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Biocontrol of early blight of tomato caused due to *Alternaria alternata* by using plant latex

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Abstract
Genus Alternaria belong to deuteromycetes having number of species and destructive plant pathogen to the families such as Solanceae, Cucurbitaceae, Brasicaceae. Tomato belong to family Solanaceae. The tomato crop grown in rabbi and kharip season having nutritional and economical value. Alternaria species cause early blight disease of tomato and lose the quality and quantity of crop. The disease is control by spraying synthetic chemicals but it creates environmental, ecological problems. Now a day's biological methods are uses to control the diseases. Biological methods are safer, biodegradable and ecofriendly, so this method is adopted by farmer rather than chemical control method. Biological agents like fungi, herbal extract and natural products are used to control diseases. In present study *Alternaria alternata* isolated from infected parts of tomato plants on PDA medium. The different concentrations of some plant latex are prepared and used to control the infection of Alternaria.

Keywords: biocontrol, early blight of tomato, *Alternaria alternata*, plant latex

1. Introduction
Alternaria species are infects the various crops belonging to the several families and reduce the quality and quantity of yield of crop plants. The genus Alternaria was first recognized by Nees in 1817. Alternaria belongs to the subdivision Deuteromycotina, class Hyphomycetes, family Dematiaceae. Species of the genus are cosmopolitan, surviving both as Saprophytes as well as weak parasites. Among the different diseases caused by the genus Alternaria, blight disease is one of the most dominant and that causes average yield loss in the range of 32-57% (Conn and Tewari, 1990) [3]. In several cases, small dark coloured spots are also formed on pods and tender twigs (Valkonen and Koponen, 1990) [17]. A comprehensive, comparative account of morphological differentiation of different Alternaria species occurring on Cucurbitaceous, Brassicaceous and Solanaceous crops are described by Khalid *et al*. (2004) [9] and Deshwal (2004) [5].
There are several methods which are being employed for management of Alternaria disease like application of chemical fungicide, herbal extract and natural product, by seed treatment, use of resistant varieties, biological control agents and other methods (Prasad and Naik, 2003) [14]. Use of chemical fungicide is an important tool in the prevention and control of crop diseases but it creates environmental, ecological and health problems so biocontrol plays important role to eco-friendly control the infection. To biological control of the diseases various plant latex and natural products are used to control the diseases because plant latex is no harmful effect on biodiversity and cheaper than a chemical fungicide.
The antifungal potency of *C. gigantea* latex extract on the *C. albicans* showed a larger diameter of clearance than that of other fungal strains (Venkatesan and Subramanian, 2010). The latex extract was screened in vitro against human pathogenic strains such as Gram positive; Staphylococcus aureus, *Bacillus subtilis*, Gram negative**;** *Salmonella typhi*, *Klebsiella phenonemia* and two fungal strains; *Aspergillus niger* and *Candida albicans*. The result agrees with that there is a need to employ broad range of extractive solvents in the extractions of possible photochemical from medicinal plants (Takazawa *et al*., 1982) [16]. The growth of four test fungi was inhibited by ethanol and chloroform extracts while the aqueous extract was the least effective on the test fungi. The mycelial growth, percentage spore's germination and germ tube extension in *Fusarium oxysporum* and *Aspergillus carbonar* is decreased when *Calotropis procera* extract concentration increases, whereas growth of *Humicola brevis* and *Penicillium lanosum* were not affected (Rizk, 2008) [15].
The water-soluble fraction of papaya latex can completely digest the conidia of many fungi including important post-harvest pathogens (Indrakeerthiand Adikaram, 1996) [6]. Other latex extracted from several plants showed a strong antifungal activity against Botryti cinerea, Fusarium sp. (Barkai-Golan, 2001) [2]. The best antifungal activity was recorded in ethanol extract of *C. procera* latex against *Candida albicans* **(**Kareem *et al*., 2008) [8]. Leaf extracts, chopped leaves and latex of *C. procera* have shown great promise as a nematicide in vitro and in vivo (Khirstova and Tissot, 1995) [10].

2. Material and methods
Samples of fungal infected parts of tomato were collected from the different areas of tehsils of Nashik district of Maharashtra. Fungal infected part samples are collected randomly and fresh infected plant materials were used for the isolation of fungus.

2.1 Isolation
Isolation of fungus was done on PDA medium because PDA plate method was most suitable for isolation of fungus. PDA was preparing by adding peeled potato (200 gm/lit.), dextrose (20 gm/lit.), agar (15 gm/lit.), pH was adjusted by pH meter. PDA medium and required glassware's are sterilized by the autoclave and are transfer in the laminar air flow cabinet. Sample are inoculating on growth medium and maintain pure culture of fungal species. Fungi are identified

by microscopic characters with the help of identification key (Mukadam *et al*., 2002) [12]. After identification of fungi pure cultures are maintain for further procedure.

2.2 Plant material and latex collection
The fresh latex of *J. curcus, C. gigentea, F. bengalensis* and *F. glomerata* were aseptically collected from the aerial parts of the healthy plants as described by Aworh *et al*. (1994) [1] in clean glass tubes containing distilled water to yield a dilution rate of 5:5 (v/v). The latex mixture was gently handled to maintain homogeneity during transport to the laboratory where it was stored at (4° C) until further use.

2.3 Preparation of latex extract
The fresh latex was selectively decanted and centrifuged at 5000 rpm for 5min. The precipitated material showing rubber aspect was pooled apart and the supernatant was decanted carefully. Finally, the samples were centrifuged as previously described and the clear soluble supernatant was collected and lyophilized. The stock solutions of latex extract were diluted suitably as required from stock solution (Juncker *et al*., 2009) [7].

2.4 Determination of antifungal activity
Plant latex aqueous extracts of each prepared with distilled water and condensed to serve as stock extract was determined by food poisoning technique (Mishra & Tiwari, 1992) [11] against tested pathogens in four different concentrations. Petriplates containing PDA medium, supplemented with different plant latex extracts at four concentrations (25, 50, 75 and 100%) with three replicates were inoculated with fresh 7 days old culture of test fungi in 8 mm discs and kept upside down. The plates were incubated in BOD incubator at 28 ± 2°C. Plates without plant latex extracts served as control. Starting two days after inoculation (DAI) radial growth was recorded daily for 8 days or until the plates were overgrown.

3. Results and Discussion
In present study different concentrations of some plant latex was tested against *A. alternata* to determine their antifungal activity. Minimum inhibitory concentration (MIC) was measured to determine the antifungal activity. *Calatropis gigantia* latex extract showed 100% reduction of radial growth of *Alternaria alternata* at 100% conc. *Jatropha curcus* (90.59%) also showed significant reduction of *Alternaria alternata* at 100% conc. However, there was significant reduction of radial growth in case of *F. bengalensis* and *F. glomerata* was also observed.

4. Tables and Figures

Table 1: Antifungal activity of plant latex extracts against *A. alternata* (AaKt)

Plant Name	Conc (%)	Radial growth of *A. alternata* (mm)	Inhibition (%)
Jatropa curcas	25	15	82.35
	50	14	83.53
	75	10	88.24
	100	8	90.59
Calotropis gigantea	25	36	57.65
	50	26	69.41
	75	20	76.47
	100	0	100.00
Ficus bengalensis	25	16	81.18
	50	14	83.53
	75	10	88.24
	100	9	89.41
Ficus glomerata	25	34	60.00
	50	28	67.06
	75	23	72.94
	100	16	81.18
Control		85	

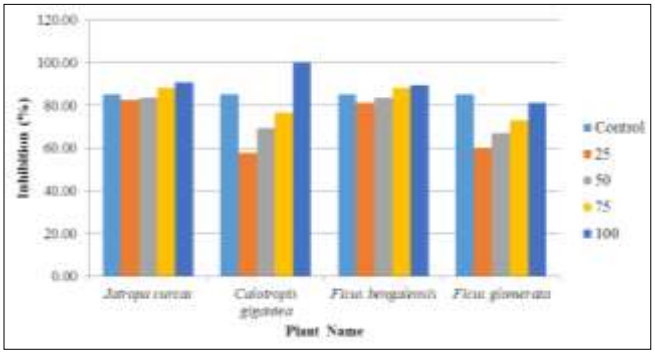

Fig 1: Antifungal activity of Plant latex extracts against *A. alternata* (AaKt)

Conclusion
Biocontrol agents like plant latex of *Calatropis gigantia* more effective against *A. alternata*. To avoid harmful effect of chemical fungicides to nature plant latex can used to ecofriendly management of diseases.

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