



Research Article

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Jasmonate Induced Inhibition of *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cavara in *Lablab purpureus* L. Sweet under *in vitro* Conditions



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Abstract

The objective of this study was to evaluate the protective role of Jasmonic acid in *Lablab purpureus* L. Sweet against Anthracnose disease. *Colletotrichum lindemuthianum* was cultured in PDA medium supplemented with 0.5 μ M, 1 μ M, 2 μ M and 5 μ M concentrations of Jasmonate. Supplementation of JA was found to inhibit radial growth of *C. lindemuthianum* after 7 days incubation. The effective concentration of JA was 1 μ M with 50% of inhibition of radial growth. When fresh pods of *Lablab purpureus* were punctured to a 0.5cm of diameter using a sterile cork borer and replaced with *Colletotrichum lindemuthianum* culture discs with or without JA solution the lesions began to appear after 7 days at 24 °C incubation. JA at 2 μ M concentration significantly reduced the lesion to 0.7cm. Thus, the *in vitro* study helps to confirm the protective role of JA.

Keywords: Anthracnose; Beans; *Colletotrichum lindemuthianum*; Jasmonic acid; Lesion

Introduction

Jasmonic acid (JA) is a member of plant growth regulators named jasmonates and its derivatives stimulate various physiological processes, including tendril twisting, formation of pollen, tubers and fruit ripening. They also enhance plant defense responses to mechanical impact, wounding caused by insects and pathogenic infections [1]. JA occurs ubiquitously in plants exhibiting hormone-like properties, regulating various physiological processes leading to growth and development, promotion of leaf senescence [2]. JA is a fatty-acid-derived signaling molecule, is involved in several aspects of plant biology including pollen and seed development, defense against wounding, ozone, insect pests and microbial pathogens [3]. The high concentrations of JA inhibits root growth and stem elongation and reduces its fresh and dry weight, as well as protein content and proline accumulation in plants [4]. Such reports are available based mainly on the studies carried out on intact differentiated leaves with exogenous application of jasmonate.

Till now it is considered that jasmonate particularly methyl esters of JA as a chemical stress agent mimicking the effect of that appear in response to external stress factors inducing senescence.

India is largest pulse producing country in the world, its having 18.45 million tonnes of pulse production from an area of 23.97 million ha [5]. Anthracnose caused by *C. lindemuthianum* is a devastating soil and air borne disease of pulse plants in India. *Colletotrichum lindemuthianum* (Sacc. and Magnus) Lams. Scrib., the causal organism of pulse anthracnose, is a serious soil born as well as seed borne pathogen of pulse crops throughout the world. Weather variables have profound impacts on the disease occurrence. Optimal temperature (22-29 °C) optimum, with high relative humidity (80%) and optimal rainfall was found favorable for anthracnose disease development Aggarwal et al. [6]. *Lablab purpureus* is consumed principally for its dry beans, shell beans and green pods and also a major source of dietary protein that complements carbohydrate-rich sources such as

rice, maize and cassava. The crop is a rich source of dietary fibers, minerals and certain vitamins [7]. Field losses in these regions, due to seedling, leaf, stem and pod infections, are up to 90% under climatic conditions favourable to the disease. Symptoms are broad ranging and include stem rot, dieback and seedling blight. Fruits are affected during the pre and post harvested period [8]. When *C. lindemuthianum* attack bean leaves, it causes dark brown necrotic lesions and decrease leaf photosynthesis activity [9]. Yield loss is due to early leaf senescence and plant death, shrunken seed and an increase in the amount of diseased seed that has lesions on its coat. Management strategies used to minimize seed borne infection include host resistance, cultural and chemical control methods. Harvesting drying and post harvest processing and storage operations can also be used to reduce the spread and to eradicate seed borne pathogens [10]. *P. fluorescens* was effective controlling of common bean anthracnose [11].

The present study is aimed to investigate the protective effects of Jasmonate in *Lablab purpureus* L. Sweet against Anthracnose tested *in vitro*.

Material and Methods

Fungal subculture|

Colletotrichum *lindemuthianum* active culture was purchased from Microbial Type Culture Collection at Chandigarh, India and subcultured into Potato dextrose agar medium. A small piece of the mycelium from the margin of the colony on PDA. The plates were incubated at 24 °C for a period of 14 days.

The effects of JA on the mycelial growth of Colletotrichum lindemuthianum

The effects of Jasmonate (Sigma-Aldrich, St Louis, U.S.A.) on the mycelial growth of Colletotrichum *lindemuthianum* were assessed *in vitro* PDA amended with 0.5µM, 1µM, 2µM and 5µM of JA. JA was initially dissolved in 100µl of acetone and further concentrations were prepared using sterile distilled water. JA solutions were autoclaved prior to use. For *in vitro* assessment a 0.5cm diameter mycelial disc from active PDA culture of Colletotrichum *lindemuthianum* was placed fungus-side facing down at the centre of petri dish. A control with acetone was used as a negative control whereas mycelial discs placed at the centre acted as a positive control. The inoculated plates were incubated at 4 °C and 24 °C for a brief up to 14 days.

Inoculation of bean pods

The mature bean pods were selected and the pods were surface sterilized using 100% ethanol and 0.1% mercuric chloride. The pods were punctured to diameter of 0.5cm using a sterile cork borer. For inoculating purpose, 0.5cm diameter disc was removed from the edge of an actively growing culture and placed mycelium-side down on the wound. Bean pods were inoculated with plugs of sterile PDA were used as control. The

inoculated pods were placed in sterile plastic container on moist filter paper and inoculated at 24 °C and 4 °C. The diameter of the lesions was measured periodically.

Statistical analysis

Differences between the concentrations of JA on the radial growth of the fungus and the differences between lesion diameters on bean pods were evaluated by analysis of variance (ANOVA). Fisher's least significance difference (LSD) at P ¼ 0.05 was used to compare the radial growth means.

Result and Discussion

Morphology of *C. lindemuthianum*

Colonies on PDA grew quickly, occupying the whole surface of the Petri dishes within 7d. They were white to dark grey with orange in colour colonies. They were powdery and well sporulated and had a few tufts associated with fruiting bodies the production of conidiomata was mainly restricted to the central areas, and the reverse was grayish-brown.

Effect of JA concentrations on colletotrichum lindemuthianum growth in vitro

The Colletotrichum *lindemuthianum* culture used in this study was progressively inhibited with increasing concentrations of JA from 0.5µM, 1µM, 2µM, 5µM. Fungal growth was significantly affected by all concentrations of Jasmonate after 7 d of incubation (Figure 1a). 1µM concentrations of JA 50% (2.5cm) inhibited the mycelial growth compared to the control. The inhibition percentages gradually decreased at 2µM and 5µM with inhibition ratios of 2.3cm and 2.0cm respectively (Figure 1b). Similar reports were the inhibitory effect of MeJA was also evident in the mycelium growth (Figure 1b). After one day, the mycelial growth was found to be reduced in the PDA medium containing MeJA at a concentration 0.1 and at 1mM Pczynska & Paulina [12]. The control solution with acetone had no effect on the growth of Colletotrichum *lindemuthianum*. Our present study reveals that the use of JA on *Lablab purpureus* is effective in reducing anthracnose in Bean pods. Beans treated with the higher volume of JA solutions showed a significantly reduction in lesion diameter compared to control plants. There were significantly differences between the effects of JA concentrations and incubation temperature on the lesion size of Colletotrichum *lindemuthianum*. JA was most effective when beans were stored at room temperature. When applied at identical concentrations, the compound was reported to inhibit, nearly at an identical level, *in vitro* germination of *A. alternata* (Fr.) Keissl. Isolated from stored apples [13]. In comparison with controls, the two JA concentrations significantly reduced lesion diameters at 24 °C. However, after storage at 4 °C, the anthracnose lesions in the control were significantly smaller and no significantly differences in lesion size were observed in bean pods treated with varying doses of JA the controls. Howe, 2005 indicate that this substance

activates JA synthesis in infected hosts. *In vitro* JA significantly inhibited fungal growth of *Colletotrichum lindemuthianum* and significant differences were observed in the mean growth rates of

Colletotrichum lindemuthianum at all concentrations compared to control. Increase in JA response to attack occur both locally and systematically [14].

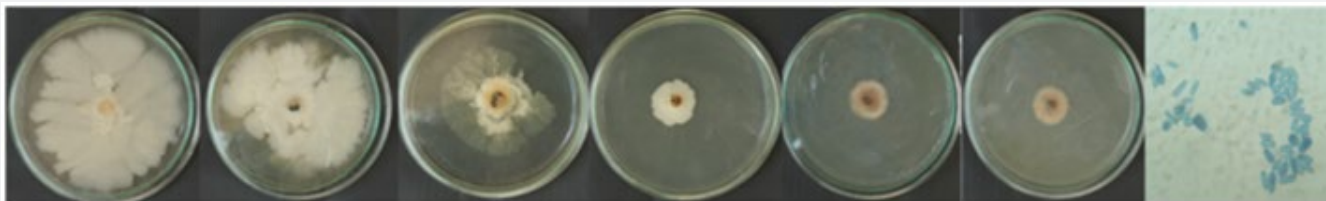


Figure 1a: Mycelial growth of *Colletotrichum lindemuthianum* on PDA supplemented with 0.5µM, 1µM, 2µM, 5µM of JA. Colony diameter was calculated 7 d after treatment.

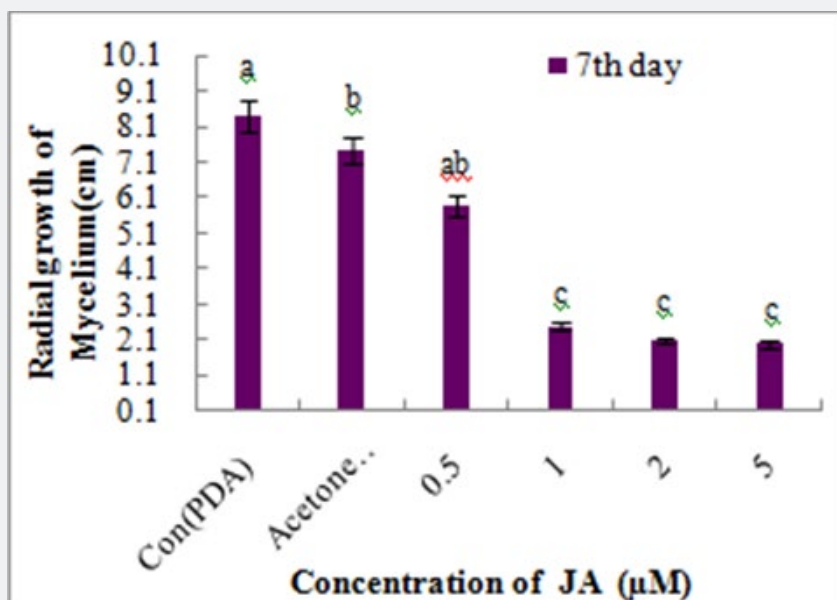


Figure 1b: Mycelial radial growth induced by four 0.5µM, 1µM, 2µM, 5µM of JA concentrations.

Effect of JA on the inoculated bean pods

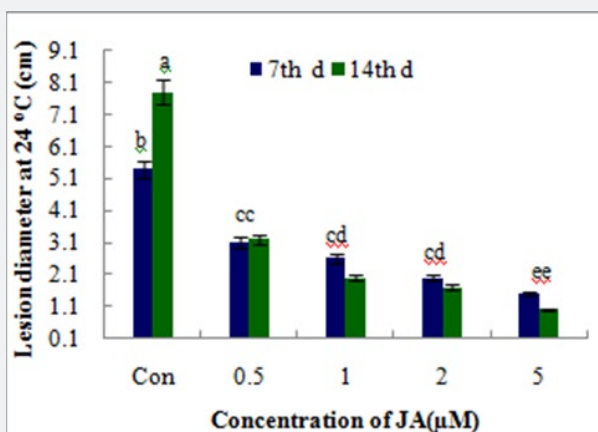


Figure 2: Lesion diameters (cm) of pods treated with JA and inoculated with *Colletotrichum lindemuthianum* 7 & 14 d after inoculation at 24 °C.



Figure 3: Anthracnose symptoms on Bean pods inoculated with *C. lindemuthianum* and stored at 4 °C & 24 °C respectively.

On the inoculated bean pods, JA solutions 2 μ M and 5 μ M significantly reduced the lesion size at 24 °C 14 d after inoculation. Lesion diameter on the inoculated bean pods reduce at 24 °C in the concentration of 2 μ M and 5 μ M of JA which are dark brown, circular infections formed. They appeared shriveled lesions were delimited by a round development symptoms and presence of conidiomata were visible after 7d incubation (Figure 2). Jasmonate significantly reduced the lesion size of bean pods treated with 2 μ M and 5 μ M lesion diameter 0.7cm and 0.6cm respectively on treated beans was significantly reduced at 24 °C compare to control (7.8cm) at 14d incubation. However, significantly differences were only detected between the 5 μ M JA concentration and the corresponding control when bean pods were stored. Anthracnose lesions on bean pods maintained at 4 °C were smaller that on stored at 24 °C and JA did not reduce

the diameter lesion at the cold storage temperature (Figure 3). Environmental factors as well as the types of pathogen infecting plants and the concentration of JA relative to each other, can affect the outcome of resulting cross talk [15]. The experimental data in this study demonstrate that the JA signaling protects against necrotrophic pathogens and insects [16-20].

Pathogenicity on the inoculated bean pods

The *Colletotrichum lindemuthianum* evaluated in this study was pathogenic to bean pod, however bean pods were more susceptible. The lesions were cut into small piece and surface sterilized into 0.1% mercuric chloride segments were placed into PDA. The plates were inoculated 7d the orange brown color colonies formed the mycelial mats were stained by lactophenol cotton blue the rod shape spore confirm the presence of *Colletotrichum lindemuthianum* (Figure 4).

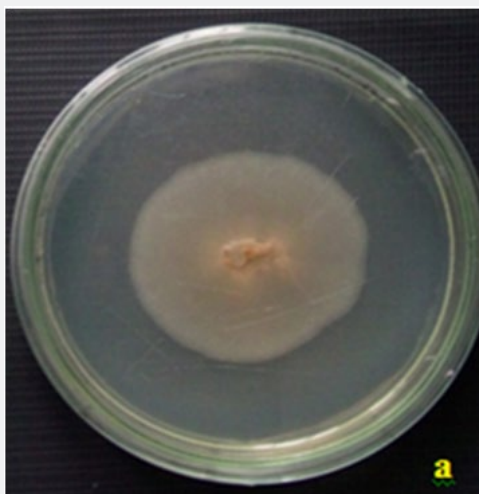


Figure 4a: Figure 4a: Pathogenicity assay of inoculated pods.

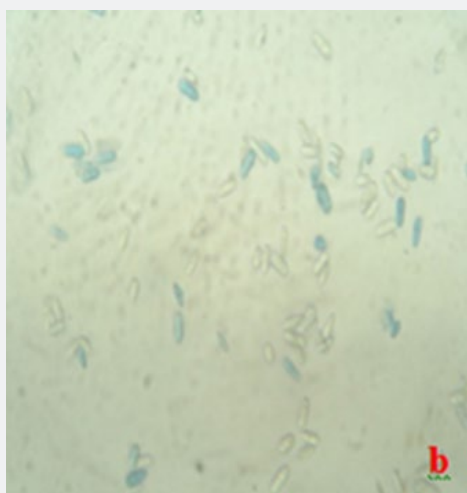


Figure 4a: Spores.

Our findings show that JA offers a safe alternative to synthetic fungicides in postharvest anthracnose disease and could be considered as a potential agrochemical of low environmental impact.

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