Nano-based formulation of *Acarous calamus* rhizome extract and its efficacy on *Aspergillus flavus*

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1. Introduction

Aflatoxin is a poisonous contaminant of food crops from *Aspergillus* fungi. It causes not only deleterious issues in humans and livestock, but also inflicts severe economic loss in agriculture production by the quality deterioration of more than 25% of food crops (Tinham, 2018). Aflatoxin is the secondary metabolites of Aspergillus species, especially *A. flavus* and *A. parasiticus* (Perrone et al., 2007). The use of fungicides to control this fungus may not be environmentally or socially acceptable due to their harmful nature. Plant extracts and secondary metabolites of microorganisms, on the other hand, have been demonstrated in multiple studies to effectively limit Aspergillus fungus growth, reproduction, and aflatoxin formation when treated (Reddy et al., 2009). The problem of plant extracts is less efficient. Nanotechnology opened an era to synthesize the Nano molecules of plant extracts using silver, copper, gold and clay. Nano molecules increase the efficacy of the active ingredients present in the plant extract through particle size reduction as well as increase surface area to volume ratio by altering the physical and chemical properties of the active ingredients (Kathiravan et al., 2015). Nano-based technologies are eco-friendly and safe but, have their own advantages and disadvantages too. The main objective of this study was to synthesise silver nanoparticles from medicinal plants with antifungal capabilities against Aspergillus and analyse their efficacy using an in vitro assay.

2. Materials and Methods

The investigations were conducted at Plant Protection & Bio Control Laboratory at the Department of Agricultural Biology, and Nanoparticles were characterized at the Chemistry Research Laboratory, Department of Chemistry, Faculty of Science, University of Jaffna.

Groundnut kernel contaminated with the *Aspergillus* fungus was surface sterilized and the infected portion was inoculated into PDA medium and pure culture was isolated by the standard tip culture method. Morphological and microscopic examination confirmed the isolate as *A. flavus*. The dry rhizome of *A. calamus* was sterilized using 3% NaOCl and dried at room temperature. *Acarous calamus* rhizome was ground to make a powder using an electric grinder. Hot water extract of *A. calamus* was prepared and was kept in the refrigerator at 4 ℃ for further studies. Silver nitrate was used for the synthesis of silver nanoparticles of *A. calamus*. Optimization of silver nitrate volume and shaking time in the mechanical shaker was conducted with 0.01 mol L^{-1} $AgNO_3$ solution. Nanoparticles were manufactured at a mass level via optimization, and nanoparticles were obtained. The poison food technique was used to investigate the efficacy of produced silver nanoparticles against *A. flavus*.

Synthesized Nano molecules were dissolved in double distilled water to prepare different concentrations [0.005 g, 0.01 g, and 0.02 g] to test their efficiency in controlling *A. flavus* growth, and reproduction. All the experiments were conducted in Complete Randomized Design (CRD), Data were subjected to ANOVA using SAS 9.1 and Duncan Multiple Range Test (DMRT) was used to identify the best treatment combination at $P < 0.05$.

3. Results and Discussion

Synthesis of Nanomolecules

Diluted *A. calamus* extract colour was yellow and immediately after the addition of a different volume of silver nitrate, it turned out to light yellow. After synthesis of nanoparticles, the colour was turned to brown and after shaking it further turned to blackish brown. Colour changes confirmed the formation of nanoparticles. Using a Jasco V-570 UV-VIS-NIR spectrophotometer, the maximum absorption peak for the green synthesised *A. calamus* nanoparticle was obtained in the wavelength range of 400 nm to 500 nm after 210 minutes of mixing with 20 mL of 0.1 mol L^{-1} $AgNO_3$. Efficacy of Nano molecules against the growth and reproduction of *A. flavus*.

The diameter of mycelial development was measured and spores were counted from the day of inoculation to fourteen days.

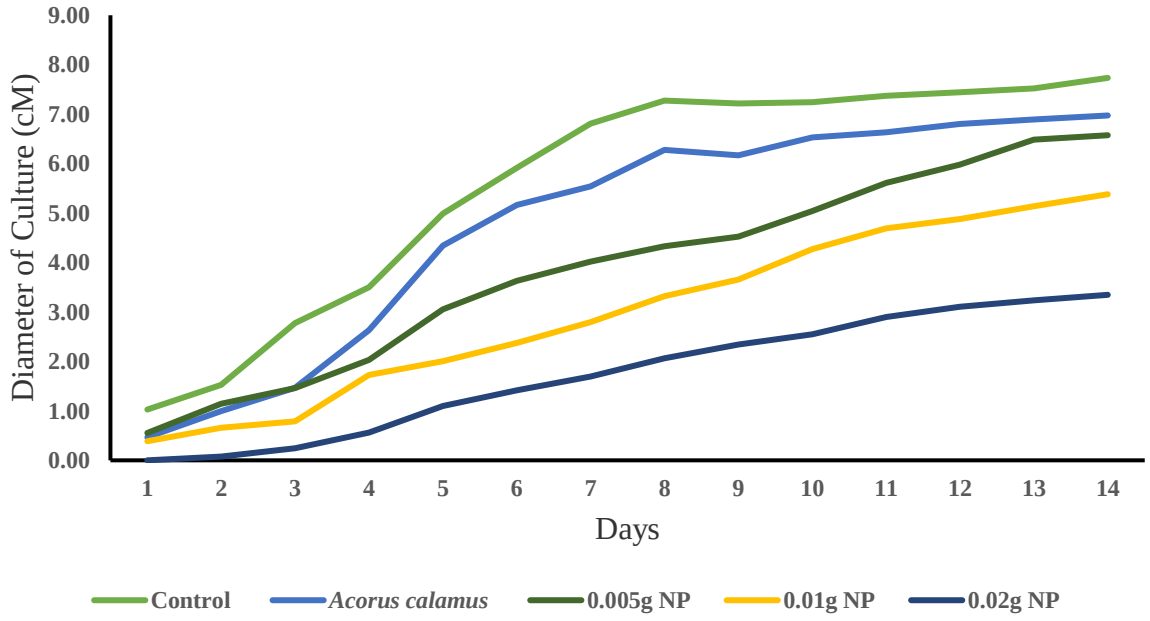

Figure 1. Comparison of Aspergillus growth in control, *A. calamus* extract and different amount of synthesized silver nanoparticles

Figure 1 illustrates the pathogen's growth rate in PDA (control), *A. calamus* water extract, and various amounts of synthesised silver nanoparticles from *A. calamus*, such as 0.05 g, 0.01 g, and 0.02 g. Nanoparticles synthesized from *A. calamus* water extract suppressed the growth of the *A. flavus* pathogen more efficiently than a direct application. In comparison to control (PDA), 0.02 g synthesized silver nanoparticles administrated trails *A. flavus* pathogen growth was significantly low (50 %) at $P < 0.05$.

The growth rate of *A. flavus* in 0.01g and 0.005g of Nanoparticle concentration were also comparably less and significant only at the concentration of 0.01g with control, *A. calamus* water extract and 0.02g concentration of Nano-synthesised *A. calamus* water extract at $P < 0.05$. Therefore, the results confirm that active ingredient(s) present in the Nano-synthesised *A. calamus* works better against test pathogen than Nano-free *A. calamus* water extract. Furthermore, when the concentration of Nanoparticles rises, the working efficacy of the active ingredient(s) rises as well.

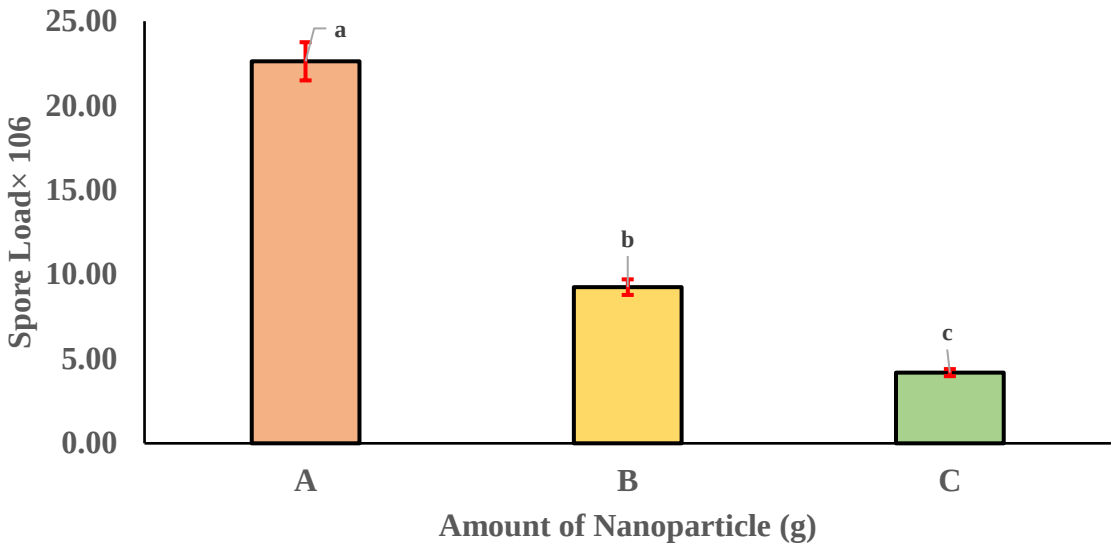

Figure 2. Number of spores produced in different amounts of nanoparticles mixed in culture media A) control B) 0.1 g, C) 0.3 g

Figure 2 shows that spore production of *A. flavus* in different amounts of synthesized silver nanoparticles present culture media. When the concentration of nanoparticles increased in the PDA media, the production of spores *A. flavus* was declined significantly at $P < 0.05$. In control (A), 0.10 g nanoparticles incorporated PDA(B), 0.30 g nanoparticles incorporated PDA(C) spore amount were 2.263×10^{7} ,9.25×10^{6},4.19×10^{6}, respectively.

Green synthesis of the metallic nanoparticle is a one-step process that reduces the metallic ion into metal by oxidizing phytochemicals that are present in the plant extract (Usha et al., 2017). Thus, this method is eco-friendly and less expensive compare to physical and chemical methods. Also, microorganisms have the ability to detoxify heavy metals. Intercellular and extracellular synthesis of metallic nanoparticles performs by using bacteria, yeast, and fungus (Singh et al., 2016).

Medda et al. (2015) green synthesized Silver nanoparticles using *Aloe vera* water extract and which was successfully controlled the plant pathogens *Rhizopus* sp. and *Aspergillus* sp. due to their antifungal activities. Jo, Kim and Jung, (2009) reported that Silver ion and Silver nanoparticles with a lower level of toxicity reduce colony formation of fungi *Bipolaris sorokiniana* and *Magnaporthe grisea*. Krishnaraj et al. (2012) reported that *Acalypha indica* leaf extract-based Silver nanoparticles inhibited the growth and reproduction of *Alternaria alternata*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Botrytis cinerea* and *Curvularia lunata* effectively at 15 mg concentration of silver nanoparticles. These findings validate the current investigation. This study clearly shows that the green synthesized silver nanoparticles suppress the growth of *A. flavus* pathogens more effectively compared to the direct application of *A. calamus* water extract. Changes in the concentration of nanoparticles have a positive relationship with the suppression of pathogens and are inversely correlated with spore production

4. Conclusions

Silver Nanoparticles were green synthesized using *A. calamus* water extract and it took 210 minutes to synthesis Nano-particles. In the Jasco V-570 UV-VIS-NIR spectrophotometer, green synthesised silver nanoparticles exhibited a greater absorption peak in the 400- 500 nm region. Synthesized silver nanoparticles have suppressed the growth of the *A. flavus* than direct application of water extract of *A. calamus*. When the concentration of nanoparticles increased

inhibition percentage of pathogen growth increased and spore formation decreased. Green synthesized silver nanoparticles using *A. calamus* may be an effective alternative to synthetic fungicides but, further extensive studies are in progress to validate the findings.

5. References

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