Antifungal Effect of Caffeine on Red Rot Disease in Indian Sugarcane

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Abstract

Caffeine is a secondary metabolite and is found in tea, coffee plants used to defend it against predators and competitors. In this study the caffeine was obtained from tea extract using liquid-solid phase transfer method. Caffeine inhibited the growth of *Colletotrichum falcatum* extracted from infected stalk of *Saccharum officinarum* on oat meal agar plates containing 25 to 250mg caffeine per 100mL. It was found that the action of caffeine on *Colletotricum falcatum* was significantly high at two concentrations 230 and 250mg. Gel exclusion method was used to study the effect of caffeine on red rot disease in Indian sugarcane. In this study 1 to 5mM of zone of inhibition was obtained. It showed that it was a reliable and eco-friendly method over chemical control methods.

Keywords: Caffeine, Secondary metabolite, *Colletotricum falcatum*, Predator, *Saccharum officinarum*, Liquid-solid phase transfer method.

Highlights

- A biological control method is developed to inhibit the growth of *C. falcatum.*
- Caffeine was obtained by liquid-solid and liquid-liquid extraction method.
- Caffeine as an antifungal at different concentration was used to kill *C. falcatum*.
- Low concentration up to 200mg of caffeine was not effective on *C. falcatum.*

Chemical Compounds

Caffeine (PubChem CID: 2519); Chloroform (PubChem CID: 6212); Ethanol (PubChem CID: 702); Sodium hypochlorite (PubChem CID: 23665760).[8-11]

Introduction

The population of world is still growing; food and energy production needs to be increased. Agriculture is the backbone of food with high yield of sugar and cereals as well as renewable source of energy. Plants need a best biological control method for higher productivity.[7] Sugarcane (*Saccharum officinarum*) is the most economical crop of the world and used as the main source of sugar from hundreds of years but recently to produce bioethanol as a renewable energy source (Menossi et al. 2007). Moreover, the bagasse of sugarcane has been largely used for animal feed and also for paper production (Pandey et al. 2000). Nevertheless, as other cash crops, sugarcane cultivation faces severe losses due to red rot disease.

The red rot disease is caused by *Colletotricum falcatum* is the most disastrous disease among sugarcanes and a big threat to both cane growers and sugar industry (Alexander et al. 1997). Due to this fungus about 30% to 40% sugarcane crops destroy per year which has no use in future. Infection of *C. falcatum* can be inhibited by use of antifungal agent in sugarcane.

Caffeine is an alkaloid and found in more than 60 plant species. It is widely consumed food constituent in the world as a nervous stimulator (Nehliq et al. 1992). Leaves of green tea contain polyphenols, caffeine (1-3%) and an enzymatic mixture thease (Pawar et al. 2011). Caffeine has various properties such as antioxidant, antibacterial, antifungal and insecticidal.

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In North America 90% adult consumes caffeine daily in form of coffee, soft drinks and energy drinks (Mendick et al. 2005). It has also been reported to be most effective antimicrobial agent against gram positive bacteria than gram negative bacteria but it shows least effectiveness against fungus. The main objective of this work is to study the antifungal effect of caffeine against *C. falcatum* that cause red rot disease among sugarcanes.

Materials and Methods

Chemicals

Chloroform, 1% sodium hypochlorite, 70% ethanol, agar, oat flakes, oatmeal were used in this experiment. Caffeine was obtained from green tea leaves.

Plant Material and Microorganism

Camellia sinensis leaves were purchased from local market in Assam (India) and an infected stalk of *Saccharum officinarum* was taken from a field that was in National Sugar Institute of Kanpur (latitude 26.510297 and longitude 80.2509504) (India). A highly virulent strain of *C. falcatum* was isolated from lesions of infected stem piece of sugarcane. Infected cane was split and opens by sterilized knife which was observed from reddish tissue along with white transverse band. One 5 mm piece of tissue was taken from margin of infected tissue, surface sterilized by dipping in 1% sodium hypochlorite for 1 min, immersed in 70% ethanol for 1min then rinsed three times with distilled water and finally dried on sterilized tissue paper.

Preparation of Sample

15g Green tea leaves were boiled with 300mL distilled water for 20min using hot water bath. Then, the solution was filtered using filter paper to get rid of the solid particles and leaves of green tea. Tea extract initially prepared were mixed with chloroform in a fume hood. Then, using reverse phase filter paper to filtered chloroform-caffeine solution by vacuum filtration that trapped water molecules only. The chloroform solution was boiled above the boiling point of water in hot water bath under fume hood to evaporate the chloroform. The crystals of caffeine were obtained after evaporation.

Inoculum Preparation

Oat meal agar media was used for growing *C. falcatum* which had the following composition: (g/mL): agar-1.5g; oat flakes-1.0g and oatmeal-1.0 at pH 7.0±0.2. The fungus *C. falcatum* was grown on oat meal agar media at 25 °C for 24H inside incubator.

Testing of Caffeine on *C. falcatum*

For the testing of Caffeine, zone exclusion method is used. Ten Oat meal agar plates were prepared. In this process all the oat meal agar plates were spread by isolates of *C. falcatum.* Wells were prepared in Petriplates after the process of spreading of *C. falcatum*. After the formation of wells caffeine solutions containing 25, 50, 75, 100, 125, 150, 175, 200, 225, 250mg per 100mL of water were poured in the wells. Now those Petriplates were kept in incubator at 25°C for 24-48H for the proper growth of *C. falcatum*. After the incubation process the Petriplates were examined carefully for the zone of inhibition from the wells to estimate the potential of extracted caffeine.

The following table shows the effect of caffeine on the *C. falcatum* growth on oat meal agar plates.

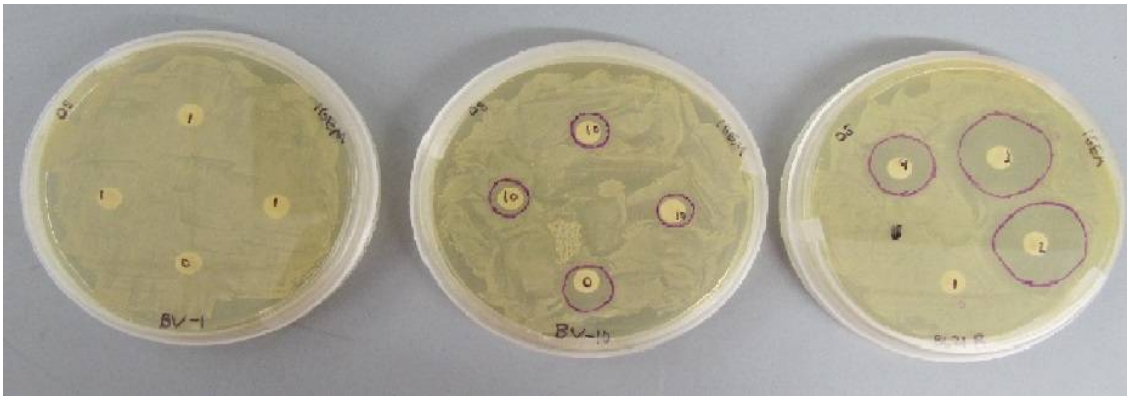
S. No.	Concentration of Caffeine (mg)	Zone of Inhibition (cm)
1.	25	No effect
2.	50	No effect
3.	75	0.01
4.	100	0.10
5.	125	0.50
6.	150	0.65
7.	200	1.00
8.	225	2.00
9.	250	2.25
10.	Control	High growth

Temperature during experiment was constant at 25°C and time of incubation is 24-48H.

Table 1.Effect of Caffeine on the *C. falcatum* Growth on Oat Meal Agar Plates

Results

Caffeine showed antifungal activity against *C. falcatum*. The highest inhibitory effect was observed against *C. falcatum* on the 250mg of caffeine concentration while no activity was seen on the 25 and 50mg concentration of caffeine against *C. falcatum*.



a) 25mg of caffeine **b) 225mg of caffeine** **c) 250mg of caffeine**

Figure 1.Zone of inhibition of *C. falcatum*

Discussion

Caffeine is an excellent natural compound because of its various used such as a nervous stimulator, including antioxidant, memory booster, antibacterial, antifungal, fertilizer and insecticide. Based on these properties, caffeine can be used as an antifungal and natural fertilizer in sugarcane field to prevent the loss of crop by the inhibition of *C. falcatum* and to increase the macronutrients in the field. On the earth, this is the most abundant and widely used compound.

In previous studies it has been shown that the caffeine only inhibits the growth of *Rhizopus stolonifer, Absidia heterospora, Penicillium* and fungi of *Aspergillus* group. However, the antifungal effect of caffeine against *C. falcatum* was not clear. By this study, the antifungal effect of caffeine against *C. falcatum* was confirmed. The antifungal effect of caffeine against *C. falcatum* may be due to the fact of that the caffeine inhibits the transcription of mRNA which are responsible for the formation of chitinase, receptor protein kinase and metallothionein in *C. falcatum*.

Conclusion

This experiment show a clear antifungal effect of caffeine against *C. falcatum* and the inhibitory effect of caffeine to *C. falcatum* can vary on caffeine concentration. *C. falcatum* was more sensitive to 250mg concentration of caffeine. The current study provides a convenient and cheapest method to stop the red rot disease among sugarcanes for farmers, sugarcane based industry and researchers.

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References

[1] Genitile A, Ferreira HT, Mattos SR et al. Effect of drought on the microtranscriptome of field grown sugarcane plants. *Planta* 2012; 237(3): 783-89.

[2] Khokhar I, Mukhtar I, Mushtaq S. Isolation and screening of amylolytic filamentous fungi. *Journal of Applied Sciences and Environmental Management* 2011; 15(1): 205-206.

[3] Mendnick CS, Cai JD, Kanady J et al. Comparing the benefits of caffeine, naps and placebo on verbal, motor and perceptual memory. *Behavioral Brain Research* 2008; 193(1): 79-86.

[4] Mohanraj D, Padmanaban P, Viswanathan R et al. Sugarcane screening for red rot resistance. *Sugarcane* 1997; 3: 18-23.

[5] Nehliq A, Daval JL, Debry G. Caffeine and the central nervous system: mechanism of action, biochemical, metabolic and psychostimulant effects. *Behavioral Brain Research* 1997; 17(2): 139-70.

[6] Pruthviraj P, Suchita B, Shital K et al. Evaluation of antibacterial activity of caffeine. *International Journal of Research in Ayurveda and Pharmacy* 2011; 2(4): 1354-57.

[7] Warsi S, Siddique A, Yadav S et al. Extraction and identification of indole-3-acetic acid synthesized by rhizospheric microorganism. *International Journal of Sciences: Basic and Applied Research* 2014; 15(1): 475-78.

[8] Caffeine. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/2519/09/17/2015.

[9] Chloroform. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/6212/09/17/2015.

[10] Ethanol. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/702/09/17/2015.

[11] PubChem Compound. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/23665760/09/17/2015.