

Original Research Article

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## **Invitro Cultural Characteristics and Effect of Botanical (Garlic) for Control of Major Fungal Diseases of Potato in Manipur**

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Potato (*Solanum tuberosum*) is the most important vegetable crop widely cultivated in Manipur. In potato crop major yield loss occurred due to fungal pathogen. In Manipur major two fungal diseases occurred *early blight* and *Fusarium* wilt responsible for yield loss. To control the losses caused by pathogens many farmers are using fungicides to obtain very good yield. The present study was carried out to recognize the effect of garlic different concentrations on control against *Alternaria* and *Fusarium* spp. under *in-vitro* condition. Per cent growth inhibitions were recorded and it was ranged from 64.90 to 90.12% and 60.91 to 92.40% for *Alternaria* and *Fusarium* spp. respectively. *Garlic* concentration 20% show best results with 90.12% and 92.40% inhibition of both the pathogen.

### **Introduction**

Potato (*Solanum tuberosum* L.) is one of the most important vegetable crop. Potato also known as ‘poor man’s friend’. It is one of the most popular crops for vegetable purposes in the country. It provides low cost energy to the human diet. It is major source of starch. In Manipur (Imphal east) potato cultivation area is 1.18 ha and production is 9.92 Mt (Agri Manipur report 2017-18)<sup>[6]</sup>.

Fungal, bacterial, viral, and also parasitic nematode are causes diseases in potato crop. In potato crop 16% losses due to microbes and out of this 70-80% due to fungal

pathogen. Major fungal diseases in potato are late blight (*Phytophthora infestans*), early blight (*Alternaria solani*), wilt of potato (*Fusarium oxysporum*). In Manipur climatic condition early blight and wilt diseases are occurred on potato crop.

Symptom of Late blight caused by *Phytophthora infestans* appeared as water-soaked spots and spots are small, light to dark green, circular to irregular shaped. Water-soaked lesions usually appear first on lower leaves.

Symptom of Early blight caused by *Alternaria solani* appeared as small, irregular, dark

brown to black spots and also "appearance of concentric rings on older leaves. The disease initially appears on the older leaves causing premature senescence and leaf area reduction (Johnson and Teng, 1990)<sup>[7]</sup>.

Symptoms of *Fusarium* wilt appeared as yellowing of the leaves, following by wilting, rolling, sometimes affecting leaves on only one side of the plant. The potatoes themselves may be blemished or decayed, often with sunken brown areas, especially at the stem end. Also plant growth stunted and developing of yellow leaves seeing in plant by Garibaldi *et al.*, (2002)<sup>[4]</sup>.

Chemicals are not only costly but also they are creating problems on the environment, human health in all areas of the world (Rahmatzai *et al.*, 2017)<sup>[14]</sup>. Now a day botanicals also used for control of many fungal diseases because those are not hazardous to crop as well as humans. Garlic (*Allium sativum* L.) derived a compound known as Ajoene inhibited spore germination of some fungi, namely, *Alternaria solani*, *Alternaria* sp., *Alternaria tenuissima*, *Alternaria triticina*, *Colletotrichum* sp., *Fusarium lini*, *Fusarium oxysporum* and *Fusarium udum*, which cause serious diseases in some important crop plant in india. (Singh *et al.*, 1990)<sup>[18]</sup>. The first of antifungal activity of garlic extracts against *N. alba* - the causal agent of bull's eye rot, one of the major diseases of apples (Daniel *et al.*, 2015)<sup>[2]</sup>. Most of the species showed inhibition of enzymes due to the effect of garlic extract. The growth of the fungal species was also remarkably reduced by the garlic extract. (Muhsin *et al.*, 2001)<sup>[12]</sup>.

The Present studied was carried out to find the best botanical (garlic) concentration on control against *Alternaria* and *Fusarium* spp. causing early blight and wilt of potato respectively.

## Materials and Methods

### Isolation of fungus cultures

The diseased plants were collected from the potato grown in the experimental field. The early blight and wilt affected leaf and stem portion was cut into small pieces and surface sterilized with 0.1% sodium hypochlorite solution then afterward three times thoroughly washed with water. Potato dextrose agar (PDA) media were prepared and sterilized in an autoclave at 120°C, 15 lbs pressure for 20 minutes. The media were poured into Petri plates and allowed to cool for sometimes. Then, the leaf pieces were inoculated into the media and kept for incubation. After seven to eight days the fungal tips were transferred to PDA slants in order to obtain pure cultures. The isolates were confirmed as *Alternaria* (Van Bruggen 1984)<sup>[20]</sup> and *Fusarium* (Majumdar *et al.*, 2007)<sup>[9]</sup> by microscopic observation of fungal culture.

### Effect of solid media on mycelial Growth

Effect of media on mycelial growth of *Alternaria* and *Fusarium* spp. were studied *in vitro*. In this studied five different media was used. Potato dextrose Agar (PDA), Rose bengal Agar (RBA), Sabouraud Agar (SDA), Richard Synthetic Agar (RSA) and Malt Extract Agar (MEA) media were used for study. After 7 days measurements were recorded.

### Preparations of plant extracts

100 g (fresh wt) of mature garlic washed in sterilized distilled water. The extract was filtered through four layers of moistened muslin cloth and the volume was adjusted to 100 ml with distilled water at final. The filtrate was centrifuged at 8000 rpm, 48°C for 15 min. The supernatant thus obtained was designated as concentrated leaf extract (Shekhawat and Prasada, 1971)<sup>[17]</sup>.

### **In-vitro effect of botanicals on the growth of fungal pathogens**

Three different concentration (10%, 20% and 25%) of botanical garlic pore in three different plates with mycelial disc of 5mm diameter was placed at the centre of the plates. Then the plates were incubated at  $25 \pm 1^\circ\text{C}$  in B.O.D. incubator. The efficacy of plant extracts on the growth of *Alternaria solani* and *Fusarium* spp. were studied by poison food technique. The percent inhibitions of mycelial growth over control were calculated by the following equation given by Edington *et al.* (1971) [3].

$$L = [(C - T)/C] \times 100$$

Where, L = Inhibition of mycelial growth, C = Radial growth measurement of the pathogen in control, T = Radial growth of the pathogen in the presence of botanical garlic concentration

### **Results and Discussion**

#### **Effect of media on growth**

The mycelial growth of *Alternaria* spp. was studied on five different media and the results

are presented in Table – 3a. The maximum radial growth of 8.5 cm was recorded in potato dextrose agar at 7 days after inoculation. The minimum radial growth of 5.15 cm was recorded in RBA at 7 days after inoculation. *Alternaria* spp. showed dark black colony with cottony puffy growth white pigment with regular margin in potato dextrose agar the cultural media used. slow to very fast growth were observed (Table – 1a). These findings are supported by Shabana *et al.*, (2015)<sup>[16]</sup> and Koley and Mahapatra (2015)<sup>[8]</sup> and Mishra and Versha (2012)<sup>[11]</sup> and Somappa *et al.*, (2013)<sup>[19]</sup> who also performed test on growth performance, and other cultural characteristics of *Alternaria* spp. by using different nutrient media.

The mycelial growth of *Fusarium* spp. was studied on five different media and the results are presented in Table – 3b. The maximum radial growth of 8.5 cm was recorded in potato dextrose agar at 7 days after inoculation. The minimum radial growth of 4.43 cm was recorded in RBA at 7 days after inoculation. *Fusarium* spp. showed Whitish puffy colony at initial stage later turns into straw colour mycelia in potato dextrose agar the cultural media used. slow to very fast growth were observed (Table – 1b).

**Table.1a** Effect of different media on growth of *Alternaria* spp.

Different media	Mycelial growth in diameter(cm)						
	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs
RBA	0.47	1.07	1.58	2.73	3.53	4.25	5.15
ME	0.86	2.10	3.23	4.08	5.15	6.37	7.72
PDA	1.48	2.45	3.73	4.80	5.73	7.20	8.50*
SA	0.62	1.85	2.83	3.78	4.61	5.93	7.58
RA	0.37	1.30	2.68	3.55	4.02	5.32	6.35
SE (d)	0.3909	0.2039	0.1912	0.1744	0.2974	0.3094	0.3304
CD at 5%	0.8333	0.4346	0.4076	0.3717	0.6340	0.6595	0.7042

\*Mean of four replications

\*\*Figures in the parenthesis are square root transformed values

**Table.1b** Effect of different media on growth of *Fusarium* spp.

Different media	Mycelial growth in diameter(cm)						
	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs	168 hrs
RA	0.80	1.53	2.9	3.8	4.70	5.08	6.10
RBA	0.23	1.35	2.13	2.58	3.2	3.6	4.43
PDA	2.13	3.23	4.23	5.3	6.43	7.6	8.50
SA	1.28	1.93	3.15	3.9	5.13	5.93	7.15
ME	1.38	2.45	3.13	3.9	5.48	6.08	7.65
SE (d)	0.3198	0.1951	0.1645	0.2187	0.1846	0.1417	0.1513
CD at 5%	0.6818	0.4159	0.3507	0.4661	0.3935	0.3020	0.3226

\*Mean of four replications

\*\*Figures in the parenthesis are square root transformed values

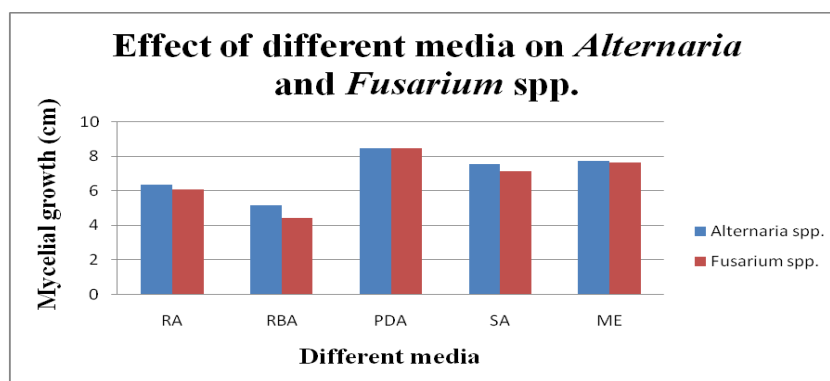
**Table.2** Percent growth inhibition of *Alternaria* spp and *Fusarium* spp. by different concentration of botanical (garlic)

Sl. no.	Botanical (garlic) concentrations	<i>Alternaria</i> spp.	<i>Fusarium</i> spp.
		Per cent growth inhibition over control (%)	Per cent growth inhibition over control (%)
1	10%	64.90* (8.08)**	60.91*(7.83)**
2	20%	90.12(9.51)	92.40(9.63)
3	25%	81.29(9.04)	75.75(8.73)
4	Control	-	-
SE (d)		4.188	4.652
CD at 5%		9.131	10.142

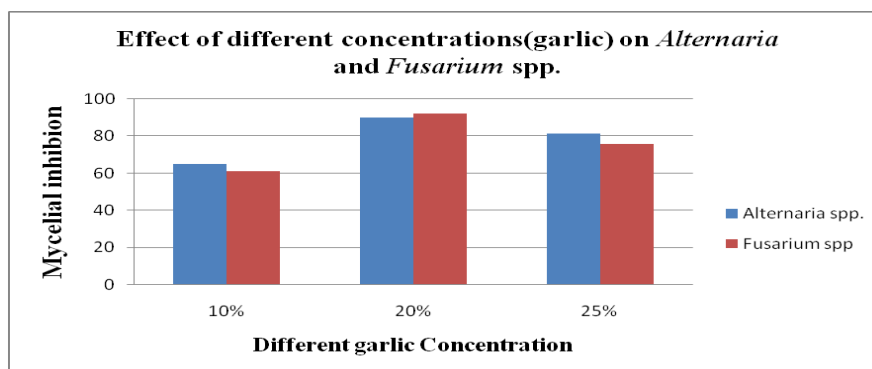
\*Mean of five replications

\*\*Figures in parenthesis are square root transformed values

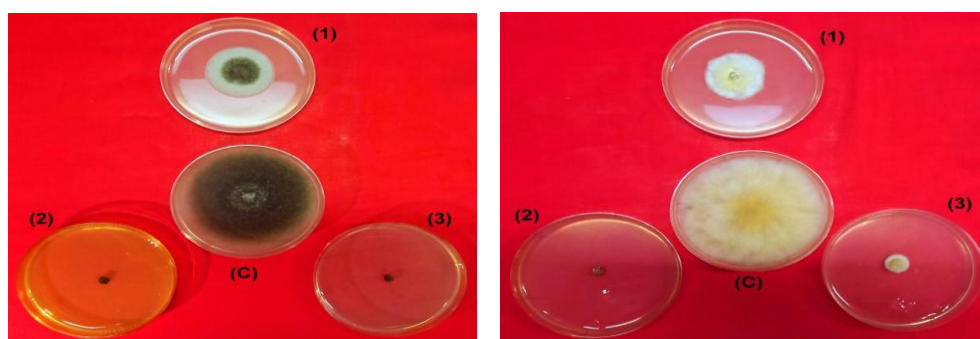
**Fig.1** Effect of different media on growth of *Alternaria* and *Fusarium* spp.



**Fig.2** Percent growth inhibition of *Alternaria* and *Fusarium* spp. by different garlic concentrations



**Plate.1a** Effect of Garlic Concentrations on *Alternaria*spp **Plate.1b** Effect of Garlic Concentrations on *Fusarium*spp



C- control  
 T1- 10% Garlic concentration  
 T2- 20% Garlic concentration  
 T3- 25% Garlic concentration

These findings are supported by Gupta *et al.*, (2010)<sup>[5]</sup> and Pradeep *et al.*, (2013)<sup>[13]</sup> and Yadav *et al.*, (2017)<sup>[21]</sup> who also performed test on growth performance, and other cultural characteristics of *Fusarium* spp. by using different nutrient media. Again, among the 5 media used, potato dextrose agar had shown best results.

**In-vitro effect of garlic concentrations on the growth of pathogens**

After 7 days of incubation, 20% concentration garlic showed maximum inhibition of 90.12%

on mycelial growth of *Alternaria* and 92.40% on mycelial of *Fusarium* spp. However, 10% concentration showed the lowest inhibition percentage which is around 64.90% (*Alternaria* spp.) and 60.91% (*Fusarium* spp.) (Table.2, Plate.1a,1b). These findings are supported by Muhsin *et al.*, (2001)<sup>[12]</sup>, Maurya *et al.*, (2016)<sup>[10]</sup>, Rex *et al.*, (2019)<sup>[15]</sup> according to work among the ten plant extracts, turmeric showed minimum mycelial growth of 9.50mm with highest inhibition over control. This was followed by garlic and eucalyptus which observed mycelial growth of 12.80 and 24.27mm with inhibition area of 85.77 and

61.60% and Ahmad *et al.*, (2017)<sup>[1]</sup>. Studied that 20% concentration of garlic extract has the potential to reduce EB disease severity, while having no noticeable phytotoxicity.

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