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# An Environment Friendly Approach for Controlling Pathogenic *Fusarium solani* (Mart.) Sacc., The causal Agent of Root Rot of Medicinal Coleus by Methyl Jasmonate

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## Abstract

Effects of 'methyl jasmonate' were studied in vitro on colony growth, sporulation, spore germination and germ tube elongation of phytopathogenic *Fusarium solani* (Mart.) Sacc., the causal organism of root rot of medicinal Coleus. Three different concentrations of methyl jasmonate- 0.05%, 0.10%, and 0.20% were used as amendment with 'potato dextrose agar' medium. At 0.10% and 0.20% concentration, methyl jasmonate was able to significantly increase percent growth inhibition of the fungal colonies, as compared to the control, in a concentration dependent manner, after 48 and 96 hours incubation. Inhibition was more severe after 48 hours than 96 hours. Highest percent growth inhibition (76.00) was with 0.20% methyl jasmonate after 48 hours. Methyl jasmonate treatments also had significant reducing effects on spore formation, spore germination frequency and germ tube growth of the pathogen in a concentration dependent manner. 0.20% methyl jasmonate had most severe effects which caused lowest spore count (8 x $10^4$/ml culture filtrate), germination percent (4.80) and germ tube length (54.52µ). In the present study, methyl jasmonate showed fungistatic and fungicidal activity against *Fusarium solani*, under in vitro conditions. This is the first report of methyl jasmonate showing inhibitory effects on this particular fungus which in turn supports the possibility of future use of methyl jasmonate as a bio-control agent against root rot of medicinal Coleus caused by *Fusarium solani*, under field conditions.

## Introduction

*Fusarium solani* is known to cause rotting of seeds, seedlings, roots, lower stems and crown of a number of plants and also of the vegetative germplasms like corms, bulbs and tubers [1]. In our earlier investigation, we reported *Fusarium solani* (Mart.) Sacc. to be the causal organism of root rot of *C. forskohlii* from lower Gangetic West Bengal [2]. This was the first report of *F. solani* causing root rot of medicinal *Coleus* from India causing severe loss [2]. *Coleus forskohlii* Briq. is a member of the family Lamiaceae and an important indigenous medicinal plant in India [3]. Tuberous roots of *C. forskohlii* produce a labdane diterpenoid 'forskolin' [4]. Forskolin, the major chemical constituent, has positive inotropic effect on heart muscle, lowers blood and intraocular pressure and is anti-inflamatory [5]. Forskolin stimulates the production of cAMP by activating 'adenyl cyclase' [6,7] and by doing so, mediates several of its effects such as lowering of blood pressure [8], stimulation of lipolysis in fat cells [9] and also acts as therapeutic agent for diseases like glaucoma, thrombosis, asthma and metastatic conditions [10] . With the present annual production of about 100 tons from 700 ha in India, cultivation of *C. forskohlii* is in the reise in several states [11]. But susceptibility to many diseases of which root rot/wilt being the most important one, is the cause behind *Coleus* becoming not very popular among the farmers [11].

Fungal pathogens are mainly controlled by chemical method but pathogen adaptability leading to fungicide resistance is a continuous problem in this system [12]. Also fungicidal application causes hazards to human health and increases environmental pollution. In view of the above situation alternative environment friendly approaches to control plant diseases are needed. One such approach is stimulating the plant's own resistance mechanism by agents which can mimic natural inducers of resistance [12]. There is increasing evidence that defence elicitors can be used as alternatives to traditional pesticides because elicitor treatments have several advantages over conventional pesticides such as reduced environmental hazards and lower acute toxicity to organisms [13].

Jasmonic acid (JA) and its methyl ester 'methyl jasmonate' (MJ) are naturally occurring plant growth regulators synthesized via the octadecanoic pathway [14]. The most intensively studied elicitor for manipulating defence pathways in plants is MJ [13]. Jasmonate induces the expression of plant defence genes in response to different pathogen attack [15]. Also antifungal potential of MJ has been demonstrated by several workers [16,17]. MJ markedly inhibits mycelial growth of *Penicillium expansum* [16]. Kepczynska [17] found MJ to reduce hyphal and mycelia growth as well as spore germination in *Alternaria alternata* (Fr) Keissl. Present work describes the effects of MJ on colony growth, spore count, spore germination and germ tube elongation of *F. solani* (Mart.) Sacc. in vitro, with a view to evaluate the future potentiality of using MJ as an alternative bio-control agent against root rot of *Coleus forskohlii* Briq. caused by the fungus.

## Materials and Methods

### Preparation of MJ solution

To incorporate into 'potato dextrose agar' (PDA) medium, 0.5% MJ stock solution was prepared according to the method of El-Khallal [18]. MJ was dissolved in sterile distilled water containing 0.01% Tween 20.

### Effects of MJ on colony growth of *F. solani*

Effects of MJ on colony growth of *F. solani* were studied by

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modifying the method of [19] on PDA plates amended with three different concentrations of MJ (0.05%, 0.10% and 0.20%). Unamended PDA was used as control (C1). A further control (C2) was PDA containing 0.01 % Tween 20 in volume similar to that of MJ stock added to obtain 0.20% final concentration.

Ten plates per MJ concentration were inoculated with small (about 2mm in diameter) mycelial clumps from periphery of 14 days old stock colony on PDA. Control plates were inoculated in similar way. Plates were incubated at room temperature under diffused light. Colony diameter was measured (in replicates of ten / treatment) after 48 and 96 hours and percentage of growth inhibition (PGI) in C2 and all the treatments was calculated in relation to the average colony diameter in PDA control (C1) according to the formula PGI= [(dc-dt) / dc] x 100, where dc = mean colony diameter in control plate (here C1), dt = mean colony diameter in test plates [20].

### Effect on MJ on spore count of *F. solani*

To determine the effects of MJ on spore count of *F. solani*, fungal colony was raised on PDA plates amended with three different concentrations of MJ as before. Controls were also similar to those of previous experiment. Ten plates per MJ concentration as well as controls were inoculated at the centre with a single mycelial clump and plates were incubated at room temperature under diffused light.

Spores were harvested from 14 days old colonies, as described by Ketabchi et al. [14]. 5ml distilled water was added to the petri dish and the surface was rubbed with sterile glass rod. Suspensions from ten plates were pooled together, filtered to remove mycelia and diluted with sterile distilled water to 100ml. Conidial number/ml suspension was determined (ten replicates/treatment) by haemocytometer.

### Effects of MJ on spore germination percentage and germ tube growth of *F. solani*

Effect of MJ on spore germination of *F. solani* was studied according to the method of [21] with modifications. 50µl aliquots of conidial suspension (prepared from 14 days old cultures on PDA) were plated evenly on petri dishes containing PDA with MJ. Concentrations of MJ and controls were similar to those described in the previous two experiments. Five plates were inoculated for each MJ concentration and controls and incubated under room temperature for 4 hours.

After 4hours, 100 spores / plate (total 500 in 5 replicates of 100 each) were observed for each treatment and control to find out the percentage of germinating spores. Lengths of ten longest germ tubes from each plate (total 50 numbers for each treatment as well as control) were measured using ocular micro meter.

### Statistical analysis

All statistical analyses were performed with Statistical Package for Social Sciences (SPSS) version 17 (SPSS Inc., Wacker Drive, Chicago, IL) [22]. Quantitative changes in different parameters were analyzed by analysis of variance (ANOVA) and mean separations were performed by post hoc analysis by Tucky's HSD method.

## Results

### Effects of MJ on colony growth of *F. solani*

MJ had inhibitory effect on colony growth of *F. solani* after 48 hours (Table 1) and 96 hours (Table 2 and Figure 1) incubation. Among three concentrations, 0.05% MJ could not reduce colony growth significantly (at P=0.05) as compared to the control (C2) but when concentrations were increased to 0.10% and 0.20%, colony growth inhibition was significant. PGI due to the effect of MJ was higher after 48 hrs incubation than 96 hrs. (Table 1 and 2).

### Effects of MJ on spore count of *F. solani*

With increasing concentrations of MJ, number of spores / ml of culture filtrate gradually decreased significantly than the controls (Table 3). For 0.05%, 0.10% and 0.20% MJ, number of spores / ml of culture filtrate were 75 x $10^4$, 33 x $10^4$ and 8 x $10^4$ respectively. Controls C1 and C2 did not differ significantly.

### Effects of MJ on spore germination percentage and germ tube elongation of *F. solani*

MJ had significant reducing effects on the spore germination percentage of *F. solani* (Table 4). Germination percentage reduced with increasing concentrations of MJ. At 0.05%, 0.10% and 0.20% MJ, respective spore germination percentages were 61.80, 39.40 and 4.80. So, 0.20% MJ had most severe effect which reduced the spore germination percentage to 4.97% and 5.02% of C1 and C2 respectively.

MJ inhibited the growth of germ tube. With the increasing concentrations of MJ, length of germ tubes reduced significantly from the controls (Table 4 and Figure 2). But the effects of MJ at 0.05% and 0.10% did not differ significantly as the difference between average lengths of germ tubes emerging at these concentrations was not significant. The highest concentration of MJ (0.20%) had most severe inhibitory effect on germ tube elongation of *F. solani*.

## Discussion

MJ can directly influence several fungal pathogens [16]. In the present study 0.10% and 0.20% MJ significantly inhibited colony growth of *F. solani* where 0.20% concentration led to higher inhibition than that affected by 0.10% concentration. PGI was higher after 48 hours than 96 hours. This was probably due to increased adaptability of the fungus towards MJ with time. In a concentration dependent manner, methyl jasmonate inhibited spore formation, spore germination and germ tube elongation. Inhibitory effects of MJ on *F. Solani* obtained in the present study are supported by previous reports which indicate that MJ can inhibit growth, sporulation and metabolism of fungi

| Growth medium | Mean colony diameter (mm) | Percent growth inhibition (PGI) [Mean ± SE] |
|---|---|---|
| PDA control (C1) | 23.00 | - |
| PDA + 0.01% Tween 20 control (C2) | 22.00 | 4.00 ± 1.04[a] |
| MJ 0.05% in PDA | 20.40 | 11.00 ± 0.91[a] |
| MJ 0.10% in PDA | 12.20 | 46.00 ± 1.66 |
| MJ 0.20% in PDA | 5.40 | 76.00 ± 1.22 |

*Means followed by same letter do not differ significantly at P=0.05

**Table 1**: Effect of MJ on colony growth of *Fusarium solani* (after 48 hrs incubation).

| Growth medium | Mean colony diameter (mm) | Percent growth inhibition (PGI) [Mean ± SE] |
|---|---|---|
| PDA control (C1) | 27.20 | - |
| PDA + 0.01% Tween 20 control (C2) | 24.40 | 9.00 ± 1.27[a] |
| MJ 0.05% in PDA | 22.40 | 13.00 ± 0.98[a] |
| MJ 0.10% in PDA | 17.40 | 35.00 ± 1.60 |
| MJ 0.20% in PDA | 10.80 | 59.00 ± 1.96 |

*Means followed by same letter do not differ significantly at P=0.05

**Table 2**: Effect of MJ on colony growth of *Fusarium solani* (after 96 hrs incubation).

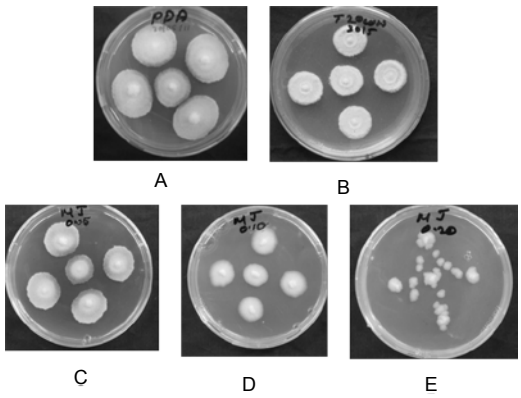


**Figure 1: (A-E):** Effects of methyl jasmonate (MJ) in PDA on colony growth of *F. solani* (Mart.) Sacc. after 96 hours incubation: A) control C1 (only PDA), B) control C2 (PDA + 0.01% Tween-20), C) 0.05% MJ in PDA, D) 0.10% MJ in PDA, E) 0.20% MJ in PDA, (Bar = 10mm).

| Growth medium | Number of spores (conidia) / ml spore suspension (Mean) |
|---|---|
| PDA control (C1) | 126 x $10^{4\,a}$ |
| PDA + 0.01% Tween 20 control (C2) | 125 x $10^{4\,a}$ |
| MJ 0.05% in PDA | 75 x $10^4$ |
| MJ 0.10% in PDA | 33 x $10^4$ |
| MJ 0.20% in PDA | 8 x $10^4$ |

*Means followed by same letter do not differ significantly at P=0.05

**Table 3**: Effect of methyl jasmonate (MJ) on spore count of *Fusarium solani*.

| Growth medium | Percentage of spore germination (Mean ± SE) | Length of germ tube (µ) (Mean ± SE) |
|---|---|---|
| PDA control (C1) | 96.40 ± 0.56[a] | 139.98 ± 3.65[a] |
| PDA + 0.01% Tween 20 control (C2) | 95.60 ± 0.51[a] | 130.89 ± 2.22[a] |
| MJ 0.05% in PDA | 61.80 ± 0.37 | 110.89 ± 3.41[b] |
| MJ 0.10% in PDA | 39.40 ± 1.08 | 99.98 ± 4.08[b] |
| MJ 0.20% in PDA | 4.80 ± 0.58 | 54.52 ± 2.97 |

*Means followed by same letter do not differ significantly at P=0.05

**Table 4:** Effect of methyl jasmonate (MJ) on spore germination percentage and germ tube length of *Fusarium solani*.

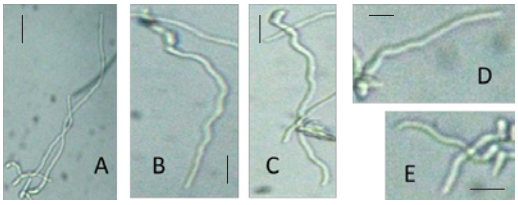


**Figure 2: (A-E):** Effects of methyl jasmonate (MJ) in PDA on germ tube length of *F. solani* (Mart.) Sacc.: A) control C1 (only PDA), B) control C2 (PDA + 0.01% Tween 20), C) 0.05% MJ in PDA, D) 0.10% MJ in PDA, E) 0.20% MJ in PDA, (Bar = 10 µ).

[16,17,23,24]. MJ in the growth medium inhibits the production of aflatoxin and mycelia pigments by *Aspergillus flavus* [23]. Al-Masri et al. [24] reported that MJ decreases mycelial growth of white mould fungus *Sclerotiana sclerotiarum* in vitro. MJ reduces spore germination and hyphal and mycelia growth of *Alternaria alternata* [17]. MJ directly inhibits the mycelial growth of *Penicillium expansum* [16]. In vitro experiment shows that MJ significantly inhibits spore germination, germ tube elongation and mycelia growth of *Colletotrichum acutatum,* the causal agent of post harvest anthracnose rot of *Eriobotrya japonica* [25].

The results obtained in the present study are significant because MJ is found to have inhibitory effects on the pathogenic fungus *Fusarium solani* under in vitro condition. This is the first report of MJ showing antifungal activity against the particular fungal species. This in turn supports the idea of probable eco-friendly use of MJ in future as a potential bio-control agent against root rot of *C. forskohlii* caused by the fungus. According to available reports MJ causes inhibition in growth of different plant organs including roots by keeping direct correlation with treatment time [26,27]. Since *C. forskohlii* (the host) is important for its root-synthesized secondary compound, any dysfunction in growth and metabolism in root organ may affect its synthesised product. Therefore it is not worthy to extend the treatment beyond the hours for peak effectiveness. Instead, for killing the pathogens (*Fusarium solani*) in this case, repeated application of MJ for short duration (by keeping safe gap in between applications) may be tried without extending the treatment time.

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