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New report of pod rot of moringa in Sri Lanka caused by *Drechslera hawaiiensis* and potential fungicides for its control

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Abstract
Moringa is not affected by any serious pests and diseases; but, can observe minor disease with the unfavorable climatic conditions. These diseases became more common with the expansion of the moringa cultivation. However, there are no reports on pod rot associated with moringa cultivation in Sri Lanka. The objectives are to isolate and characterize pathogens by morphological characterization and determine its chemical control measures. Fungal isolation was done from diseased moringa pods collected throughout the country. Isolates were identified based on their morphological characteristics such as conidia morphology, colony appearance, growth rate. Brown color patches on the pods, then splitting, and rotting the pods with the maturity have reduced the economic yield of the moringa seed industry have been observed. Based on morphological features and fungal colony characters the pathogen was identified as *Drechslera hawaiiensis* (M.B. Ellis) and pathogenicity was tested using Koch's postulates method. Subsequently, four fungicides were tested *in-vitro*. The best treatment was Tebuconazole 250 g/l EW at the rate of 3.5 ml/10l among the *in-vitro* tested fungicides in this study. Follow that; contact fungicides (Mancozeb 75% WG at the rate of 20g/10l, Propineb 70 WP at the rate of 20g/10l, Fluazinam 500 g/l SC at the rate of 10ml/10l) which partially controls the pathogen in this study can be alternatively used with seven to ten days intervals until 14 days to harvest. Maintaining of field sanitation must take place.

Keywords: *Moringa oleifera*, *Bipolaris Hawaiiensis*, *drechslera hawaiiensis*, fungal diseases

1. Introduction
Moringa oleifera belonging to the family Moringaceae is a highly valued plant distributed in many countries of the tropics and subtropics. Drumstick is popularly known as moringa. It has an impressive range of medicinal uses with high nutritional value [1]. Moringa is drought resistant and fairly disease tolerant making the crop relatively unaffected. According to Mridha and Barakah *[16]*, Moringa is resistant to most pests and diseases, although several diseases causing minor damages including a root rot caused by *Diplodia* sp**.** [20], fruit rot caused by *Drechslera hawaiiensis* (Perfect Stage: *Cochliobolus hawaiiensis*) [13, 15] and twig canker by *Fusarium pallidoroseum*, etc. Moringa tree is susceptible to *Leveillula taurica* causing powdery mildew disease reasoning for serious damage to papaya [24]. Mridha and Barakah [16] first reported six different species of fungi belonging to five genera which are *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternate*, *Fusarium oxysporum*, *Mcrophomina phaseolina*, and *Rhizopus stolonifera* associated with moringa pods. They further emphasized that the two species of *Aspergillus* are reported to be detrimental to human health. Moringa anthracnose caused by *Colletotrichum chlorophyti* was first reported in China by [5]. Gatan [8] has reported that Moringa in worldwide is affected with about 12 diseases including brown leaf spot (*Cercospora moringicola*), septoria leaf spot (*Septoria lycopersici*), alternaria leaf spot (*Alternaria solani*), powdery mildew (*Leveillula taurica*), root rot (*Diplodia* sp.), fusarium wilt (*Fusarium oxysporium* f. sp. *moringae*), fruit rot (*Cochliobolus hawaiiensis*), damping-off (*Rhizoctonia solani*), dieback (*Fusarium semitectum*), anthracnose (*Colletotrichum chlorophyti*), twig canker (*Fusarium pallidoroseum*) and rust (*Puccinia moringae*).
In Sri Lanka, Moringa is cultivated mainly in dry zone areas as a vegetable crop for both pods and green leaves. In the recent past, Moringa has been promoting in Sri Lanka as commercial cultivations using imported seeds for export and oil production purposes in addition to local consumption as a vegetable. With the rapid expansion of moringa cultivation in Sri Lanka either as an annual or as a perennial vegetable, there is a high frequency of reporting of pests and diseases in couple with the adoption of new production technologies, use of high inputs, the introduction of high yielding varieties, changes in cultural practices, the free exchange of planting materials and also changes in climatic conditions. Proper identification of diseases on various plant parts of moringa through their symptoms including their life cycle alternate host plants whether cultivated and non-cultivated are very important in the management of diseases [8].
The pod rot of moringa was reported from various parts of Sri Lanka including Putlam, Kurunegala, Pollonnaruwa, Mathale, and Anuradhapura districts. The pods are rotted and dried up prematurely with the longitudinal gummy crack on the symptoms. The similar disease caused by *Drechslera hawaiiensis* has been reported in India [14, 18]. The study was undertaken to identify the etiology of the newly reported pod rot of the moringa in Sri Lanka and to identify the potential fungicides to manage the spreading of the disease.

2. Material and Methods

2.1 Collection of samples and symptomatology

Diseased plant samples were collected from the farmer fields (Puttalam district, Polonnaruwa district, Kurunegala district, Mathale district, and Anuradhapura district in Sri Lanka) among both local and exotic moringa varieties. Initially, small brown spots appeared on the pods, which gradually enlarged and coalesced to form larger patches. Those mature pods were extensively rotted. On green pods, elliptical or elongated sunken small spots with raised light brown-reddish color margins were observed. Some brown color lesions were observed on those pods.

2.2 Isolation and identification of the causal agent (Koch's postulate)

The causal pathogen of disease was isolated from the pods that showed disease symptoms. Isolation of pathogen was obtained by the tissue segment method [21] on Potato Dextrose Agar (PDA) medium. Infected lesions of freshly collected pods were cut into sections having 1cm2 dimensions. They were surface sterilized and cultured in the PDA medium. Cultures were incubated at room temperature (27 ^{0}C – 30 ^{0}C) under normal light conditions. The pure culture was obtained by the re-culturing of isolated fungi through a single spore technique [6].

Pathogenicity was tested by Koch's postulate method with the inoculation of fresh culture to moringa pods and kept in a moisture chamber for several days until symptoms appeared.

2.3 Microscopic examinations

Morphological identification was done with the microscopic observations and colony characters [2]. Brown color conidiophores were simple and septated. Hyphae were smooth and black color fungal colonies were obtained. Conidia were straight, rounded at both ends, or cylindrical.

2.4 *In vitro* fungicide evaluation

Subsequently, fungi were grown on fungicides amended Potato Dextrose Agar (PDA) as a food poison technique used for the screen of suitable fungicide. Those fungicides and the rate of application are shown in Table 01**.**

Table 1: Different concentrations used to treat fungicides.

Treatment	Fungicide	Concentration
T1	Mancozeb 75% WG	20g/10l
T2	Propineb 70% WP	20g/10l
T3	Fluazinam 500 g/l SC	10ml/10l
T4	Tebuconazole 250g/l EW	3.5ml/10l
T5	Control	-

Mancozeb 75% WG is a broad-spectrum contact fungicide for control of the main groups' fungal diseases in many crops. It is a fungicide with multisite protective and inhibits spore germination and remains on the leaf surface and interferes with six different biochemical processes within the fungal pathogen cell. It's commonly used for the blight, different leaf spots such as anthracnose and pod rots [23]. Propineb 70% WP has a contact action and is a preventive broad-spectrum fungicide. It is a non-specific, multi-site fungicide. Conidia or germinating conidia are killed by contact. And also interferes at different locations in the metabolism of the fungi. This multi-site mode of action prevents resistance development. It is a protective fungicide that is well distributed and adheres to the plant's surface but does not penetrate inside the plant tissues. It is recommended for different Leaf and fruit spots *[23]***.** Fluazinam 500 g/l SC is a multi-site contact, broad-spectrum fungicide. It has a multi-site mode of action that disrupts energy production in fungi. And also has great protective activity, while it has little curative or systemic activity. Control every stage of the fungal life cycle. It is effective to control Ascomycota fungi. Commonly used for leaf blight such as potato late blight [23]. Tebuconazole 250g/l EW is a systemic fungicide with protective, curative, and eradicant action. And also it is a fungicidal compound with a broad – spectrum systemic action which can be used as a foliar spray or seed dressing. It provides reliable efficacy over several weeks and controls numerous pathogens in various crops. It is recommended for several leaf spots, blight, and fruit rot [23].

Fungicides amended PDA (10ml) plates were used to grow fungi by placing a 5mm diameter disc from an actively growing culture in the center of each plate. Fungi were also grown on non-fungicide PDA (10ml) as the control treatment. All fungi were incubated at room temperature (27 ^{0}C) for several days. Treatments were distributed on a Completely Randomized Design (CRD) with four replicates.

2.5 Collected data/parameters

Radial growth of each fungus was measured and relative inhibition of colony growth (%) was calculated for each isolate by using the growth data values measured after three, seven, and ten days on control plates and plates amended with fungicides [10].

2.6 Data analysis

Percentage of inhibition calculated according to the formula: [11], [3]

$$\text{Percentage of inhibition} = \frac{\text{(Colony diameter (mm) of control - Colony diameter (mm) of test plate)} * 100}{\text{Colony diameter (mm) of control}}$$

The growth inhibition by fungicides was compared by mean separation using the Duncan Multiple Range Test (DMRT). The significance of the data was determined at p = 0.05**.**

3 Results and Discussion

3.1 Disease symptoms observation

We have observed that pods reaching maturity showed extensive rot. At the beginning of the disease on green pods, elliptical or elongated sunken small spots with raised light brown-reddish color margins were observed. Upon maturity, due to unfavorable climate conditions, those patches became larger forming a brown color lesion (Pic 01). At the time of harvesting the pods as a vegetable, lesions were observed in the immature pods as well. It did not cause critical damage to the crop during this stage but in some cases, it causes severe crop loss. If the pathogen-infected to the premature pods it can result in diseased pods with a shrink to thinner dimensions at their stigmatic ends than healthy ones. In later stages of disease development, those pods can be getting rotten and dry up prematurely (Pic 01) [19]. However; when the diseased pods reached maturity an extensive rot was

observed. Symptoms of the disease are observed over the entire surface of the pods. In later stages of disease development, pods prematurely were rotten and dried, leaving uneven raised spots on the surface.

3.2 Pathogen Isolation and pathogenicity testing

The causal organism was identified as *Bipolaris hawaiiensis* (M.B. Ellis) (Syno: *Drechslera hawaiiensis* (Bugnic.); *Helminthosporium hawaiiense* (Bugnic.)) [15]. In this cultivation, *Bipolaris hawaiiensis* pod rot is identified as a severe problem. This disease appears to be newly recorded for moringa in Sri Lanka. It was identified as a new disease of edible pods of moringa s in Maharashtra caused by Drechslera [Cochliobolus] hawaiiensis, a previously unreported host [13]. Rajangam [19] reported this disease in the Maharastra region in India. According to the Mycobank [15], this fungus can be classified as Kingdom: Fungi, Sub-kingdom: Dikarya, Division: Ascomycota, Sub-division: Pezizomycotina, Class: Dothideomycetes, Sub-class: Pleosporomycetidae, Order: Pleosporales, Family: Pleosporaceae, Genus: Drechslera. Majority of seeds for planting materials imports from India. Therefore; with the emerging of the oil extraction industry in Sri Lanka those fungi maybe move to Sri Lanka with the contaminated seeds. Oil extraction from dry seeds and several values added products from mature moringa was an immerging trade in Sri Lanka. Therefore, moringa pods should place in trees until dried-off to harvest seeds. At maturity, these brown lesions split and rot the pods. It was provided a place for several secondary infections on pests and diseases.

Those symptoms were spread and getting severe under high humid rainy climatic conditions. Because they are pruning the trees for the moringa leaf market it easily destroys the new flush with the overlap of the favorable rainy season. Therefore; to prevent the disease in young pods we can schedule the pruning cycle with the climatic conditions. Even though; it is not reported, in Sri Lanka, this *Drechslera* spp can be infected with other food crops such as green grams [9].

Pic 1: Infected moringa pods

Relatively fewer diseases are reported due to *Drechslera* spp in cash crops of Sri Lanka. Rubber is one of the main export agricultural crops is Sri Lanka and Bird's eye-spots disease in rubber nurseries was reported due to *Drechslera heveae* (Petch) M. B. Ellis. (*Bipolaris heveae* (Petch) Von Arx) [12]. Brimer and Boland, [4] reported that *Drechslera heveae* plays a major role in latex yield losses of rubber plantation in Sri Lanka. Coconut is the one of other major export agricultural crops in Sri Lanka which is also infected by *Drechslera heveae*. Leaf blight disease in coconut also knows as grey blight which is infected by two parasitic fungi named *Pestalozzia palmarum* and *Bipolaris incurvata* (earlier known as *Drechslera incurvataor*, *Helminthosporium incurvatum*) [7]. Normally, leaf blight in older palms is not serious. Though, seedlings and young palms outbreaks can damage the foliage, resulting in slower growth.

3.3 *In vitro* fungicide evaluation

Growth inhibition percentage was calculated after the three, seven, and ten days. It shows that the significant growth inhibition with the Tebuconazole 250 g/l EW at the rate of 3.5 ml/l over the other fungicides which are tested (Fig: 01). As in fig 01. Shows that the lowest control of the pathogen with the propineb 70% WP after the ten days.

But it had more than 50% growth inhibition percentage at the three days old cultures. All the tested chemicals had more than 50% growth inhibition percentage until seven days after culturing in laboratory conditions. But it can be varied with the environmental conditions since they are contact fungicides. Since pathogen can develop resistance against systemic fungicides, it is not recommended for applying more than two to three times per season. Therefore; in those situations, contact fungicides that partially control the pathogen in this study can be alternatively used with the Tebuconazole 250 g/l EW with seven to ten days intervals.

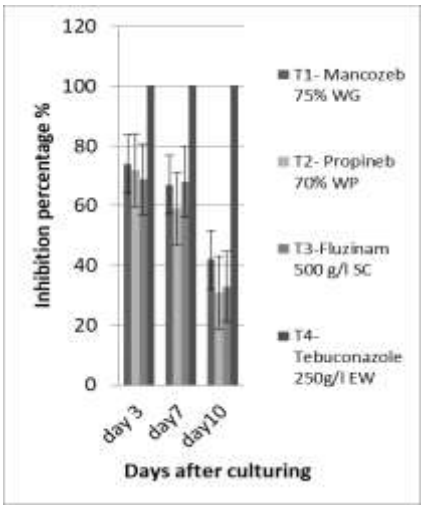
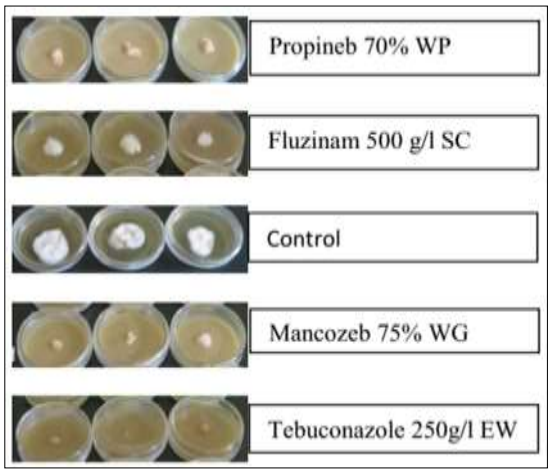
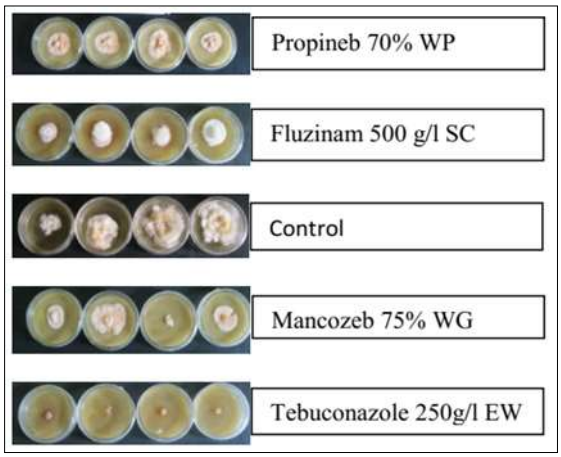


Fig 1: Growth inhibition percentage of fungi with amended fungicides

Although; in India they found Chlorothalonil, Iprodione and Maneb were effectively control the disease, the results obtained from the fungicide assay indicated that the Tebuconazole 250 g/l EW at the rate of 3.5 ml/10l was significantly (P<0.05) effective in controlling the growth of *Drechslera hawaiiensis*.



Pic 02: Fungal growth in Fungicides amended PDA plates (Day 3)



Pic 03: Fungal growth in Fungicides amended PDA plates (Day 10)

It confirmed at the Pic 02 and 03. In a future study, we can test for those fungicides. Tebuconazole 250 g/l EW is a systemic fungicide with protective and curative action. And also it is a class II fungicide. So it may be effective rather than other tested fungicides. *Drechslera heveae* was also found as a foliar pathogen in rubber cultivation in Sri Lanka where the disease was reported to be controlled by *Trichoderma* strains which showed antagonistic effects as a biological control method [22]. Therefore; there is a possibility to control *Drechslera hawaiiensis* in moringa with the biological agents in further studies.

4. Conclusion

In the moringa oil extraction seed crop, they have practiced pruning of the plant after the harvest in four months to get a new flush with new pods. When those pods were overlapped with high humid and rainy conditions the disease easily takes place. Therefore, we can recommend shifting the pruning time of the plant with climatic conditions. And also maintaining field sanitation should be a must. With the results of the laboratory testing Tebuconazole 250 g/l EW at the rate of 3.5 ml/10l was significantly controlled the disease.

Therefore; Tebuconazole 250 g/l EW at the rate of 3.5 ml/10l can be recommended for the field validation. But due to systemic fungicides, it has 21 days Post Harvest Interval (PHI). Therefore; it can be recommended for the early podding stage or at the flowering stage. Follow that; contact fungicides (Mancozeb 75% WG at the rate of 20g/10l, Propineb 70% WP at the rate of 20g/10l, Fluazinam 500 g/l SC at the rate of 10ml/10l) which partially controls the pathogen in this study can be alternatively used with seven to ten days intervals until 14 days to harvest. Since in India they found Chlorothalonil, Iprodione and Maneb were effectively control the disease, further studies can be done for those fungicides.

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