CHAPTER-IV

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

4.1 Isolation of endophytes

During the present study, endophytic microorganisms were isolated from healthy and symptomless plant tissues viz. leaves, rhizomes and roots of *Curcuma longa*. Photographs of habit sketch and plant parts are shown in photoplate 1. A total of 33 fungal isolates belonging to 5 genera (viz. *Acremonium* sp, *Curvularia lunata, Fusarium* sp, *Penicillium citrinum, Trichoderma viride*) and 55 bacterial isolates were recovered from different parts i. e. leaf, rhizome and root of *C. longa* (Photoplate 2). The fungal strains unable to produce spores were designated as white sterile mycelia (WSM) and black sterile mycelia (BSM). Photographs of the pure colonies of identified fungal endophytes were shown in the photoplate 3. The colonization frequencies of fungal endophytes were 23.3%, 18.3% and 13.4% in leaves, rhizomes and roots respectively (Fig.1). *Penicillium* sp. and White sterile mycelia were most frequently isolated endophytes (Table1).

4.2 Diversity of endophytes in the plant tissues

Endophytes are not organ specific. Endophytes present in the phylosphere may also present rhizospheric region or other parts of the plant tissues. During our investigation also, the percentage of isolation of endophytes from leaves was highest (fungi-42.4%, bacteria-47.3%). It was followed by rhizome (fungi-33.3%, bacteria-34.5%), and then root (fungi-24.2%, bacteria-18.2%) (Fig.2). Endophytes are hypothesized to help their hosts by producing bioactive substances that confer medicinal properties to the host (Ludwig-Muller, 2015). The present work, therefore, was carried out with the aim to study the endophytic microorganisms associated with *C. longa* and to screen and evaluate these microorganisms for their ability to produce bioactive secondary metabolites with antimicrobial properties. In the present study, a large number of endophytes were isolated from different parts of *C. longa* grown in greater Guwahati. Each part of the plants such as

leaves, rhizomes and roots were collected and subjected to the endophytic isolation in order to compare diversity of these organisms. The percentage of isolation was highest from leaf followed by bark and root tissues. Rodrigues (1996) in hisr studies have also observed that the leaf samples harboured more endophytes than other plat parts. Petrini (1991) found that the leaf and stem tissues of the plant were excellent reservoirs for endophytic fungi. Lodge et al. (1996) also isolated more endophytes from leaves from various angiosperms than barks and roots. Chareprasert et al. (2006) also recovered more endophytic fungi from leaf tissues of *Tectona grandis* and *Samanea saman* than other parts. More microbial species colonized the leaf tissues may be because leaves are considered as readily colonisable tissue (Arnold, 2007) and the phyllospheric region of the host plant might be more conducive for their growth than other regions. Another reason may be because leaves are short lived, bio-chemically more dynamic, grow under an environment that undergoes rapid changes, subjected to damage by sucking and chewing by herbivores. Tejesvi *et al.*, (2005) while working on the endophytic fungi of *Terminalia arjuna*, however, observed that the distribution and density of endophytes was more in inner bark segments as compared to the other plant parts. The recovery of endophytes from root was less than other plant parts. The reason for low colonization of roots by endophytes is unknown, but it might be attributed to differences in nutritional and physiological conditions of different plant parts (Huang et al., 2008). Besides, these results may also be related to the possible availability of specific growth-promoting factors present in the different plant parts. Some researchers (Schulz et al., 1993; Kumaresan et al., 2001) suggested that endophytic microorganisms show a certain degree of tissue recurrence or specificity.

Among all the endophytic strains, *Fusarium* sp and white sterile mycelia (WSM) were the most frequently isolated endophytes. Previously, few reports are available on the

isolation of endophytes from *C. longa* (Kumar et al. 2016; Gupta et al 2016). Singh et al. (2013) reported *Penicillium* sp. which was subjected to extracellular biosynthesis of silver nanoparticles. Photographs of some identified fungal species have been shown in Photoplate 4. Some Gram positive and Gram negative isolated bacterial strains were given in Photoplate 5.

4.3 Antibacterial activity of the isolated fungal endophytes

All the fungal endphytes were screened for their antimicrobial activity against both gram-positive, *Bacillus subtilis* and gram-negative bacteria, *Klebsiella pneumoniae* (Table 2). Out of the potent isolates two endophytes displayed significant antibacterial activity against both the test organisms. One of these is identified as *Fusarium* sp. on the basis of its morphology and sporulation. The other one was unable to produce spore but form white colour colony and was designated as white sterile mycelia (WSM). Zones of inhibition of endophytic fungi *Fusarium* sp and WSM were highest (18 mm in diameter) against the test organism *Klebsiella pneumoniae* (Photoplate 4) which was followed by *Curvularia lunata* and *Trichoderma viridae* (14mm in diameter in each), *Penicillium citrinum* sp (13 mm in diameter), *Acemonium* sp (12 mm in diameter), BSM (10mm in diameter) and lowest activity was shown by *Penicillium citrinum* against *B. subtilis* (4 mm in diameter) (Table 3). Fungal endophytes have been recognized as great source of novel bioactive metabolites. Besides these, endophytes are also recognized a rich source of secondary metabolites with antimicrobial activities (Tan and Zou, 2001; Strobel and Daisy, 2003). During our investigation also, fungal endophytes isolated from different parts of *C. longa* were screened for their ability to produce antimicrobial property. Among all the endophytic strains recovered from *C. longa* during our investigation, *Fusarium* sp. PDFL14 was found to exhibit a good activity against both the test organisms. In many instances endophytes isolated from different medicinal plants have shown their ability to

produce antimicrobial activity against plant and human pathogens (Raviraja et al. 2006, wang et al. 2000). Thus, the isolation of antimicrobial producing endophytic strains associated with *C. longa* indicated the importance of the medicinal plant.

Endophytes isolated from different medicinal plants have also shown their ability to show antifungal activity against a number of plant pathogenic fungi (Li et al., 2000). Several scientists isolated a number fungal endophytes showing antimicrobial activities against some human pathogens from different medicinal plants (Radu and Kqueen, 2002; Raviraja et al., 2006). In the present investigation too, crude extracts obtained from broth culture of endophytic fungi displayed considerable antimicrobial activity against a number of test organisms. However, some of the endophytes displayed low activity in the biassay. Radu and Kqueen (2002) while studying the antimicrobial activity of endophytic fungi isolated from medicinal plants, attributed the low antimicrobial activity due to less amount of active compound present in the crude extract. After purification they might have yielded compounds with good antimicrobial activity.

Table1: Occurrence of endophytic fungi isolated from different parts of *C. longa*

Endophytic Fungi	**Plant parts**			**No. of endophytes**	**CF***
	Leaves	**Rhizome**	**Roots**		**%**
Acremonium sp	0	2	0	2	3.33
Curvularia lunata	2	1	1	4	6.66
Fusarium sp	5	1	2	8	13.33
Penicilium strictum	1	0	1	2	33.3
Trichoderma viridae	0	2	1	3	5
WSM	3	4	2	9	15
BSM	3	1	1	5	8.33

*Total plant segments plated 60

Table2: Antibacterial activity of endophytic fungi against some test organisms

Endophytic Fungi	Test Organisms	
	BS	**KP**
Acremonium sp	+	++
Curvularia lunata	++	++
Fusarium sp	++	+++
Penicilium strictum	++	++
Trichoderma viridae	+	++
WSM	++	+++
BSM	+	++

+=<10mm; ++=≥10-15mm; +++=>15mm

Table3: Zone of inhibition of test orgsnisms by endophytic fungi isolated from *C. longa*

Endophytic Fungi	ZOI	
	BS	**KP**
Acremonium sp	4	3
Curvularia lunata	10	14
Fusarium sp	12	18
Penicilium strictum	12	13
Trichoderma viridae	8	14
WSM	14	18
BSM	8	10

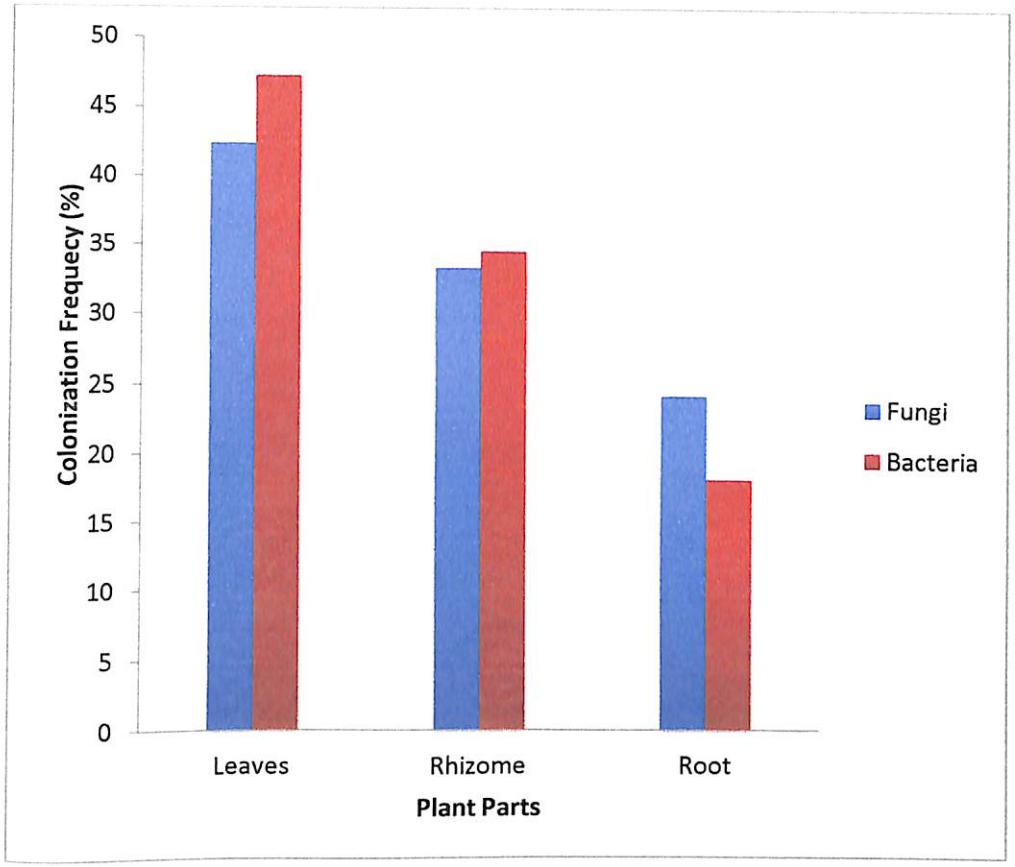


Fig. 1: Percentage of isolation of Endophytes from different plant parts of *C.longa*

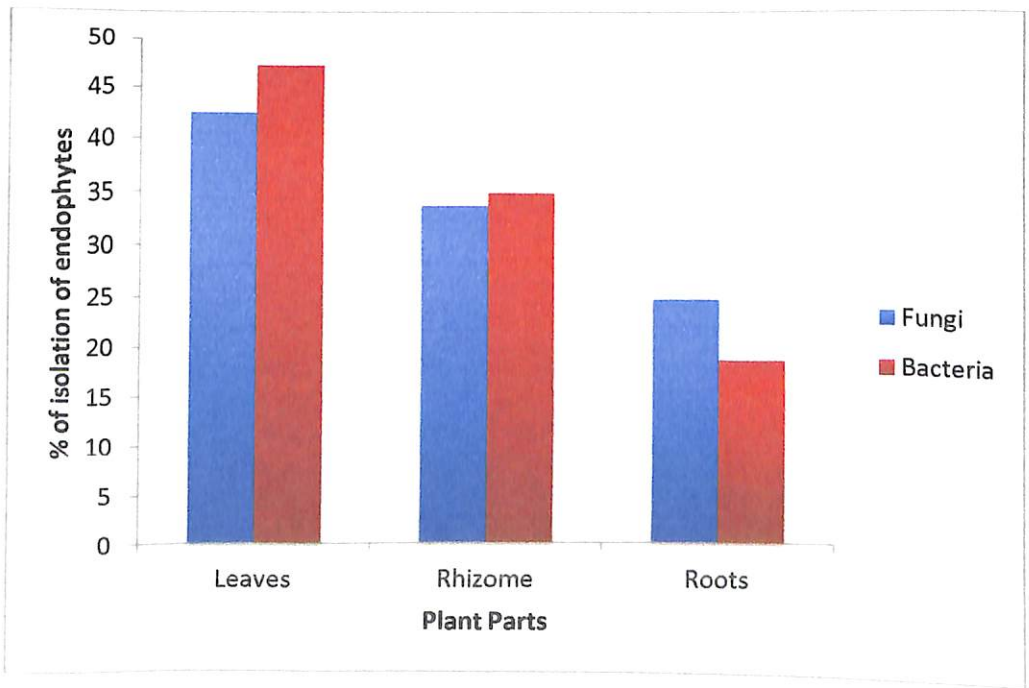
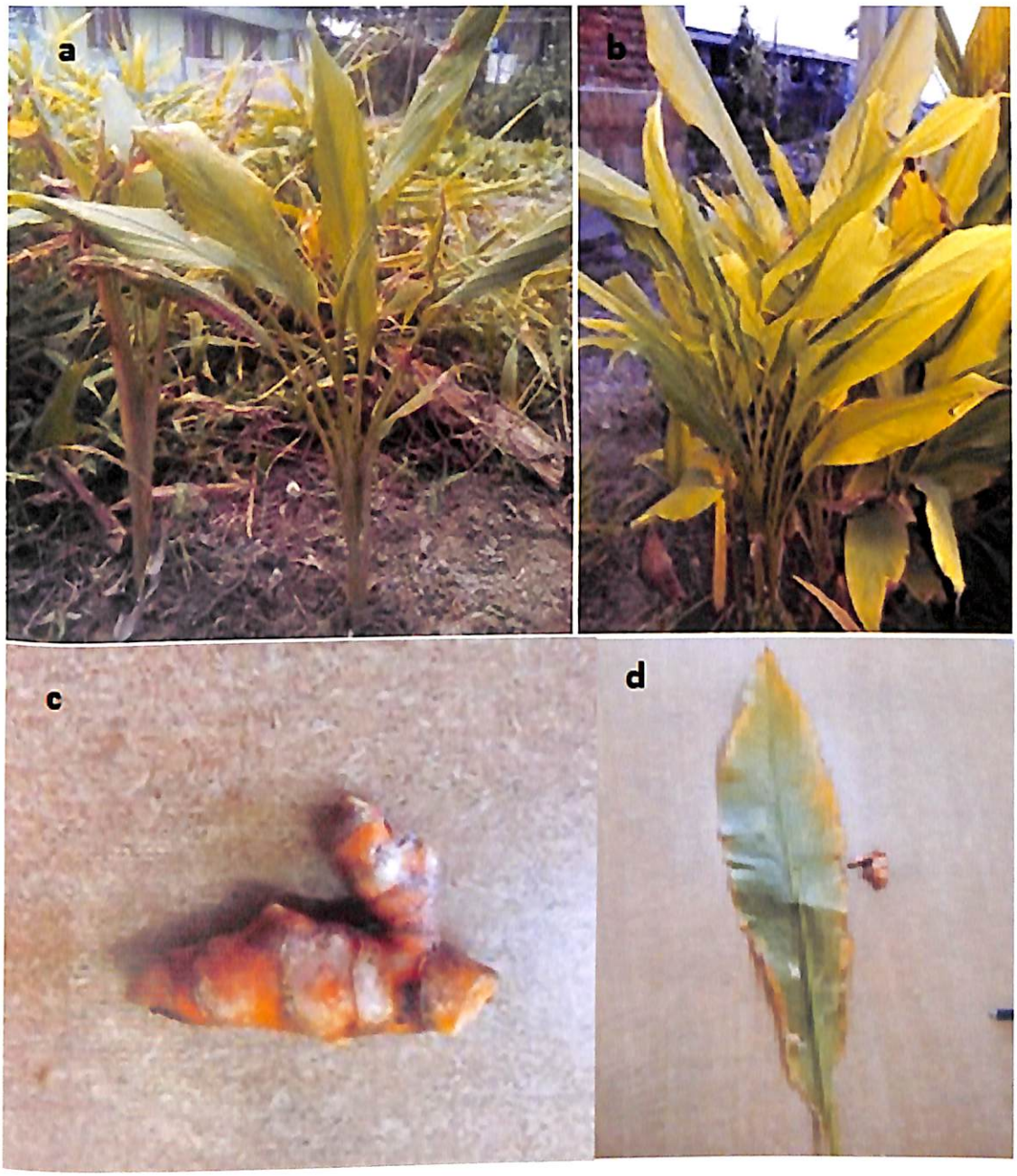
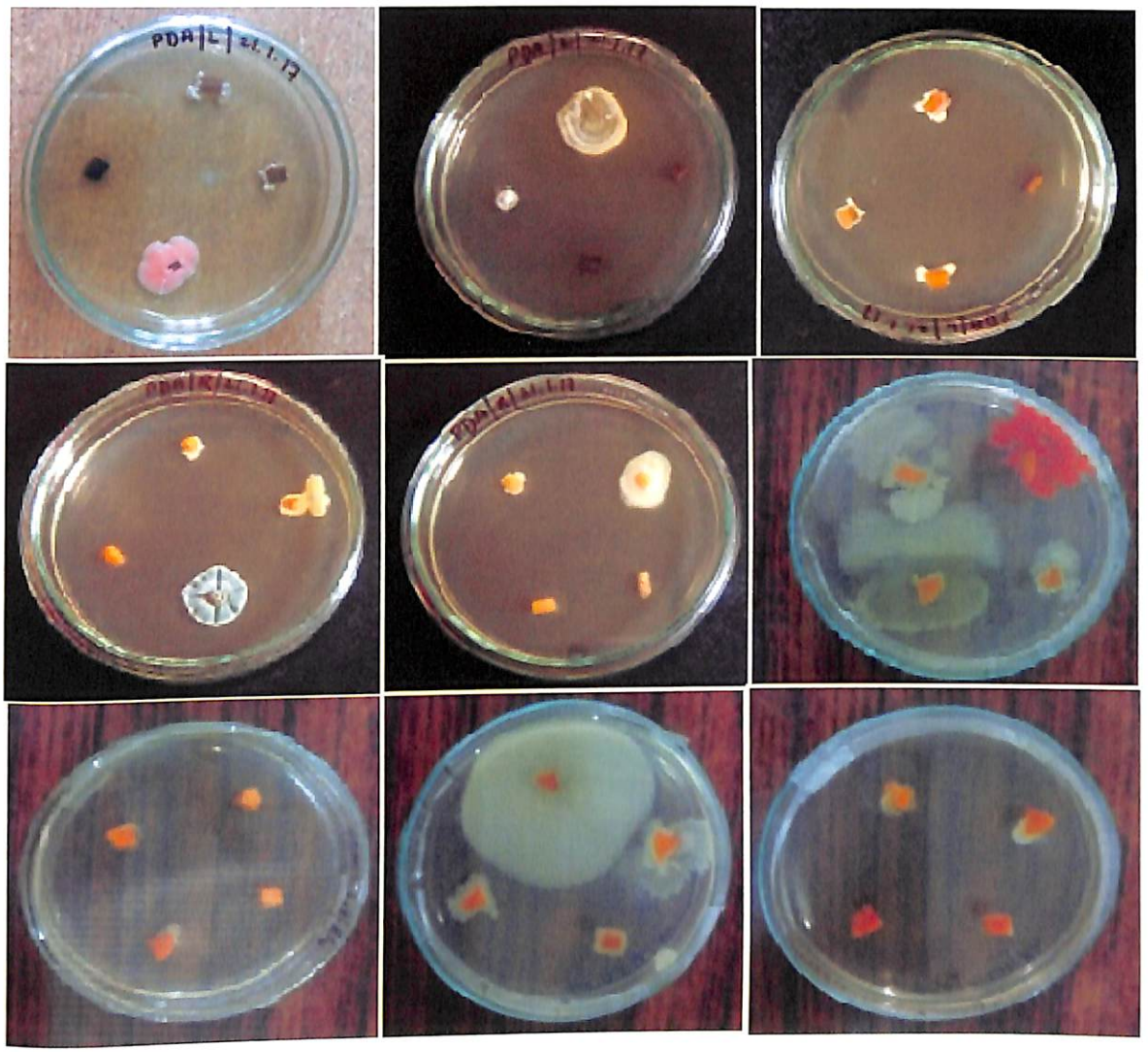


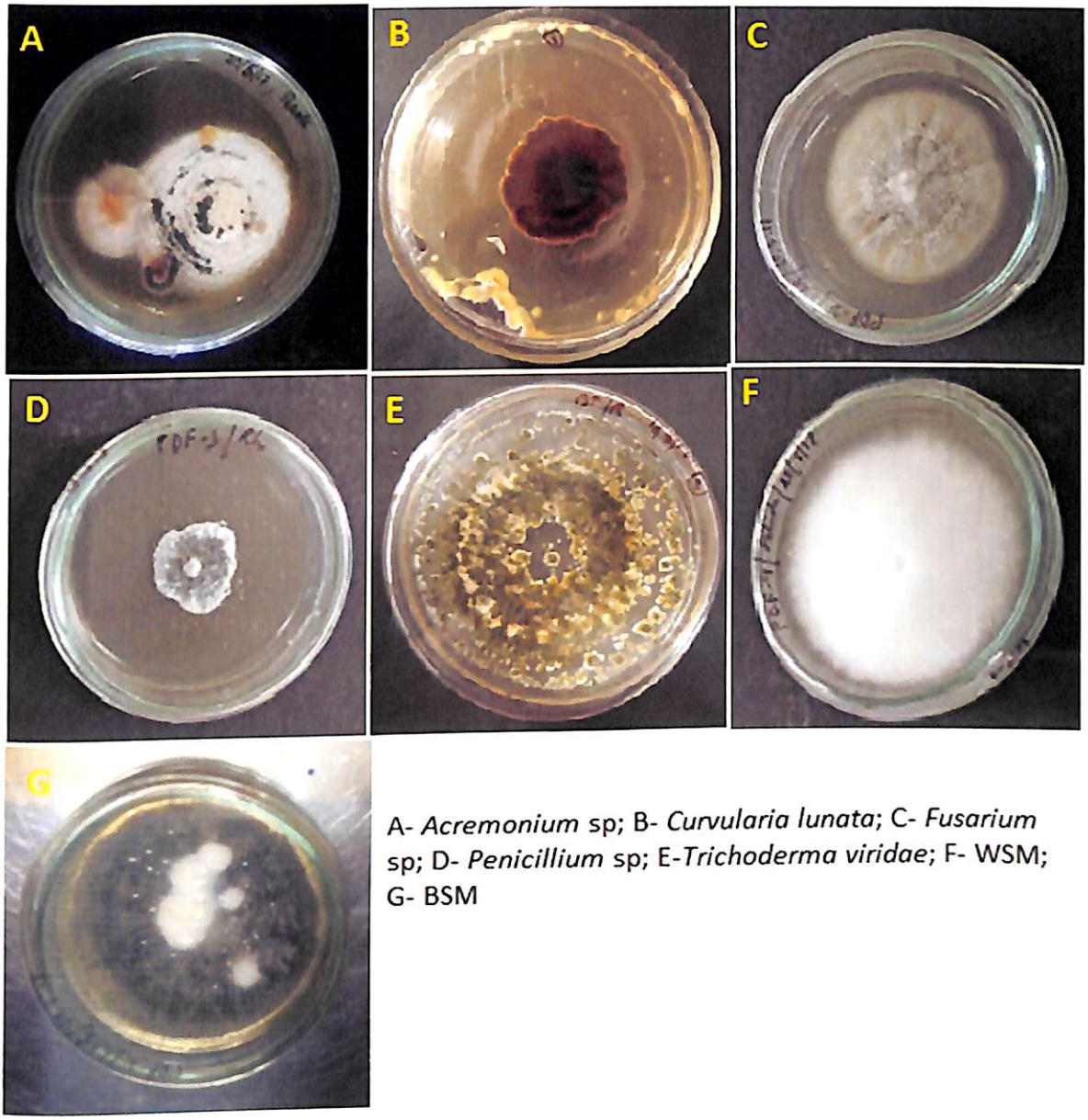
Fig. 2: Percentage of isolation of endophytes from different parts of *C. longa*



Photoplate1: *Curcuma longa* L. (a) and (b) Habit, (c) Rhizome and (d) Leaf

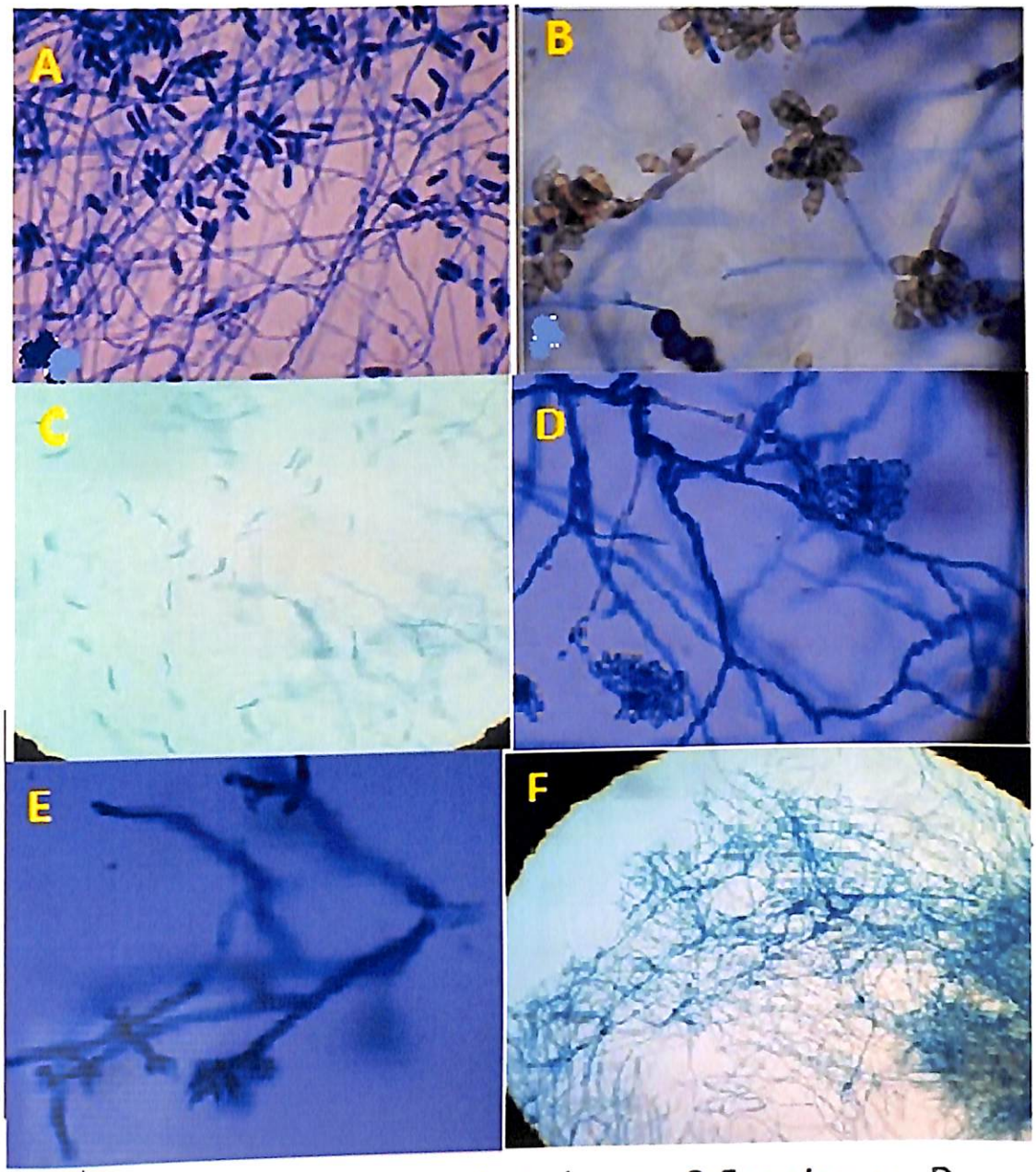


Photoplate2: Recovery of some endophytic colonies from different parts of *C. longa*



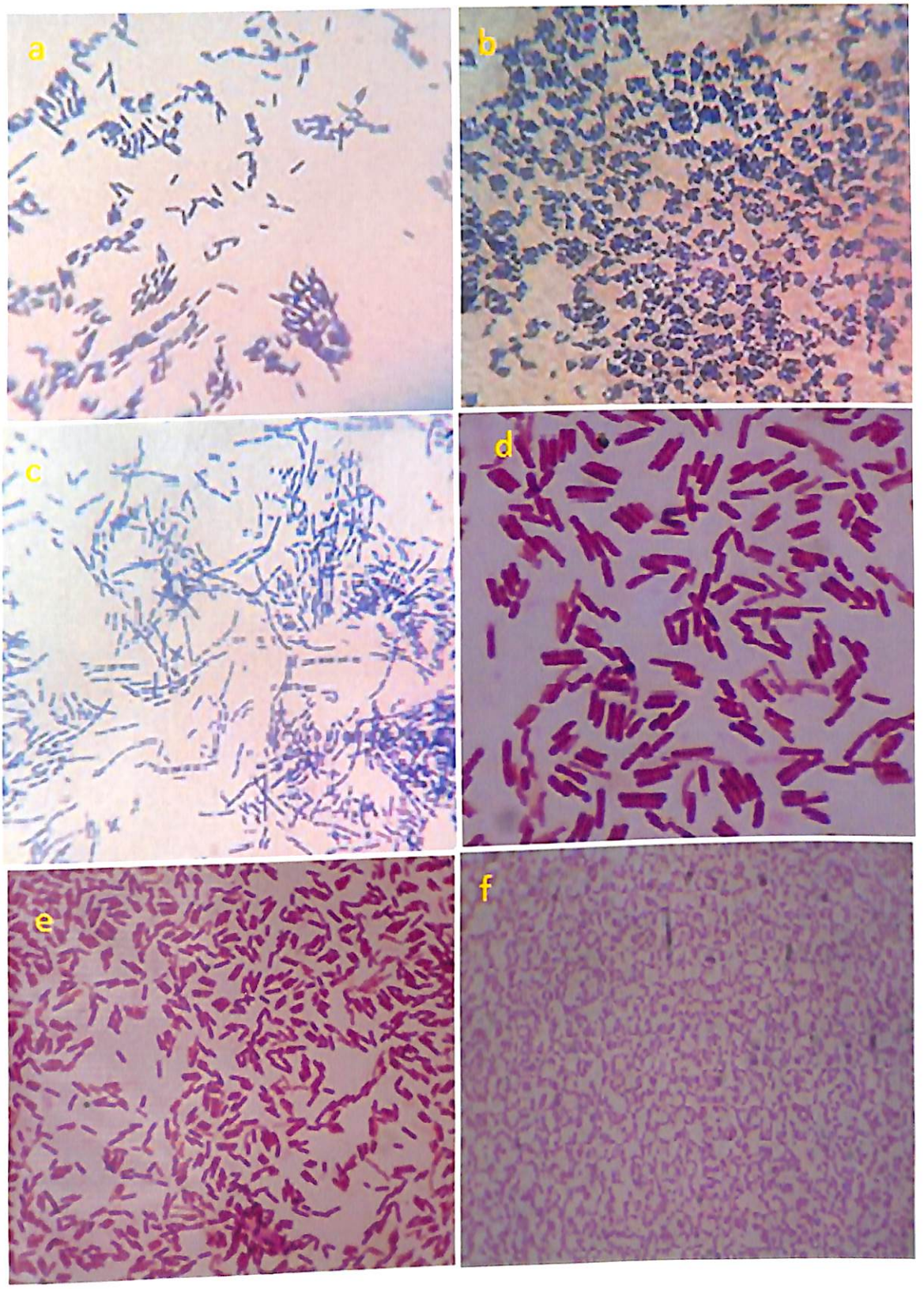
A- *Acremonium* sp; B- *Curvularia lunata*; C- *Fusarium* sp; D- *Penicillium* sp; E-*Trichoderma viridae*; F- WSM; G- BSM

Photoplate 3: Some Pure endophytic fungal colonies isolated from *C. longa*



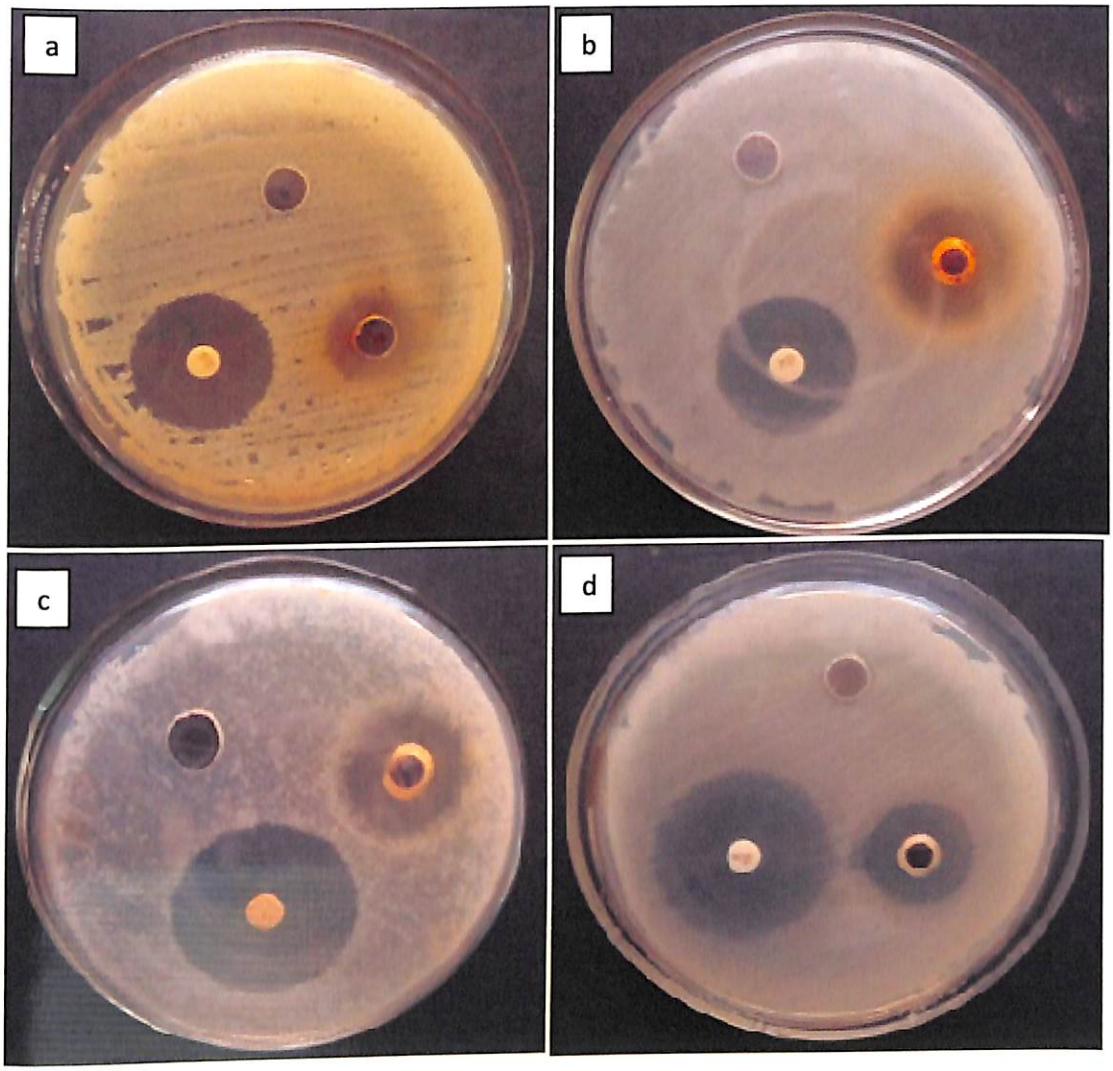
A- *Acremonium* sp; B- *Curvularia lunata*; C-*Fusarium* sp; D- *Penicillium citrinum*; E- *Trichoderma viridae*; F- Mycelia Sterile

Photoplate4: Microphotographs of identified fungal endophytes isolated from *C. longa*



Photoplate 5: Microphotographs of some bacterial endophytes isolated from *C. longa*

(a,b,c): Gram+ve and (d,e,f): Gram –ve



Photoplate 6: Zone of inhibition of (a) *Fusarium* sp, (c) WSM against *B. subtilis*, (b) *Fusarium* sp (d) WSM against *K. pneumoniae*

5. SUMMARY AND CONCLUSION

Endophytes of medicinal plants occupy a unique habitat, highly diverse and are important sources of natural metabolites of pharmaceutical importance. *Curcuma longa* L. commonly known as turmeric is known for its medicinal properties. It is a rhizomatous plant belonging to family Zingiberaceae cultivated in all over Assam. The mature dried rhizome is most common ingredient of Assamese kitchen as spice and well known antiseptic, antipyretic since ancient times. The rhizome of turmeric is very remarkable due to its metabolite richness and the physiological processes associated with these tissues. Traditionally, it has been extensively used by the people of Assam in the treatment of swelling caused by injury. In addition, turmeric also possesses antimicrobial and anticancer properties. The medicinal properties are assigned due to the presence of curcuminoid and sesquiterpenoid compounds. Plants interact with diverse communities of microorganisms for various purposes including growth promotion, yield enhancement and disease management. In turn, the endophytic microbes which reside within the healthy tisssues, derive shelter and nutrients from the host plants. *C. longa* is a rich source of endophytes.

During present investigation also we were able to recover a large number of endophytes from healthy and symptomless plant tissues viz. leaves, rhizomes and roots of the medicinal plant. A total of 33 fungal and 55 bacterial isolates were recovered from different parts i. e. leaf, rhizome and root of *C. longa*. Fungal isolates belong to genera *Acremonium, Curvularia, Fusarium, Penicillium* and *Trichoderma*. The fungal strains unable to produce spores were designated as white sterile mycelia (WSM) and black sterile mycelia (BSM). The colonization frequencies of fungal endophytes were 23.3%, 18.3% and 13.4% in leaves, rhizomes and roots respectively. *Penicillium* sp. and White sterile