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EVALUATION OF ANTIFUNGAL ACTIVITY OF SOME ORGANIC COMPOUNDS AGAINST *PHYTOPHTHORA MEADII*, THE CAUSATIVE AGENT OF FRUIT ROT IN ARECANUT *(ARECA CATECHU)*

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ABSTRACT
Arecanut *(Areca catechu L)* is a most important profitable plantation crop cultivated in Central Western Ghats of Karnataka. Among the various reported diseases in arecanut, fruit rot (koleroga / mahali) is a major devastating disease caused by *Phytophthora meadii.* It is a major constrain in arecanut production and causes heavy loss in yield. Despite the prophylactic application of both systemic and contact fungicides the incidence and severity of the disease could not be controlled. Recurrence of the disease during the monsoon and subsequent cooler months is due to survival of the fungus as oospores, chlamydospores and mycelium in soil, on fallen nuts, on dried nuts and on inflorescence remaining in the crown. Due to the limitations of conventional fungicides and to search for novel antifungal compounds with persistent and potent inhibitory activity, the present study was undertaken to evaluate the antifungal activity of 16 organic compounds belonging to different groups along with commercial fungicides (Metalaxyl Mz, Bordeaux mixture and Potassium phosphonate product, Biophite) against *P. meadii in vitro* by radial growth technique. Among them, compounds A **(**belong to triazole), H (coumarine), I (pyrimidine), M (pyrimidine) & B (benzothiophene) showed significant inhibition of the mycelial growth with an increase in their concentration. Compound A revealed highest inhibitory effect against *P.meadii* with an EC_{50} value (10 µg / ml) followed by compounds H & I (30 µg / ml), compound M (50 µg / ml) and Compound B (100 µg / ml). Further, within the five compounds, compound A exhibited promising antifungal activity against *P.meadii.* However, compared with the commercial fungicides, these compounds exhibit less inhibitory effects. The present study suggests that compound A could act as a potential fungicide to be used for further optimization. Further, this study helps in understanding the molecular mechanism of the antifungal activity of these organic compounds using molecular docking procedure. Also, these findings may be useful for farmers in managing fruit rot disease of arecanut.

KEYWORDS: Arecanut, fruit rot, *Phytophthora meadii*, Koleroga, Antifungal activity, organic compounds, fungicides.

INTRODUCTION
Plant diseases caused by fungal pathogens represent a worldwide issue and often lead to detrimental agricultural crop losses.[1] Arecanut *(Areca catechu)* is cultivated as one of the most important commercial plantation crop in Central Western Ghats of Shivamogga, Uttara Kannada and Mangalore districts of Karnataka. India is the world largest producer of arecanut with an annual production of 0.48 million tons. Arecanut is affected by a wide range of insect pests and diseases. Some of the diseases are lethal to the tree and others reduce the growth and productivity of the palm. Among the many reported diseases of arecanut, fruit rot (commonly called "Koleroga" in kannada or "Mahali" in Malayalam) caused by the fungus *Phytophthora meadii* is a major devastating disease leading to yield losses of up to 90% .[2] This disease is weather dependent and the severity, persistence and spread of fruit rot are related to the pattern of rain. The disease appears usually 15 to 20 days after the onset of regular South West monsoon (June- September) rains and may continue up to the end of the rainy season. Continuous heavy rainfall coupled with low temperature (20^0C to 22^0C), high relative humidity (90 to 100 %) and intermittent rain and sunshine hours are factors that favor the occurrence of fruit rot. Its symptoms are invariably noticed as dark

green water-soaked lesions near the perianth (calyx). The infected fruits loose their natural green color. The lesions on the fruits gradually spread covering the whole surface before or after shedding. The disease leads to heavy shedding of fruits. After a few days, a felt of white mycelial mass develops on the fallen nuts with abundant production of mycelium, sporangiospores and zoospores. Fruit rot can be controlled by cultural practices based on our understanding of the biology, ecology and chemistry of the pathogen. Over the past several decades, farmers of this region practicing both mechanical and chemical methods to control the fruit rot disease. However, still arecanut production relies mainly on fungicides such as Bordeaux mixture, Copper oxychloride, Metalaxyl Mz, aliette, Cymoxanil and Potassium phosphonate products (Biophite and Biopot).[3-4] In the early years of this century, as a means of mechanical control measure, areca bunches are covered either with 'kotta' (made of arecanut leaf sheaths) or 'karada' (made of a kind of grass) or polythene cover (200µ gauge) is in practice to control the fruit rot disease in Central Western Ghats region of Karnataka.[5] Later, several workers have evaluated the effectiveness of Bordeaux mixture alone or in combination with different adhesives and spreaders and found good results.[6-7-8] Over the past five decades, different workers conducted the field trials with regard to the management of fruit rot disease using various systemic and non-systemic fungicides along with age old practice of covering the areca bunches with polyethylene cover of 200µ thickness.[9] Besides the prophylactic application of fungicides and phytosanitory measures, the incidence and severity of the disease could not be controlled. So far no alternative has been found for copper sulphate to control the disease. There are two reasons which makes control difficult; first the fungus survives as oospores, chlamydospores and mycelium in soil, on fallen nuts, on dried nuts and on inflorescence remaining in the crown, second the constant use of fungicides induce resistance in fungus against their toxic effect. Therefore, it is quite essential to test newer chemicals and their better formulations, which may impart persistent antifungal effect to the material for a longer duration. With this view and as a part of our ongoing search for new antifungal compounds, the present study was undertaken to evaluate the antifungal activity of 16 organic compounds belonging to different classes along with commercial fungicides against *P. meadii in vitro.*

MATERIAL AND METHODS

Culture of *Phytophthora meadii*, previously isolated from infected areacanut was kindly provided by Dr. P. Chowdappa, Division of Plant Pathology, Indian Institute of Horticulture Research (IIHR), Hesaraghatta, Bangalore and maintained on Carrot Agar medium (CA) at 25^0C. The sixteen organic compounds used for antifungal activity were obtained from Dr. Basavaraj Padmasali, Department of Chemistry, Rani Channamma University, Belagavi, Karnataka. Chemical structures of these organic compounds are shown in Table 1. Commercially available agricultural fungicides Metalaxy Mz, Bordeaux mixture and Potassium phosphonate products (Biophite) were used as a positive controls. All other chemicals used in this study were of reagent grade.

In vitro antifungal assay

The antifungal activities of the chemically synthesized organic compounds were tested using radial growth technique. [10-11] appropriate volumes of the stock solutions of the synthesized compounds in dimethyl sulfoxide (DMSO) were added to the carrot agar medium immediately before it was poured into the petriplate (5 mm diameter) at 40-45˚C to obtain a series of concentrations (10, 30, 50, 70 and 100µg/ml). Each concentration was tested in triplicate. Parallel controls were maintained with DMSO mixed with carrot agar medium. The discs of mycelial felt (0.5 cm diameter) of the fungus, taken from the 8 – day - old culture were transferred aseptically to the center of the petriplates. The treatments were incubated at 27˚C in the dark. Colony growth diameter was measured after the fungal growth in the control treatments have completely covered the petriplate. Percentage of mycelial growth inhibition was calculated using the formula: Mycelial growth inhibition (%) = [DC-DT/DC] x 100 (20), where DC and DT are average diameters of fungal colony of control and treatment respectively. Bordeaux mixture, biophite and metalaxyl were used as reference fungicides (Positive controls). The 50% effective concentration (EC_{50}) was calculated for the compounds that showed significant antifungal activity.

Statistical analysis

All experiments were performed in triplicates. The data were expressed as the mean ± standard error. Wilcoxson's test was used for the statistical significance of results. Values are significant with respect to compounds at P<.005 level of significance.

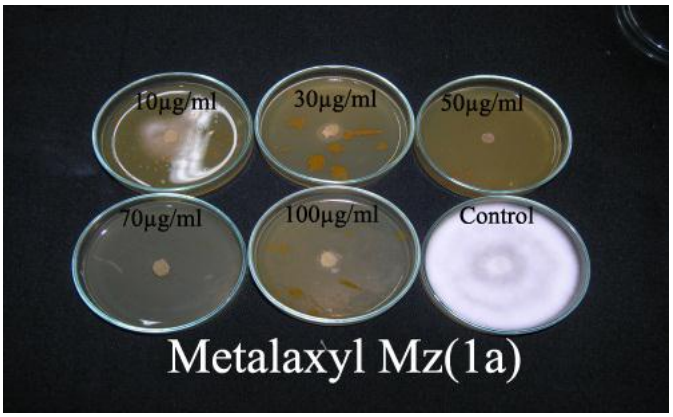
RESULTS AND DISCUSSION

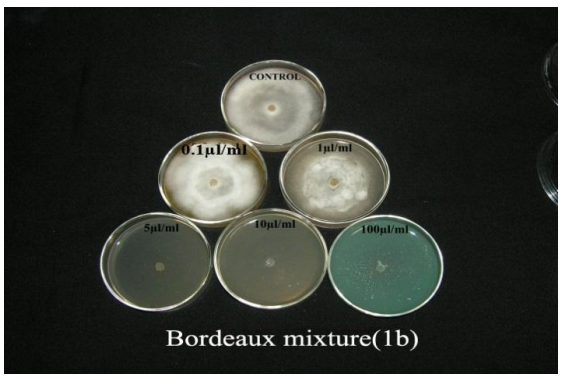
Antifungal activity of organic compounds against *Phytophthora meadii*

The inhibitory effect of 16 organic compounds (A-P) belong to triazoles, benzothiophenes, benzimidazoles, thiophenes, coumarines, chalcones, indolizines and pyrimidines along with three commercial fungicides were evaluated against *Phytophthora meadii.* All reference fungicides tested were reduced the pathogen development at all concentrations when compared with the control (Fig. 1 a-c). Inhibitory effect of Metalaxy Mz, Bordeaux mixture and Potassium phosphonate product (Biophite) against *Phytophthora meadii* has been reported by many researchers(Farih et al)[12] reported that metalaxyl at low concentrations was highly inhibitory to mycelial growth and formation of sporangia, chlamydospores, and oospores of *P. parasitica* and *P. citrophthora* suggested that metalaxyl controls the disease by affecting the pathogens at all stages of their life cycle.[13]. Sastry et al[14] showed that Bordeaux mixture (1%), copper oxychloride and metalaxyl were

found to be effective in inhibiting the growth and sporulation of *Phytophthora capsici* and *P. meadii*. Similarly, (Lokesh et al) [3] reported that the application of metalaxyl mancozeb 72WP at 0.2% twice to the arecanut bunches during monsoon drastically reduce the incidence of fruit rot / koleroga with increase in the arecanut yield. Field trials conducted by (Narayanaswamy et al) [4] found that application of either conventional or stabilized Bordeaux mixture (1%) along with proper phytosanitory measurements gives the better management of fruit rot of arecanut. Among the sixteen compounds tested, only five compounds Viz. A (belong to triazole), H (coumarine), I (pyrimidine), M (pyrimidine) & B (benzothiophene) showed significant inhibition of the mycelial growth with an increase in their concentration in the medium (Fig 2 a-e). Compound A revealed highest inhibitory effect against *P. meadii* with an EC_{50} value (10 µg / ml) followed by compounds H & I (30 µg / ml), compound M (50 µg / ml) and Compound B (100 µg / ml) (Table 2). Further, within the five compounds, compound A exhibited promising antifungal activity against *P. meadii.* However, compared with the commercial fungicides, these compounds exhibit less inhibitory effects.

In summary, the results of this study revealed that compound A, H, I, M & B are very good antifungal compounds and the strong inhibitory effect of compound A can be used to control fruit rot in arecanut.





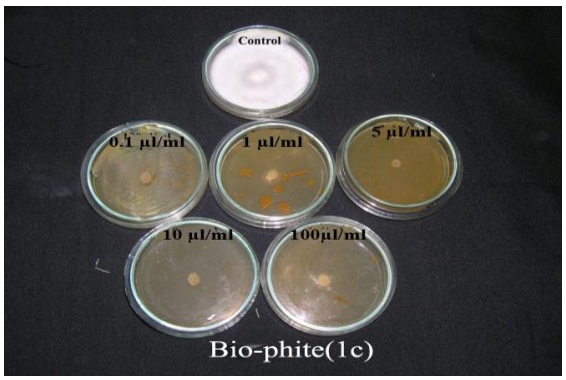
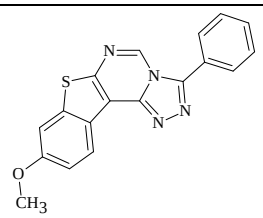
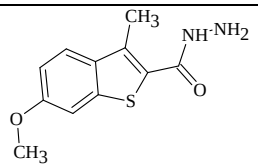
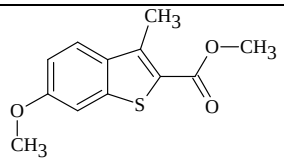
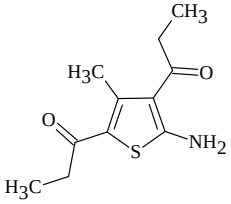
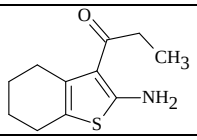
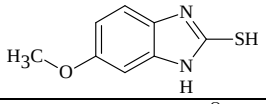
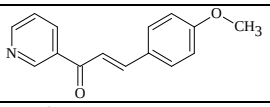
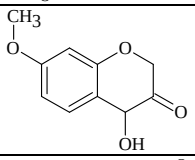
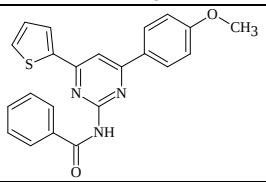
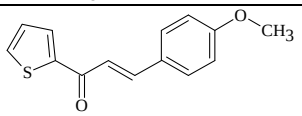
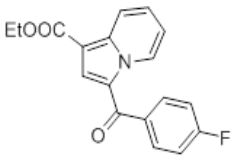
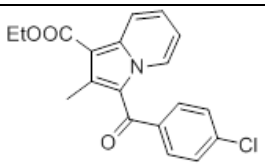
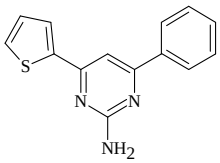
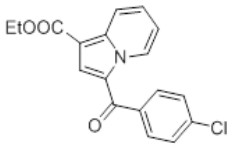
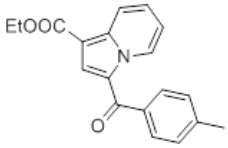
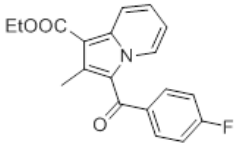


Fig. 1: *In vitro* effect of various concentrations of metalaxy Mz (1a), bordeaux mixture (1b) and biophite (1c) on mycelial growth of *Phytophthora meadii* at 7 days after incubation.

Table 1: Chemical structures of organic compounds assayed for antifungal activity against *Phytophthora meadii.*

Compound	Structure	IUPAC name	Group
A		7-methoxy-3,4-dihydro[1]benzothieno[2,3-*d*]pyrimidine- 3-phenyl[3,4-c]triazole	Triazole
B		6-methoxy-3-methyl-1-benzothiophene-2-carbohydrazide	Benzothiophene

C		methyl 6-methoxy-3-methyl-1-benzothiophene-2-carboxylate.	Benzothiophene
D		1,1'-(5-amino-3-methylthiene-2,4-diyl)dipropan-1-one	Thiophene
E		1-(2-amino-4,5,6,7-tetrahydro-1-benzothiophen-3-yl)propan-1-one	Benzothiophene
F		6-methoxy-1*H*-benzimidazole-2-thiol	Benzimidazole
G		(2*E*)-3-(4-methoxyphenyl)-1-(pyridin-3-yl)prop-2-en-1-one	Chalcone
H		4-hydroxy-7-methoxy-2*H*-chromen-3(4*H*)-one	Coumarine
I		*N*-[4-(4-methoxyphenyl)-6-(thiophen-2-yl)pyrimidine-2-yl]benzamide	Pyrimidine
J		(2*E*)-3-(4-methoxyphenyl)-1-(thiophen-2-yl)prop-2-en-1-one	Chalcone
K		ethyl 3-(4-fluorobenzoyl)indolizine-1-carboxylate	Indolizine
L		ethyl 3-(4-chlorobenzoyl)-2-methylindolizine-1-carboxylate	Indolizine
M		4-phenyl-6-(thiophen-2-yl)pyrimidine-2-amine	Pyrimidine
N		ethyl 3-(4-chlorobenzoyl)indolizine-1-carboxylate	Indolizine

O		ethyl 3-(4-methylbenzoyl)indolizine-1-carboxylate	Indolizine
P		ethyl 3-(4-fluorobenzoyl)-2-methylindolizine-1-carboxylate	Indolizine

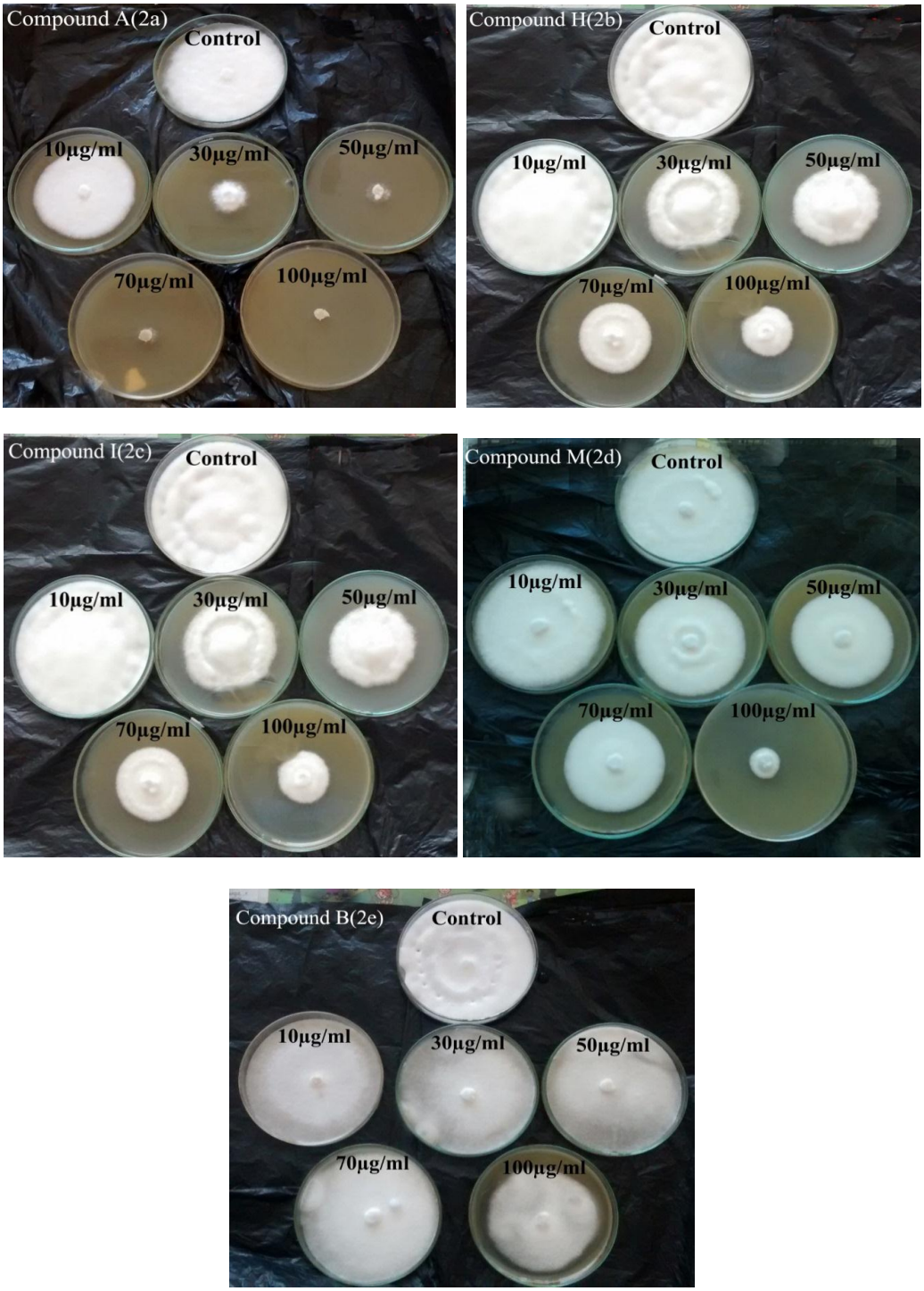


Fig 2: Effect of varying concentrations of five organic compounds A (2a), H (2b), I (2c), M (2d) & B (2e) on the mycelial growth of *Phytophthora meadii* at 7 days after incubation.

Table 2: EC_{50} values of the compounds A, H, I, M & B against *Phytophthora meadii.*

Compound	Class	EC_{50}[a] (µg/ml)
A 7-methoxy-3,4-dihydro[1]benzothieno[2,3-*d*] pyrimidine- 3-phenyl[3,4-c]triazole	Triazole	10
H 4-hydroxy-7-methoxy-2*H*-chromen-3(4*H*)-one	Coumarine	30
I *N*-[4-(4-methoxyphenyl)-6-(thiophen-2-yl) pyrimidine-2-yl] benzamide	Pyrimidine	30
M 4-phenyl-6-(thiophen-2-yl)pyrimidine-2-amine	Pyrimidine	50
B 6-methoxy-3-methyl-1-benzothiophene-2-carbohydrazide	Benzothiophene	100

[a]Values are the mean ± standard deviation (SD) of three replicates.

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