

## Isolation and evaluation of effective fungal antagonist against Northern leaf blight of maize

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### ABSTRACT

A total of ten phylloplane fungi has been isolated from healthy leaves of maize by leaf washing method viz. M. Phy-01, M. Phy-02 (*Trichoderma* sp. 1), M. Phy-03 (*Trichoderma* sp. 2), M. Phy-04 (*Penicillium* sp.), M. Phy-05 (*Fusarium* sp.), M. Phy-06 (*Aspergillus niger*), M. Phy-07, M. Phy-08 (*Trichoderma* sp. 3), M. Phy-09 (*Fusarium oxysporum*) and M. Phy-10 (*Alternaria* sp.). As well as a total of eight endophytic fungi were isolated from healthy leaves of maize by surface sterilization method and those were M. Endo-01 (*Mucor* sp.), M. Endo-02, M. Endo-03, M. Endo-04, M. Endo-05, M. Endo-06 (*Fusarium* sp.), M. Endo-07 and M. Endo-08 (*Pythium* sp.). Isolated fungi were characterized on the basis of their cultural morphological characters and microscopic view of conidia and conidiophores. Determination of their antagonistic activity against the pathogen (*E. turcicum*) causing Northern leaf blight of maize was done by dual culture method, in which phylloplane fungi *Trichoderma* sp. 1 with 56.11% has the highest inhibition percentage followed by *Trichoderma* sp. 2 with 54.04% and lowest inhibition percentage was found in genera of *Alternaria* sp. with 20.70%. Among the endophytic fungi, *Mucor* sp. with 75.55% was found to have highest inhibition percentage followed by *Fusarium* sp. with inhibition percentage of 47.79% and lowest percentage of inhibition was found in M. Endo-03 (13.96%).

### 1. Introduction

Maize (*Zea mays* L.) is one the most versatile cereal grains grown throughout the agriculture world, belongs to family Gramineae and monoecious in nature. It has the potential to adapt to wide range of environment (Kogbe and Adediran, 2003) with large number of cultivars/ variety and a highest yielding crop, the crop is known as "Queen of cereals" (Kumari *et al.*, 2016). In India, after rice and wheat, maize is the third most important cereal cultivated in the country. In India, area under maize cultivation is 8.3 million ha with a total production of 2.5 t/ha. Andhra Pradesh (20.9%) followed by Karnataka (16.5%) (DMR, 2011) are the leading state in production. Although the crop has great potentialities of production but the production is still low due to many foliar diseases in which Northern leaf blight is one of the prominent diseases caused by *Exserohilum turcicum* (Reddy *et al.*, 2013). The disease is prevalent in almost all maize growing areas and hence, results in decreasing of photosynthesis system leading to severe yield reduction of 28- 91% (Swathi *et al.*, 2021). Diseased symptom appeared

mainly after anthesis but can be found on leaves at any stage of growth (Mittal and Boora, 2005). In NEH, region after rice, maize is the second most important cereals grain crops (Singh and Devi, 2018). So as to increase yield and minimize infection of crop, strategies such as use of fungicide, insecticide and cultivation of host resistant varieties etc can be followed. But in present scenario indiscriminate use of chemical lead to more frequent occurrence of disease and also resulted in pathogen resistance build up in the region and have undesirable impact on environment (Stammler and Speakman, 2006). Hence, decreasing productivity of HYV due to low adaptability occurred due to changes in the pathogen resistant and environment (Singh and Devi, 2018). Phylloplanes fungi and endophytic fungi both have ample role in defense mechanisms, increase various physiological, bio-chemical activity and also can be used as biological control agent. Much work has been done in abroad as well as in country and countable number of work has been done in Northeast India but so far, no work has been reported from the state of Nagaland. So, by taking up present investigation

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isolation and evaluation of effective fungal antagonist against Northern leaf blight of maize caused by *E. turcicum* will be worthy and helpful in near future by finding out noble beneficial microbes. Considering all the viewed aspects, the present investigation has been planned with the isolation of fungal phylloplanes and endophytes from maize plant and also *in-vitro* evaluation of phylloplane and endophytic fungal antagonist against *Exserohilum turcicum*.

## 2. Materials and Methods

Maize diseased leaves showing the characteristic symptoms of cigar-shaped or elliptical necrotic gray-green lesions that range from 1 to 7 inches long were collected from highly infected field of School of Agricultural Sciences and Rural Development (SASRD), Nagaland University, Medziphema Campus. Diseased tissues along with adjacent healthy tissue were cut into small bits of 5 mm with a sharp flame sterilized blade. Then, those bits were surface sterilized with 1% sodium hypochlorite solution for 1 minute followed by three time rinsed in sterilized distilled water for 30 second each to remove surface sterilization agent from sample. Isolation of pathogen was done by surface sterilization method and sample bits were dried with sterilized filter paper and were placed on Petri plates containing Potato Dextrose Agar (PDA). The PDA medium used was amended with streptomycin 0.3 mg/ml to prevent bacterial growth under laminar airflow cabinet. The inoculated Petri plates were incubated at 25±1 °C. Isolated pathogen was purified by single hyphal tip method (Sangareswari *et al.*, 2016) on PDA medium. Purified pathogens were sub-cultured and maintained on PDA slants for further study.

The isolated pathogen was identified according to their cultural morphological and microscopic characters as described by Sonavane *et al.* 2015.

### Isolation of phylloplane fungi

Healthy maize leaves were collected from field of SASRD, Nagaland University, Medziphema Campus, in which maize plot were highly infected by Northern leaf blight. Leaf washing method (Lee and Hyde, 2002) was followed for isolation of phylloplane fungi. Under aseptic condition, the collected samples were cut into small bits of 5.0 x 5.0 mm<sup>2</sup> in size. Then the bits were transferred to 250 ml sterile conical flask contained 100 ml of sterilized water. The flask contained bits were shaken thoroughly for 20 minutes so that all the fungal propagules present on the leaf surface were washed onto the sterilized water. One ml of suspension was transferred by pouring method into a sterilized Petri plate which contained PDA supplemented by streptomycin 0.3 mg/ml to prevent bacterial growth (Mefteh *et al.*, 2017). The inoculated Petri plate was incubated at 25±1°C. Isolated phylloplane fungi were purified by single

hyphal tip method.

### Isolation of endophytic fungi

Healthy maize leaves from Northern leaf blight sick plots were collected from SASRD, Nagaland University, Medziphema Campus. Endophytic fungi were isolated by surface sterilization method (Mirsam *et al.*, 2016). Under aseptic condition, healthy tissue was cut into small bits of 5.0 x 5.0 mm<sup>2</sup> with a sharp flame-sterilized blade. Then, those bits were surface sterilized with 1% sodium hypochlorite solution for about 1 minute followed by three times rinsed in sterilized distilled water for 30 second each to remove surface sterilization agent. After surface sterilization samples bits were dried in sterilized filter paper and those bits were placed on Petri plates containing Potato Dextrose Agar (PDA). Inoculated Petri plates were incubated at temperature of 25±1°C. Endophytic fungi were purified by single hyphal tip method on PDA medium.

### Identification and characterization of phylloplane and endophytic fungi

The isolated pure cultures were observed under compound microscope for further identification. Photomicrographs of each phylloplanes fungi and endophytic fungi were taken and measurements of the conidia were taken at micrometer scale using the inbuilt camera software under 40X objective lens (Geeta *et al.*, 2019). The following cultural morphological characters and microscopic characters viewed were under following parameters:

- Conidia/Spore characters = color, shape, size and septation
- Conidiophores/sporangiphore characters.

### *In-vitro* antagonistic test for phylloplanes and endophytic fungi

To test the efficacy of fungal antagonist against *E. turcicum*, dual culture technique was done (Hamzah *et al.*, 2018). The fungal phylloplanes, endophytic fungi and the test pathogen (*E. turcicum*) were allowed to grow on Petri plate for 2-5 days. Fungal phylloplane or endophytic fungi and test pathogen mycelial bits were transferred aseptically and placed near the periphery of the Petri plates in opposite direction. Test pathogen alone was inoculated in the Petri plates containing PDA, which were served as control. All the inoculated Petri plates were incubated at temperature of 25±1 °C. The observation was done periodically for the growth of inoculated plates measured in mm. Three replications were maintained for each treatment. Percent inhibition (%) was calculated by using the formula given by Vincent (1947).

$$\text{Percent Inhibition (PI)} = \frac{C-T}{C} \times 100$$

Where, C = Radial growth of test pathogen in control (mm).  
T = Radial growth of test pathogen in treatment (mm).

### Statistical analysis

CRD is most suitable for laboratory experiments, pot experiments and green house experiments. So, all the laboratory experiment was laid in completely randomized design (CRD) (Sekhar *et al.*, 2019).

## 3. Results and Discussion

### Isolation and identification of pathogen (*E. turcicum*)

The isolated fungus has grey colour colony and myceliums showed profused growth, conidia were brown, elongated and curved having 3-7 septation with  $64.2 \times 11.0$ - $13.8 \mu\text{m}$  in size. So, the pathogen was identified as *E. turcicum*.

Li and Wilson, 2013 and Deshmukh *et al.* 2020 also reported that symptoms of Turcicum leaf blight may appear at any stage of crop in the form of small grey spots on the lower leaves spreading upwards as spindle shaped lesions size ranges from 1-7 inches long which are similar with the present findings.

Abebe and Singburadom, 2006; Yelgurty *et al.* 2019 reported that conidia shape were curved, spindle and elongated colony colour were gray to dark grey, and mycelium growth were moderate to profused growth, size of conidia were range from  $58.31$ - $93.97 \times 11.10$ - $13.11 \mu\text{m}$  and with a septation of 2-7 with brown colour conidia which corroborate with the present result. Present microscopic viewed findings with the used of inbuilt camera software under 40X objective lens had a close agreement with the report of (Geeta *et al.*, 2019) in which maximum number of septa and conidia found were of 3-8 with protruding hilum and  $120.78 \times 29.15 \mu\text{m}$  respectively. Kutawa *et al.* (2017) and Rajula *et al.* (2017) also characterized the causative agent of Northern leaf blight (*E. turcicum*) in maize, based on the observation colony color were grey, dark gray and light gray. Mycelial growth was found to be moderate and profuse growth and conidia shape was found to be elongated and spindle. Hence, the isolated fungi were proved to be the casual organisms of Northern leaf blight.

### Isolation and identification of phylloplanes fungi

Phylloplanes fungi were isolated from healthy maize leaves by Leaf washing method in PDA medium. Isolation of phylloplanes fungi was performed during the vegetative stage of maize plant.

For purification of isolated phylloplanes fungi a single colony were used by single hyphal tip method. Total of 10 phylloplane fungi were isolated during the

investigation. And they were denoted as M. Phy-01, M. Phy-02, M. Phy-03, M. Phy-04, M. Phy-05, M. Phy-06, M. Phy-07, M. Phy-08, M. Phy-09 and M. Phy-10.

Based on their cultural morphological characters (colony colour, colony margin, colony pattern, number of days to cover whole 90 mm Petri plate) and microscopic viewed (conidia/spore characters- shape, size and septation and conidiophores/ sporangiophores - septation) and size of conidia measured by micrometer scale (Table 1 and Plate 1). The isolated phylloplane fungi were identified as M. Phy-01 (unidentified fungi), M. Phy-02 (*Trichoderma* sp. 1), M. Phy-03 (*Trichoderma* sp. 2), M. Phy-04 (*Penicillium* sp.), M. Phy-05 (*Fusarium* sp.), M. Phy-06 (*Aspergillus niger*), M. Phy-07 (unidentified fungi), M. Phy-08 (*Trichoderma* sp. 3), M. Phy-09 (*Fusarium oxysporum*) and M. Phy-10 (*Alternaria* sp.).

The present results were in conformity with the finding of Isiaka *et al.*, 2020; Shikha *et al.*, 2020 and Chaibub *et al.*, 2020 who also reported that the isolated phylloplane fungi were identified on the basis of morphology characters like colony appearance, colony colour, fluffiness of the colony, type of growth, nature of growth cell and microscopic examination like cell shape, septation of spores/conidia and conidiophores/sporangiaophores, In present investigation isolated phylloplane fungi were identified till genus level which was found similar with Alsohailli *et al.*, 2018, identification were based on morphology characters and microscopic view like shape and size of spore, arrangement of spore hyphae characters, conidia/conidiospores arrangement.

These findings were found in close agreement with the reports of workers (Mazen *et al.*, 1985; Ahmed 1986; Bopaiah *et al.*, 1991; El Naggat and Abdel Hafez, 2003 and Swathi, 2019) as they isolated phylloplane fungi like *Alternaria* sp., *Fusarium* sp., *Aspergillus niger* and *Penicillium* sp. Those isolated fungi were found to be most frequently occurring phylloplane. Isolation of *Trichoderma* sp. was similar with the finding of Swathi, 2019. Hence, those isolated fungi were *Trichoderma* sp. 1, *Trichoderma* sp. 2, *Trichoderma* sp. 3, *Penicillium* sp., *Fusarium* sp., *Aspergillus niger*, *Fusarium oxysporum* and *Alternaria* sp.

### Isolation and identification of endophytic fungi

Endophytic fungi were isolated from healthy part of maize leaves, which were isolated by surface sterilization method. Potato Dextrose Agar (PDA) medium were used for isolation and culture. A total of eight endophytic fungi were isolated during the vegetative stage of maize plant. Isolated endophytic fungi were purified by single hyphal tip method. They were denoted as M. Endo-01, M. Endo-02, M. Endo-03, M. Endo-04, M. Endo-05, M. Endo-06, M. Endo-07 and M. Endo-08.

All the isolated endophytic fungi were attempted to characterize and identify on basis of their colony morphological characters, numbers of days to full growth of mycelium 90 mm Petri plate, conidia/spore characters (shape, size and septation) and conidiophores/sporangiaophores (septation). Size of conidia was measured by micrometer scale using inbuilt camera software under 40X objective lens. Hence, isolated fungi according to characters were depicted in Table 2 and Plate 2. As most of the isolated endophytic fungi were not producing spores but have different morphological cultural characteristic and microscopic views due to which characterization were done accordingly. Total of 8 endophytic fungi were isolated during the investigation. Only three isolates were identified till genus level during the investigation and remaining five isolates were not identified as no spores were found. Those were M. Endo-01 (*Mucor* sp.), M. Endo-02 (unidentified fungi), M. Endo-03 (unidentified fungi), M. Endo-04 (unidentified fungi), M. Endo-05 (unidentified fungi), M. Endo-06 (*Fusarium* sp.), M. Endo-07 (unidentified fungi) and M. Endo-08 (*Pythium* sp.).

Present findings were found corroborate with the reports of Paynor *et al.*, 2016 in which endophytic fungi *viz.* *A. ochraceus*, *A. niger*, *A. flavus*, *Fusarium* sp., *P. citrinum* and *C. cladosporoides* identify on the basis of morphological characters, microscopic view and colony culture. The identification of isolated endophytic fungi was done according to their morphology of their fruiting bodies, spores cultural characteristics, direct microscopic observation, micrometrical measurement and microculture techniques was found in agreement with Larran *et al.*, 2002. The present results were also found similar with Brookes, 2017 and Potshangbam *et al.*, 2017 were the isolated endophytic fungi were identified as *Pythium* sp., *Fusarium* sp. and *Mucor* sp on the basis of spore cultural characteristics.

#### ***In-vitro* evaluation of phylloplane fungi antagonist against the pathogen**

*In-vitro* evaluation of effective fungal phylloplane antagonists against Northern leaf blight of maize (*E. turcicum*) were done by dual culture method. Among the ten isolated phylloplane fungi, *Trichoderma* sp. 1 has the highest antagonists against *E. turcicum* with an inhibition percentage of 56.11% which was followed by *Trichoderma* sp. 2 (54.04%), *Penicillium* sp. (45.05%) and *Trichoderma* sp. 3 (44.63%). Lowest inhibition percentage of 20.70 % was found in *Alternaria* sp. Highest average mycelial growth of isolated phylloplane fungi was observed in *Trichoderma* sp. 1 (1.73 mm) and lowest average mycelia growth were found *Alternaria* sp. (0.50 mm). Radial mycelium growth of isolated phylloplanes fungi and data pertaining to inhibition percentage were depicted in Table 3 and Plate 3.

Present studies were found similar with the finding of (Gokulapalan *et al.*, 1992; Rao, 2005; Kumar, 2010 and Swathi, 2019) which show that highest inhibition was found in *Trichoderma* sp.(1) followed by *Trichoderma* sp.(2), most of the *Trichoderma* sp. has more than 90% inhibition percentage. Hence, they concluded that different phylloplane had different antagonist inhibition effects on others mycoflora. (Mangiarotti *et al.*, 1987; Harish *et al.*, 2007) reported that *Penicillium* sp. had the count second highest inhibition among isolated phylloplane fungi. Lowest inhibition was found in *Alternaria* sp. Sood *et al.* 2020 also reported that *Trichoderma* spp. are avirulent opportunistic plant symbionts and ecofriendly biocontrol agent, which lead to the acquisition of plant resistance to pathogens, improves developmental processes and yields, promotes absorption of nutrients and fertilizers efficiency. Mycoparasitisms, competition and antibiosis are the other biocontrol mechanism which reacts to the presence of other microorganisms.

#### ***In-vitro* evaluation of endophytic fungi antagonist against the pathogen**

Antagonistic activities between isolated endophytic fungi and *E. turcicum* were observed by dual culture method. *In-vitro* evaluation of endophytic fungi against Northern blight of maize show that among the eight isolated endophytic fungi, *Mucor* sp. has the highest inhibition percentage of M. Endo-01 (75.55%) followed by *Fusarium* sp. having an inhibition percentage of M. Endo-06 (47.6%), M. Endo-07 (31.10%) and M. Endo-05 (30.86%) respectively. The lowest inhibition percentage was found in M. Endo-04 of 11.99%. Table 4 and Plate 4 depicted the mycelium growth of isolated fungi and data pertaining to inhibition percentage.

The present findings were found in agreement with the reports of (Amzad *et al.*, 2005) which report stated that *Mucor* sp. has the highest inhibition percentage of 60.4%. Inhibitory properties of endophytic fungi were due to production of antagonistic substances, competition, direct parasitism, hyperparasitism (Araujo *et al.*, 2010) and mycoparasitisms and (De Silva *et al.*, 2019). Antagonistic activities between isolated fungi endophytes and *E. turcicum* were observed by dual cultures which were similar with the finding of (Naik *et al.*, 2009).

**Table 1.** Characterization of isolated phyloplanes fungi

Isolated phyloplane	Nos.of days for full growth of mycelium (90mm plate)	Colony characters	Hyphae	Shape of spores/conidia	Size of spores (micrometer)
M.Phy- 01	7 days	-grey mycelium with concentric ring, curled margin	-septate chlymasdospores formed, Filamentous	-no spore found	-
M.Phy -02 <i>Trichoderma</i> sp.1	3 days	-dark green, compact tufts colony, concentric rings	-alternate branch with phialides cluster at tip -hyaline -non septate	-ellipsoidal -non septate	11.74
M.Phy -03 <i>Trichoderma</i> sp.(2)	3 days	-green to whitish at maturity, compact tufts	-highly alternate branch with phialides cluster at tip -hyaline -non septate	-ellipsoidal -non septate	27.82
M.Phy- 04 <i>Penicillium</i> sp.	4 days	-olive-yellow Formed sclerotia on maturity	-brush like phialides -non septate	-single cell	8.01
M.Phy- 05 <i>Fusarium solani</i>	6 days	-white cottony mycelium, reddish to purple color	-formed chlamydospores -hyaline, filamentous branch with phialides	-oval with tapered end -septate (3-7)	*34×8 **64.45 ×11.66
M.Phy- 06 <i>Aspergillus niger</i>	4 days	-Initially white turn dark black at letter stage	-smooth colourless presence of phialides	-globose, black to dark-brown with spiny ornamentation	64
M.Phy- 07	7 days	white mycelium with lobate margin and irregular shape	-Smooth colourless branch with segmentation	-	-
M.Phy- 08 <i>Trichoderma</i> sp.(3)	3 days	-olive-green colour with white margin, compact tufts	-highly alternate branch with phialides cluster at tip, hyaline, -non-septate.	-ellipsoidal, non-septate, single cell	12.25
M.Phy- 09 <i>Fusarium oxysporum</i>	5 days	-white cottony mycelium, pinkish colour with regular shape	-formed chlamydospores, hyaline, filamentous, branch with phialides	-oval with tapered end, septation (5-9)	*36×8 **66×12
M.Phy-10 <i>Alternaria</i> sp.	6 days	-grayish at initial later greenish black with lighter border, regular border.	-branch short or elongate, brown colour present singly or acropetal	-brownish conical conidia with short beak or without beak transverse septation and longitudinal (3-4)	46×14/1 22×33.3

\*Microconidia \*\*Macroconidia

**Table 2.** Characterization of isolated endophytic fungi

Isolated endophytes	Nos.of daysfor full growth of mycelium (90mm plate)	Colony characters	Hyphae	Shape of Spores	Size of spores* (micrometer)
M.Endo-01 <i>Mucor</i> sp.	3 days	-White filamentous mycelium turn grey on maturitywith pinhead at the tip	-Sporangiospores hyaline, -no septation chlamdospores formed	-Columellae ellipsoidal, -sporangia globuse	-Columellae (46.25)** -sporangia(6x5)
M.Endo- 02	4 days	-White filamentous mycelium	-hyaline -non septate	No sporefound	–
M.Endo- 03	16 days	-White filamentous withcharcoal black at Center, filiform margin.	-hyaline -non septation	-conidia globuse withpapillae	24.98
M.Endo- 04	5 days	-Light yellow to brownish yellowat maturity	-Septation, chlamyosphores formed	No sporefound	–
M.Endo- 05	7 days	-Dark black with white mycelium, regular margin	-non septate	Conidia non-septatewith long beak.	143.32 ×14.2
M.Endo-06 <i>Fusarium</i> sp.	6 days	-White fluffy tocream filamentous mycelium, raise, circular shape	-Sporodochium hyaline Septate	Fusiform withWhip like apical cell, transverse Septa.	Macroconidia 48x2.5 Microconidia (9-10x3.4)
M.Endo-07	5 days	-White to dark grey filamentous, irregular shape	-Hyaline hyphae -No septation	-No spores found	–
M.Endo-08 <i>Pythium</i> sp.	6 days	-Milky white filamentous mycelium, Circular shape, filiform margin.	-Sporangiospores Coenocytic hyphae -non septation -Chlamyospores formed	-Sporangia are globuse with vesicle	26**

\*Average of three observation, \*\*Diameter

**Table 3.** Radial mycelial growth of isolated phylloplanes fungi and it's *in vitro* antagonistic effect against *E. turcicum*

Isolated phylloplane	Radial mycelial growth of isolated phylloplanes (mm in diameter)			Average Mean radial growth	Inhibition Percentage (%)
	Day 1	Day 2	Day 3		
M.Phy-01**	0.80	0.60	0.90	0.77	28.75 (32.37)
M.Phy-02 <i>Trichoderma</i> sp.(1)	1.80	1.80	1.60	1.73	56.11 (48.52)
M.Phy-03 <i>Trichoderma</i> sp.(2)	1.40	1.60	1.40	1.47	54.04 (46.09)
M.Phy-04 <i>Penicillium</i> sp.	1.10	1.20	1.40	1.23	45.05 (42.13)
M.Phy-05 <i>Fusarium solani</i>	1.00	0.80	0.90	0.90	26.11 (30.65)

M.Phy-06 <i>Aspergillus niger</i>	1.20	1.00	1.40	1.20	34.73 (35.65)
M.Phy-07**	0.70	0.50	0.60	0.60	22.25 (28.03)
M.Phy-08 <i>Trichoderma</i> sp.(3)	1.40	1.50	1.60	1.50	44.63 (41.42)
M.Phy-09 <i>Fusarium oxysporum</i>	1.00	0.90	1.10	1.0	28.07 (31.88)
M.Phy-10 <i>Alternaria</i> sp.	0.40	0.50	0.50	0.50	20.70 (26.75)
C.D (P = 0.05)					10.95
SEm±					3.71

\*\*unidentified phylloplanes fungi

Values under parenthesis are arc sine transformed value

**Table 4.** Radial mycelial growth of isolated endophytic fungi and it's *in vitro* antagonistic effect against *E. turcicum*

Isolated endophytes	Radial mycelial growth of endophytes (mm in diameter)			Average Mean radial growth	Inhibition Percentage (%)
	Day 1	Day 2	Day 3		
M.Endo-01 <i>Mucor</i> sp.	0.90	1.00	1.10	1.00	75.55 (60.85)
M.Endo-02**	1.50	1.80	1.90	1.73	18.6 (25.13)
M.Endo-03**	0.20	0.10	0.40	0.23	13.96 (20.84)
M.Endo-04**	0.10	0.10	0.20	0.13	11.99 (20.16)
M.Endo-05**	1.30	1.40	1.20	1.30	30.86 (33.74)
M.Endo-06 <i>Fusarium</i> sp.	0.90	0.80	1.00	0.90	47.79 (43.72)
M.Endo-07**	0.20	0.10	0.20	0.17	31.10 (33.83)
M.Endo-08 <i>Pythium</i> sp.	0.60	0.50	0.40	0.50	27.93 (31.78)
C.D (P = 0.05)					9.82
SEm±					3.27

\*\*unidentified endophytic fungi

Values under parenthesis are arc sine transformed value

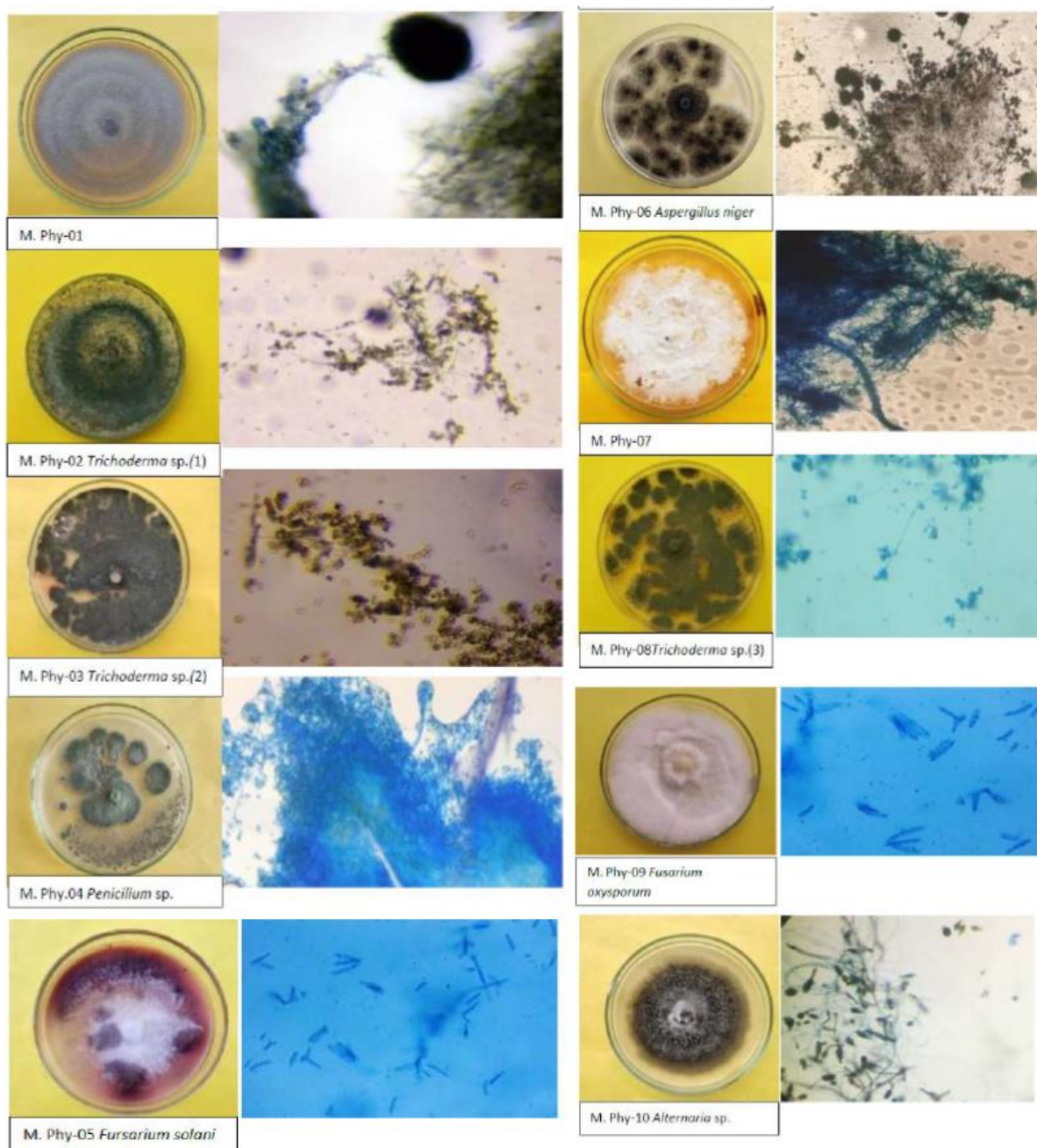


Plate 1 Left side–Pure culture colony of isolated phylloplanes fungi.  
 Right side–microscopic views of isolated phylloplanes fungi under 40X.



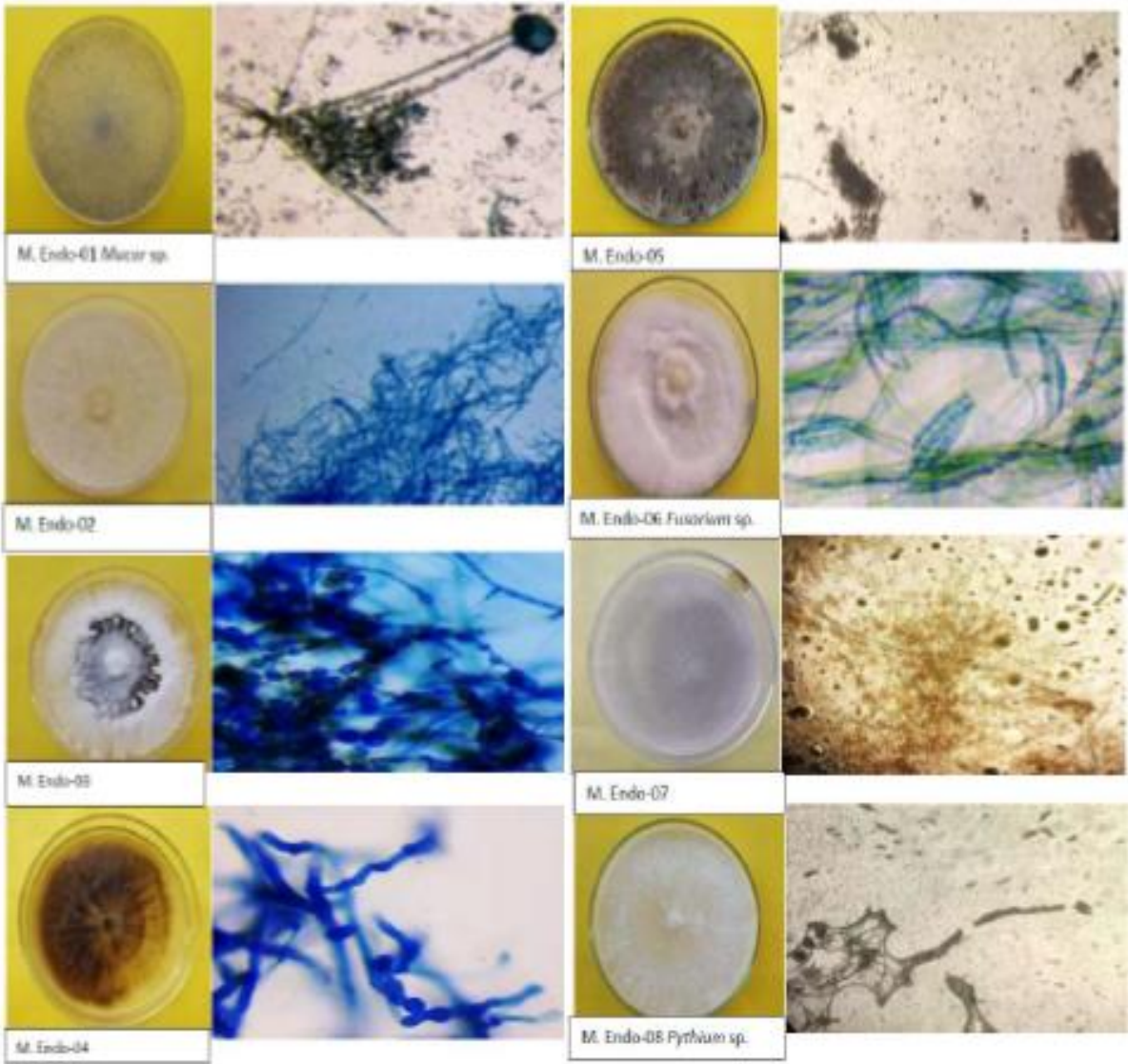


Plate 2 Left side – Pure culture colony of isolated endophytic fungi  
 Right side – microscopic views of isolated endophytic fungi under 40X.

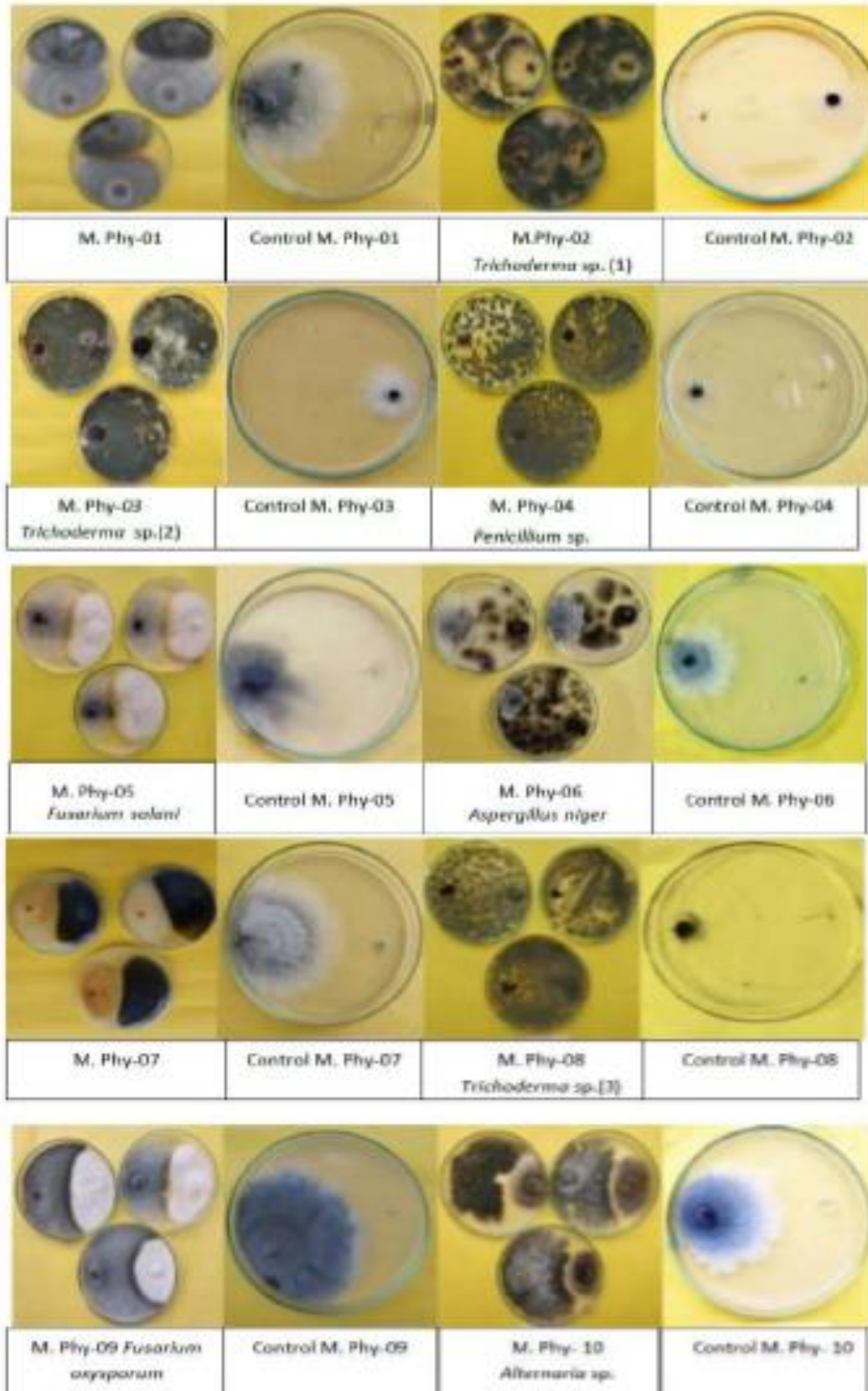
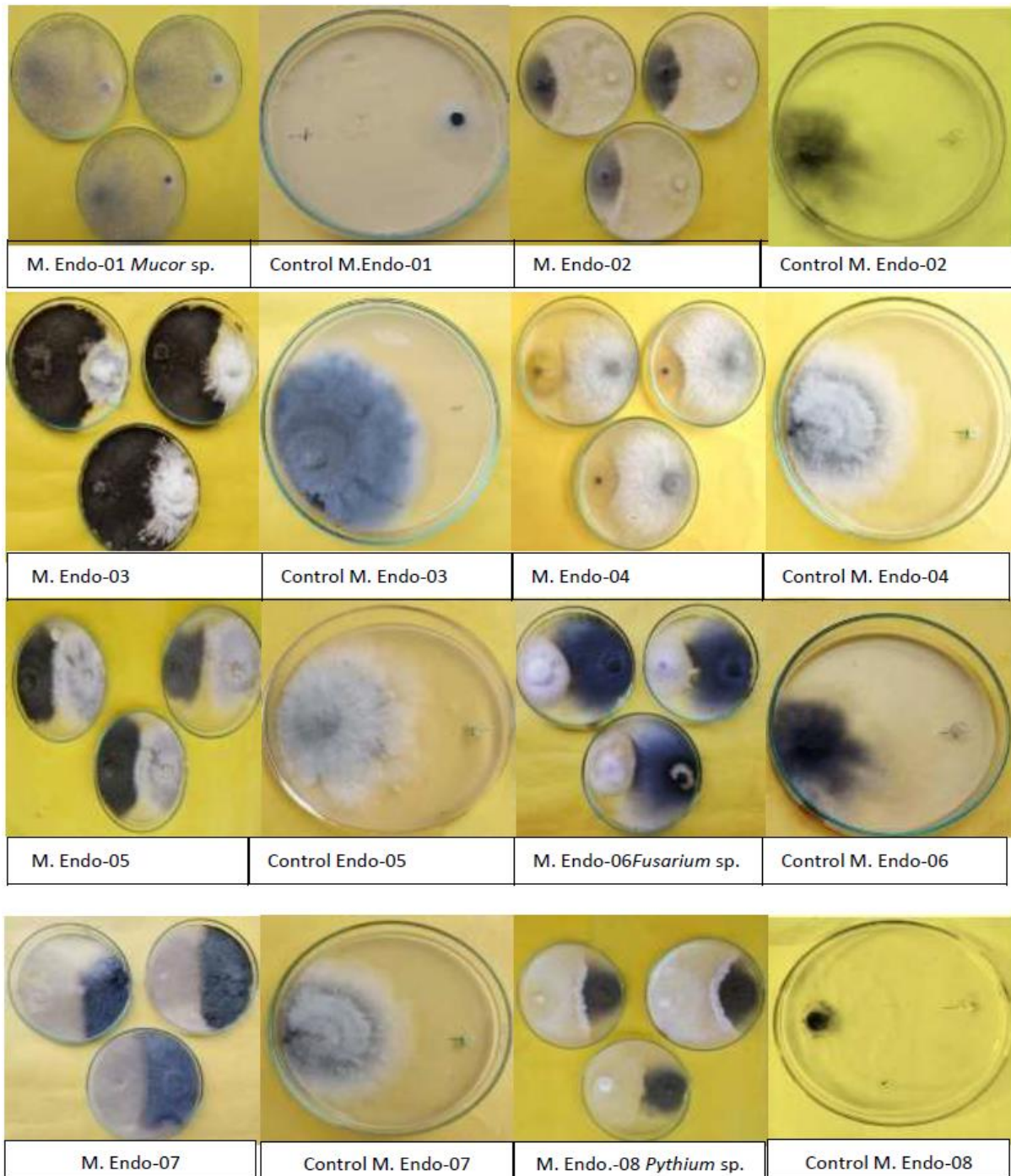


Plate 3 Left side- Antagonistic effects of isolated phylloplanes fungi against *E. turcicum*, Right side Control.



**Plate 4** Left side- Antagonistic effects of isolated fungi endophytes against *E. turcicum*, Right side- Control

#### 4. Conclusions

Considering all the above-mentioned findings of present investigation, we could conclude that PDA medium can be used for isolation of both phylloplane and endophytic mycoflora. Leaf washing method and surface sterilization method could be considered ideal for isolation of phylloplane fungi and endophytic mycoflora respectively. *In vitro* antagonistic effects of both phylloplane and endophytic

mycoflora on *Exserohilum turcicum*, can consider that both the mycoflora has a potential of being used even at field condition for management of Northern leaf blight of maize in a way of sustainable and eco-friendly farming. This will replace the adverse effects of chemical being used in present world to reach the population demand for livelihood. Both phylloplane and endophytic mycoflora not only used in management of plant disease but could enhance the plant

growth and yield production. Mentioned property can be achieved only if we could develop a greater vision towards research and technology. Since, only few successful works had been reported till date and not many phytochemicals are reported. Therefore, new milestones towards novel development have to encourage for more exploitation of both phylloplane and endophytic fungi. Hence, we could conclude that synthetic fungicide, fertilizer and weedicide can be replaced with less expensive and eco-friendly management by little involvement of phylloplane and endophytic fungi.

## 5. References

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