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Biofungicidal and Bicontrol Activity of *Cannabis sativa* Against Pytopathogenic *Phytophthora capsici* Fungi.

Rajeev Kumar[1*], Romika Bhardwaj[2], Preeti[2], Sameer[2], Jatin[2] and Sachin Kumar[2]

[1]Department of Bioscience and Bioengineering, Lovely Professional University Phagwara, Punjab, India
[2]Global Research Institute of Pharmacy, Radaur, Haryana, India

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Abstract

In vitro fungitoxic activity of Cannabis sativa leaf extracts against Phytopthora capsici isolated from (Solanum Tuberosum potato and Capsicum annuum) was studied in acetone, ethanol, methanol, and water in various solvents. The food poisoning technique was used to measure fungitoxicity, which comprised utilising different extracts at 200mg/ml and documenting their activity as radial growth and percentage inhibition. The only extracts that totally stopped the fungus from developing were ethanol and acetone extracts. The fungus was 50% suppressed by methanol, while the aqueous extract had no impact. The findings suggest that ethanol and acetone extract of Cannabis sativa can be used as a biofungicide to control pathogenic fungi.

Keywords: *Phytophthora, Cannabis, Biocontrol, Fungi, Fungicides.*

Introduction

In Developing Countries more than 800 million people do not adequate food and 10% food is lost due to plant disease which is caused by pathogen likes fungi bacteria, nematodes & viruses. Fungi affect the plants most and crop production losses. There are some medicinal plants which are naturally god gifted with invaluable bioactive compounds. These plants form the backbone of our traditional medicines. From Ancient time, many plants are used as medicines. 80% people of the earth depends on herbal plants/ medicines for their primary health care. Many diseases can be treated by the use of herbal medicines such as, In the case of many infectious diseases can be treated with herbal remedies.[1] Herbal fungicides are mostly use nowadays because they are eco-friendly. Fungi are ubiquity in the atmosphere. So, the fungal infection is become very common nowadays due to fungal pathogens. The most important method by which we can protecting the plants against the fungal attack is the use of fungicides. There are many fungicidal agents non- eco-friendly[2]. Cannabis holds an important position in Ayurveda because of their use in medicines. There are some well-known varieties of Cannabis, *Cannabis sativa, Cannabis indica* and *Cannabis ruderalis*. They are found in Central and South Asia. In the earlier cannabis was placed in the family Urticaceae or Moraceae, but after some time it includes in cannabacea.

*Corresponding author's ORCID ID: 0000-0001-6803-5101
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Cannabis sativa is also known as bhang in India. This plant has great importance in Hinduism. It is used during the worship of Lord Shiva. All the parts of these plant-like leaves, stems, bark, flowers and seeds are used in medical purpose. It is found in temperate and tropical regions of the world. It can easily/ commonly available in India in waste grounds, along road sides[3].

Cannabis sativa

Cannabis also having a number of terpenoids which makes a large percentage of its essential oil. More than 120 terpenoids have been found in this, including 58 monoterpenes, 38 sesquiterpenes, 1 diterpene, 2 triterpenes and 4 other terpenoids. The essential oil of cannabis can be obtained by process of steam

distillation[4]. The height of *Cannabis sativa* is approximately 1-2 m. About 4000 B.C, it was estimated that the cultivation of *Cannabis sativa* is first started in Russia[5]. *Phytophthora capsici* was first time introduce by Fabian Garcia. They described about Phytophthora which is Isolated from chile pepper in New Mexico by Leon H Leonion at the New Mexico Agriculture Research station in Las cruces in 1922[6].*Phytophthora* is a very harmful fungus for many crops and plants. *Phytophthora capsici* is vital oomycete phytopathogen that produces harmful effects on crop or plants foliar blight, domping –off , root, stem and wilting and fruit rot in many vegetable. It attacks mainly potato, tomato , all cucurbits and eggplant and more newly snap and lima beans[8].In India it was first introduced into Nilgiri hills. *Phytophthora* is a Greek word which indeed mean plant destroyer. This name was comes out when Anton de Bary Investigated the potato disease in 19th century. Due to this harmful effect it play a important role in Economic condition spoil. Because every country depends on profit from export of its crops and the population of the countary is also dependent on crops for food somewhere. If we take an example , The potato crop is a major source of income for millions of the country population. India scientists have detected 19 variants of the pathogen named *Phytophthora infestonts* responsible late blight disease in potato crop. Due to this disease the size of potato shrinks and it starts rotting from inside. This disease is capable of killing the entire crop in 2-3 days. If we take on example, In Ireland, the potato crop was completely destroyed between 1845 and 1849. About 40 % of the population here used to satisfy their hunger with potatoes; such was the effect of the famine that about 10 lakh people left the country and 10 lakh people died. Due to this reason the population of Ireland suddenly decreased by 20-25 % at that time. According to the website britannica, at that time the potato crop was prone to lafe blight disease. This disease is caused by a fungus called Phythophthora. There was an outbreak of this disease in the potato crop in almost all of Europe, but Ireland was must affected and now this disease has spread all over the world. Plant pathogenic oomycetes such as *Phytophthora capsici* cause destructive disease in a large range of cropsuniversal.[8]

Material & Method

Collection of plant parts: Fresh and healthy leaves of *Cannabis sativa* were collected from different localities of Yamuna Nagar (Haryana, India). These collected leaves were thoroughly washed, shade dried and converted into powdered form by the use of mixer grinder.

Preparation of plant extract: For the preparation of plant extract the concentration which was selected was 200 mg/ml, so 20 gm leaf powder were mixed with 100 ml of respective solvents (methanol, acetone, ethanol and aqueous). The extraction was carried out by soxhlet extraction method and followed by filtering using Whatman's filter paper No. 1. After filtration it was evaporated until 1/5th of the total volume remained and the final content was stored at 4°c for further use in airtight bottles.

Isolation of Test Fungus: *Phytophthora* were isolated from the infected plant of capsicum. The media used was Potato Dextrose agar (PDA). The pure culture of fungus was maintained on PDA at 27±2°C.

Antifungal Assay: Antifungal activity of plants was determined by Food Poison Technique. 3ml of standard extracts was mixed with 50 ml of potato dextrose agar (PDA) and autoclaved. Autoclaved media was transferred into petriplates aseptically. After solidification of media, they were inoculated with 3 mm inoculum plug of the 7 days old culture of test fungus and incubated at 27+2°C for 7 days. After the period of incubation the radial diameter was measured in mm. Petriplates without the test extracts but with same amount of sterilized water served as negative control while the petriplate along with antifungal griseofulvin (5 mg/ml) served as positive control. Radial mycelium growth on different extracts was transformed into inhibition percentage by using the following formula[9,10,11]

Inhibition percentage = (Gc-Gt/Gc) x 100

Gc= Radial diameter of control - diameter of inoculum plug; Gt = Radial diameter of plate with extract – diameter of inoculum plug.

The experiment was carried out in triplicates. The result presented in table are based on the mean values of all replications.

Result & Discussion

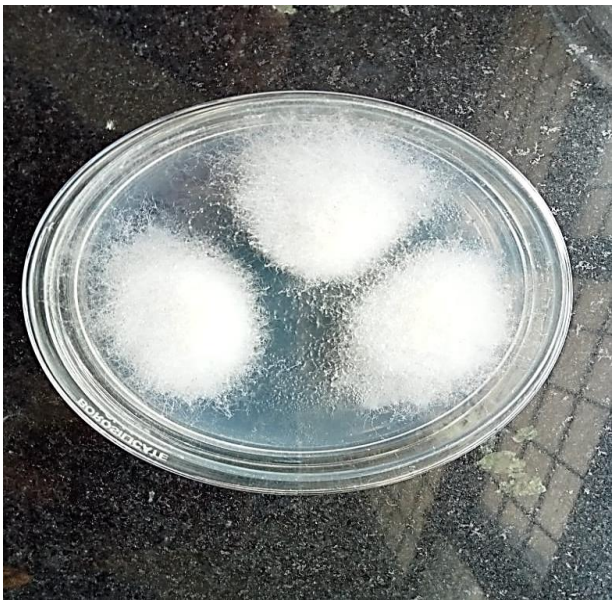
Various extracts of *Cannabis sativa* yielded different outcomes in studies. The antifungal activity of *Cannabis sativa* against the test fungus is shown in Table A& B. The aqueous extract of the leaves of *Cannabis sativa* found to be almost inactive against test fungus. The percentage inhibition given by aqueous extract was 0.88% in *Capsicum annuum* with radial diameter of 50 ± 0.3mm and 0% in *Solanum tuberosum* with radial diameter of 52 ± 0.1mm. The methanol extract showed approximately 50% inhibition i.e. 50.99% in *Capsicum annuum* and 53.18% in *Solanum tuberosum*, while inhibition percentage with ethanolic and acetonic extract was found to be 100%. The petriplates showing negative control gave the radial diameter of 0±0.0 mm in *Capsicum annuum* while 0±0.0 mm in *Solanum tuberosum*. The antifungal activities of ethanolic and acetonic extracts of leaf powder of *Parthenium hysterophorus* were significantly active against the tested organism, while methanolic extract had lower antifungal activity and aqueous extract had no activity against the tested organism, according to the above experimental results.

Table 1

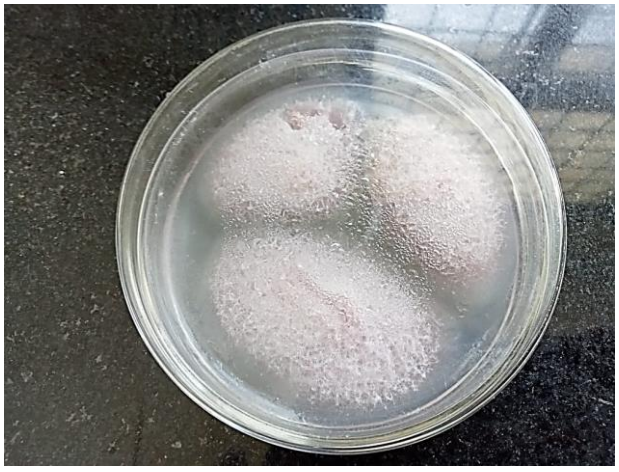
Radial Growth (mm)		
Extracts	*Capsicum annuum*	*Solanum tuberosum*
Aqueous	50 ± 0.3	52 ± 0.1
Methanol	22 ± 0.15	21 ± 0.12
Ethanol	0 ± 0.0	0 ± 0.0
Acetone	0 ± 0.0	0 ± 0.0
Control	58.5 ± 0.1	54 ± 0.15

Table 2

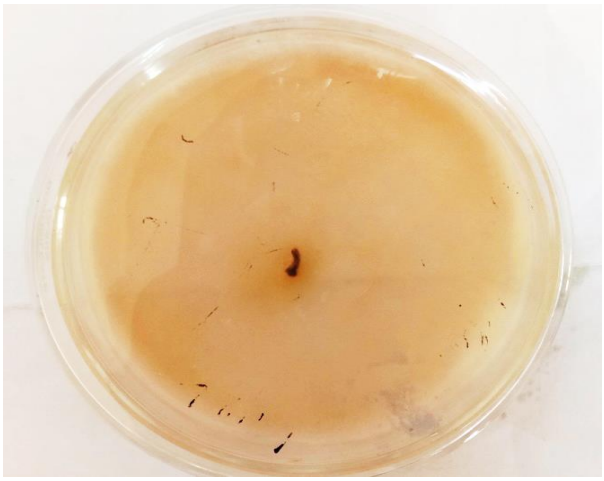
Percentage Inhibition (%)		
Extracts	*C. annuum*	*S. tuberosum*
Aqueous	0.88	0%
Methanol	50.99	53.18
Ethanol	100	100
Acetone	100	100
Control	____	____



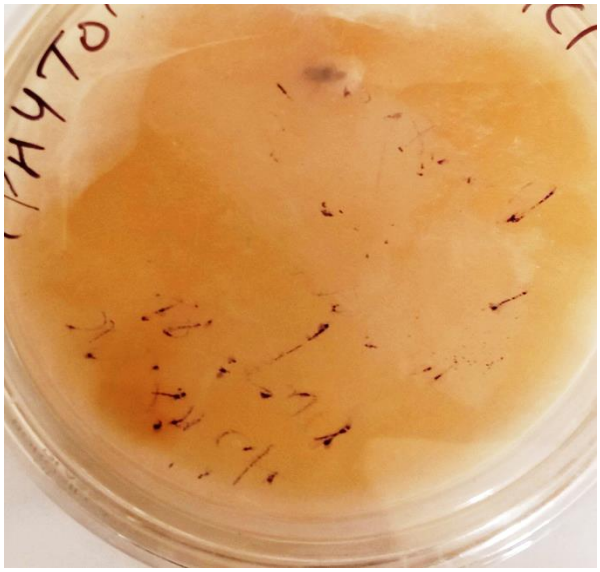
(A) Acetone



(B) Ethanol



(A) Acetone



(B) Ethanol

(*Cannabis Sativa* shows 100% inhibition against *Phytophthora capsici*)

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