Effect of ergosterol and arecanut husk extracts on *Phytophthora arecae*, the causal organism of Koleroga of Arecanut

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ABSTRACT

Phytophthora arecae an oomycetes fungus causes a devastating disease known as Koleroga of arecanut. This fungus reproduces asexually by producing sporangia. Koleroga disease spreads in the plantation through zoospores released from sporangia. Hence understanding the requirements for growth and sporulation becomes important for developing better control measures. With this background, few nutritional supplements were used to study the growth and sporulation of this fungal pathogen isolated from Koleroga affected arecanut. V8 agar, a recommended growth medium used in the present investigation is supplemented with areca nut husk extracts (both ripened and unripened) and ergosterol separately. Enhancement of growth was observed in V8 juice medium supplemented with both ergosterol and arecanut husk extracts. Sporangia formation was observed in the presence of ergosterol.

Keywords: Koleroga, *Phytophthora arecae*, ergosterol, V8 agar

India is the traditional arecanut growing country in the world and contributes to 55 % of the world's arecanut production (www.utkrishhta.com). "Koleroga disease" (also known as Mahali) of areca nut, caused by the fungus *Phytophthora arecae* (L.C. Coleman) Pethybr. (www.mycobank.org) is the most devastating and destructive disease predominantly affecting areca plantations of coastal Karnataka and Kerala state. With more than 60 species, all being pathogenic, the genus *Phytophthora* is reported to infect many important crop plants and accounts for severe losses in the agricultural sector. *Phytophthora* species reproduces sexually by producing oospores and asexually by producing sporangia (www.q-bank.eu). These sporangia produce motile zoospores which swim in water and spread to other plants causing the infection. Hence, sporulation is very crucial for spreading the disease. Understanding the nutritional factors which are going to induce the sporulation is very much important to combat the disease effectively. According to Hendrix, Sterols are reported to promote the sporulation in some *Phytophthora* species [1]. According to Kheng-Hoy Chee, steroid constituents isolated from garden peas promoted growth and sporulation *Phytophthora cinnamomi* [2]. Sterols are important for the fungus to maintain the membrane fluidity and integrity. *Phytophthora* species are well known auxotrophs for sterols and they obtain the sterols from their host plants [3]. Since this pathogen infects young arecanuts, extracts from arecanuts should support the growth of the fungus. Hence in the present study, the effect of ergosterol and arecanut husk extract was analyzed on the growth and sporulation of *Phytophthora arecae.*

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Phytophthora isolates which are used in this study were isolated from the Dakshina Kannada district of Karnataka. Isolates of *Phytophthora arecae*, i.e, UJ-G and UJ-F were inoculated to 3 different media, i.e., V8 agar, clarified V8 agar and clarified V8 agar supplemented with ergosterol (http://fhm.fs.fed.us/sp/sod/misc/culturing_species_phytophthora.pdf). Cultures were incubated at 24±3°C. Colony diameter was measured for 10 days. Mycelium was observed under the microscope for the sporulation. Composition of the above mentioned three media are given here. V8 agar medium: V8 juice-100ml, $CaCO_3$ - 1g, Agar- 15g, DH_20 - 900ml; Clarified V8 agar medium: Clarified/Buffered V8 juice - 100ml, Tryptophan - 20mg, $CaCl_2.2H_2O$ - 100mg, Thiamine HCl - 1mg, Agar - 15g, DH_2O - 900ml; Clarified V8 agar medium supplemented with ergosterol: Clarified Buffered V8 juice - 100ml, Ergosterol - 30mg, Tryptophan - 20mg, $CaCl_2.2H_2O$ - 100mg, Thiamine HCl - 1mg, Agar - 15 g, DH_2O - 900 ml.

P. arecae culture was inoculated to clarified V8 medium with different combinations of arecanut husk extracts. Clarified V8 agar medium is used as control. This medium was supplemented with arecanut husk extracts in four different ways, viz., ripened heat sterilized, ripened filter sterilized, unripened heat sterilized and unripened filter sterilized. Inoculated Petri plates were incubated at 24±3°C. Colony diameter was measured for 10 days. Mycelia was observed under the microscope for the sporulation. Results were subjected to statistical analysis (ANOVA test) by using GraphPad Prism 6.

In the present study, maximum mycelial growth was observed in V8 agar medium as it contains more nutrients than other media. Clarified V8 agar medium supplemented with ergosterol enhanced the growth of the fungus when compared to clarified V8 agar medium without ergosterol (Figure 1). Significant difference in the growth was observed when the results were analysed statistically. Further, sporangia production was seen in V8 agar medium and Clarified V8 agar medium supplemented with ergosterol. Similar observations were made by Medina with β-sitosterol in *P. infestans* [4]. Sporangia were absent in clarified V8 agar medium without ergosterol. Thus, present investigation revealed the importance of sterol for both mycelial growth and sporangia production of *P. arecae*.

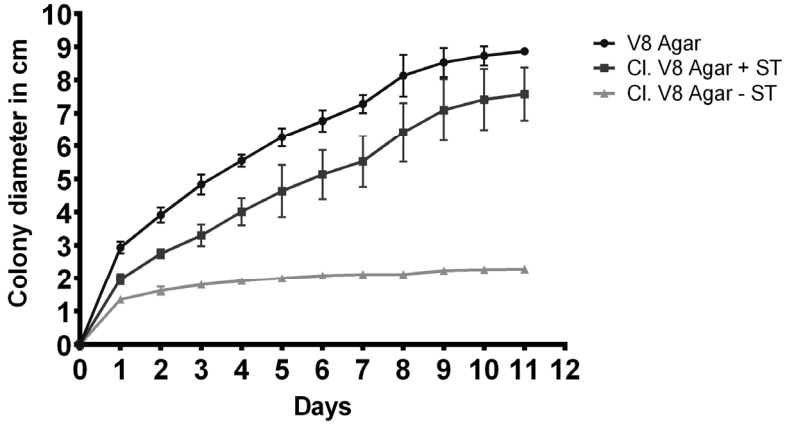

Figure 1. Effect of ergosterol on the growth of *Phytophthora arecae* isolate UJ-G.

Clarified V8 agar medium supplemented with Arecanut husk extracts promoted mycelia growth of *Phytophthora arecae* (Figure 2). Growth rate was enhanced in heat sterilized ripened and unripened arecanut husk extract supplemented media compared to filter sterilized ripened and unripened arecanut husk extract supplemented media. However significant difference in the growth

was not observed when the results were analyzed statistically. Sporulation was not observed in the above 10 day-old cultures. However, in nature enormous sporulation on the young arecanut under suitable environmental conditions results in fast spread of the disease. This study has given preliminary understanding of the effect of arecanut husk extract on the growth of the fungus. Further work is in progress to understand the factors affecting the sporulation of *P. arecae.*

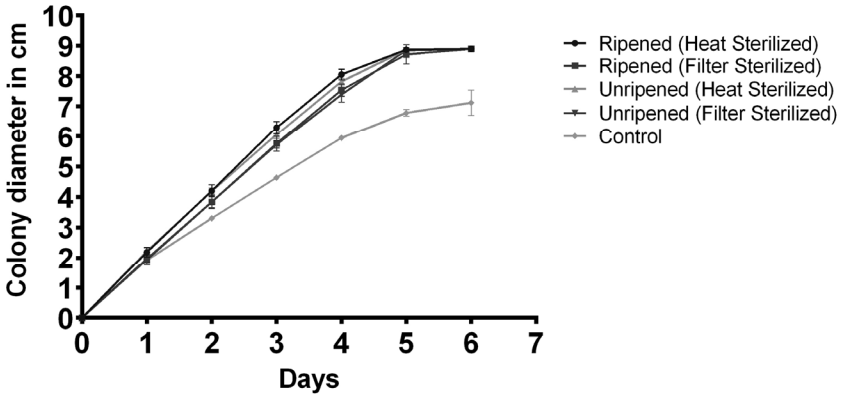

Figure 2. Effect of arecanut husk extracts on the growth of *Phytophthora arecae* isolate UJ-F.

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