

In vitro Evaluation of Some Selected Fungicides against *Pestalotia palmarum* (Cooke.) Causal Agent of Grey Leaf Spot of Coconut

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Abstract

To evaluate the efficacy of some selected contact, systemic and mixed fungicides against *Pestalotia palmarum* causal organism of grey leaf spot of coconut. Naturally infected leaf sample of coconut having characteristic symptoms of grey leaf spot diseases was collected from Khulna University campus. The pathogen was isolated, purified and identified as *P. palmarum*. The fungicides were tested against *P. palmarum* at 1000, 2000 and 3000 ppm concentrations. All fungicides at all concentrations inhibited the growth of the pathogen and their effect differed significantly (≤ 0.01). No growth was observed in Hexaconazol, Propiconazol, Hepridion and Carbendazim at any concentrations. Mancozeb at all concentrations and Mancozeb+Metalexil at 2000 and 3000 ppm inhibited more than 70%. Mancozeb+Metalexil 1000 ppm inhibited the lowest (60.49%).

Keywords: Fungicides; *Pestalotia palmarum*; Grey leaf spot of coconut

Introduction

Coconut is (*Cocos nucifera* Linn.) the most important perennial fruit plant in the world belonging to the family Arecaceae (Palmaceae). Coconut palms are successfully grown in the tropics and hence referred to as 'King of the tropical palms'. The South Pacific and South Africa are often cited as the possible centre of origin.

The importance of the palm lies in fact that not only does it supply food, drink and shelter but it also provides raw materials for a number of industries. Coconut is one of the important nut crops in Bangladesh. Its production in Bangladesh is 907255 Metric tons from 12825 acres of land in 2004-2005 [1]. It is mostly grown in the southern part of the country.

The coconut requires an equable climate which is neither very hot nor very cold (temperature ranges from 20 to 32°C), light sandy and sandy loam soil and well distributed rainfall. But the optimum temperature is 27°C for maximum growth and nut yield. In places where the mean temperature is less than 15 to 20°C, the palm does not flourish due to both physiological and morphological changes and cold temperature upsets fruiting. Clayey soil is unfavorable for coconut cultivation because of the impermeability indispensable drainage system.

Yield of the coconut also reduces day by day due to the causes of various diseases. Such as, sooty mould, stem bleeding, leaf spot, white thread blight, root rot, brown root rot and bud rot disease which are caused by different fungus. (<http://www.learn-grow.org/uploads/file/2Diseases%20of%20PNG%20foods.pdf>). Among the diseases every year grey leaf spot disease caused by *Pestalotia palmarum* (Cooke.) attacks the gardens and decreases the growth and development of the tree as well as the yield of the fruit. The symptom is only developed in the mature leaves in the form of grayish white spots surrounded by brown margin. Several of the spots coalesce together and form irregular grey necrotic patches and show burnt or blighted appearances. The upper surface of the affected leaves reveals dark grey eruptions like pin heads. This disease is a serious problem all over the coconut growing regions of Bangladesh.

It has been demonstrated that the fungus usually requires wounds for the plant penetration and disease development [2].

P. palmarum can be controlled by different way such as using fungicides, botanical extracts and antagonistic agents [2], but among these ways fungicide is most effective way of controlling *P. palmarum*. In previous investigations on the fungicidal effect against grey leaf spot of coconut have been recommend some effective fungicides like Tilt 250 EC, cupravit-50 WP, Dithane M-45, Bavistin, Macuprax etc. But there is no considerable work on other fungicides to control *P. palmarum* on coconut in Bangladesh [3-5]. So, the information regarding the effect of different fungicides against *P. palmarum*, the causal organism of grey leaf spot of coconut is not enough which demand more investigation on it. The present study was undertaken to evaluate the efficacy of some selected fungicides against *P. palmarum*, the causal agent of grey leaf spot of coconut.

Materials and Methods

Collection of sample

Diseased plant parts of Coconut (*Cocos nucifera*) showing typical leaf spot symptoms were collected from the Khulna University campus, Bangladesh.

Preparation of potato dextrose agar (PDA) medium

The basic medium, PDA was prepared following the standard procedure [6]. At first 200 g peeled potato is cut into slice and then boiled in 1000 ml water. After that it was sieved and 15 gm agar were mixed with it in a water bath, after few minutes 20 g dextrose were mixed with it and stirred properly so that it cannot be coagulated. The pH was adjusted to 6.5 of the media by using pH meter with the help of 1N HCL and sterilized in autoclave at 121°C temperature for 20 minutes.

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Isolation of the fungus

The fungus was isolated from the infected leaf of coconut following tissue planting technique [7]. The infected diseased samples along with healthy tissues were cut into small pieces and surface sterilized by dipping in 0.1% sodium hypochloride (NaOCl) solution for two minutes. NaOCl on the surface of the leaf pieces was decanted by soaking with sterilized blotting paper. The cut pieces were then placed onto sterilized potato dextrose agar (PDA) in glass petridishes (20 mL/ petridish) and incubated in an incubator at $27 \pm 1^\circ\text{C}$ until mycelium formation. The hyphal tips were transferred onto PDA plate after growing the mycelium. The new plates were incubated at $27 \pm 1^\circ\text{C}$ for acervuli production.

Identification of fungus isolates upto species

The fungus was then identified on the basis the morphological of characteristics with the help of identifying key book [8].

Pathogenicity test of *P. palmarum*: Pathogenicity test was done using healthy excised leaf of coconut. Healthy leaves of Coconut samples were cut into 4 cm² pieces and surface sterilized with 70% ethanol for ten second. Just after ten seconds the leaf pieces were moved over the flame to burn excess ethanol on the surface of the leaf. They were transferred onto sterilized water agar in sterilized petridishes. Sample pieces were injured softly by flame sterilized pointed needles. Advanced hyphae and acervuli were cut from the margin of pure cultures carefully with the help of flame burned inoculation needle and placed at three places (two at both margins and one at the center) onto the injured excised leaf of the host and incubated at $27 \pm 1^\circ\text{C}$ for ten days. After fifteen days of incubation the samples were checked for appearance of characteristics symptoms.

Purification and preservation

To obtain pure culture of the pathogen, the hyphal tips were transferred aseptically onto PDA plate by using the flame sterilized tip of an inoculation needle. The plate was incubated at room temperature for seven days. Advanced hyphae were collected and transferred into the test tube slants containing PDA and incubated at room temperature for seven days. After incubation, the slants were carefully checked for contamination and then preserved at 4°C in a refrigerator for further use.

Multiplication of *P. palmarum*

PDA was poured in sterilized petridishes, 25 ml in each. After solidification, the plates were inoculated by placing 5 mm discs of three days old PDA culture of *P. palmarum*. The discs were cut with flame sterilized cork borer (5 mm diameter). The inoculated petridishes were kept in the growth chamber at a temperature of $28 \pm 1^\circ\text{C}$ for few days. All the works were undertaken under the laminar air flow cabinet.

Different Fungicides used in this experiment

The fungicides used in the experiment are listed in the Table 1.

Preparation of different concentration of fungicides mixed with PDA

Different fungicides were evaluated in *in vitro* condition against *P. palmarum* following poison food technique [9]. Concentrations of the fungicides were selected based on the recommended dose. One is lower, other one is recommended and other higher dose. Required amount of

Chemical name	Mode of action	Trade name	Concentration (ppm)
Mancozeb	Contact	Nemispore 80 WP	1000, 2000 and 3000
Carbendazim	Systemic	Amcozim 50 WP	1000, 2000 and 3000
Hexaconazole	Systemic	Conza 5 EC	1000, 2000 and 3000
Difenoconazole+ Propiconazole	Systemic	X-tra care 300 EC	1000, 2000 and 3000
Mancozeb+Metalexil	Contact	Ridomil 72 WP	1000, 2000 and 3000
Hepridion	Contact	Hepridion 70 WP	1000, 2000 and 3000

Table 1: List of fungicides that are used in the experiment.

fungicides were mixed with 100 ml PDA, then mixed by shaking and finally autoclaved. For control treatment no fungicide was added.

Adjustment of pH

The pH of the medium was adjusted to 6.5.

Pouring and solidification

100 ml PDA was poured in sterilized petridishes 20 ml each. Each plate was considered as a replication. Five plates were used for each treatment.

Inoculation and incubation

The plates were inoculated at the centre with 5 mm disc taken from 3 days old PDA culture one disc at centre per plate. The mycelial discs were taken from the edge of the colony. The plates were incubated at $27 \pm 1^\circ\text{C}$.

Measurement of radial growth and calculation of percent inhibition

The radial growth of mycelium in each plate was recorded as an average of two diameters measured at right angles to one another.

Percentage inhibition of growth was calculated using the following formula [10]:

$$\text{Percent of inhibition} = \frac{X-Y}{X} \times 100$$

Where,

X=Average growth of *P. palmarum* in control petridishes.

Y=Average growth of *P. palmarum* in each fungicide treated petridishes.

Experimental design and data analysis

The experiment was laid out in CRD with five replications. The data were analyzed statistically using MSTAT-C computer program and means were compared for difference following Duncan's Multiple Range Test (DMRT).

Results and Discussion

Identification of fungus

The pathogen was identified based on morphology of reproductive structures that is, acervuli, conidial characteristics and found *P. palmarum*.

Pathogenicity test of *P. palmarum*

It was found that *P. palmarum* produced characteristic symptoms of grey leaf spot of coconut.

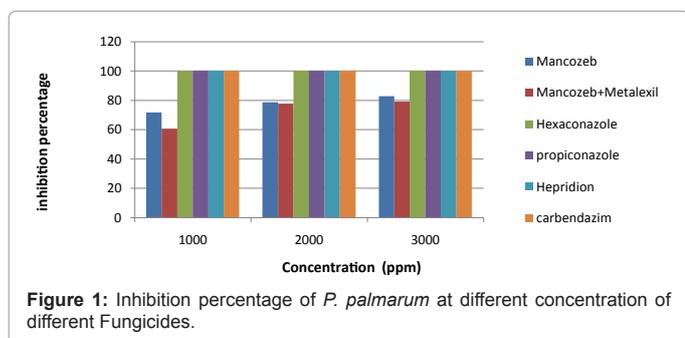
Effect of fungicides against *P. palmarum*

Six fungicides namely, Mancozeb, Mancozeb+Metalexil, Hexaconazole, Difenconazole+Propiconazole, Hepridion and Carbendazim at three concentrations each were tested against *P. palmarum*. The results are presented in Table 2. Effect of different fungicides at different concentrations was found significantly different at 1% level. Hexaconazole, Difenconazole+Propiconazole, Hepridion and Carbendazim inhibited the growth of *P. palmarum* totally. No growth was found at any concentration. Mancozeb at 3000 ppm inhibited the growth of *P. palmarum* 82.86% which differed from all fungicides at all concentrations. Effect of Mancozeb 2000 ppm and Mancozeb+Metalexil 2000 and 3000 ppm was statistically similar but differed from other concentrations of different fungicides. They inhibited 78.67%, 79.02% and 77.62% respectively. Mancozeb inhibited 71.64% which differed from other concentrations at all fungicides. Mancozeb+Metalexil 1000 ppm inhibited the lowest (60.49%) which also differed from others.

Carbendazim was found effective fungicide against *P. palmarum* by

Fungicides	Concentration (ppm)	Inhibition Percentage (%)
Mancozeb	1000	71.636d
	2000	78.672c
	3000	82.864e
Mancozeb+Metalexil	1000	60.488e
	2000	77.622c
	3000	79.020c
Hexaconazole	1000	100a
	2000	100a
	3000	100a
Difenconazole+Propiconazole	1000	100a
	2000	100a
	3000	100a
Hepridion	1000	100a
	2000	100a
	3000	100a
Carbendazim	1000	100a
	2000	100a
	3000	100a
Co-efficient of Variation		2.31%
Level of Significance		0.01

Table 2: Inhibition percentage of *P. palmarum* at different concentration of different fungicides.



Sanjay et al. [3]. Sharma et al. [4] found Mancozeb as the most effective fungicide against *P. palmarum*. Propiconazole was found very effective in the inhibition of *P. palmarum* in the study by Parveen and Kachapur [5] (Figure 1).

Conclusion

Hexaconazole, Difenconazole+Propiconazole, Hepridion and Carbendazim were very effective even at low concentration. Mancozeb alone found better than Mancozeb+Metalexil mixture.

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