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In vitro evaluation of bio control agents against *Alternaria alternata* causing leaf blight disease of *Adhatoda zeylanica* Medic

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ABSTRACT

The experiment was conducted to know the efficacy of different bio control agents at different concentrations against growth of Alternaria alternata, causing leaf blight of Adhatoda zeylanica Medic. Different bio control agents in different solvents were tested in vitro, among all leaves extracts Polyalthiya longifolia was found to be more effective and inhibited cent per cent fungal growth i.e. 100% at 3.0 % concentration in alcoholic form followed by acetone leaves extract at 4.5 % concentration. However, least mycelial growth inhibition was recorded in aqueous leaves extract concentration.

Key words*: Adhatoda zeylanica , Alternaria alternate, Biocontrol, Polyalthiya longifolia.*

INTRODUCTION

Adhatoda zeylanica Medic. is an important medicinal plant, good source of phenols, tannins, alkaloids, anthroquinones, saponins, flavonoids, amino acids, reducing sugars, vasicine and vasicinone etc[5]. Hence, entire plant parts are used as medicine. The leaves, flowers, fruits and roots are used to treat cold, cough, whooping cough, chronic bronchitis, asthma[6]. Besides, it increases WBC, blood lymphocytes, splenic, lymphocytes and peritoneal macrophages[9].

Such important medicinal plant is affected by many diseases among which leaf blight caused by *Alternaria alternata* (Fr. Keissler) is most devasting diseases in India[8] and the pathogens are responsible for decreasing potential yield of the plant[1]. The chemical fungicides, which are traditionally used have high cost and hazardous effects. The use of fungicides, has helped in increasing the yield of plants but one of the major problems with constant use of the chemicals is that the resistance can be induced in target organisms and contaminate the environment with toxic substances. Hence, the possible alternative to solve such problem is the use of plants, which are able to produce antifungal substances. Fungicides of plant origin are environmentally safe and nonphytoxic. Farmers can easily prepare the extract of such plant materials[3]. Keeping in views the importance of disease, the present investigation was carried out to test the efficacy of different bio control agents against *Alternaria alternata.*

MATERIALS AND METHODS

The experiment was conducted under *in vitro* condition. Nine different leaves extract concentrations were prepared i.e. 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0 and 4.5 % by using three different solvents i.e. acetone, alcohol and aqueous.

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The leaves were dipped in different concentrations for 15 min. Then they were kept on filter paper in sterile petriplates, sterilised water was added in the petriplates time to time to maintain moisture content in the leaves[2]. 5 mm disc of pure fungal culture was added in 1 ml diluted water in the test tube and suspension is prepared (Plate 1). This suspension is applied over the leaves by brush and incubated at room temperature for 8 days. Effect of acetone, alcohol and aqueous leaves extract was calculated by using formula –

$$I = 100(C-T) / C$$

Where I = Percent Inhibition

C = Control reading

T = Treated reading

Plate 1: Pure culture of *Alternaria alternate* and mycelial structure

RESULTS

In vitro evaluation of bio control agent provides useful and preliminary information about efficacy against the pathogen within shortest period of time. In the present investigation, *in vitro* evaluation of bio control agent i.e. *Polyalthiya longifolia* was undertaken and results are presented (Table 1 & 2).

Among the different concentrations studied 0.5 % acetone leaves extract shows % inhibition from 01.81 to 68.12 % from 1st to 8th day of incubation period. The treatment of 1% concentration shows 09.09 to 86.00, 1.5 % shows 20.00 to 92.15 %, 2.0 % concentration shows 30.90 to 93.72 %, 2.5 % shows 47.27 to 94.43 %, 3.0 % shows 64.54 to 95.25 %, 4.0 % concentration shows 90.09 to 98.78 % inhibition, during 1st to 8th day. However, 4.5 % acetone leaves extract concentration shows cent percent inhibition of mycelia growth from 1st to 8th day of incubation period.On the other hand, alcoholic leaves extract concentration at 0.5 % concentration shows 27.27 to 63.43, 1.0 % shows 43.63 to 82.71, 1.5 % shows 62.72 to 87.75, 2.0 % shows 80.90 to 97.40, 2.5 % shows 91.81 to 98.65 and 3.0 % concentration shows cent percent mycelial growth inhibition from 1st to 8th day of incubation period.Besides this, 4.0 % aqueous leaves extract concentration is found to be 100 % inhibitory.

From above results alcohol leaves extract concentration was found superior in inhibiting the growth of pathogen.

Table 1: Efficacy of Acetone, Alcohol & Aqueous leaves extract of Polyalthia Longifolia on *Alternaria alternata*

I.P days	Concentration (%) Percentage inhibition (%)											
	0.5			**1.0**			**1.5**			**2.0**		
	Ac	**Al**	**Aq**	**Ac**	**Al**	**Aq**	**Ac**	**Al**	**Aq**	**Ac**	**Al**	**Aq**
1day	1.81	27.27	8.18	9.09	43.63	10.90	20.00	62.72	20.90	30.90	80.90	38.18
2day	2.42	38.18	22.42	15.15	46.66	38.18	26.06	67.87	43.63	39.39	82.42	53.93
3day	9.67	41.29	28.70	38.70	65.16	42.58	52.25	79.35	55.48	56.77	89.35	70.00
4day	31.42	45.00	30.95	52.85	68.57	51.90	59.52	79.52	62.38	61.42	89.76	71.19
5day	56.84	51.57	54.73	69.36	78.73	69.26	79.15	86.31	78.73	81.47	94.42	84.42
6day	59.76	55.82	55.15	72.08	79.36	74.07	85.38	86.64	83.59	88.09	96.09	85.78
7day	63.39	60.40	60.75	85.66	80.40	80.71	90.26	86.91	86.20	91.42	96.91	87.58
8day	68.12	67.43	66.18	86.00	82.71	80.87	92.12	87.75	87.12	93.72	97.40	90.25
SE	0.61	0.61	0.81	0.61	0.61	0.82	0.41	0.6	0.61	0.61	0.61	0.61
CD@ 0.5	1.84	1.82	2.45	1.83	1.84	2.45	1.22	1.82	1.84	1.82	1.83	1.82

Table 2: Efficacy of Acetone, Alcohol & Aqueous leaves extract of Polyalthia Longifolia on *Alternaria alternate*

I.P days	Concentration (%) Percentage inhibition (%)														
	2.5			**3.0**			**3.5**			**4.0**			**4.5**		
	Ac	**Al**	**Aq**	**Ac**	**Al**	**Aq**	**Ac**	**Al**	**Aq**	**Ac**	**Al**	**Aq**	**Ac**	**Al**	**Aq**
1day	47.27	91.81	62.72	64.54	100	80.00	60.00	100	91.81	90.09	100	100	100	100	100
2day	57.57	92.72	67.87	64.48	100	83.63	78.78	100	93.93	90.30	100	100	100	100	100
3day	73.87	93.22	80.96	79.35	100	90.32	87.74	100	96.45	94.19	100	100	100	100	100
4day	78.57	94.28	85.00	81.19	100	90.95	89.76	100	96.66	95.00	100	100	100	100	100
5day	88.42	97.15	92.21	90.73	100	95.47	94.73	100	98.31	97.47	100	100	100	100	100
6day	90.21	97.75	94.64	92.06	100	96.62	94.70	100	98.80	98.14	100	100	100	100	100
7day	93.16	98.34	95.89	94.46	100	97.18	95.98	100	99.15	98.57	100	100	100	100	100
8day	94.43	98.65	96.90	95.25	100	97.84	96.81	100	99.34	98.78	100	100	100	100	100
SE	0..61	0.60	0.61	0.61	-	0.61	0.60	-	0.61	0.61	-	-	-	-	-
CD @ 0.5	1.83	1.82	1.84	1.82	-	1.83	1.82	-	1.84	1.82	-	-	-	-	-

Ip=Incubation Period, Ac=Acetone, Al=Alcohol, Aq=Aqueous.

DISCUSSION

Dhavle *et. al,.*[3] also observed various plant leaves extracts including *Polyalthiya longifolia* as most effective in inhibiting growth of fungal pathogen. Rajmanickam *et. al.,* [7] also tested the activity of crude extracts of *Azadiracta indica, Dattura metal, Polyalthiya longifolia* against mycelial growth of fungal pathogens. Kareppa,[4] studied the biological control of fungal diseases of medicinal plants. He also reported that different pests and diseases infect medicinal plants to same extent. Hence, he investigated the biological control of fungi pathogens of Sonmukhi i.e. *Asperagus officinalis* and Turmeric i.e. *Curcuma longa*. He used different plants leaves extracts viz. *Azadirchata indica, Eucalyptus citriodora, Euphorbia hirta, Glassocardia bosvella, Polyalthia logifolia, Solanum nigrum* and *Vinca rosea*. After evaluation of efficacy of these plants leaves extracts, *Azadirchata indica, Solanum nigrum* , *Polyalthia longifolia* and *Vinca sosea* were found most effective than other plant leaves extracts to control fungal pathogens. The present work recommends to increase the use of biocontrol agents instead of chemical pesticides, to avoid harm effects caused by them.

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