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DIMOCARPUS LONGAN (JUNGLI LYCHEE) EXTRACT ACTS AGAINST *MAGNAPORTHE ORYZAE TRITICUM*, CASUAL AGENT OF WHEAT BLAST

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ABSTRACT

Mondol MAM (2021) *Dimocarpus longan* (jungli lychee) extract acts against *Magnaporthe oryzae Triticum*, casual agent of wheat blast. *Int. J. Sustain. Crop Prod.* 16(1), 24-28.

Rice blast, caused by a fungus, *Magnaporthe oryzae Triticum* (*MoT*) pathotype, is an important and serious disease of wheat worldwide. The disease has been reported to cause yield losses up to 100%. Extensive use of persistent synthetic pesticides to control notorious wheat blast imposing enormous threat to human health and environment as well as developing resistance in fungal population to fungicides. Additionally, increasing demand for organically produced foods, stimulated search for alternative approaches. Nowadays, botanical pesticides are gaining popularity due to effective in managing crop pests, inexpensive, biodegradable, easily available and have low toxicity to non-target organisms. With a view to identify active plant extracts, 11 extracts (five ethyl acetate and six methanol extracts) were subjected to *in vitro* screening test against *Magnaporthe Oryzae Triticum (MoT)* pathotype. The marked inhibitory activity (20 mm zone of inhibition) of methanol extract (1 mg/disk) obtained from *Dimocarpus longan* (jungli lychee) was shown against *MoT.* This plant extract can thus be used for developing natural fungicides against wheat blast. Other medicinal plant extracts showed little or no activity at all.

Key words: *activity, extracts, fungicides, medicinal plants, wheat blast, zone of inhibition*

INTRODUCTION

The world's population is expected to increase by 2 billion in the next 30 years, from presently 7.7 billion to 9.7 billion in 2050 according to a report of United Nations. To feed this huge growing population, we need to raise overall food production by some 70 percent. Increased demand for foods to feed the ever-growing population led to development and adoption of synthetic chemicals as a quick and effective strategy of managing crop pests and diseases (Geraldin *et al.* 2020).

In the last 70 years, synthetic pesticides had played a major role in huge production of crops by controlling pests and mankind was immensely benefited. Nevertheless, overuse and misuse of synthetic pesticides have resulted in the development of resistance by pests (bacteria, fungi, insects, weeds, etc), resurgence and outbreak of new pests, toxicity to non-target organisms and hazardous effects on the environment endangering the sustainability of ecosystems (Jeyasankar *et al.* 2005).

Wheat, a stable food of many nations, forms the base of global food security, providing 20% of proteins and calories of a vast population of developing countries. Wheat is cultivated across the world under varied climatic conditions ranging from sub-tropical to temperate. However, due to widespread cultivation in variable conditions, wheat has always faced a number of constraints from biotic and abiotic factors. Among these, the most devastating disease of wheat is blast.

The actual meaning of blast is explosion. Rice, wheat, barley etc crops are affected by blast diseases caused by distinct plant pathogenic fungi. The diagnostic symptom of rice blast includes diamond shaped lesions on leaves caused by *Magnaporthe oryzae* (TeBeest *et al.* 2007). Wheat blast, caused by *Magnaporthe Oryzae Triticum* (*MoT*) pathotype, directly infects wheat ear and renders grains shrunken, shriveled and deformed within a week of initial symptoms. A combination of high temperatures, excessive rain, long and frequent leaf wetness, and poor fungicide efficacy has favored the outbreak of this disease (Goulart *et al.* 2007).

Rice blast has been one of the most important and damaging, whereas wheat blast is of relatively recent occurrence (Maciel 2016). It was first reported in 1985 in Brazil and later quickly spread to other iso-climatic neighboring countries of South America. Wheat blast is now a serious production constraint in the tropic and sub-tropic regions causing yield losses of up to 100% (Peng *et al.* 2011).

In February 2016, the first outbreak of worrisome wheat blast disease was reported outside of South America in Kushtia, Meherpur, Chuadanga, Jhenaidah, Jessore, Barisal, Bhola, Magura, Narail, and Faridpur districts of Bangladesh (Malaker *et al.* 2016) affecting about 15% of the total wheat cultivated area with production losses up to 100%. Rapidly spreading notorious wheat blast diseases are imposing enormous threat to wheat production and thereby food security. Presently, wheat blast disease has emerged as a threat for global wheat production (Cruz and Valent, 2017).

Wheat is the second staple food of Bangladeshi population after rice. Currently, farmers heavily use synthetic persistent (not easily biodegradable) fungicides against wheat blast pathogens leading to the wide spread emergence of resistance which reduces fungicide efficacy (Castroagudin *et al.* 2015). Additionally, accumulation of these fungicides' residues in the environment (soil, water and air) is imposing enormous threat to the human health (cause cancer, neurological defects, asthma, allergies, birth defects, etc) and ecosystem. Many birds, aquatic and soil organisms, animals and soil fertility are under threat of toxic effect of synthetic and persistent fungicides which challenges food security of Bangladesh (Mahmood *et al*. 2016). However, antifungal resistance and environmental toxicity issues of conventional fungicides necessitate searching for safer and environmental friendly fungicides from natural sources against notorious wheat blast pathogen, *M. oryzae Triticum* (*MoT*).

Plants, as long-lived stationary organisms, defense attackers by producing secondary metabolites. These bioactive secondary metabolites can be extracted using suitable organic solvents. Management of pests using plant extracts was practiced since ancient time until synthetic pesticides were developed (Mahmood *et al.* 2016). The synthetic pesticides were immediately accepted after development due to their effectiveness and efficacy in managing serious crop diseases (Raja 2014). Consequently, the use of natural products of plant origin slowly faded until recently when use of synthetic pesticides started threatening human health and environmental safety (Nikkhah *et al*. 2017). Currently, organic foods produced by using plant-based pesticides are gaining popularity due to detection of hazardous residues of synthetic pesticides in foods and increased awareness among consumer on food safety (Karaca *et al.* 2017; Mishra *et al*. 2018).

Natural products which are safe for the environment and have low toxicity to living organisms are gaining interest as important sources for the development of fungicides, and these may serve as effective substitutes for synthetic fungicides. The main focus of this study was to evaluate *in vitro* efficacy of 11 medicinal plant extracts (six methanol extracts and five ethyl acetate extracts) against wheat blast caused by notorious fungal pathogen, *M. oryzae Triticum* (*MoT*) pathotype.

MATERIALS AND METHODS

General experimental procedures

Ethyl acetate (Scharlau, Spain), methanol (Scharlau, Spain), *n*-hexane (Daejung Chemicals and Metals Company Ltd, Korea), Mueller Hinton Agar (HiMedia, India), sterile filter paper disk (BioMaxima S.A., Poland), filter paper (Whatman Int. Ltd, Maid Stone, England), heavy duty blender (Havells, India) and iprodione (Auto Crop Care Ltd, Dhaka, Bangladesh) were bought from local suppliers. All used solvents and reagents were analytical and reagent grades, respectively.

Collection and storage of medicinal plants

Healthy stems and leaves of 11 medicinal plants (Table 1) were collected through an expedition in 2019 from Mirzapur under Tangali districts. These plants were collected in plastic bags. The plant parts (leaves and stems) were thoroughly washed under running tap water, chopped into small species and dried under shade for 2 weeks in the biological science lab, Bangladesh Open University. After drying, the plant materials were grinded into fine powdered form by using a blender, kept in plastic bags and stored in refrigerator.

Table 1. List of medicinal plants (local, English and scientific names) used for activity screening against *MoT* pathotype

SL	Local Name	Common Name	Scientific Name
1	Uggrogandha/Boch	Sweet flag	*Acorus calamus*
2	Kumari lata	Chinese moon crepper	*Paederia foetida*
3	Shate chita	Leadworts	*Plumbago auriculata*
4	Snake cactus	Snake plant	*Acanthocereus tetrogonus*
5	Chatim	Devil’s tree	*Alstonia scholaris*
6	Jungli lychee	Longan	*Dimocarpus longan*
7	Kunch	Rosary pea	*Abrus precatorius*
8	Ram tulsi	Hoary basil	*Ocimum americanum*
9	Agar	Agar wood	*Aquilaria malaccensis*
10	Gorachakra	Bowstring hemp	*Sansevieria roxburghiana*
11	Kalokashi/Kashowraj	False Daisy	*Eclipta alba*

Extraction of medicinal plants

Lately, powdered form of the above 11 medicinal plants (Table 1) was taken out from storage and subjected to solvent extraction. 100 g powder of each medicinal plant was taken in 2 L conical flasks; 1 L ethyl acetate was added to five of them (plant number 4, 7, 8, 9 and 10) and 1 L methanol was added to another six of them (plant number 1, 2, 3, 5, 6 and 11) and all were left for overnight. Then the plant material was removed by filtration using Whatman filter paper and the filtrate was concentrated to dryness using rotary evaporator at reduced temperature (40°C). Both ethyl acetate and methanol extracts were partitioned between methanol and *n*-hexane; the *n*-hexane phase, mainly contains fats, was discarded and methanol phase was concentrated to dryness using rotary evaporator at reduced temperature (40°C). These extracts were subjected to activity screening against *MoT* pathotype.

Collection of test pathogens

The test pathogen, *M. oryzae Triticum* (*MoT*) pathotype, was called from Bangladesh Wheat and Maize Research Institute (BWMRI), Nashipur, Dinajpur, Bangladesh. This test pathogen was isolated from infected wheat.

Seed culture of the test pathogen

To study activity of medicinal plants (Table 1), first the test pathogen (*MoT* pathotype) was streaked on the sterilized potato dextrose agar (PDA) medium (prepared according to the manufacturer's guideline) (3.9% w/v) from stock culture and then incubated at 28°C for five days. This seed culture was used for activity screening of medicinal plant extracts. All the microbial culture works were done under aseptic condition.

Activity assay against *MoT*

To prepare the activity assay plate, PDA medium was sterilized at 121°C for 20 min by autoclave machine. The medium was poured on the sterilized Petri dish (120 mm) and left to solidify in laminar airflow cabinet. Antifungal activity against *MoT* of the medicinal extracts was determined by agar disk diffusion assay method (Bauer *et al.* 1966). In brief, some mycelia of *MoT* was taken out from seed culture plate by sterilized cotton swab and spread on sterilized PDA medium. Each medicinal plant extract (Table 2) was dissolved and diluted with appropriate solvent combination. From diluted each plant extract, a specific amount of sample was taken out by the micropipette so that it contained 1 mg extract and impregnated in the sterile microbial susceptibility testing paper disk (6 mm). After drying, all the disks containing test samples were transferred about 1 cm apart by sterile forceps on the surface of the previously *MoT* pathogen spread agar plate. Then this plate was incubated at 28°C for five days. After incubation, zone of growth inhibition for each extract was measured in mm. Iprodione (1 mg/disk) and one sterile empty paper disk (6 mm) were used as positive (standard) and negative controls in this experiment, respectively.

RESULTS AND DISCUSSION

In vitro antifungal activity of 11 medicinal plants was evaluated against notorious wheat blast causing pathogen, *MoT*. The zones of inhibition (mm) exhibited by plant extracts are listed in Table 2. Among the studied plants, methanol extract obtained from jungli lyche (*D. longan)* showed a significant inhibition zone (20 mm) among all the extracts (methanol and ethyl acetate). Other extracts (1, 2 and 8) showed zone of inhibition ranged between 8-9 mm (Fig. 1 and Table 2). Unfortunately, extracts 3, 4, 5, 7, 9, 10 and 11 did not show any activity against tested pathogen.

Wheat is the second important staple food crop in Bangladesh after rice. Presently, wheat production in Bangladesh is under threat due to outbreak of devastating blast disease caused by *MoT* (Peng *et al*. 2011). Although some synthetic fungicides are being used for controlling wheat blast but heavily and frequent use of these fungicides imposing enormous threat to human and plant health as well as beneficial microorganisms, and ultimately leading to develop resistance by the pathogens.

Table 2. Yield of extracts of different medicinal plants and their activity against *MoT*

SL	Local Name	Scientific Name	Yield (in g)	Zone of inhibition (in mm) against *MoT*
1	Uggrogandha/Boch	*A. calamus*	2.98	9
2	Kumari lata	*P. foetida*	7.05	8
3	Shate chita	*P. auriculata*	1.28	0
4	Snake cactus	*A. tetrogonus*	0.67	0
5	Chatim	*A. scholaris*	2.32	0
6	Jungli lychee	*D. longan*	5.85	20
7	Kunch	*A. precatorius*	9.44	0
8	Ram tulsi	*O. americanum*	2.00	8
9	Agar	*A. malaccensis*	4.00	0
10	Gorachakra	*S. roxburghiana*	4.31	0
11	Kalokashi/Kashowraj	*E. alba*	2.00	0
12	Iprodione (+ve control)	-	-	30
13	Blank disk (-ve control)	-	-	0

One of the sustainable approaches to manage wheat blast by the application of natural pesticides especially originated from plant sources. Plant extracts have been well-known for their medicinal and antimicrobial properties since the history of mankind. They offer a greater scope for sustainable agriculture than synthetic chemicals as they are relatively safe, easily biodegradable and eco-friendly. Natural pesticides from plants are cheap, readily available and cost-effective for poor farmers in developing countries where synthetic fungicides are scarce and expensive (Mossini *et al.* 2004).

Fig. 1. Antifungal activity of 11 (serial numbers 1 to 11 of Table 2) extracts against notorious wheat blast casual agent, *MoT*.

Fungi are the main casual agents of plant diseases which cause a considerable loss of crop yield across the world. In an *in vitro* experiment, garlic clove extract (1:10 dilution) completely inhibited mycelia growth (up to 93.33%) of *MoT* and minimized disease incidence and severity with promotion of yield parameters (Zohura *et al.* 2018). The plant extracts obtained from processed coffee (*C. arabica*) exhibited 81.12% and 89.40% inhibitory effect at concentrations of 10% and 25% (w/v), respectively against *Pyricularia grisea*-the causal agent of rice blast disease with no phytotoxic to rice seedlings in applied concentration (Hubert *et al.* 2015).

The methanol extract obtained from stems of a tree, *Catalpa ovata,* exhibited potent *in vivo* antifungal activity against the most severe rice blast disease causal agent, *Magnaporthe grisea* (Cho *et al*. 2006). In an *in vivo* experiment with methanol extracts of invasive plants, *Amorpha fruticosa* and *Phytolacca americana*, showed potent antifungal activity at 3,000 ppm against severe crop fungal diseases *viz*., rice blast, tomato gray mold, pepper anthracnose and tomato late blight (Bajpai *et al.* 2012). Methanol extract of *Lawsonia inermis leaves* exhibited potent antifungal activity against major plant pathogenic fungi (Khan and Nasreen, 2010). *Pythium debaryanum* is a fungal species causes diseases many wild and cultivated plants against which methanol extracts of *Lawsonia inermis*, *Mimosa pudica* and *Phyllanthus niruri* revealed marked activity (25 mm, 20 mm and 20 mm inhibition zone, respectively) at a concentration of 40 mg/disk (Ambikapathy *et al.* 2011).

When zone of inhibition against *MoT* is compared in same concentration (1 mg/disk) between the most active extract (jungli lychee) and standard (iprodione), it has been shown that iprodione produced more zone of inhibition (30 mm) than extract of jungli lychee (20 mm). But the point to be noted in here that iprodione is a pure compound whereas extract of jungli lychee may contain many compounds out of which 2-3 compounds may be active. The pure form of these 2-3 compounds of jungli lychee extract may be more active than standard, iprodione.

There are several approaches in developing plant-derived natural pesticides in a cost-effective way: (1) Traditional extraction of bioactive secondary metabolites by solvents (e.g., water, methanol etc) under conditions from the field-grown plants that produce the highest levels of the compounds, determination of the dose of the bioactive extracts against target pests through lab and field level *in vivo* activity test; (2) Bioassay guided isolation and characterization of the plant-derived active secondary metabolites and subsequently their production through synthesis from inexpensive precursors or through fermentation by gene transfer to microorganisms and fixing dose through lab and field level experiments; (3) Enhancing biosynthesis of the target plant-derived bioactive compounds through inexpensively synthesized metabolic precursors; (4) Using plant growth regulators, elicitors, and metabolic blockers with a view to increase production of plant-derived target pesticides and so on. Active compounds of jungli lychee against wheat blast may be subjected to developing eco-friendly and cost-effective natural fungicides for sustainable agriculture practices following any one of the above appropriate approach.

CONCLUSION

Wheat production in Bangladesh is under threat due to outbreak of devastating wheat blast as currently available fungicides are partially effective against disease causal agents. Additionally, environmental toxicities of these fungicides further limit their application. The current findings suggest that extract obtained from leaves and stems of jungli lychee possess significant antifungal properties against *MoT* pathotype. This plant extract is safe and eco-friendly. Additional studies are required to identify and characterize the active antifungal compounds in the extract and their role in wheat blast disease control in order to develop natural and eco-friendly fungicides.

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CONFLICT OF INTEREST

There is no conflict of interest to declare.

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