5. Discussion

The Reactive Oxygen Species (ROS) or free radicals generated in the normal metabolically active cells as by-products or in cells under stress situation(s) when overcome the cellular balance are suggested to be causative agents of various degenerating diseases resulting to aging and death. The antioxidants often reducing molecules can terminate the chain reactions that initiated and propagated by free radicals by being oxidized themselves.

Fungi are the immense source of biologically active compounds and these make an extraordinarily important contribution in the food and agriculture industries like drug production, food processing, bio-control agents, enzyme biotechnology and other research tools. They can be cultivated in fermentors and various scale-up techniques can be utilized to increase their yield and potentials. Many researches had reported significant antioxidants (Table 2.1 and 2.4) from different fungi. In the present study some of the mushroom species and endophytic fungi were collected from forest and tested for total phenolic contents and antioxidant capacity.

Forest of Uttarakhand (India) are well known for diversity of mushroom species (Harsh et al., 1983; Harsh and Bisht, 1997; Vishwakarma et al., 2011) and in recent years some mushroom species collected from this state were studied for their antioxidant capacity (Mohsin et al., 2011; Kamra and Bhatt, 2012; Mishra et al., 2013; Rawat et al., 2013; Jeena et al., 2014). In the present study 16 mushroom species including wood decaying fungi (Armillaria mellea, Auricularia auricula, Ganoderma applanatum, G. lucidum, Laetiporus sulphureus, Lentinus tigrinus, Phellinus linteus, Pleurotus ostreatus, P. sajor-caju, Polyporus arcularius, Schizophyllum commune, Sparassiss crispa and Spongipellis unicolor) and mycorrhizal mushrooms (Agaricus bisporus, Lycoperdon pyriforme and Russula brevipes) mushrooms were collected from forest nearby Dehradun.
(Uttarakhand) of which *Spongipellis unicolor* was studied first time for their antioxidant capacity.

Many researchers (Fisher *et al.*, 1994; Nagaraja, 2011a, 2011b; Kumar and Hyde, 2004; Zeng *et al.*, 2011; Dhankhar *et al.*, 2012; Sunayana and Prakash, 2012) had reported wide range of fungal endophytes in different plants. In the present study *Alternaria alternate*, *Aspergillus fumigates*, *A. humicola*, *A. niger*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Colletotrichum dematium*, *C. gloeosporioides*, *Curvularia lunata*, *Cercospora tageticola*, *Fusarium lateritium*, *F. oxysporum*, *F. solani*, *Macrophomina phaseolina*, *Mucor varans*, *Penicillium decumbens*, *P. frequentans*, *Pestalotiopsis disseminate*, *Rhizoctonia solani*, *Scytalidium lignicola* and two sterile mycelia i.e., Mycelia sterilia sp. 1 and Mycelia sterilia sp. 2 were isolated as endophytes from healthy plants in preliminary test, however, on the basis of pathogenicity test conducted on their healthy host plants in glass house only *A. fumigatus*, *A. humicola*, *A. niger*, *Chaetomium globosum*, *S. lignicola*, *Mucor varans*, *Penicillium frequentans*, Mycelia sterilia sp.1 and Mycelia sterilia sp. 2 were confirmed as true endophytes and rest other isolates found as pathogenic. Strobel and Daisy (2003) had also suggested a term “opportunistic parasites” for such fungi which present in healthy plants without showing any symptoms for certain period but become pathogenic in favourable condition.

5.1. **Extract yields of fungi**

The yields of extracts obtained from the fruiting bodies were found higher than that of their mycelial biomass except *Auricularia auricula*, *Schizophyllum commune* and *Sparassis crispa*. Lesser yields i.e., 7.47% and 13.47% from fruiting bodies of *Agaricus blazei* and *Lentinus edodes*, respectively have also been reported by Silva and Jorsge (2011). Yim *et al*. (2009) had found 10.30% of yield from cultivated fruiting body of *Pleurotus ostreatus* in Methanolic extracts. Simsek *et al*. (2008) had reported 11.22 to 23.01% yield from fruiting bodies extracts of *Agaricus bisporus* when grown on different compost
and activator materials. Puttaraju et al. (2006) had reported little higher yields i.e., 32±2.07, 30±2.58 and 20±2.58% from methanolic extracts of fruiting bodies of Pleurotus sajor-caju, Sparassis crispa and Russula brevipes, respectively. Mau et al. (2004) had also reported high yields i.e., 37.8±0.07 and 27.5±0.06% from red and white mycelia of Antrodia camphorata cultivated in PDB, respectively. Mycelial biomass of Phellinus igniarius cultivated in potato dextrose broth (PDB) extracted with absolute methanol was reported 20.31% extract yield (Lung et al., 2010). The yield of Armillaria mellea cultivated in PDB medium was found to be i.e., 24.52% (Lung and Chang, 2011) similar to present study. Kalyoncu et al. (2010) had reported higher yield from mycelia of Auricularia auricula (21.47%) but lower yields from Ganoderma lucidum (4.94%) and Lentinus sajor-caju (8.40%) cultivated in liquid Hagem medium.

Contrary to with mushroom species all isolates of endophytic fungi gave higher yields than 11% whereas the mycelia of Chaetomium globosum and Aspergillus niger yielded more than 21%. Yen et al. (2002) had reported only 9.70% yield from mycelia of Aspergillus candidus.

5.2. TPC of fugal extracts
In the present study significant variation in the TPC values of fruiting bodies and mycelia of mushroom species (Table 4.3) was observed and it was found that the fruiting bodies contained more phenolic compounds than the mycelia. Silva and Jorge (2011) and Carvajal et al. (2012) had reported higher values of TPC from the fruiting body extract of Agaricus blazei i.e., 34.44±0.23 and 20.00±0.70 mg GAE g⁻¹, respectively. However, similar range as in present study were reported from the fruiting body extract of Pleurotus sajor-caju (7.4 mg GAE g⁻¹), Russula brevipes (0.7 mg GAE g⁻¹) and Sparassis crispa (1.7 mg GAE g⁻¹); P. ostreatus (6.03±0.67 mg GAE g⁻¹); A. bisporus (4.49±0.16 mg GAE g⁻¹) and Lycoperdon perlatum (10.57±0.17 mg GAE g⁻¹); and Lentinus tigrinus (17.30±1.70 mg GAE g⁻¹) by Puttaraju et al. (2006); Yim et al. (2009); Lillian et al. (2009); and Filipa et al. (2011);
respectively. Dubost et al. (2007) had also reported similar TPC value from fruiting body extracts of *A. bisporus* (8.00±0.48 mg GAE g⁻¹) and *P. ostreatus* (4.27±0.69 mg GAE g⁻¹). Lower TPC value was reported by Mirfat et al. (2010) from the methanolic extract of fruiting body of *Schizophyllum commune* (1.72±0.05 mg GAE g⁻¹) as compared to the present study. The mycelia of *L. edodes* cultivated in PDB and harvested after 15 days of incubation was reported to have TPCs value of 11.0±1.0 mg GAE g⁻¹ (Wu and Hansen, 2008). Carvajal et al. (2012) had reported TPC value of 20.8±0.9 mg GAE g⁻¹ from methanolic extract of cultivated mycelia of *A. blazei*.

Variable values of TPC were also found in the extracts of endophytic fungi. *Aspergillus fumigatus*, *A. humicola*, *A. niger*, *Scytalidium lignicola*, *Penicillium frequentans*, *Mycelia sterilia* sp.1 and *Mycelia sterilia* sp. 2 were between 10 to 15 mg GAE g⁻¹ except *Chaetomium globosum* which showed highest TPC value of 17.48±0.21 mg GAE g⁻¹. Similar value of TPC was reported by Heba (2014) from mycelial extract of *C. madrasense* (17.43 mg GAE g⁻¹) cultivated in Czapek dox’s broth (CDB) but lower value was reported by Ravindran and Naveenan (2011) from *C. globosum* (10.64±0.02 mg GAE g⁻¹) cultivated in Malt extract broth. However, slightly higher TPC values were reported in methanolic extracts of mycelia of *Penicillium citrinum* (28.0 mg GAE g⁻¹), *P. granulatum* (15.3 mg GAE g⁻¹) and *Aspergillus wentii* (18.6 mg GAE g⁻¹) cultivated in enriched CDB medium by Arora and Chandra (2011).

### 5.3. Antioxidant capacity of extracts

It was noticed that TPC and antioxidant capacity of fruiting bodies of mushroom species were found better than their mycelia. Such variations suspected the high contents of soluble compounds present in fruiting bodies that may be absent or present in low concentration in cultivated mycelia. It may be also predicted that active compounds synthesized in fungal mycelia may be secreted extracellularly in culture medium although their estimation and purification is complicated. Muszynska et al. (2012) had reported almost double β-
carotene in fruiting body (7.5±0.15 μg g⁻¹ dry weight) extract of *Calocera viscosa* than its mycelia (3.5±0.10 μg g⁻¹ dry weight) grown in ideal liquid medium (Oddoux medium). Mau *et al.* (2004) had also compared quantity of some antioxidant components detected in methanolic extract of cultivated mycelia of *Antrodia camphorata* in their experiment with methanolic extract of fruiting body of same fungus previously studied by Huang (2000) separately and found that extract of white mycelia contained significantly lesser amount of β-carotene, tocopherols and total phenolic contents. Tsai *et al.* (2006) had reported higher values of percent yield and EC₅₀ values of DSC and REP of hot water extract of *Agrocybe cylindracea* fruiting body than that of their cultivated mycelia. Similar comparison was also reported in the study of Lee *et al.* (2008). They had reported higher percentage of DPPH scavenging at a concentration of 5 mg ml⁻¹ i.e., 53.3% from ethanolic extract of fruiting body of *Hypsizygus marmoreus* than 24.5% from its mycelial extract at same concentration and agreed to conclude that fruiting bodies of a mushroom have higher antioxidant capacity than its mycelia.

### 5.3.1. DPPH radical-scavenging assay

The antioxigenic compounds rapidly react with DPPH radicals and reduce most of the DPPH radical molecules corresponding to available hydroxy groups and showed a dose-dependent activity (Brand-Williams *et al.*, 1995). In the present study scavenging of DPPH• was increased with increasing of concentrations of fungal extracts and the corresponding absorbance were decreased at 517 nm. The lower EC₅₀ values of fruiting bodies (0.56 to 1.24 mg ml⁻¹) showed that these were more potent to scavenge DPPH• than the corresponding mycelia (2.51 to 8.39 mg ml⁻¹) irrespective of the mushroom species (Table 4.3). Different researchers had used different quantity of extract sample and DPPH solution of different molarities in their protocols and some researchers had used different solvents for extract preparation, hence there were variations in the values of EC₅₀ of DSC of fungal extracts. Puttaraju *et al.* (2006) had used lower sample quantity (5 to 25 µl)
with 1 ml of DPPH (500 µM) and reported higher EC\textsubscript{50} values of DSC of methanolic extracts of Pleurotus sajor-caju (2.50±0.37 mg ml\textsuperscript{-1}), Russula brevipes (1.60±0.36 mg ml\textsuperscript{-1}) and Sparassis crispa (4.54±0.30 mg ml\textsuperscript{-1}). Similarly Keles \textit{et al.} (2011) and Filipa \textit{et al.} (2011) individually had used 50 µl and 30 µl of the diluted sample with 6×10\textsuperscript{-5} M of DPPH solution in their methods and reported higher values of EC\textsubscript{50} from the extract of Agaricus bisporus (19.51 mg ml\textsuperscript{-1}) and Lentinus tigrinus (17.30±1.70 mg ml\textsuperscript{-1}), respectively. Jean-Michel \textit{et al.} (2008) and Rawat \textit{et al.} (2013) had used equal amount (1 ml) of sample and DPPH (0.4 mmol) solution and reported slightly higher EC\textsubscript{50} values from A. bisporus (1.69±0.04 mg ml\textsuperscript{-1}) and (1.162±0.016 mg ml\textsuperscript{-1}) which were in the range of present study. However, Türkoğlu \textit{et al.} (2007) had reported very low EC\textsubscript{50} values from the extract of R. delica (207.09 µg ml\textsuperscript{-1}) when using 1 ml of sample with 4 ml of DPPH (0.01mM) in their method. Sheena \textit{et al.} (2005) had reported very low EC\textsubscript{50} values from extract of fruiting body of Ganoderma lucidum (61.0±2.5 µg ml\textsuperscript{-1}) yet quantity of sample and DPPH were not mentioned in their methodology. Mirfat \textit{et al.} (2010) used 0.1 ml of sample and 3.9 ml of DPPH (0.06 mM) solution and reported lower EC\textsubscript{50} values from Schizophyllum commune (0.145 ± 0.01 mg ml\textsuperscript{-1}). Tsai \textit{et al.} (2008) used same proportion of sample and DPPH radial solution in their method and reported similar range of EC\textsubscript{50} value from ethanolic extract of cultivated fruiting body (fully matured) of A. bisporus (0.70 ± 0.03 mg ml\textsuperscript{-1}). Mau \textit{et al.} (2005) reported EC\textsubscript{50} values of DSC from extract of young fruiting body of G. tsugae as 5.89 mg ml\textsuperscript{-1} using same quantity of sample and DPPH solution as found in present study.

Among the mycelial extracts of mushroom species only the extract of Lentinus tigrinus and Armillaria mellea were found with EC\textsubscript{50} values below than 3 mg ml\textsuperscript{-1} and extracts of other species showed EC\textsubscript{50} values between 3 to 9 mg ml\textsuperscript{-1}. The extracts of Lycoperdon pyriforme, Russula brevipes, Auricularia auricula and Spongipellis unicolor showed EC\textsubscript{50} between 6 to 9 mg ml\textsuperscript{-1}. Mau \textit{et al.} (2004);
Asatiani et al. (2007b); and Lung and Chang (2011) had reported similar range of EC$_{50}$ from mycelial extracts of *Antrodia camphorata* (2.06±0.03 and 1.70±0.01 mg ml$^{-1}$ from red and white mycelia, respectively); *Armillaria mellea* (1.7±0.05 mg ml$^{-1}$), *Auricularia auricula* (2.7±0.3 mg ml$^{-1}$) and *Ganoderma lucidum* (2.2±0.3 mg ml$^{-1}$); and *Armillaria mellea* (2.96±0.15 mg ml$^{-1}$); respectively. The quantity of samples and DPPH solution in their methods were similar to present study. Carvajal et al. (2012) had reported lower EC$_{50}$ value from extract of young and old mycelia of *Agaricus blazei* (1413±52 and 599±35 µg ml$^{-1}$, respectively) when they had used 150 µl sample with 2850 µl of the DPPH solution (final concentration of 0.1 m mol l$^{-1}$).

The EC$_{50}$ values of DSC of extract of *Chaetomium globosum* (1.59±0.04 mg ml$^{-1}$) and *Aspergillus niger* (2.32±0.08 mg ml$^{-1}$) and Mycelia sterilia sp. 2 (2.23±0.09 mg ml$^{-1}$) although were found lower among the edophytic fungi, but were higher than that of any fruiting body extracts and lower than all of the mycelial extracts of mushroom species. EC$_{50}$ values of DSC of other endophytic fungi found in the range of 3.29±0.08 to 6.23±0.11 mg ml$^{-1}$ which were in the range of EC$_{50}$ values of most of the mycelia extracts of mushroom species. Ravindran and Naveenan (2011) reported lower EC$_{50}$ values from ethyl acetate extract of mycelia of *C. globosum* (403.9±4.24 µg ml$^{-1}$) cultivated in Malt extract broth. Murthy et al. (2011) had used 0.1 ml of sample with 2 ml of DPPH solution (100 µM) and reported very low EC$_{50}$ values from *Aspergillus* sp. (220±1.07 µg ml$^{-1}$), *Penicillium* sp. (320±3.72 µg ml$^{-1}$) and *Mucor* sp. (240±2.33 µg ml$^{-1}$). Arora and Chandra (2011) had used 0.5 ml sample with 3 ml of DPPH solution (0.02 mM) in their method and reported slightly lower EC$_{50}$ values from mycelial extracts of *Aspergillus* wentii (0.30 mg ml$^{-1}$), *Penicillium citrinum* (0.10 mg ml$^{-1}$) and *P. granulatum* (0.12 mg ml$^{-1}$) cultivated in Czapek-Dox's broth. Yen et al. (2002) had reported EC$_{50}$ value of 9.5 mg ml$^{-1}$ (calculated from their data) from acetone extract of mycelial extract of *A. candidus*. 
5.3.2. Reducing power
Antioxidant capacity is also concomitant with reducing power assay. It is believed that reductones present in extracts react with free radicals to stabilize and terminate radical chain reactions (Dorman et al., 2003). The reducing power of the extracts also increased with concentration and fungal extracts in the present study showed moderate to good reducing ability in comparison to BHT. Among the mushroom species the reducing power of fruiting bodies were found better than that of their mycelia except mycelial extract of *Schizophyllum commune* which showed lower EC\textsubscript{50} values of REP than its fruiting body extract. It was also observed that EC\textsubscript{50} values of REP of fruiting body extracts of *Armillaria mellea* and *Lentinus tigrinus* showed less than 1 mg ml\textsuperscript{-1}. Whereas fruiting body and mycelial extracts of other fungi showed EC\textsubscript{50} values of REP between 1 to 6 mg ml\textsuperscript{-1} except mycelial extracts of *Polyporus arcularius* which showed higher EC\textsubscript{50} values than 6 mg ml\textsuperscript{-1}. Similar range of EC\textsubscript{50} values of REP were reported by Lillian et al. (2008b) from the fruiting body extracts of *Hypholoma fasciculare* (0.95±0.01 mg ml\textsuperscript{-1}), *Lepista nuda* (3.53±0.09 mg ml\textsuperscript{-1}), *Lycoperdon molle* (2.27±0.01 mg ml\textsuperscript{-1}), *L. perlatum* (2.96±0.01 mg ml\textsuperscript{-1}), *Ramaria botrytis* (0.68±0.00 mg ml\textsuperscript{-1}) and *Tricholoma acerbum* (3.27±0.02 mg ml\textsuperscript{-1}). The EC\textsubscript{50} value of methanolic extracts of fruiting body of *Agaricus bisporus* (Bs0118H a wild strain) (1.76±0.27 mg ml\textsuperscript{-1}) was reported by Jean-Michel et al. (2008) was nearly similar to present study. Lillian et al. (2008a); and Filipa et al. (2011) had found EC\textsubscript{50} value of REP of 3.63±0.02; and 2.23±0.35 mg ml\textsuperscript{-1} from methanolic extracts of fruiting body of *A. bisporus*; and *Lentinus tigrinus*; respectively that were little higher but in the range of values found from other species in present study. Isabel et al. (2007) had reported EC\textsubscript{50} value of REP in similar range from fruiting body extracts of *Lactarius deliciosus* (3.42 mg ml\textsuperscript{-1}) and *Tricholoma portentosum* (3.12 mg ml\textsuperscript{-1}). Jaita et al. (2010) had found EC\textsubscript{50} values 13.00±0.07 mg ml\textsuperscript{-1} from fruiting body extract of *Pleurotus squarrulosus* that was higher in comparison to mushroom species studied in present study.
Contrary to these, Mau et al. (2004) had reported EC$_{50}$ value for REP 2.06±0.03 mg ml$^{-1}$ and 1.70±0.01 mg ml$^{-1}$ from extracts of red and white mycelia of *Antrodia camphorata*, respectively that were in the lower range of results of the present study. Mau et al. (2005) had reported similar range of EC$_{50}$ value REP from mycelial extract of *Ganoderma tsugae* (2.48 mg ml$^{-1}$).

Among the endophytic fungi EC$_{50}$ values of *Chaetomium globosum*, *Aspergillus fumigatus*, *A. humicola*, *A. niger*, *Penicillium frequentans*, *Mycelia sterilia* sp.1 and *Mycelia sterilia* sp. 2; and *Scytalidium lignicola* and *Mucor varians* showed below 2 mg ml$^{-1}$; between 2 to 5 mg ml$^{-1}$; and higher than 5 mg ml$^{-1}$; respectively. Arora and Chandra (2011) had reported EC$_{50}$ values of REP of mycelial extracts of *A. wentii*, *Penicillium citrinum* and *P. granulatum* cultivated in Czapek-Dox’s broth as 0.8, 0.1, 0.4 mg ml$^{-1}$, respectively. Lung et al. (2010) had reported EC$_{50}$ values of REP from mycelial extract of *Phellinus igniarius* (9.97±0.13 mg ml$^{-1}$). There were no reports in the literature for the reducing power of these fungal species and have been tested first time.

### 5.3.3. β-carotene bleaching inhibition assay

In the INBCB assay, coupled oxidation of β-carotene and linoleic acid produced hydroperoxides as free radicals. The rate of β-carotene bleaching can be slowed down with the presence of antioxidants. Thus lower bleaching of β-carotene indicates higher antioxidant capacity of an extract. Fungal extracts showed a dose-dependent activity in this assay also. It was observed that fruiting body extracts of *Armillaria mellea*, *Ganoderma applanatum*, *G. lucidum*, *Pleurotus ostreatus* and *P. sajor-caju*; fruiting body and mycelial extracts of *Agaricus bisporus*, *Auricularia auricula*, *Laetiporus sulphureus*, *Lentinus tigrinus*, *Phellinus linteus*, *Polyporus arcularius* and *Sparassis crispa*, only mycelial extracts of *Lycoperdon pyriforme*, *Russula brevipes*, *Schizophyllum commune* and *Spongipellis unicolor* showed EC$_{50}$ values of INBCB ≤ 1 mg ml$^{-1}$; between 1 to 3 mg ml$^{-1}$; and higher than 3 mg ml$^{-1}$;
respectively. Sheena et al. (2005) had reported similar value of EC$_{50}$ values of INBCB of fruiting body extract of *G. lucidum* (0.62±0.004 mg ml$^{-1}$). Filipa et al. (2011) reported slightly higher value of EC$_{50}$ of INBCB of fruiting body extract of *Lentinus tigrinus* (4.95±0.42 mg ml$^{-1}$) but in the same range of value of mycelial extracts of *Schizophyllum commune* tested in present study. However, Lillian et al. (2008a) had reported higher value of EC$_{50}$ from extract of commercial sample of fruiting body of *Agaricus bisporus* (21.39±0.45 mg ml$^{-1}$). The EC$_{50}$ values of INBCB were reported from the fruiting body extracts of mushrooms of other species by Lillian et al. (2007), Pal et al. (2010) and Lillian et al. (2008b) were in the same range as in present study i.e., *Leucopaxillus giganteus* (2.00 mg ml$^{-1}$) and *Sarcodon imbricatus* (3.97 mg ml$^{-1}$); *Pleurotus squarrosulus* (3.79±0.28 mg ml$^{-1}$); and *Hypholoma fasciculare* (0.86±0.02 mg ml$^{-1}$), *Lepista nuda* (4.21±0.0.09 mg ml$^{-1}$), *Lycoperdon molle* (1.92±0.05 mg ml$^{-1}$), *L. perlatum* (2.49±0.06 mg ml$^{-1}$), *Ramaria botrytis* (0.67±0.01 mg ml$^{-1}$) and *Tricholoma acerbum* (5.89±0.28 mg ml$^{-1}$); respectively. Asatiani et al. (2007a) reported similar range EC$_{50}$ values of INBCB from the mycelial extract of *Agrocybe aegerita* (2.4 mg ml$^{-1}$), *Daedalea gibbosa* (2.2 mg ml$^{-1}$), *Macrolepiota excoriata* (2.0 mg ml$^{-1}$) and *Phellinus robustus* (4.6 mg ml$^{-1}$).

EC$_{50}$ values of INBCB of mycelial extracts of *Chaetomium globosum*, Mycelia sterilia sp.1 and Mycelia sterilia sp. 2 showed between 1 to 3 mg ml$^{-1}$, however, extracts of other endophytic fungi showed higher EC$_{50}$ values between 3 to 7 mg ml$^{-1}$. Ravindran and Naveenan (2011) had reported EC$_{50}$ values of INBCB from mycelia extract of *C. globosum* as 47.75±1.00 µg ml$^{-1}$ and except this no report was found for INBCB assay (EC50 values) of other fungi and have been tested for the first time.

It was observed that EC$_{50}$ values of DSC, REP and INBCB obtained from fungal extracts were found interrelated in most of the cases like lowest EC$_{50}$ were found in all the three tests for fruiting body and mycelial extract of *Armillaria mellea* and mycelial extract of
Chaetomium globosum. Similarly, fruiting body and mycelial extracts of Pleurotus ostreatus, P. sajor-caju, Agaricus bisporus, Sparassis crispa and Ganoderma lucidum and mycelial extracts of Aspergillus niger and Mycelia sterilia sp. 2 have lower and interrelated EC$_{50}$ values. This has suggested presence of certain compounds in them that actively participated in all tests. Such interrelation can also be predicted with the comparatively higher EC$_{50}$ values of all three tests of mycelial extracts of mushroom species Schizophyllum commune and Spongipellis unicolor and endophytic fungi Aspergillus fumigatus, A. humicola, Scytalidium lignicola, Mucor varians, Penicillium frequentans. Contrary to above, lower EC$_{50}$ values were found only for INBCB; and DCS tests from fruiting body extract of Phellinus linteus; and G. applanatum, Polyporus arcularius, Russula brevipes and Lycoperdon pyriforme, respectively, and have comparatively higher values in other tests. This has suggested that antioxidant compounds present in them were not a universal radical scavenging or able to scavenge particular group(s) of free radicals.

5.4. **Correlation between TPC and antioxidant capacity**

TPCs values of all extracts i.e., of fruiting body and mycelia of mushroom species and mycelia of endophytic fungi were significantly negatively correlated with the corresponding EC$_{50}$ values of antioxidants assays (Table 4.5 and Fig 4.4 to 4.12) which are in accordance with the early reported findings of Lung et al. (2010); Lung and Chang (2011); Puttaraju et al. (2006) while testing the TPCs and antioxidants capacity of fruiting body extracts of Phellinus igniarius (r= 0.996); Armillaria mellea (r= 0.957); and 23 saprophytic mushroom species (r=0.950); respectively. Beside the mushroom species, such positive correlation between TPC and antioxidant capacity has also been reported in plants (Zheng and Wang, 2001; Kaur and Kapoor, 2002; Kriengsak et al. 2006; Guleria et al., 2010; Memnune et al., 2011). It is well known that phenolic compounds exert antioxidant activity in biological systems (Husain et al., 1989; Rice-Evans et al.,
Higher Pearson correlation coefficient (-0.9188, -0.8574 and -0.8393) calculated between the values of TPC and corresponding DSC, REP and INBCB of mycelial extracts of endophytic fungi predicted that phenolic compounds in them have considerable contribution in scavenging of DPPH radical, reducing of Fe$^{3+}$/ferrocyanide complex and inhibition of bleaching of β-carotene. Phenolic compounds in mycelial extracts of mushroom species have slightly weaker correlation coefficient with DSC ($r\approx-0.8155$) and nearly same with REP ($r\approx-0.8472$) presuming their moderate good and good contribution in corresponding assays of antioxidant capacity. However, significantly weaker correlation coefficient ($r\approx-0.5201$) was found between the values of TPC and EC$_{50}$ of INBCB of these extracts as well their EC$_{50}$ values were lesser. This suggested the possibility of presence of other compounds which might have participated in inhibition of bleaching of β-carotene. Similar prediction can also be made for overall antioxidant capacities of fruiting body extract of mushrooms because they showed significantly better EC$_{50}$ values of DSC, REP and INBCB but comparatively lesser correlation coefficients (-0.7557, -0.6965 and -0.7026) were determined with the corresponding values of TPC.

### 5.5. Growth enhancing properties of extracts

All plants synthesize an amazing diversity of biomolecules including flavonoids, polysaccharides, proteins, enzymes, etc. having antioxidant capacity that not only balance free radicals generated by metabolic pathways and during stress conditions but also regulate growth and disease directly or indirectly. However, in stress situations (biological and non biological) when the balance become disturbed and prooxidant activity elicited, the role of antioxidants become important especially when an external source can make any effort. Many researchers (Simonetti et al., 2002; Nesci et al., 2003; Vasar, 2004; Hala et al., 2005; Hajiboland and Amjad, 2007; Sakr and Arafa, 2009; Fayed et al., 2010) had studied growth promotive roles of synthetic antioxidants in their experiments on different plants. In the present
study first time the influence of fungal extracts of *Armillaria mellea* and *Chaetomium globosum* having potential in *in vitro* antioxidantive capacities were tested on seed germination, growth and callus proliferation of seedlings of *Dalbergia sissoo*, *Valeriana wallichii* and *Withania somnifera*.

### 5.5.1. Effect on seed germination

It was noticed that extracts of both fungi i.e., MEAM and MECG showed neutral effects at lower concentration (0.05 and 0.1%) but showed negative and dose dependent effects when concentrations increased on the germination of seeds and germination index of all tested plants. Increasing doses also caused delay in seed germination. These results are similar to the findings of Djanaguiraman *et al.* (2005) who reported that phenolic compounds like gallic acid, p-coumaric and phydroxy benzoic acids inhibit seed germination of *Vigna mungo*. They also reported that inhibition of seed germination was increased with increase in concentrations. Chaudhuri and Kar (2008) had also reported similar findings in seed germination of *Vigna radiata* which was significantly inhibited by increasing concentration of Propyl Gallate (a synthetic antioxidant). The correlation study in the present work suggested that excellent antioxidant capacity of fungal extracts were due to the phenolic contents in them. Consequently, at the same time higher proportion of phenolic compounds in the mycelial extracts of *Armillaria mellea* and *Chaetomium globosum* can be attributed to their allelopathic effects on tested seedlings of plants. Siddiqui *et al.* (2009) reported that aqueous extracts of five plants i.e., *Leucaena leucocephala*, *Azadirachta indica*, *Tectona grandis*, *Dalbergia sissoo* and *Ficus bengalensis* contained high phenolic and contents inhibited the seed germination in *Pisum sativum*. Kumar and Singhal (2009) had found that methanolic extracts of *Calotropis procera* latex inhibit seed germination of *Vigna radiata*. Contrary to them El-Maarouf-Bouteau and Bailly (2008) suggested that hydration of seeds and then initial metabolic reactions in mitochondria produces
free radicals that help to trigger-on the genes responsible for synthesis of regulative enzymes for seed germination. It may be possible that higher concentrations of fungal extracts in present study scavenged free radicals and inhibited/delayed seed germination.

5.5.2. **Effect on disease control**

It was observed that neither MEAM and MECG was found effective against wilt disease caused by *Fusarium solani* and the percent survival reduced equally with control in the seedlings of *Dalbergia sissoo*, *Valeriana wallichii* and *Withania somnifera*. Simonetti *et al.* (2002) had reported *in vitro* antimicrobial property of phenolic antioxidants, such as butylated hydroxyanisole (BHA) against human pathogen *Candida albicans* and *Escherichia coli*. Nesci *et al.* (2003) reported that BHT, BHA, trihydroxybutyrophenone (THB) and propyl paraben (PP) delayed the germination of spores of *Aspergillus flavus* and *A. parasiticus* in *in vitro* condition, however, no report is available similar to present study. It was noticed that total phenolic contents were increased in leaves of infected or resistant seedlings of tested plant species which is in concurrence to findings of Lattanzio *et al.* (2006), Qin *et al.* (2006), and Jabeen *et al.* (2009) for the critical relationship of indigenous antioxidants with fungal infection in plants.

5.5.3. **Effect on seedling growth**

The results revealed that MEAM improved height, collar diameter, fresh and dry weight of shoot and root and MECG improved only height, fresh and dry weight of shoot and dry weight of root of seedlings of *Dalbergia sissoo* at lower concentration (0.1%), however, their higher concentrations reversed the influences. Similar results were also observed with seedlings of *Withania somnifera*. The seedlings of *Valeriana wallichii* remained unaffected by treatments of MEAM and MECG. Many researchers (Desaint *et al.*, 2004; Kwak *et al.*, 2006; Shulaev and Oliver, 2006; Torres *et al.*, 2006; El Maarouf-Bouteau and Bailly, 2008) believed that free radicals and antioxidants both regulate signalling pathways and cellular growth in plants and on the
basis of their hypothesis the higher concentration of active extracts of MEAM and MECG in the present study probably obstructed free radical signalling pathways in the seedlings of *D. sissoo* and *W. somifera* and caused negative growth response. Moreover, allelopathic effects of fungal extracts may also be considered for their inverse effects. Cao and Cutler (1993) suggested that at higher concentration radical absorbing capacity of an antioxidant decreases and can lead to the formation of additional free radicals in *in vivo* and *in vitro* conditions. Sanchez-Fernandez *et al.* (1997) reported similar effects of exogenous application of micromolar concentrations of synthetic antioxidant glutathione (GSH) that increases the number of meristematic root cells undergoing mitosis, whereas depleted on over doses. El-Lethy *et al.* (2010) reported similar effects i.e., lower concentration of two synthetic antioxidants Putrescine (120 mg l\(^{-1}\)) and of α-tocopherol (200 mg l\(^{-1}\)) increased the height and fresh and dry weight of *Linum usitatissimum* L., but remained unaffected at higher concentrations. No report was found for biological source of antioxidant studied on plant growth promotion as in present study. However, Vasar (2004) had reported that formation of shoots of *Prunus avium* (sweet cherry) were increased 27% by synthetic antioxidants dithiothreitol (0.25 mM) on *in vitro* rooting in greenhouse. Hala *et al.* (2005) had reported growth enhancing (increase in number of leaves, branches and fresh and dry weight of shoot) activity of tocopherol and ascorbic acid both at 400 mg l\(^{-1}\) of in faba bean plants. Similarly, Sakr and Arafa (2009) found two synthetic antioxidants spermine (10 mg l\(^{-1}\)) and ascorbic (200 mg l\(^{-1}\)) acid significantly increasing the height, leaves number, dry weight of root and shoot of canola plants.

**5.5.4. Effect on callus regeneratoin**

From the results (Table 4.10, 4.15 and 4.20) it can be suggested that increasing concentrations of fungal extracts have species specific effects on weight ratio of proliferated callus. The lower concentrations (0.1% of MEAM and 0.1 and 0.2% of MECG) of both extracts had
greater effect on pre-treated callus of *Dalbergia sissoo*. All concentrations of both extracts remained neutral on callus of *Valeriana wallichii*, however, showed negative effects on weight ratio of callus of *Withania somnifera* except at 0.05%. It was also observed that callus in all test plants in control degenerated after 4 weeks, whereas the callus treated with active extracts of both fungi reduced necrosis and browning and remained viable even after 7 weeks. Anthony *et al.* (2004) had reported that the pre-treatments of two synthetic antioxidants i.e., tripotassium citrate (0.75 g l⁻¹) and citric acid (0.25 g l⁻¹) on the explants of *Conostephium pendulum* reduced somatic embryo production but prevented from necrosis and browning up to 10 days. Abdelwahd *et al.* (2008) reported that synthetic antioxidant ascorbic acid (1 mg l⁻¹) mixed in MS medium reduced lethal browning and improved shoot regeneration of explants of faba bean. Similarly, Tang *et al.* (2004) found that polyvinylpolypyrrolidone (5 mg l⁻¹) and 1, 4-dithio-dl-threitol (2 mg l⁻¹) improved callus formation by 26 % and 19%, respectively. No report was found for natural antioxidant tested on callus of any plants as in present study.

In conclusion, *Armillaria mellea* and *Chaetomium globosum* were found as excellent source of TPC and antioxidants among the all tested fungi. Other mushroom species i.e., *Agaricus bisporus*, *Ganoderma applanatum*, *G. lucidum*, *Lentinus tigrinus*, *Pleurotus ostreatus* and *P. sajor-caju* and endophytic fungi i.e., *Aspergillus humicola*, *A. niger*, *Chaetomium globosum* and *Mycelia sterilia* sp. 2 were also showed good TPC and antioxigenic activities. The correlation coefficient suggested that antioxidant potentials of tested fungi were assumed primarily due to their phenolic contents which also encourage a new area of investigation. Furthermore, the mycelial biomass of fungi (mushrooms and endophytic species) were equally important and can be explored as a sustainable source of antioxidants which also have ability to reduce chemical input into the environment. The present study also projected the role of fungal extracts in plant growth management, however, it cannot be fully linked with of free
radicals, instead there were negative effects on seed germination, survival of seedlings in sick soil and growth at higher dose. On the other hand their positive influence like improvement of callus weight ratio of *D. sissoo* and seedling growth of *D. sissoo* and *W. somnifera* at lower concentration cannot be undermined and these results look forward as an alternate approach in the field of agriculture and forestry, however, more elucidative studied are needed. Such extracts (natural antioxidants) can also be proposed in food industry using seed sprouts to regulate seed germination. Present study showed that the antioxidants can be used effectively to preserve/maintain callus for longer period in plant tissue culture industry.