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In Vitro Evaluation of Bacterial Bioagents Against SorghumTurcicum Leaf Blight Caused by *Exserohilum turcicum* (Pass.) K. J. Leonard & Suggs

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ABSTRACT

Efficacy of different bacterial isolates *viz.*, endophytes, phylloplane and methylotrophs against *Exserohilum turcicum* was evaluated by employing dual culture technique. Six bacterial isolates SLSE-05 (60.73%), SLSE-04 (54.97%), HMP A 1902 (54.45%), PPFM-5 (30.89%), HMP *Bc* 1903 (27.27%) and PPFM-8 (22.51%) were found most effective in suppressing the pathogen's radial growth *in vitro.* Antagonist interactions resulted in hyphal morphological alterations in *E. turcicum* which included hyphal thinning, shortening of hyphal septa, swelling, wrinkling, protoplasm disintegration, clustering of hyphae and hyphal tip shearing in fungal hyphae that expanded towards bacterial colonies. Growth inhibition associated with hyphal morphological changes indicated antagonistic activity against *E. turcicum.*

Keywords: *Bacterial bio-control agents and Exserohilum turcicum.*

Sorghum [*Sorghum bicolor* (L.) Moench] is popularly known as jowar, has originated from Africa. It is widely grown across the world for its diverse usage options and also being relatively an economically important cash and food crop. It is also referred to as 'poor man's crop' for its performance in marginal lands with low fertilizers and inputs compared to other cereals. It is used as food, feed, syrup and bio-fuel crop with excellent drought resistance compared to other cereals and is considered as a "fail safe crop" (Burke *et al*., 2010). *Rabi* sorghum is an important crop grown under residual moisture condition in Andhra Pradesh (Vijaya Kumar *et al.*, 2014). Global sorghum production of 62.24 M metric tons was recorded from 40.99 M ha. India ranks fifth in total sorghum production (4.78 M metric tons) (USDA, 2020). In Andhra Pradesh, it occupies an area of 1.11 lakh ha with an annual production and productivity of 3.25 lakh tons and 2928 kg ha^{-1}, respectively (Third Advance Estimates, 2020-21, DES-AP).

It has the potentiality of contributing to increased food production both in developing and developed countries. The major diseases that affect sorghum include anthracnose, Turcicum leaf blight, downy mildew, rust, charcoal rot and smuts (covered kernel smut, loose smut, long smut and head smut). Of them, Turcicum leaf blight (TLB) is one of the most destructive foliar diseases of sorghum caused by *Exserohilum turcicum.* But, the disease management is still dependent on agrochemicals. Keeping in view of the hazards associated with agrochemicals, a thought to identify the efficacy of bacterial bio-agents such as endophytes, methylotrophs and phylloplane group of bacteria in managing *Exserohilum turcicum* causing leaf blight disease was given.

MATERIAL AND METHODS

Four isolates of endophytes and three isolates of phylloplane bacteria available in the Department of Plant Pathology, Agricultural College, Bapatla and eight isolates of methylotrophs from sorghum phyllosphere were evaluated *in vitro* for their antagonistic activity against mycelial growth of *E. turcicum* by using dual culture technique (Dennis and Webster, 1971). Sterilized potato dextrose agar medium was poured aseptically into sterile Petri plates under laminar air flow chamber and allowed for solidification. Seven days old actively growing culture of *E. turcicum*, was cut using five mm sterile cork borer. The mycelial disc was placed at the center of the PDA plate. With the help of an inoculation loop, test bacterial isolate from a 72 h old actively growing culture was streaked parallely on both sides of fungal disc in such a way that it is 2.5 cm away from the fungal disc, while plates inoculated with *E. turcicum* alone served as control and the plates were incubated at 28±2ºC for 96 h, and replicated thrice. The extent of antagonistic activity of the bacterial isolates was calculated on 4th day by measuring the radial growth of *E. turcicum*. The per cent inhibition of *E. turcicum* was calculated as suggested by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

Where,

I= Per cent inhibition over control

C= Growth of the pathogen in control plate

T= Growth of the pathogen in dual cultured plate

RESULTS AND DISCUSSION

Effect of bacterial bio-agents on radial growth of *E. turcicum*

Significant inhibitory effect on test pathogen was observed with all the bacterial isolates tested. Inhibition over control ranged from 4.71 per cent (PPFM-02) to 60.73 per cent (SLSE-05). Clear zone of inhibition (ZI) was observed with endophytic bacteria SLSE-05 (0.9 cm), SLSE-04 (0.6 cm) and phylloplane bacteria HMP A 1902 (0.5 cm). Of all the isolates, SLSE-05 was the most promising antagonist over other isolates and recorded the least radial growth of *E. turcicum* (2.50 cm), which was followed by SLSE-04 (2.87 cm) and was found on par with HMP A 1902 (2.90 cm). Endophytes were found to have significant antagonistic property followed by phylloplane bacteria and methylotrophic bacteria respectively on *E. turcicum* (Table 1, Fig.1 and Plate1).

Microscopic observation at interaction zone of antagonistic bacteria and fungi had characteristic morphological alterations like hyphal thinning, shortening of hyphal septa, swelling, wrinkling, protoplasm disintegration, clustering of hyphae and hyphal tip shearing were noticed.

The endophytic isolate SLSE-05 caused hyphal thinning, wrinkling, shrunken hyphae and moderate hyphal tip shearing at the zone of inhibition (Plate 2). While interactions with endophytic isolate SLSE-04 resulted in shortening of hyphal septa, protoplasm disintegration along with hyphal thinning (Plate 3). Hyphal tip shearing, swelling, hyphal thinning and clustering of hyphae resembling the formation of prosenchyma were noticed when *E. turcicum* encountered antagonistic effects from HMP A 1902 (Plate 4). Hyphal thinning was commonly noticed whenever *E. turcicum* hyphae headed towards bacterial colonies. Formation of chlamydospores intercellularly and terminally at five days after inoculation was another characteristic feature observed.

Our results are in accordance with earlier findings of Strunnikova *et al*. (2007), who found chlamydospore formation due to biotic and abiotic

Table 1. Radial growth and per cent inhibition of *E. turcicum* dual cultured with different bacterial bio-agents *in vitro*

S. No.	Treatments	Radial growth (cm)	Per cent inhibition	Zone of Inhibition (cm)
1	SLSE-03	5.70[i]	10.47	-
2	SLSE-04	2.87[b]	54.97	0.6
3	SLSE-05	2.50[a]	60.73	0.9
4	SRSE-01	5.67[i]	10.99	-
5	HMP A 1902	2.90[b]	54.45	0.5
6	HMP *Bc* 1903	4.63[d]	27.27	-
7	HMP *Pf* 1901	5.07[f]	20.41	-
8	PPFM-01	5.73[i]	9.94	-
9	PPFM-02	6.07[j]	4.71	-
10	PPFM-03	5.53[h]	13.08	-
11	PPFM-04	5.17[g]	18.84	-
12	PPFM-05	4.40[c]	30.89	-
13	PPFM-06	5.57[h]	12.56	-
14	PPFM-07	5.53[h]	13.08	-
15	PPFM-08	4.93[e]	22.51	-
16	Control	6.37[k]	-	
	SEm ±	0.03		
	CD (P ≤ 0.05)	0.09		
	CV (%)	1.17		

Numbers with same superscript are statistically not significant.

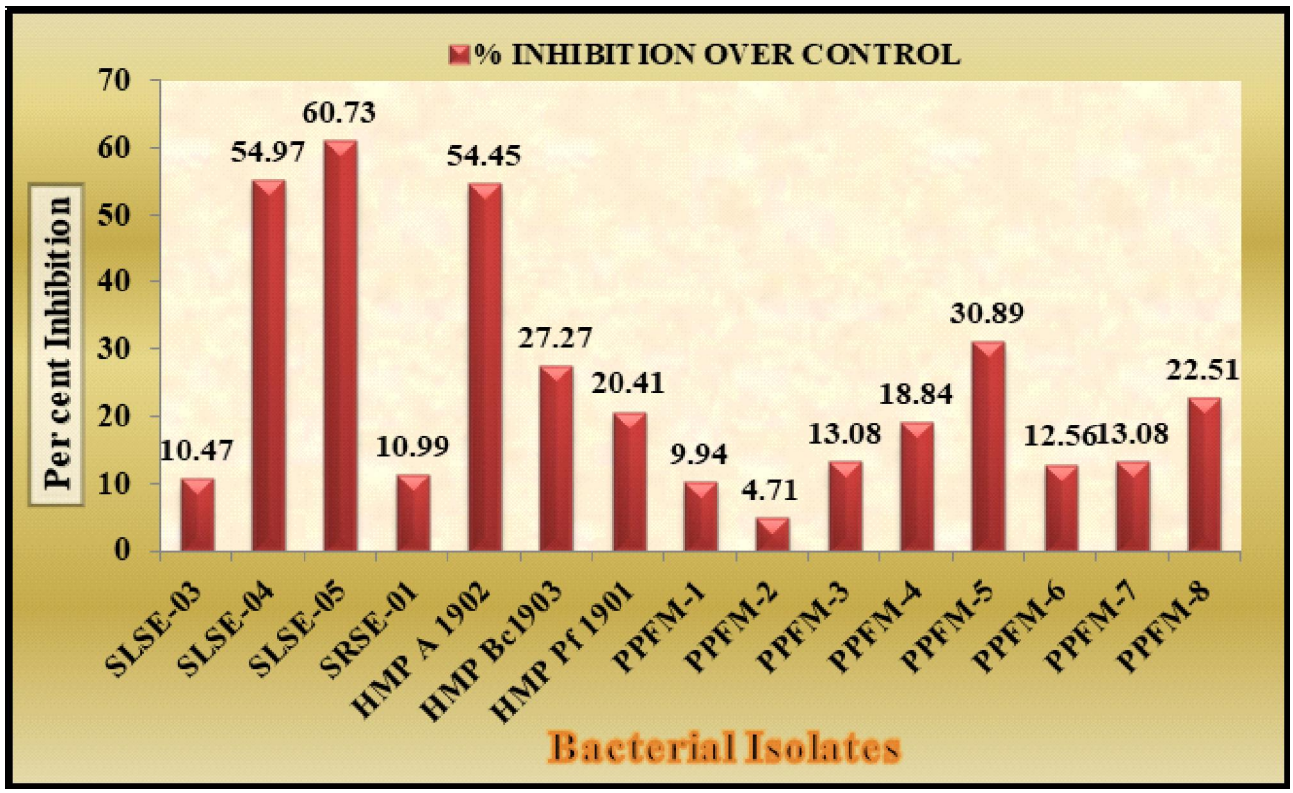

Fig 1. Inhibition per cent of *E. turcicum* dual cultured with different bacterial bio-agents *in vitro*

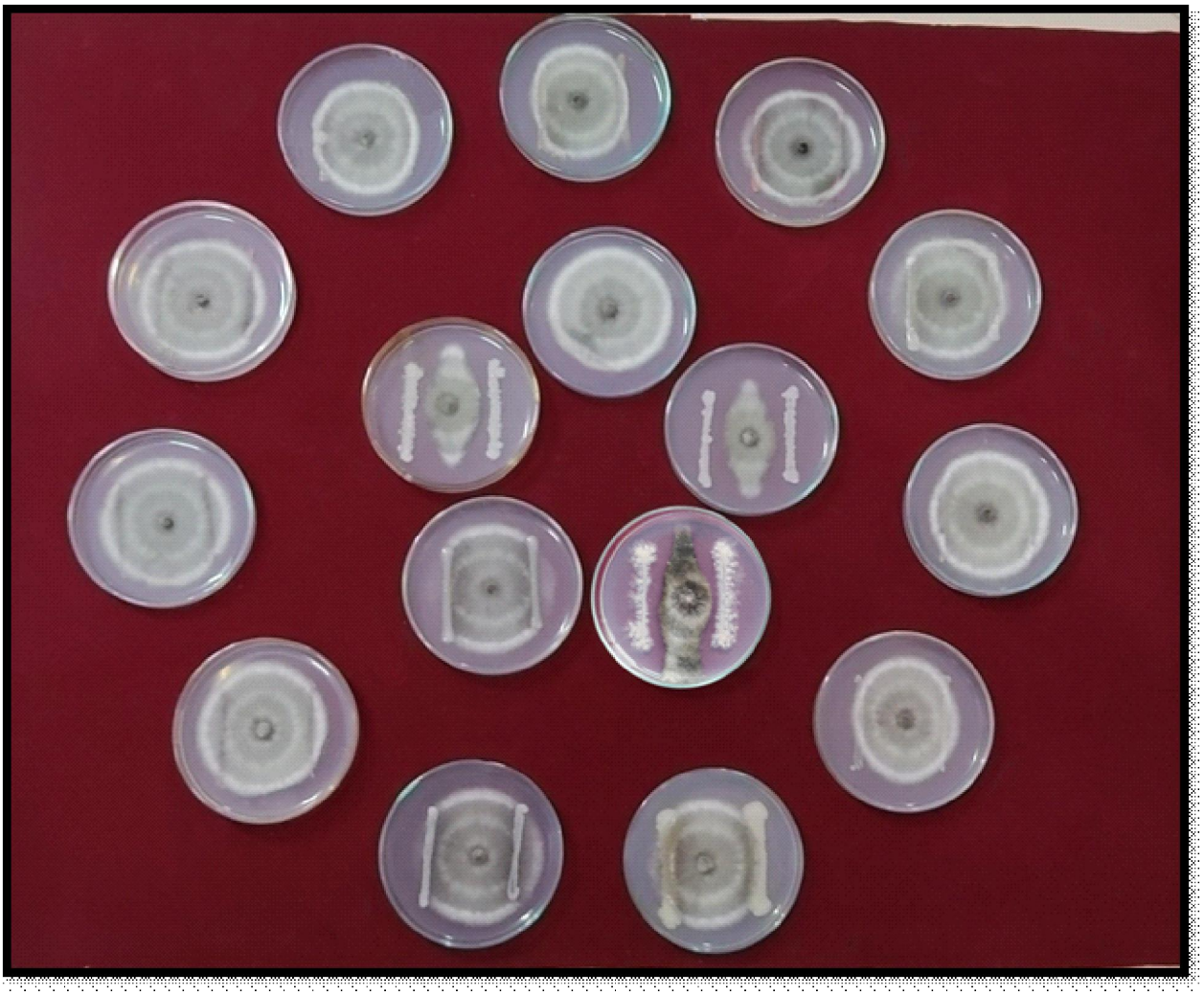

Plate 1. Effect of different bacterial isolates on radial growth of *E. turcicum* using dual culture technique.

stresses. Similarly, Goh *et al.* (2009) reported *Bacillus amyloliquefaciens* SMCD 518 as a biological control agent which triggered chlamydospore formation in *Fusarium graminearum* and *F. sporotrichioides*.

Arunasri *et al*. (2013) reported among three rhizobacterial isolates tested, *Pseudomonas* spp., was effective in inhibiting mycelial growth of *Sclerotium rolfsii* and sclerotial inhibition upto 43.1% and 71% respectively. Liu *et al*. (2019) observed endophytic strain NEAU-S7GS2 with significant inhibitory effect on the mycelial growth of *E. turcicum* (67.6%) when compared to untreated control. Honey dew (2020) screened antagonistic activity of ten bacterial isolates against *E. turcicum* from maize and reported HMP A 1902, HMP *Bc* 1903 and HMP *Pf* 01 as effective isolates. Similarly, Kavya (2020) stated that, out of 13 endophytic bacterial isolates, three isolates SRSE-01, SLSE-04 and SLSE-05 as more effective in inhibiting the mycelial growth of *E. turcicum*.

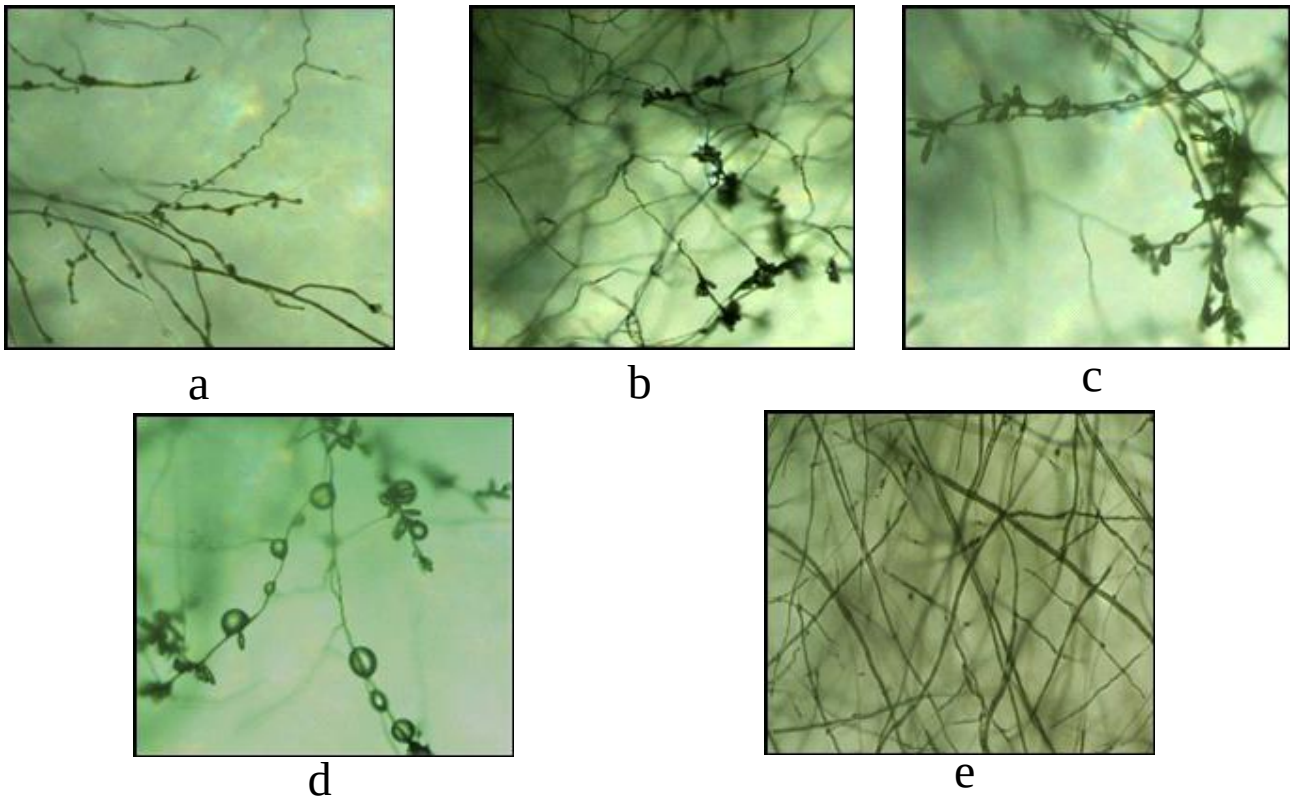

Plate 2 Microscopic view of interaction zone between SLSE-05 and *E. turcicum*.
a-Initial stage of chlamydospores, b-Hyphal wrinkling, c-Less chlamydospore formation at peripheral end of (ZI), d-Hyphal thinning and chlamydospore formation, e-Control.

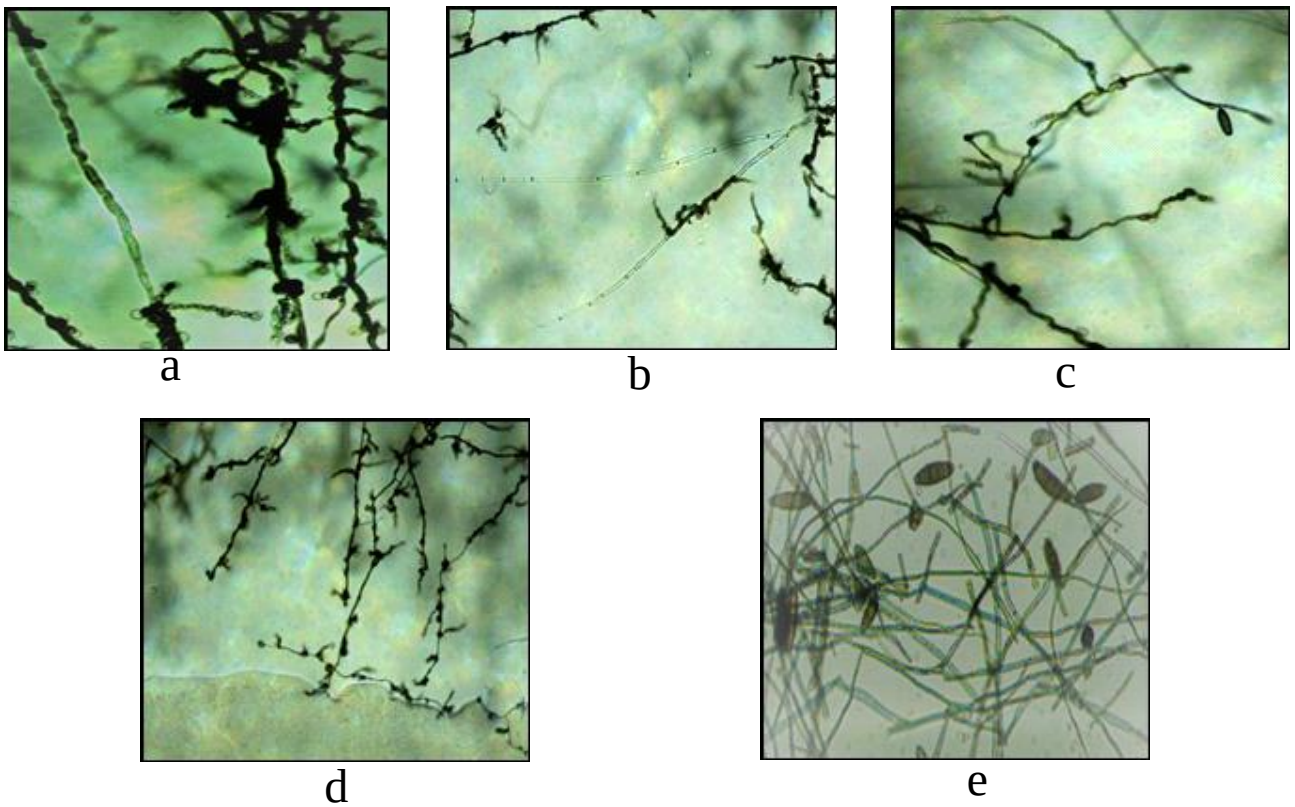

Plate 3 Microscopic view of interaction zone between SLSE-04 and *E. turcicum*.
a-**Shortening of hyphal septa, b-Protoplasm disintegration, c-Hyphal wrinkling, d-Chlamydospores formation at (ZI), e-Control.**

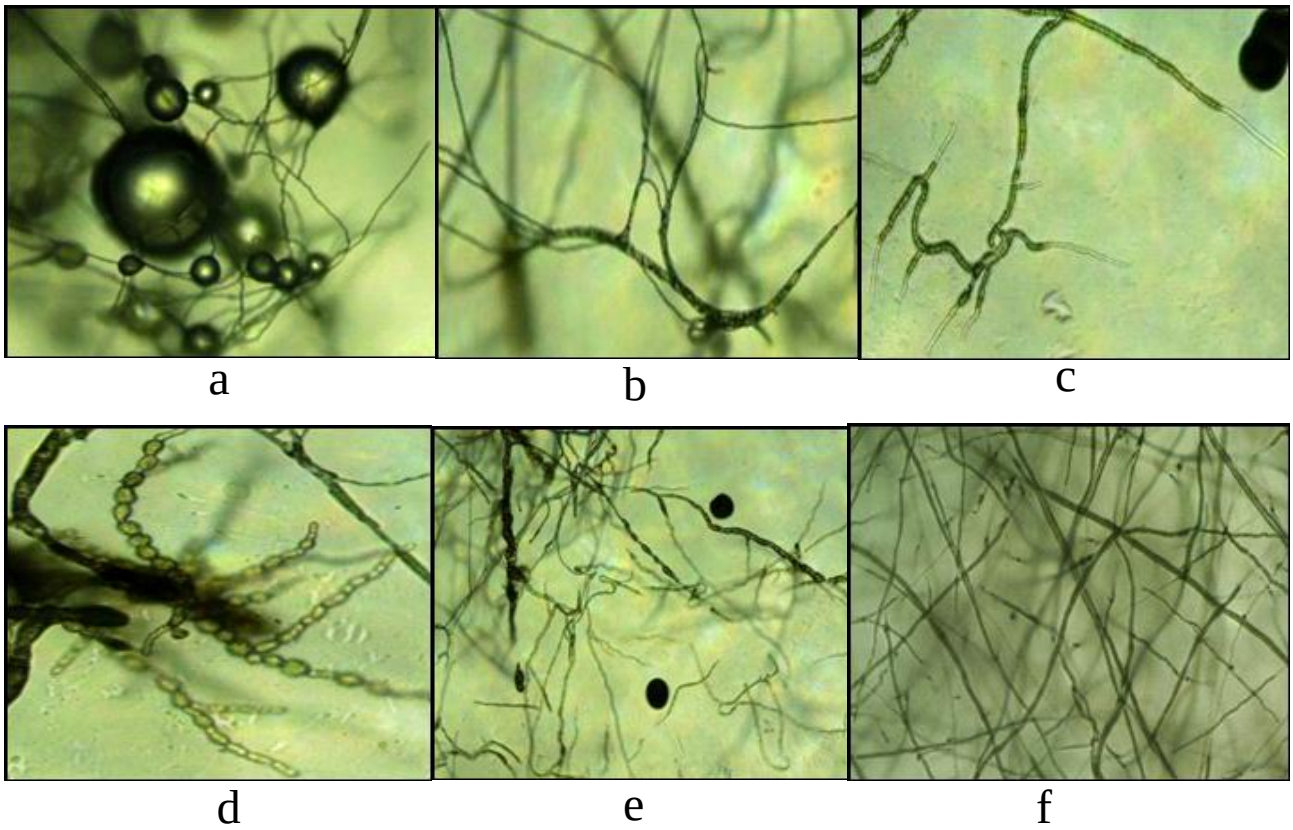

Plate 4 Microscopic view of interaction zone between HMP A 1902 and*E. turcicum*. a-Chlamydospores formation at (ZI), b-Hyphal clustering, c-Hyphal tip shearing, d-Hyphal swelling, e-Hyphal wrinkling, f-Control.

CONCLUSION

Dual culture studiesrevealed that, among 15 different bacterial isolates tested for antagonism, six isolates *viz*. SLSE-05, SLSE-04, HMP A 1902, PPFM-5, HMP *Bc*1903 and PPFM-8 were the promising isolates with maximum antagonistic potential by significantly restricting the growth of *E. turcicum* to 2.50, 2.87, 2.90, 4.40, 4.63 and 4.93 cm respectively in comparison to monocultured *E. turcicum* (6.37 cm) with an inhibition rates of 60.73%, 54.97%, 54.45%, 30.89%, 27.27% and 22.51%, respectively. However, significant inhibitory effect on test pathogen over control was observed with all the bacterial groups tested. Therefore, the isolates used in the study have enough ability to limit pathogen growth, thereby the isolates can be used in future consortia development studies.

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