International Journal of Pharmacy and Biological Sciences-IJPBS™ (2021) 11 (3): 27-31
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | OA Journal | MCI Approved | Index Copernicus

Evaluation of antifungal activity of Hexanoic acid (Hx) against *Fusarium oxysporum* f. sp. *Lycopersici*

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Received: 12 Mar 2021 / Accepted: 10 Apr 2021 / Published online: 1 Jul 2021
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Abstract
Hexanoic acid (Hx) is recognised as a potential plant disease resistance inducer as well as an antifungal agent. In the present study, we have studied antimicrobial activity of Hx against *Fusarium oxysporum* f. sp. *Lycopersici* (FOL) by *in vitro* disc diffusion method. FOL is a causal agent of vascular wilt disease of tomato (*Solanum lycopersicum L.*) *and account for significant yield loss. Different concentrations of Hx* (0.01, 0.05, 0.1, 0.15, 0.2 %, w/v) *were tested against FOL and t*he maximum *mycelial* growth inhibition of 65.7 % was recorded in 0.2 % against FOL. *It suggests that Hx is an antifungal agent and its application control vascular wilt disease on tomato crop.*

Keywords
Hexanoic acid (Hx), Scanning electron microscopy (SEM), antifungal activity, *Fusarium oxysporum* f. sp. *Lycopersici* (FOL), tomato.

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INTRODUCTION
Tomato (*Solanum lycopersicum* L.) is one of the widely cultivating vegetable crops in the world belongs to family *Solanaceace*. The estimated annual production of tomato is about 180 million tonnes [1]. It is a regular component of Indian diet and grown for its edible fruit that contain major source of nutrients, minerals, vitamin A and C [2]. India is the major tomato producing and exporting country in the world [3]. However, the crop is susceptible to many pests and pathogens and account for significant yield loss. Of the many plant pathogens, *Fusarium oxysporum* f. sp. *Lycopersici* is a major soil-inhabiting fungus cause vascular wilt disease in tomato. The symptoms of *FOL* on tomato include stunting of plant, browning of vascular symptoms, yellowing, and drooping of the leaves [4]. *Fusarium oxysporum* is a soil borne fungus split into divisions called formae specialis. Each formae specialis are host specific and produce different symptoms and limit the production of several economically important crops such as banana [5], potato [6], cucumber [7] and sweet potato [8]. In order to control plant pathogens, misuse and overuse of pesticides associated with environmental and food chain contamination, health issues and development of resistance microbes [9]. Hence, many studies have focused to develop high performing pesticides that are less harmful to the health and environment [10]. Fatty acids (FAs) are organic acids categorized by the presence of a methyl group ($-CH_3$) at one end and a carboxyl group (-COOH) at another end. FAs are ubiquitous in nature, belongs to physiological important class of molecule involved in membrane structure, cell energy storage and several signalling pathways. Several studies have indicated that the large number of fatty acids have antimicrobial activity, and hence, getting attention to replace the harmful chemicals with FAs to control plants diseases [11]. Among fatty acids, hexanoic acid or caproic acid is a short chain monocarboxylic acid naturally found in several plants and animal oils and fats [12]. Studies have revealed that Hx is a

potential antimicrobial component effectively reduced the growth of several microbes including F. *oxysporum* f. *sp. Cucumerinum*, *Colletotrichum lagenarium*, *Alternaria solani, Fusarium oxysporum* f. *sp. Lycopersici*, *Botrytis cinerea*, *Trichoderma viride* and *Myrothecium verrucaria* [11, 12, 13, 14]. In the present study we have studied the *in vitro* antifungal activity of hexanoic acid against FOL.

MATERIALS AND METHODS

Fungal Culture

The FOL strain (MTCC No. 10270) was purchased from MTCC, Chandigarh, India. The required chemicals for experiments were of analytical grade purchased from Himedia and SRL, India.

Microscopic observations of *FOL*

Pure culture of FOL was maintained on Potato Dextrose agar (PDA) medium. The morphological characteristics of FOL culture was initially examined under simple microscope (stain with lactophenol cotton blue), later under SEM (JEOL JSM-IT500).

In vitro antifungal activity of Hx

Different concentrations (0.01, 0.05, 0.1, 0.15, 0.2 % w/v) of Hx were prepared in sterile distilled water and used for antifungal activity against *F. oxysporum* f. *sp. lycopersici*. *In vitro* antifungal activity was evaluated by food poisoning technique. The Potato dextrose agar (PDA) medium was sterilised at 121^0C for 15 mins and poured into sterile petridishes. After solidification of agar medium, 100 µl of Hx spread evenly over the medium by a sterile glass spreader (different concentrations on different PDA plates). Later a small bit of mycelium was taken from 7 days old *FOL* culture and placed on the centre of the Hx spread PDA plates. Similarly, the same volume of mycelium was placed in PDA plate (without Hx) used as control. Further the petriplates were incubated for 4 days at 25^0C and after the colony diameter was measured in centimetre (cm). Duplicates were maintained for each Hx concentration and percent inhibition of mycelia growth was calculated by using the following formula [15].

$$\text{Percent Inhibition} = \frac{\text{mycelial growth in control} - \text{mycelial growth in treatment} \times 100}{\text{mycelial growth in control}}$$

RESULTS AND DISCUSSION

Fatty acids are known to possess antibacterial, antimalarial, and antifungal activity [14]. The precise mechanism for antifungal activity of fatty acids is remain unclear, however based on some reports suspecting that the fatty acids interact with the cell membrane of the fungal cells and increase membrane fluidity, which further leads to leakage of the intracellular components and subsequent death of pathogens [16]. In a study, the antifungal activities of nine fatty acids (butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, oleic acid, and linoleic acid) against four phytopathogenic fungi: *Alternaria solani*, *Colletotrichum lagenarium*, *Fusarium oxysporum* f. sp. *Cucumerinum*, and *Fusarium oxysporum* f. sp. *lycopersici*, were assessed via petridish assay and found inhibition of mycelial growth and spore germination [11]. Similarly, essential oil of *Cinnamomum tamala* and *Lantana camara* showed effective antifungal activity against *Colletotrichum gloeosporioides*, *Fusarium oxysporum* and *Alternaria alternata* at different concentrations [17,18,19, 20]. This is because of the presence of different bioactive constituents like β-ionone, hexanoic acid, caryophyllene oxide, Eicosane, squalene, β-caryophyllene and tiglic acid [21]. Studies have identified that Hexanoic acid is a potential antifungal agent which has controlled an economically important plant fungal pathogen *Botrytis cinerea*, [12, 13]. Similarly, Pohl et al., [14] noticed that Hx was effectively controlled *Trichoderma viride* and *Myrothecium verrucaria.* Instead of using toxic fungicides for the control of plant pathogens, supplementing with fatty acids is an eco-friendly approach and they might offer new strategies to control plant-pathogenic fungi in future sustainable agriculture.

Fusarium oxysporum (F. oxysporum) is a fungal plant pathogen infects wide range of hosts and cause significant yield loss upto 45 % [22]. It infects hosts such as tomato [23], sweet potato [8], tobacco [24], potato [6], cucurbits [25], banana [5], legumes [26] and other herbaceous plants. The present study is to evaluate the antimicrobial effect of Hx against FOL *in vitro* petriplate method. The pure culture of *FOL* has shown white cottony aerial mycelium with pinkish pigmentation on the PDA plates. Microscopic observations (SEM) of the culture have shown features including 3 septate macroconidia with monophialides on conidiophores and spherical chlamydospores with smooth or rough walled formed singly are pairs (Fig1a & 1b). Similar characteristic features of *FOL* were reported by Kee et al., (27). In order to reveal the antifungal activity of Hx, five different concentrations (0.01, 0.05, 0.1, 0.15, 0.2 %, w/v) of Hx were tested against *FOL*. The results indicating a significant positive relation between concentrations of Hx and the mean percentage inhibition of fungus *F. oxysporum* f. *sp.*

lycopersici. Data presented in table 1 indicates that the percentage of antifungal activity was varied between 45-67.8%. Among Hx treatments, the maximum mycelium growth inhibition was 67.8 % at 0.2 % concentration whereas the minimum inhibition was 45 % at 0.05 % concentration (Fig.2). The remaining concentrations viz. 0.01, 0.1, and 0.15 % showed 58.5 %, 62.8 % and 61.4% respectively. Hexanoic acid treatment induced a phosphate efflux from treated mycelium and also alters the polyamine metabolism suggesting an alteration of membrane that trigger rapid cell death (12). In a similar study, linolenic acid has shown antimicrobial activity against *R. solani* and *P. ultimum* and *C. perniciosa* at different concentrations [28].

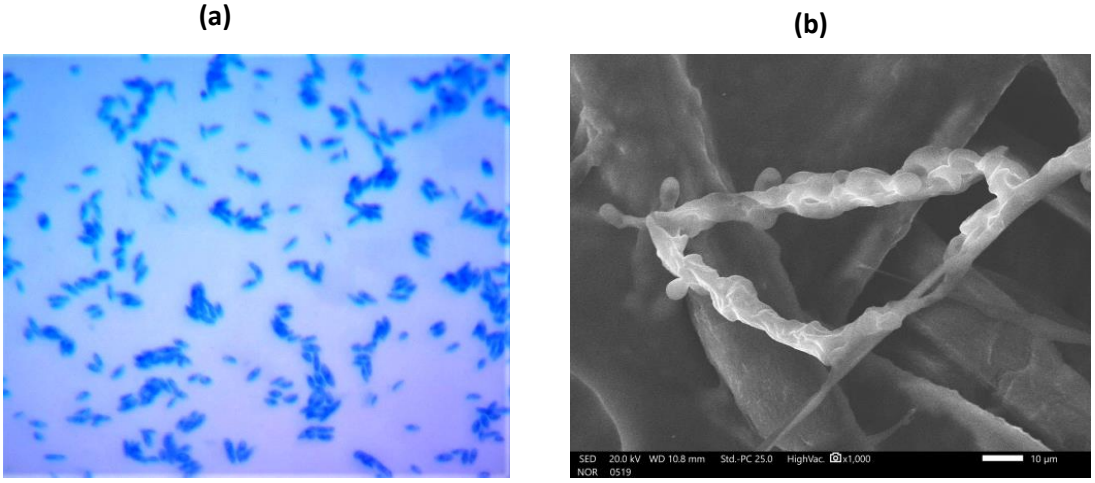

Fig1. *Fusarium oxysporum .sp.* L image under a) Light microscopy b) SEM

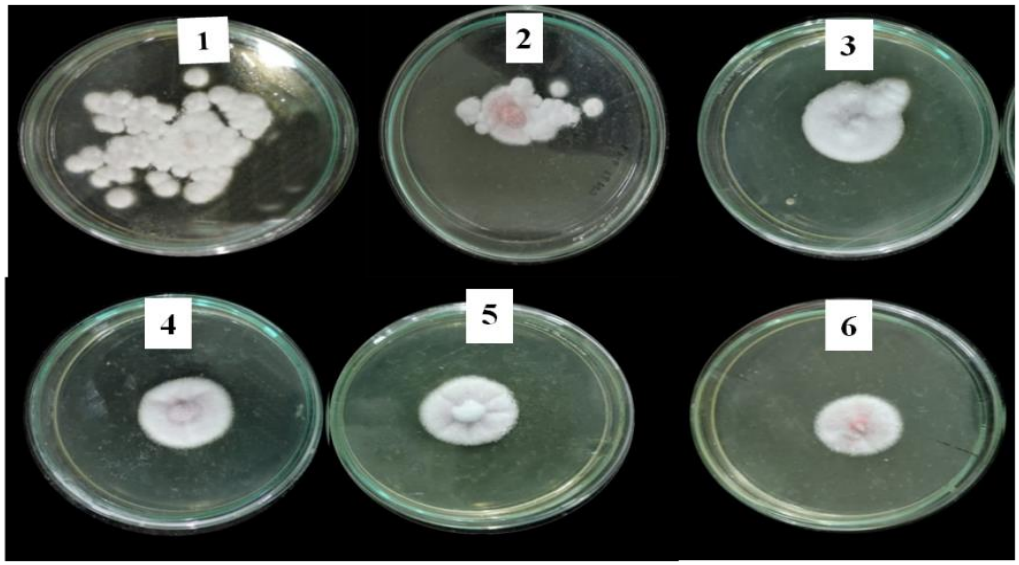

Fig2. Effect of different concentrations of HX on mycelia growth of *F. oxysporum* Control (1), HX 0.01 % (2), HX 0.05 % (3), HX 0.1 % (4), HX 0.15 % (5), and HX 0.2 % (6)

Table 1. Effect of different concentrations of Hx on mycelial growth of *FOL.*

Hx concentrations	Growth of mycelia (cm)	Inhibition of fungal growth (%)
Control	7.5	-
0.01 %	2.9	58.5 %
0.05 %	3.85	45%
0.1 %	2.6	62.8%
0.15 %	2.7	61.4%
0.2 %	2.4	65.7%

CONCLUSION

Fusarium oxysporum f. sp. *Lycopersici*, a fungal plant pathogen causes vascular wilt disease in tomato. In the present study it was identified that hexanoic acid had antifungal activity against the *FOL*. Among the Hx concentrations, 0.2 % has shown higher antifungal activity compared to other concentrations against *FOL*. Therefore, Hx application for control of *FOL* at field level might be an eco-friendly and healthy approach. However, comprehensive field-based studies remain needed to use Hx as a antifungal component at fields levels.

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