SUMMARY

In the present investigation, the selection of Botanical Garden, where many important plants are maintained. Among those eight medicinal plants namely *Garcinia indica*; *Saraca indica*; *Vitis quadrangularis*; *Mimosa pudica*; *Oxalis corniculata*; *Ruta graveolens*; *Piper betle*; *Basella rubra* were selected for the experimental plants.

The study revealed that selection of plants, isolation and identification of different endophytic fungi in monsoon, winter and summer seasons during 2011-2014. This study also brought antagonistic property of endophytic fungi against *C. capsici* by dual culture method. The diversity of endophytic fungi showed the ubiquitous forms along with some rare species. Variation in colonization pattern of endophytic fungi strengthens the view that endophytic microbial populations positively correlated with various environmental factors. Although the present study deals with the diversity of fungal communities of some medicinal plants, the actual diversity may depend on methods used for gathering and handling of leaf samples, size of the leaf fragment and most importantly the culture medium. The total number of species and frequency of colonization can be further worked out on morphological methods, since many fungi do not grow on artificial culture medium and are difficult to identify in culture.

In the present investigation, a significant variation was detected based on the colonization frequency of endophytic species at different seasons of the year, indicating the environmental factors such as rainfall and atmospheric humidity and their effect on host plant. Therefore, surveys of endophytic fungal communities at different seasons of the year might favour a higher recovery of particular species. Further, our result also showed that the samples collected in monsoon harboured more abundant endophytic populations than that in summer, implying a seasonal fluctuation for the endophytes.

In the present study, 18 different fungal genera with 30 different species were isolated. Among the *Alternaria* genus was isolated with 3 different species, the genus *Aspergillus* was isolated with 6 different species, the genus *Fusarium* was isolated with 3 different species, the genus *Nigrospora* was isolated with 2 different species and *Penicillium* genus was isolated with 3 different species. Therefore, this study revealed that fungal endophytes had multiple hosts showing the wide range of host specificity in all the eight medicinal plants.

Seasonal variation in the colonization rate (CR) of endophytic fungi in both *Garcinia indica* and *Saraca indica* showed that CR (%) was high during monsoon, least during
summer and moderate during winter. % of Colonization rate in *Garcinia indica* was (53.1%CR) during monsoon, (43.1%CR) least during summer and (22.1%CR) moderate during winter season. Similarly % of Colonization rate in *Saraca indica* was (43.7%CR) during monsoon, (22.2%CR) least during summer and (17.3%CR) moderate during winter season. Comparing the two tree species % of CR was maximum 39.4% in *Garcinia indica* and minimum 27.1% in *Saraca indica*. *Nigrospora padwickii* and *Rhizopus nigricans* were found to be the dominant endophytic fungi. Colonization frequency of endophytic fungi in *Garcinia indica* L. leaves with endophytic fungus *Nigrospora padwickii* had the maximum (3.3% CF) colonization frequency and endophytic fungus *Fusarium niveum* had the minimum (1.1% CF) colonization frequency during monsoon season. During winter endophytic fungus *Fusarium elegans* had the maximum (2.2% CF) colonization frequency and *Alternaria chlamydospora* had minimum (0.7% CF) colonization frequency. During the summer endophytic fungus *Bipolaris nodulosa* had maximum (1.9% CF) colonization frequency and *Cladosporium epiphyllum* had minimum (0.4%CF) colonization frequency, where as in case of *Saraca indica* L. leaves with endophytic fungus *Rhizopus nigricans* had maximum (2.9% CF) colonization frequency and endophytic fungus *Aspergillus flavipes* had minimum (0.7% CF) colonization frequency during monsoon season. During winter endophytic fungus *Rhizopus nigricans* had the maximum (2.2% CF) colonization frequency and *Fusarium niveum* had minimum (0.4% CF) colonization frequency. During the summer endophytic fungus *Aspergillus fumigatus* had maximum (1.8% CF) colonization frequency and minimum had *Aspergillus sydowii* (0.0%CF). Compared to others endophytes quite interestingly there was a record of *Verticillium terrestre* was isolated only from host *Garcinia indica* revealing the host specificity of the fungi.

Seasonal variation in the colonization rate (CR) of endophytic fungi in both *Vitis quadrangularies* and *Mimosa pudica* showed that CR (%) was high during monsoon, least during summer and moderate during winter. % of Colonization rate in *Vitis quadrangularies* was (46.2%CR) during monsoon, (15.1%CR) least during summer and (31.8%CR) moderate during winter season. Similarly % of Colonization rate in *Mimosa pudica* was (32.8%CR) during monsoon, (10.6%CR) least during summer and (24.4%CR) moderate during winter season. Comprising of the two shrub species % of CR was maximum 31.1% in *Vitis quadrangularies* and minimum of 22.6% in *Mimosa pudica*. *Aspergillus awamori* and *Penicillium citrinum* were found to be the dominant endophytic fungi. Colonization frequency of endophytic fungi in *Vitis quadrangularies* L. leaves with endophytic fungus *Aspergillus awamori* had the maximum (2.9% CF) colonization frequency and endophytic
fungus *Nigrospora padwickii* had the minimum (0.7% CF) colonization frequency during monsoon season. During winter endophytic fungus *Geotrichum candidum* had the maximum (1.9% CF) colonization frequency and *Aspergillus fumigatus* had minimum (0.4% CF) colonization frequency. During the summer endophytic fungus *Aspergillus awamori* had maximum (1.5% CF) colonization frequency and *Penicillium rubrum* had minimum (0.4% CF) colonization frequency. In case of *Mimosa pudica* L. leaves with endophytic fungus *Penicillium citrinum* had maximum (2.9% CF) colonization frequency and endophytic fungus *Nigrospora padwickii* had minimum (0.7% CF) colonization frequency during monsoon season. During winter endophytic fungus *Penicillium citrinum* had the maximum (1.9% CF) colonization frequency and *Aspergillus fumigatus* had minimum (0.4% CF) colonization frequency. During the summer endophytic fungus *Penicillium citrinum* was maximum (1.5% CF) colonization frequency and minimum had *Alternaria burnsii* (0.0% CF).

There was no significant difference between monsoon and winter season with respect to fungal colonization rate among the herbs. But during summer season, the colonization rate was found to be least compared to winter and monsoon season. In the monsoon season colonization was found to be high compared to winter and monsoon. Comparing of the two herb species % of CR was maximum 25.6% in *Oxalis corniculata* and minimum of 18.8% in *Ruta graveolens*. Colonization frequency of endophytic fungi in *Oxalis corniculata* L. leaves with endophytic fungus *Aspergillus flavipes* had the maximum (2.2% CF) colonization frequency and endophytic fungus *Aspergillus awamori* had the minimum (0.7% CF) colonization frequency during monsoon season. During winter endophytic fungus *Aspergillus flavipes* had the maximum (2.9% CF) colonization frequency and *Cladosporium epiphyllum* had minimum (0.4% CF) colonization frequency. During the summer endophytic fungus *Aspergillus flavipes* had maximum (1.1% CF) colonization frequency and *Cladosporium epiphyllum* had minimum (0.0% CF) colonization frequency. In case of *Ruta graveolens* L. leaves with endophytic fungus *Nigrospora sphaerica* had maximum (2.2% CF) colonization frequency and endophytic fungus *Aspergillus awamori* had minimum (0.7% CF) colonization frequency during monsoon season. During winter endophytic fungus *Nigrospora sphaerica* had the maximum (1.5% CF) colonization frequency and *Aspergillus awamori* had minimum (0.2% CF) colonization frequency. During the summer endophytic fungus *Nigrospora sphaerica* had maximum (1.1% CF) colonization frequency and minimum had *Aureobasidium pullulans* (0.3% CF). Compared to others endophytes quite interestingly there was a record of *Papulaspora sepedoniodes* and *Petrakia cochinensis* was isolated only from
host *Ruta graveolens* revealing the host specificity of the fungi. And also *Humicola insolens* was only isolated from the host *Oxalis corniculata* revealing the host specificity of the fungi.

Similarly the colonization rate among the climber species in all seasons *Basella rubra* showed least 17.7% colonization rate and *Piper betle* showed highest 30.3% colonization rate. The total colonization rate (CR) was maximum in monsoon, least during summer and moderate during winter. Colonization frequency of endophytic fungi in *Piper betle* L. leaves with endophytic fungus *Penicillium purpureogenum* had the maximum (2.6% CF) colonization frequency and endophytic fungus *Aspergillus clavatus* had the minimum (1.1% CF) colonization frequency during monsoon season. During winter endophytic fungus *Penicillium purpureogenum* had the maximum (2.2% CF) colonization frequency and *Nigrospora sphaerica* had minimum (0.7% CF) colonization frequency. During the summer endophytic fungus *Alternaria chlamydospora* had maximum (1.5% CF) colonization frequency and minimum was *Alternaria geophila* (0.0%CF). In case of *Basella rubra* L. it was recorded that the colonization frequency (CF %) of endophytic fungi was maximum of (1.8% CF) for *Bipolaris nodulosa* and minimum of (0.7%) CF was *Nigrospora sphaerica* during monsoon season. There was a maximum of (1.1% CF) with the presence of *Cunninghamella blackesleean* and a minimum of (0.4% CF) in *Nigrospora sphaerica* during winter season. The (%) CF was maximum of (1.5% CF) with *Fusarium elengans* and minimum of (0.4% CF) with *Aspergillus clavatus* during the summer season. Compared to others endophytes quite interestingly there was a record of *Penicillium purpureogenum* and *Periconia hispidula* was isolated only from host *Piper betle* revealing the host specificity of the fungi.

On the eight experimental plant leaves, in all the seasons the total colonization rate was ranged from maximum of 39.4% in *Garcinia indica* and a minimum of 17.7% in *Basella rubra*. Scheffe post hoc and pears correlation in all the three season on eight experimental plants the total colonization frequency of dominant endophytic fungi, had a minimum of 14.8% of *Nigrospora sphaerica* in *Ruta graveolens*, herb had maximum 34.4% *Aspergillus flavipes* in *Oxalis corniculata* there results indicated that *Nigrospora sphaerica* was the least frequency and *Aspergillus flavipes* had the high frequency as a most dominant endophytic fungi.

The total colonization rate ranged from a minimum of 17.7% in *Basella rubra* L. a climber and maximum of 39.4% in *Garcinia indica* which is a tree species. Scheffe Post hoc test clearly showed that in selected eight medicinal plants of all the three seasons *Basella*
rubra had minimum rate of colonization frequency (17.1% CR) and Garcinia indica had maximum colonization rate compared to other medicinal selected plants.

Total Colonization frequency of dominant endophytic fungi Nigrospora sphaerica was 14.8% minimum CFD in Ruta graveolens, where as colonization frequency of dominant endophytic fungi Aspergillus flavipes was 34.4% maximum CFD in Oxalis corniculata. Sheffe Post hoc test was shown the significant variation in the dominant colonization frequency among the selected eight medicinal plants.

To understand the relation between eight medicinal plant species for their colonization rate of endophytic fungi, the to Pearson’s Correlation matrix showed the Pearson’s Correlation matrix high significance at 0.05 levels was observed among the eight medicinal plants. Pearson correlation was observed in between Garcinia indica and Mimosa pudica; Saraca indica and Ruta graveolens, similarly in the Basella rubra and Vitis quadrangulararies indicates positive Pearson correlation of colonization rate shown. The results revealed that all the six plants viz; Garcinia indica and Mimosa pudica; Saraca indica and Ruta graveolens; Basella rubra and Vitis quadrangulararies had significantly colonized (harbours) with endophytic fungi at equal rate. All six hosts support the growth of endophytic fungi irrespective of the seasons.

Bary- Curtis similarity was used to describe the taxonomic affinity of endophytic mycobiota among the hosts. In order to understand similarities between the endophytic fungi, the data was subjected to Bray-Curtis Similarity index. A fixed stopping rule at 85% similarity was applied. It was observed that, Aureobasidium epiphyllum and Bipolaris nodulosa were at closest distance with 86% similarity; Penicillium rubrum and Rhizopus nigricans were at the next closest distance. However 95% similarity was seen between Fusarium poae and Geotrichum candidum in trees species. In case of shrubs, according to the Bray Crutis similarity index, Penicillium rubrum and Rhizopus nigricans were at the closest distance with 87% similarity; Fusarium poae and Nigrospora padwickii were at the closest distance with 82% similarity. In case of herb species Bray Crutis similarity index showed that Cladosporium epiphyllum and Nigrospora sphaerica were at closest distance with 78% similarity; Papulaspora sepedoniodes and Humicola insolens were at the next closest distance at 80%. However 82% similarity was seen between Aspergillus flavipes and Aureobasidium pullulans but at far distance from the axis. In the case of climber species Bray-Curtis similarity index was observed that Fusarium elegans and Fusarium niveum were
at closest distance with 92% similarity; *Penicillium purpureogenum* and *Periconia hispidula* were at the next closest distance at 84%. However 88% similarity was seen between *Nigrospora sphaerica* and *Torula alli*.

The antagonistic activity of *Nigrospora sphaerica* isolated from, *Ruta graveolens* leaf segments showed on higher percentage of inhibition zone against *Colletotrichum capsici*, whereas, as the *Penicillium citrinum* isolated from *Mimosa pudica* and *Aspergillus awamori* isolated from *Vitis quadrangularies* inhibit the minimum percentage of inhibit the wilt pathogen, *Colletotrichum capsici* compared to other isolates in vitro.

The identification of bioactive compounds in *Nigrospora sphaerica* methanol shows 15 compounds. These compounds contain extract exhibited strong antifungal activity. Therefore, this strategy may significantly accelerate the discovery process in natural products research and their role in pharmacology. The separation of each compound individually needed and their role should be identified in the future.

The effect of arbuscular mycorrhizal fungi (*Glomus mosseae*) and *Nigrospora sphaerica* in combination showed a significant growth response on *Ocimum sanctum* in a green house pot experiments. The inoculated plants showed improved growth over control. The different growth parameters increased significantly after 45 and 90 days respectively. The dual inoculation of *Glomus mosseae* + *Nigrospora sphaerica* was most effective in increasing shoot length, leaf area, shoot biomass, root length and AM (arbuscular mycorrhizal) root colonization over control. Maximum spore number and colonization was found in dual combination of *Glomus mosseae* + *Nigrospora sphaerica*. The maximum increase in root weight was found in combination of *Glomus mosseae* + *Nigrospora sphaerica*. In single inoculation *Glomus mosseae* provide to be more effective. These studies brought synergetic effect of two organisms.