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Leaf rust of teak caused by *Olivea tectonae* in Bangladesh and its control

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Abstract

Teak leaf rust caused by *Olivea tectonae* is a serious disease in all teak growing areas of Bangladesh. In this region this rust was found in abundance and severely affects the leaves of teak plants and damages the activity of leaves. In this context, a survey was conducted to assess the teak rust disease incidence in four Thana of Chattogram and one Thana of Faridpur district of Bangladesh since 2017-2018. During the survey, the highest disease incidence (85 %) were recorded at Badarpur, Faridpur sadar Thana, Faridpur District and the lowest (65 %) were recorded at Fatikchari Thana, Chattogram district. Throughout the observation time, pustules of orange yellow color uredospores appeared profusely on the under surface of matured leaves especially on the adaxial surface near the mid rib leaf. Later the infected leaves dry up and appear burned, with severe to complete defoliation of young trees in 20-30 days from the onset of symptoms. Developed fungus was identified as *O. tectonae*. Pathogenicity test confirmed efficiency of *O. tectonae* to induce typical symptoms on teak plant. According to the available literature, this is the first record of leaf rust on teak in Bangladesh under natural infection in the field. Photoperiod and temperature experiments indicated better germination of uredospore in the dark at 22 - 26 °C. The maximum uredospore germination (65.53 %) was observed at 95 percent RH and at 65 percent RH no germination (0%) was observed. On testing the fungicides effectiveness some chemicals showed in some cases the inhibiting of uredospores germination. Diathene-M 45 showed the highest conidial inhibition % and Thiovit showed the lowest conidial inhibition percentages (21.38 %). Finally, it can be concluded that teak leaf rust caused by *O. tectonae* is a severe disease in all teak growing areas of Bangladesh that causes serious damages of teak plants. It is the first time detected in Bangladesh. A certain fungicides were effective in suppressing uredospore germination of *O. tectonae* under laboratory conditions. Field trials should be undertaken using chemicals which are effective and economical. So, this work may encourage other workers to study these diseases and their proper management.

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Introduction

Teak (*Tectona grandis* Linn. f.) is one of the most preferential timber all over the world, meanwhile it has been used for many centuries for a range of products and services. It is known for its strength, durability and maintain attractive appearance. Obviously, it constitutes high-class furniture and is one of the most sought after hardwoods in the international market. The species is native to the Indian-Burmese floristic region and found naturally in India, Myanmar, Thailand and Lao (Kaosa-ard, 1986). Due to its high timber qualities, market demand, ease of domestication and cultivation, teak plantations have been widely established throughout the tropics from the 1850s (FAO, 1957). At the present time, Teak has been successfully established as an exotic tree species in Bangladesh. Government and non-government organization have taken mass program to cultivate this plant in to the open ground and road side throughout the Bangladesh. During cultivation this plant is facing serious problems of insect pests and diseases. In nurseries and plantation, bacterial wilt, leaf spots, and leaf rust can causes serious problems. At present-day, Teak leaf rust caused by *O. tectonae* occurs throughout the range of distribution of the host in warmer areas of the world, such as India, Pakistan, Sri Lanka, Taiwan, Myanmar, Indonesia and Thailand (Gibson, 1992). Recently, the outbreak of this disease has been also seen in many Teak growing areas of Bangladesh.

It attacks trees growing in the nurseries, young plantations and also road side plantings. It is most prevalent during August to February. The severity of disease is favored by hot, dry weather and it causes an early defoliation and serious loss in increment of the tree. Teak has been adopted as an exotic plantation species in a number of tropical areas and teak rust is a potentially important disease in this veneration. In difference with many other diseases, published evidence on teak rust is very insufficient. Therefore, the purpose of this study was to survey for the present status of leaf rust disease incidence in different location of teak growing areas in Bangladesh and to identify the causal pathogen and its control.

Materials and methods

Disease Survey and Collection of Rust Samples

A survey was conducted to assess the teak rust disease incidence in Chattogram and Faridpur district of Bangladesh since 2017 to 2018. Disease assessments were done in four Thana viz. Hathazari, Fatikchhary, Raozan and Rangunia of Chattogram District and one Faridpur sadar Thana of Faridpur District, Bangladesh. Teak plants showing typical symptoms of leaf rust were collected from surveyed areas using paper bags and envelopes. The surveyed areas were the most important for teak growing areas of Bangladesh. This region has a subtropical monsoon climate characterized by wide seasonal variations in rainfall, moderately warm temperatures and high humidity. Three seasons are generally recognized in this area: a hot, humid summer from March to June; a cool, rainy monsoon season from June to October; and a cool, dry winter from October to March. In general, maximum summer temperatures range between 32°C and 38°C. April is the warmest month in this region. January is the coldest month, when the average temperature for most of the country is 10°C.

During survey, tress were randomly selected and the infected leaves were examined. Height and spacing of the trees were measured. Age of the trees were also obtained from the local forest office.

The disease incidence was assessed in different planting type's viz. Nursery teak plant, Road-side teak plantation, Group teak plantation and Single teak plantation.

The disease incidence (%) was calculated by following formula.

$$\text{Disease incidence (\%)} = \frac{\text{Number of plant affected}}{\text{Total number of plants observed}} \times 100$$

Study of Symptom and morphology

Rust infected leaves were collected and symptoms of rust on the leaves were systematically studied. Uredial morphology was checked under the microscope. For isolation of pathogen, the length and breadth of 100 spores were measured at random by

using an ocular micrometer under high power magnification (400 x).

Pathogenecity Test

Pathogenecity test of teak rust fungus was carried out at Forest pathology laboratory, BFRI, Bangladesh. Experiments were conducted using the detached leaf method (Amadi, 2003). Healthy mature leaves were collected from Seed orchard Division Nursery, BFRI, Chattogram, Bangladesh in sterile polyethylene bags and taken to the laboratory for artificial inoculation. Samples were surface-sterilized in 70 % ethanol for 3 minutes and rinsed in 2 changes of sterile distilled water. The leaves were pricked (injured) at four points with sterile needle and then placed on layers of sterile moist filter papers in Petri dishes. Spore suspensions (10^3 spores/mL) of the uredospores were prepared and 1 ml of fungal suspension was used separately to inoculate each test leaf. Inoculated leaves were incubated at 25° C in relative humidity chamber and observed frequently for symptom development. Re-isolations were carried out from inoculated leaves showing symptoms. Control plates were inoculated with sterile distilled water and also incubated at humidity chamber.

Determination of Uredospore Germination at Different Temperature and Photoperiods

Uredospores from the infected teak leaf were dusted on TEB (Teak leaf Extract Broth) plates. The plates were incubated in dark under eight different temperatures (5, 10, 15, 20, 25, 30, 35, and 40°C). The other plates were placed under three different conditions of photoperiod, namely (a) in the dark (b) under the fluorescent (2000 lux) and (c) 12 hours dark and 12 hours light alternately. The experiment was carried out with 4 replicates. Observations were made under the microscope after 24 hours of incubation.

Effect of Relative Humidity (RH) on Uredospore Germination

Two hundred µl of spore suspension prepared in TEB containing two per cent sucrose solution was taken on a cavity slide. The cavity slides were incubated at 25°

C in relative humidity chamber by maintaining different relative humidity levels viz., 60, 65, 70, 75, 80, 85, 90 and 95 %. Three replications were maintained for each treatment. Uredospore germination was observed after 24 h of incubation and the per cent germination was calculated.

Effects of Sucrose on Uredospore Germination

The germination of uredospores was studied in TEB (2.5 %) containing sucrose (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 %) and dextrose (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 %) using cavity slide method. Uredospores from the infected teak leaf dust were transferred on respective media. Later 200 µl of suspension was transferred to the cavity slides. The ambient temperature of 25° C and 100 percent RH was maintained throughout the experiment. The observations recorded after 24 hours by counting number of spores germinated per microscopic field (10 X magnification) and four microscopic fields were counted. The spores producing the germ tube twice their size was considered as germinated. The per cent germination was calculated by using the formula,

$$\text{Per cent spore germination} = \frac{\text{No of uredospores germinated}}{\text{Total no of uredospores observed}} \times 100$$

In Virto Inhibition of Different Fungicides on Uredospore Germination of O. tectonae

Fourteen fungicides (Table 1) were tested *in vitro* for their effects on spore germination inhibition of *O. tectonae*. The concentrations used for fungicides were 0.01, 0.10, 10, 100, and 1000 ppm. Firstly the fungicides were prepared as concentrated suspensions or solutions. Sterile distilled water was added separately in warm sterile 1.25 % TEB to obtain the required concentration. Fifteen ml of TEB medium incorporated with fungicides were then poured into 9 cm diameter petridish. Rust infected teak leaves were collected from Seed orchard Division Nursery, BFRI, Chattogram, Bangladesh.

The uredospores were dusted on the TEB medium on the plate from infected leaves. The plates were then incubated in the dark for twenty four hours at 25°C. Then a drop of lactophenol and cotton blue was

placed on the uredospores suspension on the slides. The slides were examined under high power microscope ($\times 400$) for recording the percentage of conidial germination. Percentages inhibition of uredospore germination (PIEG) using the formula by Skidmore and Dickinson (1976). Where, $PIEG = \frac{E_1}{E_2} \times 100$

$$\frac{E_1}{E_2} \times 100$$

E_1 = Total number of uredospore in control treatment.
 E_2 = Germination of uredospore in fungicidal treatment.

The experiment was carried out with 3 replicates. Control plates consisted of the media without fungicides. Observation was made after 24 hours of incubation.

Experimental design and statistical analysis

All experiment was of a Completely Randomized Design (CRD) with 4 replications. The statistical analysis was performed and mean data were compared by Duncan's Multiple Range Test (DMRT) using SPSS software.

Results and discussion

In Bangladesh, the disease has been observed for a long time, but there is little information about the disease. As a result, the experiment was conducted to survey of disease incidence in different planting types in different teak growing areas of Bangladesh, observation of disease symptoms, morphology and pathogenicity of *O. tectonae* and chemical control of pathogen under *in vitro* condition.

Table 1. The details of the fungicides used against the pathogen are given in the following table.

SL. No.	Trade Name	Common Name	Chemical Name
1.	Indofil	Indofil	Mencozeb
2.	Knowing	Knowing	Carbendazim
3.	Ridomil	Ridomil gold	Mancozeb
4.	Oxyvit	Oxyvit 50 WP	Copper oxychloride
5.	Cupravit	Cupravit 50 WP	Copper oxychloride
6.	Aimcozim	Aimcozim	Carbandazim
7.	Champion	Champion	Copper hydroxide
8.	Sunvit	Sunvit	Copper oxichloride
9.	Diathane M 45	Diathane M 45	Mancozeb
10.	Thiovit	Thiovit 80 WG	Sulpher
11.	Autostin	Autostin	Carbendazim
12.	Amivit	Amivit	Copper oxychloride
13.	Rovral	Rovral	Eprodion
14.	ABRA	ABRA	Carbendazim

Survey of disease incidence in different teak growing areas of Bangladesh

The incidence of teak rust in different planting types were showed in Table 2. The highest infection percentage assessed in group planting was at Badarpur, Faridpur sadar, Faridpur District (85 %) and the lowest incidence percentages (63 %) was recorded at Fatikchari Thana, Chattogram district.

The observation of the present disease survey agreed with the reports of many authors (Thakur *et al.*, 2015; Cabral *et al.*, 2010; Daly *et al.*, 2006), who stated that the prevalence of teak leaf rust in regions with hot and dry climatic conditions. *Symptom and morphology of pathogen* the infected leaves had a gray flecked appearance on the upper surface and there were masses of yellowish orange uredia on the

underside, in corresponding position. Color of uredospores changed from yellowish to tan color (Fig. 1). Uredinia were powdery, orange, sub-epidermal at first, later erumpent. Paraphyses were cylindrical, curved and swollen at the apex, 3-5 μ m thick. Uredospores were ovoid, orange-yellow, echinulate, 20-23 x 16-22 μ m, with germ pores present and cell walls 2-5 μ m thick. Teliospores were absent. The uredospore size of different isolates was shown in Table 3. Significant difference was found in length of uredospores in different isolates of *O. tectonae*, however, they were different in breadth among the isolates tested.

The symptoms and fungal characteristics agree with the description by Mulder and Gibson (1973) of *O. tectonae*, teak leaf rust.

Table 2. The incidence of teak rust in different planting types.

Planting type	Locations		Age	Average Height (m)	Spacing (m)	No. of plants observed	Disease incidence (%)
	Thana	District					
Nursery	Seed orchard Division Nursery, BFRI campus, Chattogram	Chattogram	6 months	0.8-0.9	-	100	78 c
Road-side plantation	Rangunia	Chattogram	5-13 years	4.8	8.6 x 9.1	50	65 d
Road side plantation	Rauzan	Chattogram	8-12 years	6.9	5.3 x 7.6	40	72 c
Road side plantation	Fatikchari	Chattogram	10-15 years	7.3	7.4 x 7.9	45	63 d
Group plantation	Badarpur, Faridpur sadar	Faridpur	5-7 years	2.8	3.5 x 3.8	60	85 a
Single plantation	Badarpur, Faridpur sadar	Faridpur	5-8 years	2.0	2.7 x 2.7	50	82 b

In a column different letters are significantly different by DMRT at 5% level.

Pathogenicity Test

Inoculated leaves showed soaked brown lesions along the leaf veins and extended to other parts of the leaves. Symptoms started from the injured portions of the leaves and spread throughout the leaf surface.

Inoculated leaves also showed white cottony growth covering the initial points of entry of the pathogen.

This symptom corresponds to that observed in the field during disease survey.

Table 3. Morphology of uredospores from different isolates of *O. tectonae*.

Isolates	Location		Shape	Length (µm)		Breadth (µm)	
	Thana	District		Range	Mean	Range	Mean
Nursery	Seed orchard Division, BFRI campus, Chattogram	Chattogram	Globose, Ovoid	13.0-24.8	22.6 ab	12.5-20.0	17.3 ab
Road-side plantation	Rangunia	Chattogram	Globose, Ovoid	12.0-23.6	21.8 ab	11.3-20.0	14.86 d
Road-side plantation	Rauzan	Chattogram	Globose, Ovoid	13.3-21.7	20.85 bc	10.86-15.32	15.63 cd
Road-side plantation	Fatikchari	Chattogram	Globose, Ovoid	11.4-19.75	23.62 a	12.53-12.64	18.72 a
Group plantation	Hathajari	Chattogram	Globose, Ovoid	18.5-24.1	19.28 cd	12.5-22.5	16.51 bcd
Group plantation	Badarpur, Faridpur sadar	Faridpur sadar	Globose, Ovoid	20.5-21.92	18.53 d	11.54-13.73	17.95 ab
Single teak plantation	Badarpur, Faridpur sadar	Faridpur sadar	Globose, Ovoid	17.5-25.0	19.8 cd	12.5-20.0	16.3 bcd

In a column same letters are not significantly different by DMRT at 5 % level.

The artificially inoculated organisms were later re-isolated from the inoculated teak leaves. In this study the rust infected teak plants showed the typical symptom as described by many workers (Mulder &

Gibson, 1973; Sharma *et al.*, 1985). There was significantly difference in uredial morphology of rust pathogen except the size of breadth among the isolates tests.

Table 4. Percent inhibition of uredospore germination of *O. tectonae* at different concentrations of fungicides.

SL. No.	Fungicide Name	Chemical Name	Percentages inhibition of uredospore germination (PIUG)					
			Concentrations (ppm)					
			0.01	0.10	1.0	10	100	1000
1.	Indofil	Mencozeb	24.74 ab	32.18 b	39.63b	95.53b	-	-
2.	Knowing	Carbendazim	22.31 cd	30.75 bc	32.63e	59.84e	-	-
3.	Ridomil	Mancozeb	22.26 cd	28.93cd	36.72d	92.42c	-	-
4.	Oxyvit	Copper oxychloride	18.64 e	23.74 e	29.53f	47.29g	58.63a	64.79a
5.	Cupravit	Copper oxychloride	16.58 f	21.63 f	20.62h	35.51h	46.93d	49.82d
6.	Aimcozim	Carbandazim	21.53 d	27.96 d	37.85cd	89.95d	-	-
7.	Champion	Copper hydroxide	19.32e	24.53 e	32.83e	49.63f	52.12b	59.18b
8.	Sunvit	Copper oxichloride	17.52ef	23.52 e	29.64f	46.28g	49.82c	56.34c
9.	Diathane M 45	Mancozeb	26.32a	36.74 a	43.92a	100a	-	-
10.	Thiovit	Sulpher	10.62g	15.38 g	18.93i	21.38j	31.32f	38.49f
11.	Autostin	Carbendazim	23.53bc	29.82 c	38.73bc	89.49d	-	-
12.	Amivit	Copper oxychloride	18.52e	24.42 e	26.25g	30.73i	41.93e	45.63e
13.	Rovral	Eprodion	8.42h	16.83 g	18.63i	22.31j	24.94g	29.42g
14.	ABRA	Carbendazim	21.49d	28.95 cd	36.75d	93.73c	-	-

In a column same letters are not significantly different by DMRT at 5 % level.

Uredospore Germination at Different Temperatures and Photoperiods

Percentage germination of uredospores at different incubated temperatures was shown in Fig. 2. Temperature plays an important role among the physical factors, which influences distribution,

growth and reproduction of the fungus. The highest uredospore germination was found at the temperature range of 20-25°C, followed closely by 15 and 25°C. No uredospore germinated at 5, 10, 35 and 40°C. Percentage germination of uredospores for different photoperiod test was shown in Fig. 3.

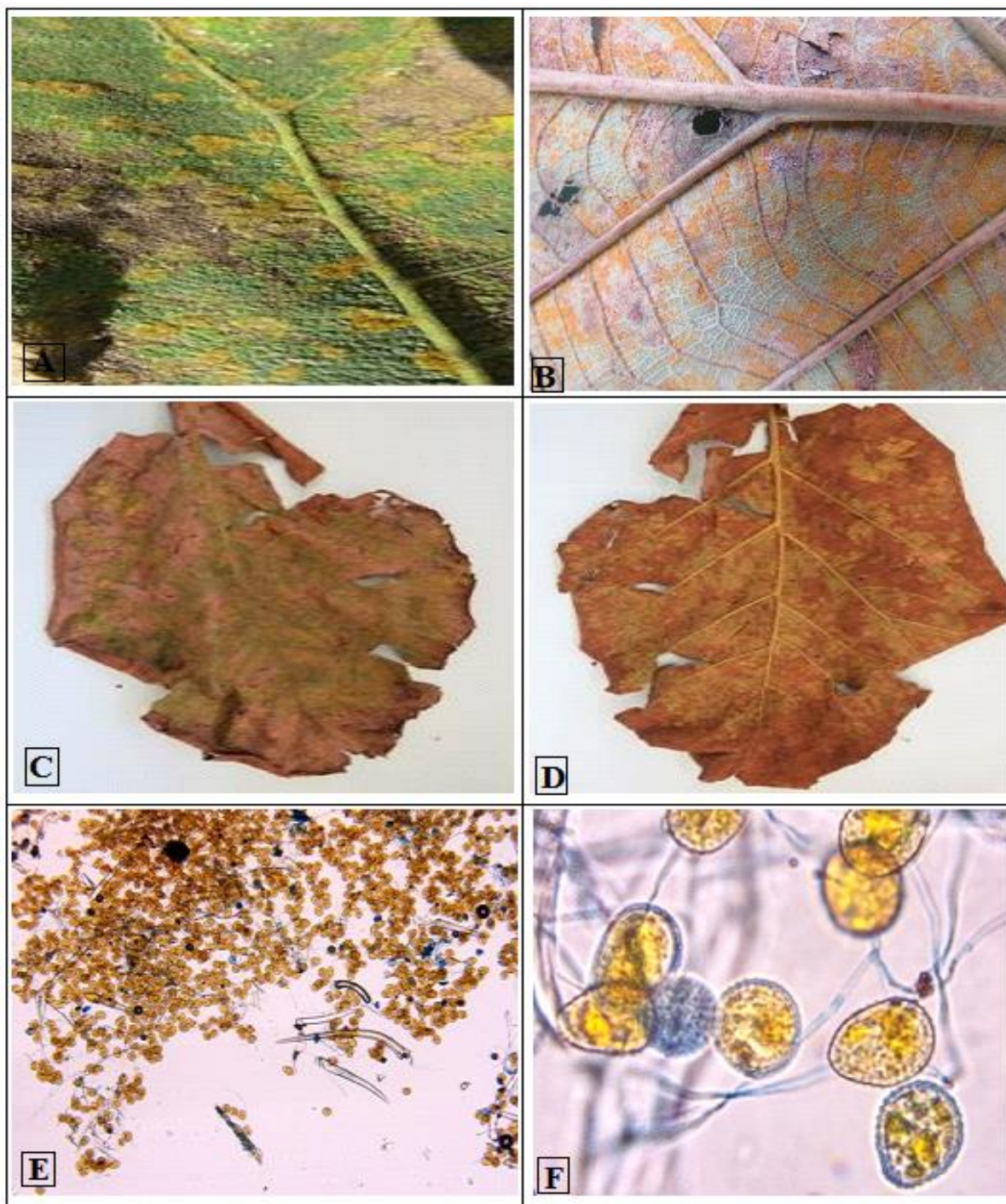
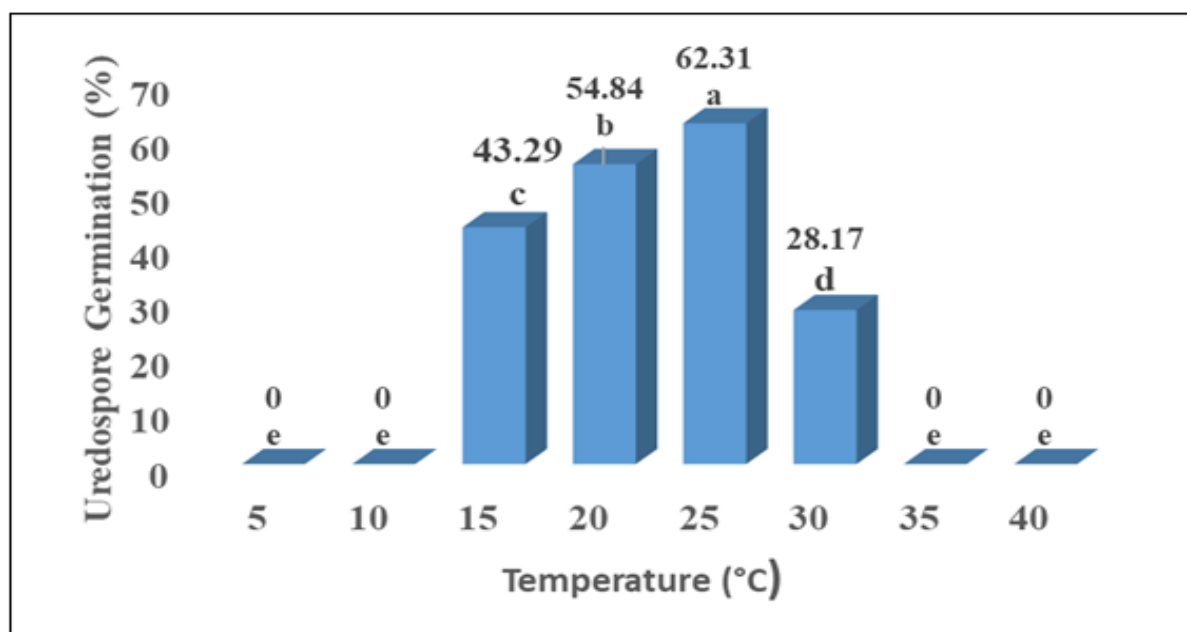


Fig. 1. Leaf of *Tectona grandis* (Teak) showing leaf rust symptoms and microscopic view of *Olivea tectonae*. (A) Necrosis on adaxial surface of the leaf, (B) urediospores on abaxial surface, (C) Dry leaf (upper surface), (D) Dry leaf (Lower surface), (E) Urediniospores and uredinial paraphyses, (F) Urediniospores of *Olivea tectonae* (10 μ m).

The highest germination was found in dark, moderate in dark and light and minimal under light. These findings are in agreement with Anusha *et al.*, (2018) who has reported that uredospore germination of *Cerotelium fici* (Cast.) Arth. is affected by the nutrient

availability and environmental factors like temperature and relative humidity. The optimum temperature estimated for *Cerotelium fici* uredospore germination found was 25°C after 24 hrs of incubation.



Bar marked by same letters are not significantly different by DMRT analysis at 5% level

Fig. 2. Uredospore germination of Badarpur, Faridpur isolate after 24 hours incubation on 1.25% TEA at different temperatures.

Effect of Relative Humidity on Uredospore Germination

Relative humidity markedly significantly affected the *O. tectonae* uredospore germination. In the present investigation, the maximum uredospore germination (65.53 %) was observed at 95 percent RH and at 65 percent RH no germination (0%) was observed (Fig. 4). Anusha *et al.* (2018) reported the highest uredospore germination of *Cerotelium fici* at 100 percent RH and least at 65 percent RH.

Effect of Sucrose and Glucose on Uredospore Germination

The sucrose at 2.5 percent concentration recorded the maximum uredospore germination (62.79%) and was found to be significantly superior to the other concentrations tested (Fig. 5).

The glucose at 2.5 percent was also found as best treatment showing 56.23 percent spore germination,

which was followed by sucrose at 2 percent concentration (54.68) and sucrose at 3 percent (53.53). In a similar study, Sumangala *et al.*, (2014) found that, the uredospore germination of brown rust pathogen of sugar cane was maximum in sucrose at 2.0 per cent and least in 0.5 per cent sucrose.

In Vitro Inhibition of Different Fungicides on Uredospore Germination of O. tectonae

The percentage inhibition of uredospore germination of *O. tectonae* by each fungicide at different concentration was shown in Table 4.

Amongst the tested fungicides Diathane M 45 was found to be the most effective and its 10 ppm concentration could cause 100 percent uredospore germination inhibition. Next to Indofil followed by ABRA and Ridomil was found to be the most effective at 10 ppm concentration. Thiovit showed the lowest conidial inhibition percentages (21.38 %).

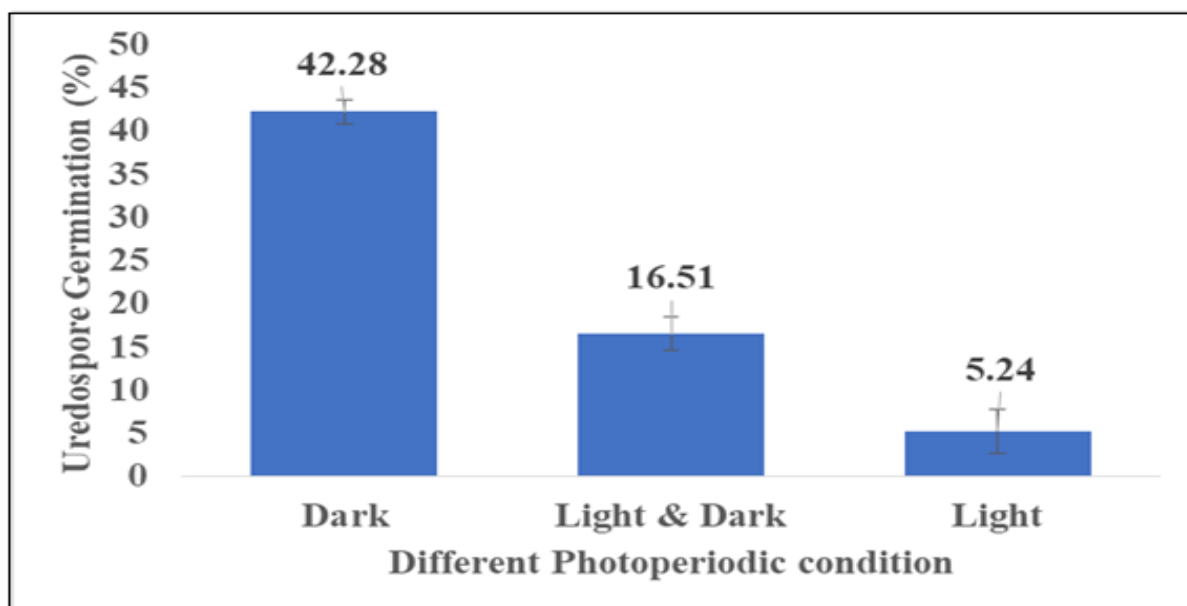
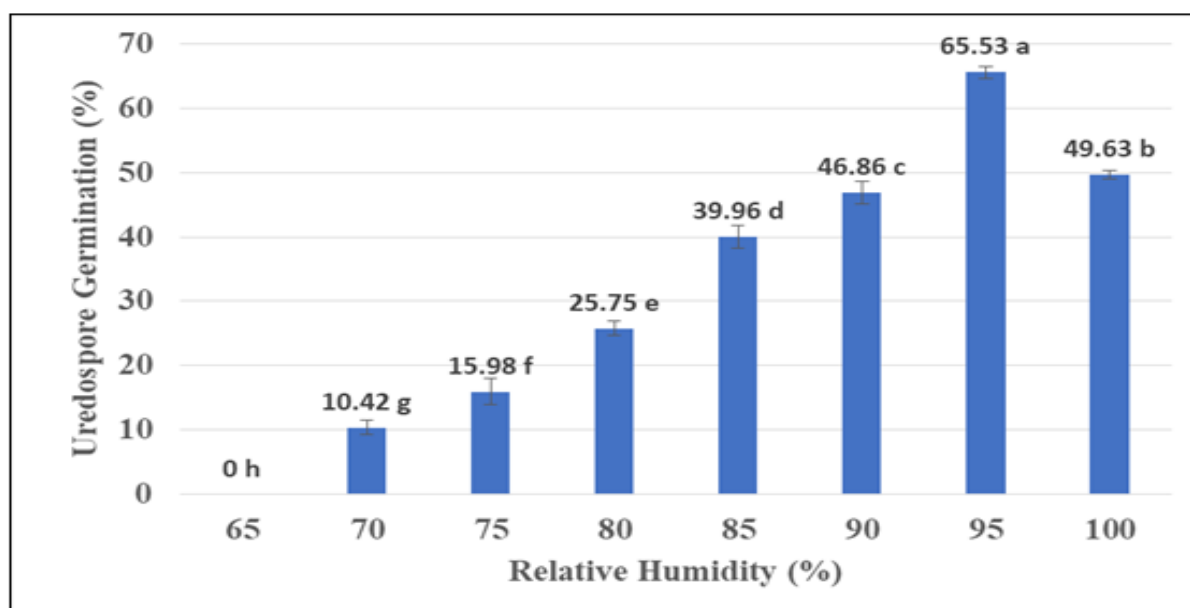


Fig. 3. Uredospore germination (%) of Badarpur, Faridpur isolate after 24 hours incubation on 1.25 % TEA at different photoperiods.

In a similar study, Shrivastava, *et al.* (1983) observed that Dithane M – 45 was more effective in reducing the severity of black and brown rust of wheat. In another study, Tamuli *et al.*, (2012) was carried out to study the efficacy of fungicides against leaf rust of *Cymbopogon* caused by *Puccinia nakanishikii* (Diet). They were evaluated five fungicides, viz., Bayer 5072 70 WP, Bayleton 25 WP, Vitavax 75% wp, Bayton 15 SD and Furavax 7.5% WP at two different

concentrations (0.1% & 0.2%) to control rust diseases of *Cymbopogon martinii* (Roxb.) Wats and *Cymbopogon citratus* (DC) Stapf.

Their results indicated that Bayleton at 0.1% exhibited good control of the disease with 3.25% and 6.25% disease intensity in *C. martinii* and *C. citratus* respectively. Other fungicides were not effective in controlling the disease.

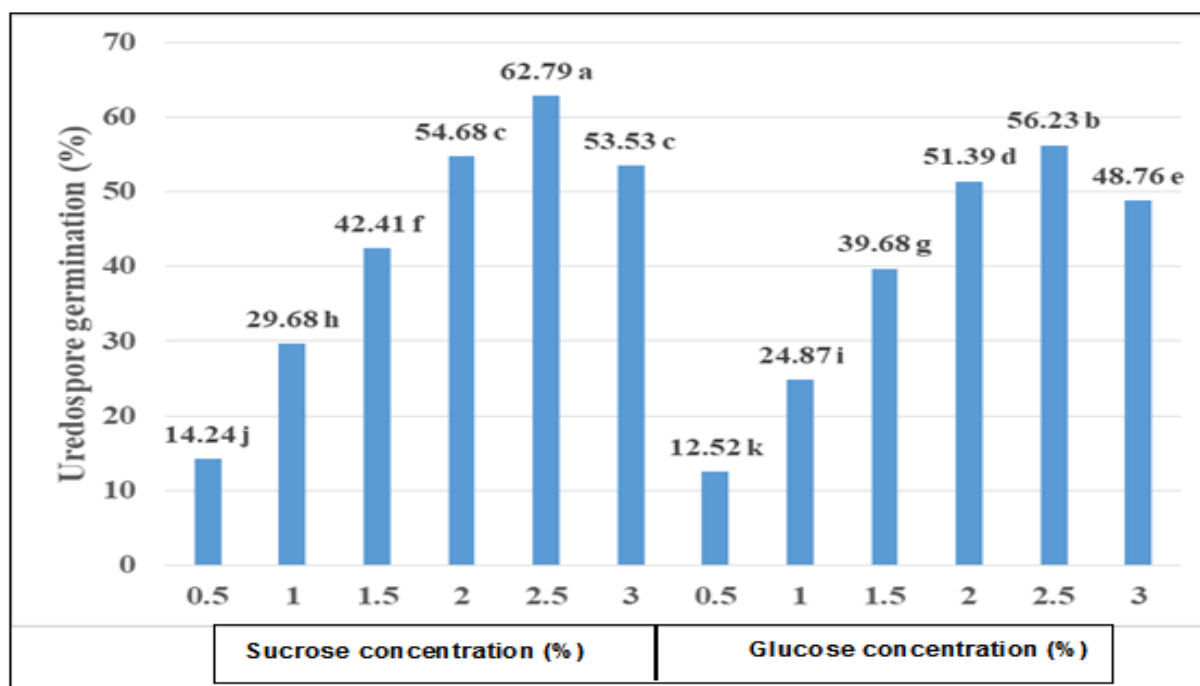


Bar marked by same letters are not significantly different by DMRT analysis at 5% level

Fig. 4. Uredospore germination of Badarpur, Faridpur isolate after 24 hours incubation on 1.25% TEA at different relative humidity.

In this study, all fungicides tests were toxic to uredospore germination of *O. tectonae*. However, Dithane M – 45 was most effective with low concentration. Finally, it can be concluded that a certain fungicides were effective in suppressing

uredospore germination of *O. tectonae* under laboratory conditions. Field trials should be undertaken using chemicals which are effective and economical.



Bar marked by same letters are not significantly different by DMRT analysis at 5% level

Fig. 5. Uredospore germination of *O. tectonae* after 24 hours incubation on 1.25% TEA at different sucrose and glucose concentration.

Conclusion

Teak leaf rust caused by *Olivea tectonae* is a severe disease in all teak growing areas of Bangladesh that causes serious damages of teak plants. The severity of disease is favored by hot, dry weather and it causes an early defoliation and serious loss in increment of the tree. According to the available literature, this is the first record of leaf rust on teak in Bangladesh. Photoperiod and temperature experiments indicated better germination of uredospore in the dark at 22 - 26 °C.

The maximum uredospore germination (65.53 %) was observed at 95 percent RH. On testing the fungicides effectiveness, Diathene-M 45 showed the highest conidial inhibition % (93.73) and Thiovit showed the lowest conidial inhibition percentages (21.38 %). Field trials should be undertaken using chemicals which are effective and economical. So, this work may

encourage other workers to study these diseases and their proper management.

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