**Bioscience Discovery, 5**(1):55-59, Jan. 2014

ISSN: 2229-3469 (Print); ISSN: 2231-024X Online
**Received: 15-10-2013, Revised: 02-12-2013, Accepted: 22-12-2013**

***Full Length Article***

# Selective inhibitory *in vitro* activity of *Aegle marmelos* (L.) extracts on plant fungal pathogen *Colletotrichum acutatum*

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**ABSTRACT**

Methanol and petroleum ether extracts of seeds of *Aegle marmelos* were tested against anthracnose causing plant fungal pathogen *Colletotrichum acutatum.* The antifungal potential of same extracts was also tested against three fungal strains, *Metarrhizium anisopliae*, *Trichoderma harzianum* and *Penicillium* spp. Both methanol and petroleum ether extracts showed antifungal activity at tested concentrations (1:1, 1:2, and 1:3). Activity exhibited by methanol extract was greater compared to petroleum ether extract. None of the extracts showed any effect on the growth of *Metarrhizium anisopliae*, *Trichoderma harzianum* and *Penicillium* spp. The use of active principles of *Aegle marmelos* as natural pesticide and their applications in formulationscontaining biological control agents has been discussed.

**Key words :***Aegle marmelos,* anthracnose, *Colletotrichum acutatum,* selective inhibition

## INTRODUCTION

*Colletotrichum* species are one of the important plant fungal pathogens in tropical and sub tropical countries responsible for causing disease syndrome called as anthracnose, Shivprakash *et al.*, (2011). The pathogenesis of the disease is unique through formation of appresoria that are associated with penetration into host cells, and tissue destruction, O'Conell *et al.,* (2012). The anthracnose is characterized by tissue necrosis, corm root, leaf crinkles and characteristic spiral twisting of floral peduncles, Freeman et al. (2000). Host range includes wide variety of plants such as apple, almond, anemone, citrus, lupin, peach, pecan, strawberry, cassava, sorghum, bananas and others (Freeman, 2008). Inclusion of *Colletotrichum* species (8$^{th}$ rank) among top ten fungal pathogens is sufficient to prove its devastating effects on crops, herbaceous and woody plants, Dean *et al.* (2012).

In India, *Colletotrichum* has been attributed for major loss in horticultural crops such as mango, Lakshmi *et al.,* 2011; sorghum, Pande *et al.,* 1991; chilli, Sharma and Shenoy, 2013. *Colletotrichum truncatum* is also known to cause the rare human infections of mycotic keratitis and endophthalmitis, Shivparakash *et al.* (2011). Thus, the management and control of Colletotrichum has a significant importance to prevent losses due to diseases and reduction in post-harvest quality. Integrated pest management, use of chemicals fungicides and products from natural origin has been used for treatment of anthracnose. Plant extracts possess wide range of biological activities; have been explored as source of natural pesticides and used against plant diseases.

Bhardwaj (2012) investigated the potential of twenty plant extracts against plant fungal pathogen *Fusarium solani*. Satish *et al.* (2007) studied the antifungal potential of aqueous extracts of 52 plant species against eight important species of *Aspergillus* that are responsible for causing seed deterioration during storage.

*Aegle marmelos* (L.), a native plant of India, belonging to family Rutaceae, commonly known as wood apple is one of the important plants in ayurvedic and traditional folk medicine. Its fruits, roots, barks, leaves, rind and flowers are mainly used for medicinal purpose, Swami Sadashiv Tirth (2005). Along with ayurvedic medicinal system *Aegle marmelos* has also been described by traditional folk and Chinese medicine system, Sekar *et al.* (2011). In recent years, *Aegle marmelos* has been extensively investigated, and found to contain number of pharmacologically active compounds possessing antimicrobial activity, Dabur *et al.,* 2004; Dabur *et al.,* 2007; Poonkothai and Saravanan, 2008; Tambekar *et al.,* 2012.

Considering the pharmacological significance of *Aegle marmelos*, present study was undertaken to evaluate the antifungal potential of extracts of *Aegle marmelos* against *Colletotrichum acutatum.* Further, to check whether the extracts exhibit any harmful effect on fungal species that are non pathogenic to plants, proved to be members of plant growth promoting rhizobacteria and effective biological control agents, the activity was also tested against *Metarhizium anisopliae, Trichoderma harzianum* and *Penicillium* spp. To the authors' knowledge, this is first report demonstrating the effect of antifungal potential of seed extracts of *Aegle marmelos* on *Colletotrichum acutatum.*

## MATERIALS AND METHODS

Seeds and leaves of *Aegle marmelos* selected for present study were obtained from botanical garden of college of horticulture, Shahada (District-Dhule). Authentication of plant material was done at department of Botany, Ahmednagar College, Ahmednagar.Plant material was thoroughly washed with distilled water and air dried.

### Preparation of extracts

Air dried material (leaves and seeds) were ground into fine powder and stored at dry place until use. Active principles were extracted by warm extraction using soxhlet apparatus. 20 grams of dry powder was packed in Whatman filter paper no.1 and subjected to extraction process using solvent for 15-20 cycles. Extracts were prepared in methanol and petroleum ether. Aqueous extract was prepared by dissolving the plant material in warm water for 24 hrs (with constant stirring) followed by filtration. Extracts obtained in different solvents were concentrated, dried, dissolved in 20 ml of dimethyl sulfoxide (DMSO) and stored in refrigerator at $4^{o}$C until further use.

### Microorganisms used

Throughout studies, three fungal strains *Colletotrichum acutatum, Metarrhizium anisopliae, Trichoderma harzianum* and *Penicillium spp* were used. Fungal strains were maintained on Sabouraud dextrose medium agar slants supplemented with streptomycin (100 µg/ml).

### In-vitro antifungal activity

Inoculum for each test fungus was prepared by growing it 48 hours on Sabouraud dextrose medium agar. Count of spores was adjusted to obtain $10^{8}$ spores per ml of suspension (Distilled water containing 0.1% tween 80). Antifungal activity was performed by disc diffusion method according to Cavanagh 1972. For this, Sabouraud dextrose medium agar was seeded with 1 ml of spore suspension of test fungi and plates were poured and solidified. Disc (diameter 8mm, made from whatman filter paper no. 1) loaded with 20 µl of extract were placed on seeded agar, kept for pre-diffusion in refrigerator and incubated at $28^{o}$C for 48 hrs. Disc loaded with 20 µl DMSO was used as control. All experiments were carried out in triplicate. Same procedure was repeated for two fold and three fold diluted extracts. Antifungal activity was measured in terms of zone of inhibition formed around the disc after incubation. The inhibition zone was measured in nearest of millimeter as mean of three readings using zone reader (Hi-media, Mumbai).

## RESULTS AND DISCUSSION

Due to their safety as compared to chemical pesticides, large number of active principles derived from plant sources such as alkaloids, phenols and terpenoids have been explored as natural pesticides against various pests, Mann and Kaufmann (2012). Present study carried out to determine *in vitro* activity of seed extracts of *Aegle marmelos* on *Colletotrichum acutatum* showed that both methanol and petroleum ether extracts are capable of inhibiting the fungal growth.

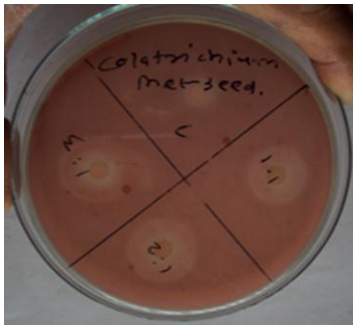


Fig. 1a (*C. acutatum*)

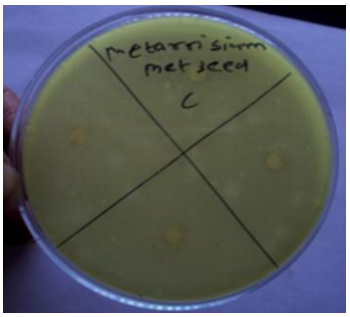


Fig. 1b (*M. anisopliae*)

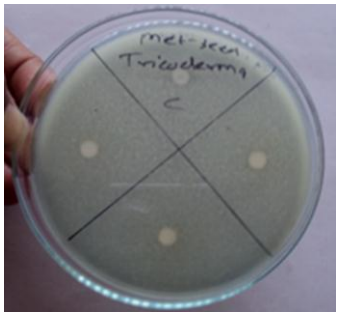


Fig. 1c (*T. harzianum*)


Fig. 1d (*Penicillium spp*.)


Fig. 2a (*C. acutatum*)

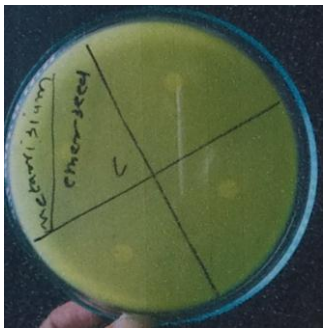


Fig. 2b (*M. anisopliae*)

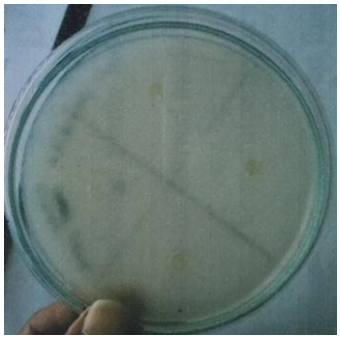

Fig. 2c (*T. harzianum*)

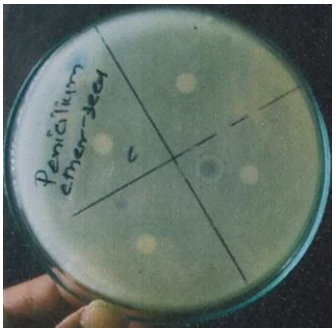


Fig. 2d (*Penicillium spp*.)

**Figure 1 (a-d) showing antifungal activity of methanol extract on test fungi.**
**Figure 2 (a-d) showing antifungal activity of petroleum ether extract on test fungi**

Growth of *Trichoderma harzianum*, *Metarrhizium anisopliae* and *Penicllium* spp. was not inhibited by any of the extracts tested in the study. The zones of inhibitions of extracts are shown in shown in table 1 and table 2 while pictures in figure 1 and figure 2 demonstrate the results of antifungal assay. Singh et al. (2008) investigated the activity of securinine against some plant pathogens including *Colletotrichum* spp and showed that it substantially inhibits the spore germination in fungus. Similar kind of study is reported in which Bangniwar et al. (2012) demonstrated the effect of leaf aqueous extracts of various plants including *Aegle marmelos* against *Colletotrichum dematium*. However both studies have not mentioned any aspect of inhibition caused to the fungi that are important members of rhizospheric flora of plant and known to exhibit benevolent interaction in rhizosphere. *Trichoderma harzianum, Metarrhizium anisopliae* are established biological control agents while *Penicillium* spp produces the compounds that promote the growth of plants. The observation that growth of these three fungi is not inhibited, tend to the conclusion that methanol and petroleum ether extracts of seeds of *Aegle marmelos* has selective action on *Colletotrichum acutatum*.

**Table 1: Antifungal activity of methanol extract against test fungi**

| Sr. | Test organism | Zone of Inhibition (mm) | | |
|---|---|---|---|---|
| | | **1:1** | **1:2** | **1:3** |
| **01** | *Colletotrichum acutatum* | 22 | 19 | 13 |
| **02** | *Metarrhizium anisopliae* | -- | -- | -- |
| **03** | *Trichoderma harzianum* | -- | -- | -- |
| **04** | *Penicillium spp.* | -- | -- | -- |

**Table 2. Antifungal activity of petroleum extract against test fungi**

| Sr. | Test organism | Zone of Inhibition (mm) | | |
|---|---|---|---|---|
| | | **1:1** | **1:2** | **1:3** |
| **01** | *Colletotrichum acutatum* | 19 | 13 | 07 |
| **02** | *Metarrhizium anisopliae* | -- | --- | -- |
| **03** | *Trichoderma harzianum* | -- | -- | -- |
| **04** | *Penicillium spp.* | -- | -- | -- |

Number of studies has shown the presence of alkaloids such as aegeline and skimmianine in methanol and petroleum ether extracts of *Aegle marmelos* which may be responsible for antimicrobial activity. However the mechanism of selective action remains unclear.

*Colletotrichum acutatum* is responsible for devastating effects on economically important crop plants in temperate and sub tropics, every plant species being susceptible to the infection. *Colletotrichum* affects fruit, causing disease on immature and developing fruit in the field and damage mature fruit at harvest and during storage,thus bringing about pre-harvest as well as post harvest loss of crop, Wharton et al. (2004). Use of plant derived products could be one of the means for effective treatment and control of anthracnose. The extracts of seeds of *Aegle marmelos* tested in present study clearly demonstrate that active components possess potential activity against *Colletotrichum acutatum*and could thus be used for the formulation of products based on natural compounds. Moreover, these compounds could also be used to fortify the formulations containing biological control such as entamopathogenic fungi *Metarrhizium anisopliae*, mycoparasitic fungi *Trichoderma harzianum* and *Penicillium* spp thus adding up to efficacy of the formulation.

*In vitro* antifungal activity against anthracnose causing fungus *Colletotrichum acutatum* revealed that, seeds of *Aegle marmelos* contain active principles that can cause inhibition of fungal growth and thus could be used efficiently for management of *Colletotrichum*. Also, selective inhibition of growth of *Colletotrichum acutatum,* while no harm to beneficial fungi makes *Aegle marmelos*, the potential source of natural pesticide. Furtherwork may be extended to purify, identify and characterize the antifungal compounds, elucidate their mechanism of action, carry out safety and toxicity tests so that they prove to be compounds of agricultural importance.

**ACKNOWLEDGMENT**

Authors are thankful to Head, Department of Botany and Principal, Ahmednagar College, Ahmednagar for providing facilities towards successful completion of present study.

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How to Cite this Article:

**Avinash E Gawade, Nitin S. Gaikwad and Sudhir R Bale, 2014.** Selective inhibitory *in vitro* activity of *Aegle marmelos* (L.) extracts on plant fungal pathogen *Colletotrichum acutatum*. *Biosci. Disc.,* **5**(1):55-59.

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