International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(5): 1346-1350

Received: 05-07-2017
Accepted: 07-08-2017

SB Mahantesh
Department of Plant Pathology, College of Agriculture, University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India

C Karegowda
Department of Plant Pathology, College of Agriculture, University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India

SV Kavitha
Department of Plant Pathology, College of Agriculture, University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India

TH Kavita
Department of Plant Pathology, College of Agriculture, University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India

ND Punith Kumar
Department of Plant Pathology, College of Agriculture, University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India

Correspondence
SB Mahantesh
Department of Plant Pathology, College of Agriculture, University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka, India

In vitro evaluation of fungicides, bio agents and natural plant extracts against early blight caused by *A. solani*

SB Mahantesh, C Karegowda, SV Kavitha, TH Kavita and ND Punith Kumar

Abstract
Early blight (*Alternaria solani*) of tomato is most destructive disease in tropical and subtropical countries. It is a potential disease of tomato that reduces its production globally both in conventional and tunnel cultivations. In present study, fungicides, bio agents and botanicals were screened against early blight disease causing pathogen *A. solani* on tomato. Among all the tested fungicides, contact fungicide Mancozeb @ 1000 ppm, systemic fungicide, Hexaconazole @ 1000 ppm and the combi fungicide Carbendazim 12 % + Mancozeb 63 % @ 0.2% recorded the maximum inhibition of 88.42 %, 90.58 % 88.07 % mycelial growth respectively. Among the bio agents tested *Trichoderma harzianum* (UAHS 1) found effective in inhibiting the mycelial growth (80.36 %) followed by *T. harzianum* (UAHS 2) (78.33 %). Among the nine plant extracts evaluated, Pongamia leaf extract @ 10 per cent was found to be effective in inhibiting the mycelial growth of *A. solani* (54.76 %). The efficacy of fungicides, bio agents and botanicals can be further evaluated in combinations of spray options under field conditions.

Keywords: early blight, *Alternaria,* fungicides, bio agents, plant extracts.

Introduction
Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular and widely grown vegetable crops of both tropics and subtropics of the world, belongs to the family Solanaceae. It is grown for its edible fruit and is consumed in diverse ways, including raw, as an ingredient in many dishes, sauces, salads and drinks and an esteemed source of vitamin A and C. Early blight also known as target spot disease caused by *Alternaria solani* (Ellis and Martin) Jones and Grout is one of the world's most catastrophic disease incurring loss both at pre and post-harvest stages in tomato growing tracks of India. Diseases are traditionally managed by chemical fungicides. The aim of the present study is to compare the efficacy of antagonistic bio control agents with the botanicals and chemical products under *In vitro* and *In vivo* conditions in order to use such a strategy in an eco-friendly and sustainable integrated disease management practices.

Material and Methods
In vitro evaluation of fungicides, bio agents and botanicals against *Alternaria solani* (Ellis and Martin) Jones and Grout was carried out through dual culture and poisoned food technique, respectively (Ganie *et al*., 2013) [4].

In vitro evaluation of fungicides and plant extracts against *Alternaria solani*
Poison food technique was used in present assay. The study was conducted at Plant Pathology Department, UAHS, Shivamogga during 2015-16. The efficacy of four non-systemic, five systemic and five combi fungicides were tested against *A. solani* at 100, 250, 500 and 1000 ppm concentrations on Potato dextrose agar medium using poisoned food technique under *in vitro* condition viz., Chlorothalonil, Mancozeb, Propineb, Azoxystobin, Difenconazole, Hexaconazole, Tebuconazole Propiconazole, Carbendizim + Mancozeb, Carbendazim + Iprodione, Hexzconazole + Zineb, and Tebuconazole + Trifloxystrobin were assyed. The poison food technique (Shravelle, 1961) was followed to evaluate the efficacy of non-systemic, systemic and combi products in inhibiting the mycelial growth of *A. solani*. The fungus was grown on PDA medium for nine days prior to setting up the experiment.

The PDA medium was prepared and melted. The required concentration of fungicidal suspension was added to the molten medium. Twenty ml of poisoned medium was poured in each sterilized Petriplates. Suitable check was maintained without addition of fungicide. Mycelial disc of 5 mm was taken from the periphery of nine days old culture and placed in the center of Petriplates and incubated at 27 ±1°C for nine days. The diameter of the colony was measured in two directions and average was recorded. Three replications were maintained for each treatment and CRD was followed. Data obtained were subjected for statistical analysis.

For evaluation of plant extracts, Water hyacinth, Lantana, Marigold, Neem, Parthenium, Tulasi, Onion, Pongamia and Garlic were selected for the study.

Fresh leaves of nine plants were collected from various locations of UAHS, Shivamogga and confirmed their taxonomical identification. These samples were washed thoroughly with tap water and surface sterilized with 0.1 per cent sodium hypochlorite and repeatedly washed with distilled water. Hundred grams of leaf materials was taken and cut into small pieces, 100 ml water was added and the leaf materials were crushed using a grinder. The stock solution of all the leaf extracts was collected by filtering with muslin cloth. 2.5, 5 and 10 per cent of leaf extracts were prepared by adding 2.5, 5 and 10 ml of stock solution to 97.5, 95 and 90 ml of PDA medium, respectively. The PDA medium with plant extracts was sterilized in autoclave. The sterilized amended medium was poured into petri plates, for each treatment three replications were maintained, these plates were inoculated with 5 mm disc of nine days old culture of *A. solani*. Suitable control plates were maintained where in culture discs were inoculated into the center of potato dextrose agar plates without plant extracts. Radial growth of the fungus was measured after seven days of inoculation and the per cent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1947) [11]

$$I = \frac{(C-T)}{C} X\ 100$$

Where,
I = Per cent inhibition
C = Radial growth in control
T = Radial growth in treatment

In vitro evaluation of bio agents against pathogen _A. solani_ using dual culture technique

The efficacy of six bio agents *viz*., *Trichoderma harzianum*,(UAHS 1, 2) *Trichoderma viride* (GKVK,DWD) *Pseudomonas fluorescens* and *Bacillus subtillis* were tested in inhibiting the radial growth of *A. solani* on potato dextrose agar using the dual culture technique under *In vitro* condition. Bio agents were evaluated for their efficacy through dual culture technique twenty ml of PDA was poured in to 90 mm diameter Petridishes and allowed to solidify. 5 mm discs of *A. solani* taken from nine days old culture was placed at one end of Petridish and respective antagonistic organisms were inoculated at the opposite side. In case of bacterial antagonist *A. solani* was placed at both ends of Petriplates and bacterial culture was inoculated at the centre of Petriplates. Each treatment was replicated four times and incubated for six days at 27±1°C. The activity of antagonistic organisms was recorded by measuring the colony diameter of *A. solani* in each treatment and compared with control. Three replications were maintained for each treatment and CRD was followed. Data obtained were subjected for statistical analysis.

Result and Discussion

In vitro* evaluation of fungicides against *Alternaria solani

Three non-systemic, five systemic fungicides and four combi products were evaluated against *A. solani* under *in vitro* at three different concentrations. Poisoned food technique was followed as detailed in material and methods. The data are presented in the table 1.

The results indicated that there was a significant difference among contact fungicides in inhibiting the growth of *A. solani*. Among the three contact fungicides evaluated, Mancozeb (79.80 %) was significantly superior over other treatments followed by Chlorothalonil (71.62 %). Least inhibition was observed in Zineb (66.94 %). Among the different concentration of non-systemic fungicides tested against *A. solani*, Mancozeb at 1000 ppm (88.42 %) was found very effective. Least inhibition of mycelial growth of pathogen was observed in Zineb (60.67 %) at 100 ppm concentration. The efficacy of Mancozeb was previously reported by several workers Roopa *et al*. (2014) [9]; Kamble *et al.* (2000) [5].

Among the systemic fungicides evaluated against *A. solani*, Propiconazole (85.14 %) gave maximum inhibition of the mycelial growth of pathogen. This was followed by Hexaconazole (84.21 %). Least inhibition of mycelial growth was observed in Azoxystrobin (79.78 %). Among the different concentration of systemic fungicides tested, Propiconazole at 1000 ppm concentration (90.58 %) gave maximum inhibition of mycelial growth of pathogen. This was followed by Difenconazole at 1000 ppm (88.07 %) concentration. Least effective fungicide was Azoxystrobin at 100 ppm (75.36 %) concentration**.** Efficacy of these fungicides was previously reported by Roopa *et al*. (2014) [9]; Arunakumar (2006) [2]; Amaresh (2000) [1] and Patel and Choudhary (2010) [7].

Among the four combi-fungicides evaluated against *A. solani*, SAAF 75 WP (Carbendazim 12 % + Mancozeb 63 %) (82.87 %) gave maximum inhibition of the mycelial growth of pathogen. This was followed by Nativo (Tebuconazole 50 % +Trifloxystrobin 25 % WG) (80.09 %). Least inhibition of mycelial growth was observed in Quintal 50 WP (Carbendazim 25 % + Iprodione 25 %) (77.51 %). Among the different concentration of combi-fungicides tested, SAAF 75 WP at 1000 ppm concentration (88.07 %) gave maximum inhibition of mycelial growth of pathogen. This was followed by Nativo 75 WG (87.07 %). Least inhibition of mycelial growth of pathogen was observed in Quintal 50 WP at 100 ppm (67.82 %) concentration. The efficacy of SAAF 75 WP was previously reported by several workers Arunkumar (2008) [3] and Roopa *et al*. (2014) [9].

Table 1: *In vitro* evaluation of fungicides against *A. solani*

S. No.	Fungicides	Per cent inhibition of mycelial growth				Mean
		Concentration (ppm)				
		100	250	500	1000	
1	Mancozeb 75 WP	72.69 (58.53)*	76.75 (61.32)	81.30 (64.48)	88.42 (70.17)	79.80 (63.62)
2	Chlorothalonil 75 WP	68.17 (55.76)	70.82 (57.35)	73.31 (58.92)	74.21 (59.51)	71.62 (57.88)
3	Zineb 75 WP	60.67 (51.20)	64.08 (53.23)	70.36 (57.05	72.65 (58.50)	66.94 (54.99)
4	Hexaconazole 5 EC	80.75 (64.03)	83.18 (65.84)	85.12 (67.36)	87.80 (69.65)	84.21 (66.72)
5	Azoxystrobin 23 EC	75.36 (60.28)	78.39 (62.36)	81.86 (64.84)	83.53 (66.14)	79.78 (63.40)
6	Propiconazole25 EC	80.08 (63.57)	83.48 (66.07)	86.42 (68.43)	90.58 (72.21)	85.14 (67.58)
7	Difenconazole25EC	78.24 (62.24)	81.20 (64.36)	84.63 (66.97)	88.07 (69.87)	83.03 (65.85)
8	Tebuconazole25 EC	78.24 (62.24)	81.20 (64.36)	84.63 (66.97)	88.07 (69.87)	83.03 (65.85)
9	Nativo 75 WG	72.75 (59.18)	76.48 (61.89)	84.08 (66.47)	87.07 (69.87)	80.09 (64.27)
10	SAAF 75 WP	75.11 (60.10)	82.00 (64.99)	86.33 (68.36)	88.07 (69.87)	82.87 (65.83)
11	Avatar 72WP	72.67 (58.52)	76.58 (61.09)	82.73 (65.52)	85.80 (67.93)	79.44 (63.26)
12	Quintal 50 WP	67.82 (55.47)	75.25 (60.26)	81.00 (64.22)	86.00 (68.09)	77.51 (62.01)
		Fungicides		Concentration		F×C
S.Em.±		0.54		0.31		1.09
C.D. at 1 %		1.52		0.88		3.05

*The values in the parenthesis are arc sign transformed

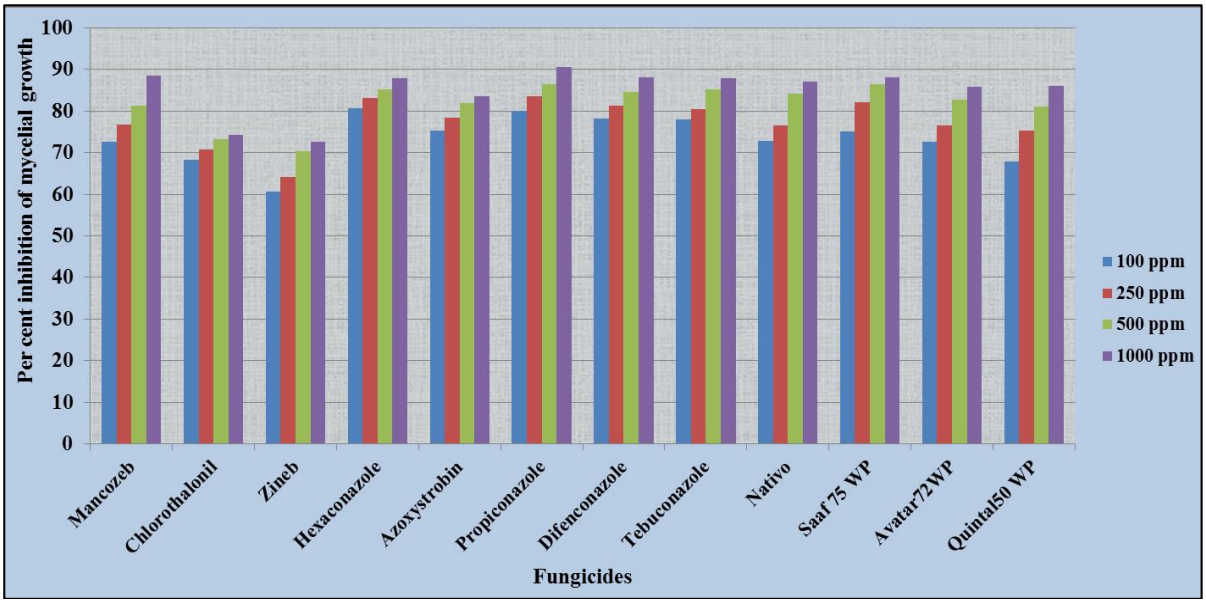

Fig 1: *In vitro* evaluation of fungicides against *A. solani*

In vitro* evaluation of bio agents against *Alternaria solani

The effect of fungal and bacterial bio agents was studied *in vitro* against *A. solani*. Two fungal bioagents *viz.*, *Trichoderma viride*, (GKVK & DWD) and *Trichoderma harzianum* (UAHS-1 & UAHS-2) and two bacterial bio control agents *viz.*, *Bacillus subtilis* and *Pseudomonas fluorescens* were evaluated through dual culture technique as described in material and methods and the results obtained are presented in the table 2.Among six bioagents, *T. harzianum* (UAHS-1) showed maximum per cent inhibition (80.36 %) followed by UAHS-2 (78.33 %), *T. viride* (GKVK) (75.86 %), (DWD) (73.88 %), *Bacillus subtilis* (45.00 %) and *Pseudomonas fluorescens* (60.36 %) were found effective in inhibition of mycelial growth of fungus. Similar results wherein efficacy of *Trichoderma* spp. against *Alternaria* species was previously reported by Leifort *et al*, (1992) [6], Amaresh (2000) [1] and Pramod Kumar (2007) [8].

Table 2: *In vitro* evaluation of bioagents against *A. solani*

S. No.	Bio agents	Per cent inhibition
1.	*Trichoderma harzianum*- UAHS-1	80.36 (63.71)*
2.	*Trichoderma harzianum* -UAHS-2	78.33 (62.26)
3.	*Trichoderma viridae*-GKVK	75.86 (60.57)
4.	*Trichoderma viridae*-DWD	73.88 (59.26)
5.	*Pseudomonas fluorescens*	60.36 (50.98)
6.	*Bacillus subtilis*	45.00 (42.12)
	S.Em.±	0.52
	C.D. at 1 %	1.61

*The values in the parenthesis are arc sign transformed

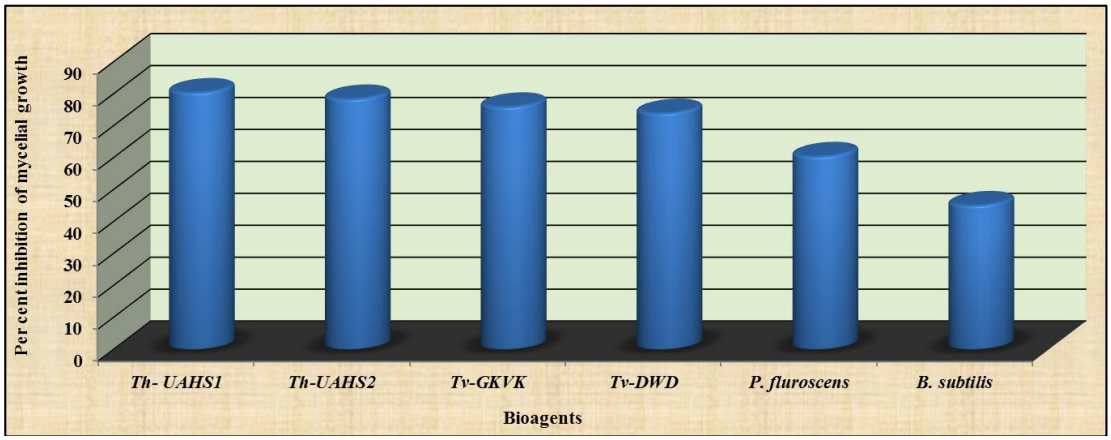

Fig 2: *In vitro* evaluation of bio agents against *A. solani*

In vitro* evaluation of botanicals against *A. solani

The antifungal activity of nine plant extracts were evaluated against *A. solani* at three different concentrations under *In vitro* by using poisoned food technique as described in material and methods and the results obtained are presented in the table 3.

Among nine plant extracts, 10 Per cent concentration of Pongamia leaf extract was found best in inhibiting the mycelial growth (54.76 %), significantly superior over all other leaf extracts which was followed by Neem leaf extract (48.27 %), Lantana leaf extract (47.42%), and Garlic bulbs (46.42 %) respectively. Whereas, least inhibition of mycelial growth was recorded in Water hyacinth (36.75 %).

Similarly, at 5 per cent concentration, Pongamia leaf extract (46.25 %) was found significantly inhibited the mycelial growth followed by Lantana (43.58 %) and Neem (42.92 %).Whereas, least inhibition of mycelial growth of *A. solani* were recorded in Water hyacinth (34.33 %), Onion (35.08 %) and Partheneum (36.25 %). Similar trend was recorded for 2.5 Per cent concentration of plant extracts. The present investigation of various botanicals inhibiting the growth of *A. solani* is in line with the earlier findings Shekhawat and Prasad, 1971 [10]; Amaresh, 2000 [1] and Pramod Kumar, 2007 [8].

Table 3: *In vitro* evaluation of botanicals against *A. solani*

S. No.	Botanicals	Per cent inhibition of mycelial growth Concentration (%)			Mean
		2.5	5	10	
1.	Water hyacinth (*Eichornia crassipes*)	32.00 (34.4)*	34.33 (35.87)	36.75 (37.31)	34.36 (35.87)
2.	Garlic (*Allium sativum* L.)	36.57 (37.2)	40.50 (39.52)	46.42 (42.94)	41.16 (39.89)
3.	Marigold (*Tagetus sp.*)	33.50 (35.3)	37.83 (37.95)	40.42 (39.46)	37.25 (37.59)
4.	Neem (*Azadirachta indica*)	37.50 (37.7)	42.92 (40.92)	48.27 (44.01)	42.89 (40.89)
5.	Onion (*Allium cepa*)	30.74 (33.6)	35.08 (36.29)	42.83 (40.88)	36.22 (36.94)
6.	Lantana (Lantana camara)	38.58 (38.4)	43.58 (41.31)	47.42 (43.51)	43.19 (41.07)
7.	Partheneum (*Parthenium hysterophorus* L)	33.17 (35.1)	36.25 (37.00)	38.83 (38.53)	36.08 (36.90)
8.	Tulasi (*Ocimum tenuiflorum*)	35.25 (36.4)	38.83 (38.53)	40.57 (39.56)	38.22 (38.17)
9.	*Pongamia (Pongamia pinnata)*	42.17 (40.4)	46.25 (42.85)	54.76 (47.73)	47.73 (43.69)
			Botanicals	Concentration	Botanicals*Concentration
	S.Em.±		0.61	0.35	1.06
	CD at 1 %		1.75	1.01	3.04

*The values in the parenthesis are arc sign transformed

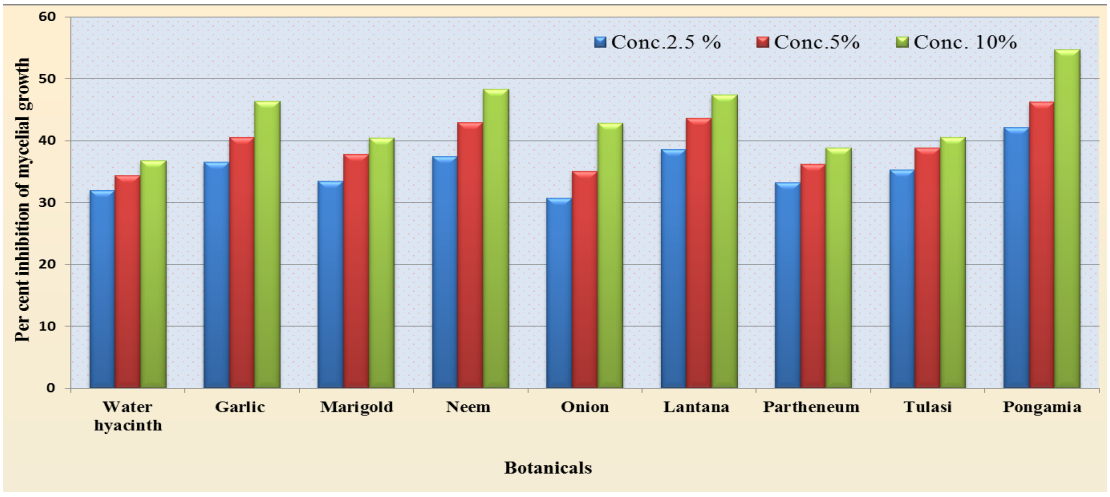

Fig 3: *In vitro* evaluation of Botanicals against *A. solani*

References

1. Amaresh YS. Epidemiology and management of *Alternaria* leaf blight and rust of sunflower (*Helianthus annuus* L.). Ph.D. *(Agri.)* Thesis, Univ. Agric. Sci., Dharwad, 2000.
2. Arunakumara KT. Studies on *Alternaria solani* (Ellis and Martin) Jones and Grout causing early blight of tomato. *M. Sc. (Agri.)* Thesis. Unvi. Agri. Sci., Dharwad (India). 2006, 70.
3. Arunkumar GS. Studies on leaf blight of chrysanthemum caused by Alternaria alternata (Fr.) Keissler. *M. Sc. (Agri.)* Thesis, Univ. Agri. Sci., Dharwad (India), 2008, 85.
4. Ganie SA, Ghani MY, Qaisar Anjum, Qazi Nissar, Shabir UR, Dar WA. Bio efficacy of plant extracts and bio control agents against *Alernaria solani*. African J. Microbiology Research. 2013; 7(34):4397-4402.
5. Kamble PU, Ramiah M, Patil DV. Efficacy of fungicides in controlling leaf spot disease of tomato caused by *Alternaria alternata* (Fr.) Kessiler. J. Soils and Crops. 2000; 10:36-38.
6. Leifort C, Sigee DC, Epton HAS, Stanley R, Knight C. Isolation of bacterial antagonistic to post harvest fungal disease of cold stored Brassica spp. Phytoparastica, 1992; 20:158-163.
7. Patel RL, Choudhary RF. Management of *Alternaria solani* causing early blight of tomato with fungicides. J. Plant Dis. Sci. 2010; 5:65-67.
8. Pramodkumar T. Biological management of Alternaria blight of onion. MSc. *(Agri.)* Thesis. Univ. Agric. Sci. Dharwad (India), 2007.
9. Roopa RS. Yadahalli KB, Kavyashree MC. Evaluation of natural plant extracts, antagonists and fungicides against early blight caused by A. solani *In Vitro.* The Bioscan. 2014; 9(3):1309-1312.
10. Shekhawat PS, Prasad R. Antifungal properties of some plant extracts in inhibition of spore germination. Indian Phytopath. 1971; 24:800-802.
11. Vincent JM. Distribution of fungal hyphe in the presence of certain inhibitor. Nature. 1947; 159:239-241.