Research Area 2

Plant based Antimicrobial compounds against Aspergillus flavus and A. parasiticus in Peanuts

In addition to gene manipulation to control Aspergillus sps in peanuts, my research also focuses on use of plant based antimicrobial compounds such as essential oils/vapors and probiots to prevent Aspergillus contamination. According to FAO, approximately 25 million dollars are lost per year due to the contamination of peanuts by Aspergillus spp. and aflatoxins. *A. flavus* is the common fungus contaminating peanuts and destroying peanuts shells before they are harvested and produce aflatoxins which are known to be both highly toxic and carcinogenic, threatening humans, livestock and crops worldwide. Prevention and decontamination of the mycotoxigenic Aspergillus species and aflatoxins in peanuts is of paramount importance because, it is the world's fourth most important source of edible oil and third most important source of vegetable protein. At present, disease management in crop fields is practiced solely through adaptation of suitable cultural practices such as rotation, use of quality seed and fungicides and altering the time of planting. In addition to post-harvest procedures through use of chemicals for aflatoxin prevention, several other viable means to prevent the contamination process in crops before harvest are being undertaken in several laboratories around the world. Use of chemicals to control Aspergillus species has proven to be unsuccessful and detrimental to the environment. Further, many disadvantages are associated with the use of chemical preservatives as antifungal agents. Extensive use of these substances might produce several side effects such as carcinogenicity, teratogenecity and toxicity to consumers.

There is an increasing interest in use of alternative antimicrobial agents from plants to control toxigenic Aspergillus species. One such alternative is the use of natural plant products with antifungal properties. Recently, there has been considerable interest in the use of essential oils and their vapors from aromatic plants with antimicrobial activities for controlling Aspergillus species in peanuts. The main reason for considering the essential oils and the vapors, as antifungal agents, is that the majority of the essential oils are classified as Generally Recognized As Safe (GRAS), their use in foods as preservatives is often limited due to flavor considerations. This will obviously reduce exposure to the legal, environmental, and public safety hazards . While the quest to control Aspergillus spp., using both conventional and advanced techniques continues here in Georgia and US as a whole, the aflatoxin contamination in peanuts is far from over. This study identifies edible plants based essential oils for their antifungal properties against *A. flavus* and *A parasiticus*. This research is a novel idea to pest management program and aflatoxin contamination peanuts. Moreover, the use of essential oil as antimicrobial agents, over protectant fungicides or developing a resistant cultivar either by conventional or genetic engineering may apply directly to the growers' margin of profit and at the same time keeping peanut consumers healthy.

Specific Objectives:

1. To screen and select plant-based essential oils for their antifungal activity to prevent the growth of aflatoxigenic A. flavus.

2. To chemically characterize the active principles of essential oils for their antifungal properties.

3. To determine Minimal Inhibitory Concentration (MIC) and Minimal Fungicidal Concentration (MFC) of the essential oil components.

4. To quantify aflatoxin (B1) production by *A. flavus* exposed to specific essential oils.

5. To understand the mechanism of action of active principles of essential oil components against *A. flavus.*

Potential contributions of the study include the following:

- The present study will shed light on efficacy of essential oils to control A. flavus in peanut from different agro-climatic zones of Georgia and elsewhere in the US.

- The study may form part of further research to ease the management practices for the prevention of aflatoxins by Aspergillus species in peanut, other nuts, and food grains in theUS.

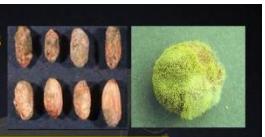
- Our research findings can be used to overcome growers' problems with Aspergillus species in peanut growing countries around the globe and to enhance consumer value in peanut and peanut products.

- The efficacy of essential oils as antifungal agents against *A. flavus* in our study may become suitable for applications in food industry. The purpose for their suitability is their natural origin, which consumers find comforting, and eco-friendly and which is beneficial to the environment. These beneficial characteristics could increase food safety and shelf life.

- In view of Kennesaw State University's commitment to Global Learning, this partnership will strengthen the existing international research collaboration between the US and India and promote transfer of technology.

Plant Based as Antifungal Agent Against *Aspergillus* species in Georgia peanuts

Reesheda Gilbert, Premila N. Achar and C Phillips*
Department of Biology and Physics, Kennesaw State University, Kennesaw, GA
*College of Health sciences, University of Northampton, UK



Abstract

A. flavus is the most common strain of *Aspergillus* that causes crop contamination and a common threat to peanut industries worldwide. In Georgia *A. flavus* continues to be a major setback in the peanut industry. Safe and ecological friendly methods for controlling *A. flavus* with antimicrobial compounds such as essential oils are being explored to replace chemically based fungicides. Although genetically engineered proteins are being persuaded as another method of control, it is not cost-effective. Essential oils derived from aromatic plants such as cinnamon and clove have clinically displayed antifungal characteristics. Our study tested the synergistic effects of both cinnamon and clove oil vapors. *A. flavus* spores were exposed to different concentrations at 24, 72, and 96 h and incubated for seven days. Exposure time correlates with growth of *A. flavus*. Further studies will focus on active ingredients these oils vapors to show their potential as biological control agents.

Introduction

Essential oils are highly regarded as being general recognized and safe (GRAS) and uniquely characterized for their weakness not to facilitate microbial resistance. EO vapors have been reported as antimicrobial agents (Maruzzella and Sicurella 1960). Mycelial growth and spore germination of *P. chrysogenum*, *A.niger*, and *A.alternata* decreased when exposed to Citri-V™ (Phillips et al., 2011). There are several reports on the antifungal activities of essential oils against fungi. Essential oil vapors provide a natural approach to decrease the susceptibility of aflatoxin producing *Aspergillus* from proliferating at an exponential rate. Recently, Mungai and Achar, 2011, reported the Minimum Inhibition Concentration (MIC) of cinnamon oil (500ppm) and clove oil (1000ppm) and established that these oils have synergistic effect against *A. flavus* and *A. parasiticus*. This study aims to isolate and characterize *A. flavus* from contaminated peanuts, and determine the efficacy of plant based essential oils vapors (EOV) as antifungal agents against *A. flavus* in peanuts. It is hypothesized that essential oil vapor can be used as antifungal agents against *A. flavus*.

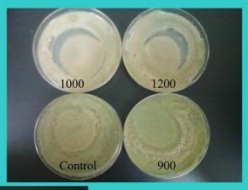


Fig. 1. MIC of clove oil vapor against *A. flavus* at 1000 ppm, 24h exposure.

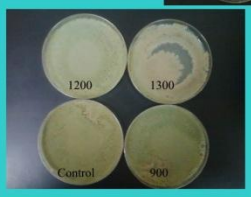


Fig. 2. MIC of cinnamon oil vapor against *A. flavus* at 1300ppm, 24h exposure.

Materials and Methods

Materials: Potato Dextrose Agar, Clove oil, Cinnamon, Tween 80, Ampicillin. *A. flavus* spores. All tests were replicated twice.

Determination of Minimum Inhibitory Concentration (MIC) of essential oil vapors: *A. flavus* from peanuts in Georgia, subcultured in PDA (Potato Dextrose Agar) for seven days at 37 °C. Essential oils tested for antifungal activities at various concentrations. Plate diffusion wells: 1 ml mold suspension inoculated on sabouraud agar Petri dishes (Hadeacek and Greger, 2000). Clove oil vapor tested for MIC at 800, 900, 1000, 1200 and Cinnamon at 900, 1100, 1200, and 1300 ppm. Each concentration added on sterile filter discs (5mm) and inverted underneath the top cover of the plate. Tween 80 served as controls. Concentrations indicating zones of inhibition (cms) greater than 10 mm diameter considered to be MIC.

Determination of the Minimal Fungicidal Concentration (MFC) of essential oil vapors: The Minimal Fungicidal Concentration (MFC) of the selected oil vapors determined (Pinto et al., 2009). Agar plates inoculated with 1ml of mold spores. Serial dilution and incubation done as above. The concentration indicating inhibition (cms) halos equal or higher than 10 mm diameter considered as MFC.

Vapor Chamber Method: paper discs (5 mm), inoculated with different concentration of essential oil, each was placed separately on evaporating crucibles. PDA plate with colonies of *A. flavus* placed inverted on the crucible. A 600 ml beaker was then inverted on top of the crucible-PDA- *Aspergillus* set up to form a growth chamber, sealed with Para film and foil, incubated at 37 °C for 24, 72, 96 h. Inhibition zone was measured as above.

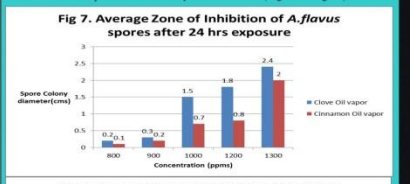
Results

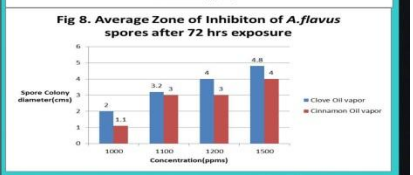
Fig. 3. & Fig. 4. *A.flavus* spores exposed to clove and cinnamon vapors, 72h followed by days incubation: clove oil vapor exhibit a greater antifungal property at 1000ppm compared to 1100ppm cinnamon; 1500 ppm of clove, zone of inhibition is greater than 2300ppm of cinnamon oil vapor.

Fig. 5. & Fig 6. *A.flavus* spores exposed to clove vapors, 96 h followed by 7 days incubation: an increase in inhibition zone is observed after exposure to 2000ppm and 2100ppm clove and cinnamon, respectively and exposure to 2600ppm and 2700ppm clove and cinnamon, few spores still present.

Discussion

Variations in the zones of inhibition after exposure to clove and cinnamon oil vapors were related to concentration and time. Clove oil vapors inhibited mycelial growth faster than cinnamon oil vapors and shows a greater zone of inhibition at concentration as low as 1000ppm at 24 hours of exposure compared to 1300ppm of cinnamon oil vapors after seven days of incubation (Fig. 7. & Fig. 8.)





Conclusion

Our results indicate that essential oil vapors can be potentially used as antifungal agents against *A. flavus* in contaminated peanuts. Since plant based essential oils are considered having GRAS status, replacing chemical fungicides, currently used by the peanut industries, with theses antifungal agents will be beneficial to the environment and the consumer's health. The isolation and purification of active ingredients of both these oils should be further investigated to establish their antifungal properties and an alternate to pesticides. As the study of essential oil evolves, these biocontrol agents may become part of Integrated Pest Management (IPM) against *Aspergillus* species in peanuts and all other edible nuts.

References

- Hadaceck, F. and Greger, H. 2000. Inhibitory effect of some spice essential oils on *Aspergillus ochraceus* NRRL 3174 growth and ochratoxin production. *Let. Appl. Microbiol.*, 29: 238-241.
- Mungai P. and Achar, P.N. *Directed Study*, CSM, KSU 2011.
- Pinto, E., Vale-Silva, L., Cavaleiro, C. and Salgueiro, L. 2009. Antifungal activity of the clove essential oil from *Syzygium aromaticum* (*Eugenia caryophyllus*) on *Candida*, *Aspergillus* and dermatophyte species. *J. Med. Microbiol.*, DOI: 10.1099/jmm.0.010538-0
- Maruzzella, J.C. and Sicurella, N.A. 1960 Antibacterial activity of essential Oil vapors. *J Am Pharm Assoc*, 49: 692–694.

The authors would like to thank the Department of Biology and Physics, KSU for financial support and Drs. MY Sreenivasa, Univ of Mysore, India & C. Phillips, Univ.of Northampton, UK. for their expertise in antimicrobial compounds.

R Gilbert -Oral Presentation Dept Symposium 2014-1.pdf