undecane, 3,7-dimethyl-, Dihydrooaramisinin, Benzaldehyde, 3,4-dimethyl-, Heptadecane, 2,6,10,15-tetramethyl-, 2,4-Di- tert-butyl-phenol, 1-Propanamine, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-,1,2-Benzenediacarboxylic acid, butyl octyl ester, n-Hexadecanoic acid, Dibutyl phthalate,2,2,4-Trimethyl-3-(3,8,12,16-tetramethylheptadeca-3,7,11,15-tetraenyl)-cyclohexanol, Methyl steareate, Cyclohexane, Octadecanoic acid, Deoxyspergualin, Methyl glycocholate, 3TMS derivative, 1,25-dihydroxyvitamin D3, TMS derivative, Spirost-8-en-11-one, 3-hydroxy-, 1H-Indene, 1-hexadecyl-2,3-dihydro- and Bis(2-Ethylhexyl) phthalate. Among these, Dibutyl phthalate recorded the highest peak area of 15.57 percent, expressed at 19.92 RT (Retention time).

**Discussion**

Though it is well proven that mushrooms apart from being consumed as food, synthesize numerous molecules that are known to be bioactive. These bioactive compounds found in fruiting bodies, mycelial mat, and cell-free culture broth are polysaccharides, proteins, fats, minerals, glycosides, alkaloids, volatile oils, terpenoids, tocopherols, phenolic, flavonoids, carotenoids, folates, lectins, enzymes, ascorbic, and an organic acid which possess antifungal, antimicrobial, antibacterial, antiviral, anti-inflammatory, antioxidant, insecticidal and nematicidal, anticancer, prebiotic, and immunomodulation properties[25]. Extensive studies have shown that among mushroom molecules tested, the biomolecules extracted from CFC culture filtrate of *G. lucidum* exhibited the highest egg hatching inhibition and juvenile mortality recorded in 72 hours at the concentration of 1000 ppm followed by 750 ppm. Subsequently, the CFC condensate of *L. edodes* and *S. commune* also significantly caused hatching inhibition and juvenile mortality in 72 hrs exposure at the concentration of 1000 ppm. The results are in line with the findings of [23] who reported that the bioactive molecules of *O. sinensis* exhibited maximum inhibition of egg hatching (94%) and juvenile mortality (92%) of *M. incognita* at 72 hours of incubation. In the same way, [3] stated that the culture filtrate of Amuroder maeacera, Laccaria tortillis, Peziza spp., Omphalotus mucida, Pleurotus palmutus, and Tyloplus striatus exhibited nematicidal activity against the pinewood nematode *Bursaphelenchus xylophilus*, with over 80 percent mortality within 72 h of exposure. Similarly, bioactive compounds of luminescent mushroom *Neonothopanus nambi* at the concentration 500mg/l caused 99% juvenile mortality [3]. In parallel, basidiomycete fungi *Coprinus comatus* was found to be a nematophagous fungus, which showed nematicidal activity against the free-living nematode, *Paragrellus redivivus*, and the root-knot nematode, *M. arenaria*[23]. The CFC condensate of *G. lucidum* exhibited more nematicidal activity as compared to *L. edodes* and *S. commune* (Fig 1 and Fig 2). The reason behind the anti-nematic effect of CFC filtrate of *G. lucidum* on eggs and juveniles of *M. incognita* is due to the innate nature and the presence of toxic metabolites such as phenols, esters, and fatty acid which has been confirmed through GC-MS analysis and the presence of 23 compounds expressed in Table 3. Among these compounds, 2, 4-Di-tert-butyl-phenol, n-Hexadecanoic acid and Dibutyl phthalate might have been responsible for anti-nematic activity. Correspondingly, [29] revealed the presence of esters and phenolic derivatives including the compounds L- ascorbyl 2, 6-dipalmitate, dibutyl phthalate, dimethyl phthalate, and 2, 6-di-tert-butyl-p-cresol which suppressed egg hatching and also repelled juvenile mortality in *M. incognita*. The supporting evidence [19] reported that acetic acid, hexadecanoic acid (n-decanoic acid derivatives) and 4H-Pyrane-4-one, 2, 3-dihydro-3,5-dihydroxy-6-methyl also exhibited nematicidal activities against root-knot nematode. Consequently, the lipophilic phenol 2, 4-Di-tert-butyl-phenol showed nematicidal activity against *Caenorhabditis elegans* during fumigation or soil treatment [27].

**Table 1:** Effect of Ethyl acetate extract of CFC filtrate of mushrooms on egg hatching of *M. incognita*

| Treatments                   | Concentration / Incubation period |
|------------------------------|----------------------------------|
|                              | 250 ppm | 500 ppm | 750 ppm | 1000 ppm |
|                              | 24h     | 48h     | 72h     | 24h     | 48h     | 72h     | 24h     | 48h     | 72h     | 24h     | 48h     | 72h     |
| CFC Condensate of *L. edodes* | Ethyl acetate fraction | 35.6<sup>a</sup> | 38.6<sup>d</sup> | 42.1<sup>d</sup> | 49.6<sup>c</sup> | 52.1<sup>c</sup> | 60.3<sup>d</sup> | 66.3<sup>d</sup> | 71.6<sup>d</sup> | 78.3<sup>d</sup> | 80.3<sup>a</sup> | 83.6<sup>d</sup> | 85.6<sup>d</sup> |
| CFC Condensate of *G. lucidum*| Ethyl acetate fraction | 40.5<sup>c</sup> | 44.1<sup>e</sup> | 48.3<sup>c</sup> | 58.6<sup>b</sup> | 61.2<sup>c</sup> | 65.6<sup>c</sup> | 78.6<sup>c</sup> | 81.3<sup>c</sup> | 84.3<sup>c</sup> | 88.5<sup>d</sup> | 90.1<sup>e</sup> | 92.6<sup>d</sup> |
| CFC Condensate of *S. commute*| Ethyl acetate fraction | 29.5<sup>c</sup> | 32.6<sup>c</sup> | 36.1<sup>c</sup> | 44.5<sup>b</sup> | 48.6<sup>c</sup> | 50.1<sup>c</sup> | 59.2<sup>c</sup> | 63.1<sup>c</sup> | 68.3<sup>c</sup> | 70.1<sup>c</sup> | 72.6<sup>c</sup> | 74.1<sup>c</sup> |
| Methanol control             |        |         |         | 53.1<sup>c</sup> | 26.3<sup>e</sup> | 10.3<sup>c</sup> | 53.1<sup>c</sup> | 26.3<sup>e</sup> | 10.3<sup>c</sup> | 53.1<sup>c</sup> | 26.3<sup>e</sup> | 10.3<sup>c</sup> |
| Control (Sterile water)      |        |         |         | 41.8<sup>c</sup> | 12.5<sup>c</sup> | 1.3<sup>b</sup> | 41.8<sup>c</sup> | 12.5<sup>c</sup> | 1.3<sup>b</sup> | 41.8<sup>c</sup> | 12.5<sup>c</sup> | 1.3<sup>b</sup> |
| SED                          | 0.56   | 0.31    | 0.17    | 0.36   | 0.19    | 0.32    | 0.59   | 0.36    | 0.72    | 1.14   | 3.64    | 1.40    |
| CD (0.05)                    | 1.19   | 0.66    | 0.38    | 0.76   | 0.42    | 0.70    | 1.25   | 0.76    | 1.55    | 2.42   | 8.11    | 2.99    |

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Table 2: Effect of Ethyl acetate extract of CFC filtrate of mushrooms on juvenile mortality of *M. incognita*

| Treatments                                      | Concentration / Incubation period |
|------------------------------------------------|----------------------------------|
|                                                 | 250 ppm  | 500 ppm  | 750 ppm  | 1000 ppm |
|                                                 | 24h | 48h | 72h | 24h | 48h | 72h | 24h | 48h | 72h | 24h | 48h | 72h |
| CFC Condensate of *L. edodes* (Ethyl acetate fraction) | 8.75b  | 17.2c | 26.5c | 17.0b | 32.8c | 36.2c | 27.5c | 45.7d | 64.5d | 35.7d | 56.7d | 81.2c |
| CFC Condensate of *G. lucidum* (Ethyl acetate fraction) | 20.2a  | 25.0a | 35.7c | 33.5c | 45.7d | 49.0d | 40.7c | 57.7c | 78.7c | 51.2c | 73.7c | 93.2c |
| CFC Condensate of *S. commune* (Ethyl acetate fraction) | 3.25b  | 12.7c | 18.7c | 11.5c | 23.0b | 30.5c | 16.5b | 34.7c | 45.0c | 29.7c | 44.7c | 60.2c |
| Methanol control                                  | 18.2b  | 27.2c | 36.7d | 18.2b | 27.2c | 36.7c | 18.2b | 27.2c | 36.7c | 18.2b | 27.2c | 36.7c |
| Control (Sterile Water)                          | 0.0a   | 0.0a  | 0.0a  | 0.0a  | 0.0a  | 0.0a  | 0.0a  | 0.0a  | 0.0a  | 0.0a  | 0.0a  | 0.0a  |
| SED                                             | 1.57c  | 1.21c | 1.55c | 2.70c | 1.55c | 1.33c | 2.53c | 2.34c | 4.08c | 3.50c | 2.59c | 3.30c |
| CD (0.05)                                       | 3.50b  | 2.59c | 3.30c | 5.76c | 3.30c | 2.83c | 4.98c | 4.08c | 4.58c | 4.43c | 4.24c | 4.08c |

Table 3: Biomolecules separated from CFC filtrate condensate (Ethyl acetate fraction) of *G. lucidum* by GCMS analysis.

| Retention Time | Compounds                                      | Chemical Formula | Molecular Weight (g/mol) | Structure | Area Percentage |
|----------------|------------------------------------------------|------------------|--------------------------|-----------|----------------|
| 3.153          | Octadecane, 3-ethyl-5-(2 ethylbutyl)           | C<sub>18</sub>H<sub>38</sub> | 254.51                  | ![Structure](image) | 0.300          |
| 3.639          | Decane                                         | C<sub>10</sub>H<sub>22</sub> | 142.28                  | ![Structure](image) | 1.007          |
| 4.409          | Undecane, 3,7-dimethyl-                         | C<sub>11</sub>H<sub>20</sub> | 184.36                  | ![Structure](image) | 0.607          |
| 6.400          | Dihydroartemisinin                              | C<sub>13</sub>H<sub>22</sub>O | 284.34                  | ![Structure](image) | 1.004          |
| 6.830          | Benzaldehyde, 3,4-dimethyl-                     | C<sub>8</sub>H<sub>16</sub>O | 134.17                  | ![Structure](image) | 0.36           |
| 10.53          | Heptadecane, 2,6,10,15-tetramethyl-             | C<sub>21</sub>H<sub>44</sub>N | 296.57                  | ![Structure](image) | 0.647          |
| 10.87          | 2,4-Di-tert-butylphenol                         | C<sub>14</sub>H<sub>22</sub> | 206.32                  | ![Structure](image) | 0.631          |
| 14.96          | 1-Propanamine                                   |                  | -                       | -         | 1.506          |
| 16.06          | Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-    | C<sub>10</sub>H<sub>10</sub>N | 154.16                  | ![Structure](image) | 2.279          |
| 18.05          | 1,2-Benzenedicarboxylic acid, butyl octyl ester|                  | -                       | -         | 0.723          |
| 19.43          | Hexadecanoic acid, methyl ester                 | C<sub>16</sub>H<sub>32</sub> | 256.42                  | ![Structure](image) | 2.814          |
| 19.92          | Dibutyl phthalate                                | C<sub>10</sub>H<sub>22</sub> | 278.34                  | ![Structure](image) | 15.57          |
| 23.05          | 2,2,4-Trimethyl-3-((3,8,12,16-tetramethyl heptadeca-3,7,11,15-tetraenyl)-cyclohexan |                  | -                       | -         | 1.568          |
| 23.36          | Methyl stearate                                 | C<sub>18</sub>H<sub>38</sub> | 298.50                  | ![Structure](image) | 6.291          |
| 23.36          | Cyclohexane                                     | C<sub>9</sub>H<sub>12</sub> | 84.16                   | ![Structure](image) | 2.247          |
| 23.80          | Octadecanoic acid                               | C<sub>18</sub>H<sub>36</sub> | 284.47                  | ![Structure](image) | 4.763          |
| Compound                      | Structure                          | Formula | Molecular Weight | Activity (%) |
|-------------------------------|------------------------------------|---------|------------------|--------------|
| Deoxyspergualin               | ![Deoxyspergualin](image)          | C_17H_37 | 387.52           | 0.545        |
| Methyl glycocholate, 3TMS derivative | ![Methyl glycocholate](image) | -       | -                | 0.285        |
| 1,25-Dihydroxyvitamin D3, TMS derivative | ![1,25-Dihydroxyvitamin D3](image) | -       | -                | 0.354        |
| Spirostan-8-en-1-one, 3-hydroxy- | ![Spirostan-8-en-1-one](image) | C_27H_40 | 428.60           | 0.283        |
| 1H-Indene, 1-hexadecyl-2,3-dihydro- | ![1H-Indene, 1-hexadecyl-2,3-dihydro](image) | C_25H_42 | 342.60           | 1.116        |
| Bis(2-ethylhexyl) phthalate   | ![Bis(2-ethylhexyl) phthalate](image) | C_24H_38 | 390.55           | 0.796        |

**Concentrations and Time intervals**

**Fig 1**: Effect of CFC filtrate of mushrooms on egg hatching of *M. incognita*

**Fig 2**: Effect of CFC filtrate of mushrooms on juvenile mortality of *M. incognita*
Fig 3: Chromatogram depicting the GC-MS studies of ethyl acetate fraction of *G. lucidum*

**Conclusion**
To summarize the present findings of ethyl acetate fraction of the cell-free culture filtrate of *G. lucidum* is known to produce plentiful bioactive compounds that could be potentially used for the management of root-knot nematode, *Meloidogyne incognita*.

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**References**
1. Agbenin NO. Biological control of plant parasitic nematodes: prospects and challenges for the poor Africa farmer. A review. Plant Protection Science-UZEI (Czech Republic) 2011.
2. Bernard GC, Egnin M, Bonsi C. The impact of plant-parasitic nematodes on agriculture and methods of control. Nematology-Concepts, Diagnosis and Control 2017.
3. Bua-art S, Sakirsirat W, Hiransalee A, Kanokmedhakul S, Lekphrom R. Effect of bioactive compound from luminescent mushroom (Neonothopanusnambi Speg.) on root-knot nematode (*Meloidogyne incognita* Chitwood) and non-target organisms. Asia-Pacific Journal of Science and Technology 2011;16(4):331-341.
4. De Waele D, Elsen A. Challenges in tropical plant nematology. Annual Review Phytopathology 2007;45:457-485.
5. Dong JY, Li XP, Li L, Li GH, Liu YJ, Zhang KQ. Preliminary results on nematicidal activity from culture filtrates of Basidiomycetes against the pine wood nematode, *Bursaphelenchus xylophilus* (Aphelegchoidae). Annals of microbiology 2006;56(2):163.
6. Eisenback JD, Triantaphyllou HH. Root-knot nematodes: *Meloidogyne* species and races. Manual of Agricultural Nematology 1991;1:191-274.
7. Freire ES, Campos VP, Pinho RSC, Oliveira DF, Faria MR, Pohlit AM et al. Volatile substances produced by *Fusarium oxysporum* from coffee rhizosphere and other microbes affect *Meloidogyne incognita* and Arthrobotryosconoides. Journal of nematology 2012;44(4):321.
8. Gomez KA, Gomez KA, Gomez AA. Statistical procedures for agricultural research. 2nd Edn. John Wiley and Sons, New York 2010.
9. Hatvani N. Antibacterial effect of the culture fluid of *Lentinus edodes* mycelium grown in submerged liquid culture. International Journal of Antimicrobial Agents 2001;17(1):71-74.
10. Jones DK, Slazas RR, Feller III FR, Codman, ShurtleffInc. Vascular occlusion device with an embolic mesh ribbon. U.S. Patent 2013;8:361-104.
11. Jones JT, Haegeman A, Danchin EG, Gaur HS, Helder J, Jones MG et al. Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular plant Pathology 2013;14(9):946-961.
12. Kaloshian I, Williamson V, Miyao G, Lawn D, Westerdahl B. “Resistance-breaking” nematodes identified in California tomatoes. California Agriculture 1996;50(6):18-19.
13. Karssen G, Moens M. Taxonomy and Principal General Root-Knot Nematodes. Plant Nematology (Perry, R. y Moens, M. Eds). CAB International, Wallingford, UK, (Part I) 2006, 60-90.
14. Lindequist U, Niedermeyer TH, Julich WD. The pharmacological potential of mushrooms. Evidence-Based Complementary and Alternative Medicine 2005;2(3):285-299.
15. Liu B, Liu X, Liu Y, Xue S, Cai Y, Yang S et al. The infection of cucumber (*Cucumis sativus* L.) roots by *Meloidogyne incognita* alters the expression of actin-depolymerizing factor (ADF) genes, particularly in association with giant cell formation. Frontiers in plant science 2016;7:1393.
16. Luo H, Liu Y, Fang L, Li X, Tang N, Zhang K. *Coprinus comatus* damages nematode cuticles mechanically with spiny balls and produces potent toxins to immobilize nematodes. Applied and Environmental Microbiology 2007;73(12):3916-3923.

17. Luo H, Mo M, Huang X, Li X, Zhang K. *Coprinus comatus*: A Basidiomycete fungus forms novel spiny structures and infects nematode. Mycologia 2004;96(6):1218-1224.

18. Molinari S, Fanelli E, Leonetti P. Expression of tomato salicylic acid (SA) -responsive pathogenesis-related genes in Mi-1-mediated and SA-induced resistance to root-knot nematodes. Molecular Plant Pathology 2014;15(3):255-264.

19. Ntalli NG, Vargiu S, Menkissoglou-Spiroudi U, Caboni P. Nematicidal carboxylic acids and aldehydes from Melia azedarach fruits. Journal of agricultural and food chemistry 2010;58(21):11390-11394.

20. Perry RN, Moens M. Plant Nematology. Wallingford, UK: CABI Publisher 2006, 447.

21. Reis FS, Pereira E, Barros L, Sousa MJ, Martins A, Ferreira IC. Biomolecule profiles in inedible wild mushrooms with antioxidant value. Molecules 2011;16(6):4328-4338.

22. Rouhana-Toubi A, Wasser SP, Fares F. The shaggy ink cap medicinal mushroom, *Coprinus comatus* (higher Basidiomycetes) extract induces apoptosis in ovarian cancer cells via extrinsic and intrinsic apoptotic pathways. International Journal of Medicinal Mushrooms 2015;17(12):1127-36.

23. Sangeetha C, Krishnamoorthy AS, Ramakrishnan S. Testing bioactive compounds of Chinese caterpillar fungus, *Ophiocordyceps* spp against root knot nematode (*Meloidogyne incognita*). Research Journal of Agricultural Sciences 2015a;6(5):1129-1133.

24. Sasser JN, Carter CC. Root-knot nematodes (*Meloidogyne* spp.) Identification, morphological and physiological variation, host range, ecology, and control. Nematology in the southern region of the United States. Southern Cooperative Series Bulletin 1982;276:21-32.

25. Sivanandhan S, Khusro A, Paulraj MG, Ignacimuthu S AL-Dhabi NA. Biocontrol properties of Basidiomycetes: An overview. Journal of Fungi 2017;3(1):2.

26. Susana SA, Anguina CR, Reglera G, Rivas CS. Improvement of antimicrobial activity of edible mushroom extracts by inhibition of oxidative enzymes. International Journal of Food and Science Technology, 2009;44:1057-1064.

27. Wang ZQ, Wu YX, Zhou H, He YQ. Nematicidal Activity of 2, 4-Di-tert-butylphenol against *Caenorhabditis elegans*. Agrochemicals 2014;53:298-300.

28. Wasser SP. Review of medicinal mushrooms advances: good news from old allies. Herbal Gram 2002.

29. Yang G, Zhou B, Zhang X, Zhang Z, Wu Y, Zhang Y Lü, *et al.* Effects of tomato root exudates on *Meloidogyne incognita*. PLoS One 2016;11(4).