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Research Paper

Plant tonic, a plant-derived bioactive natural product, exhibits antifungal activity against rice blast disease

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ABSTRACT

The tendency towards application of natural products and botanical extracts as safer antimicrobial agents against plant pathogens has recently been increased. Plant Tonic9 (EOX-SOV) is an environmentally friendly product and by its application there is no concern of resistance as it is with conventional pesticides. The goal of the present research was to determine the effect of application of this product against *Magnaporthe oryzae*, the causal agent of rice blast. The efficacy of plant tonic against *M. oryzae* was evaluated through in vitro and in vivo experiments. Under in vitro conditions, application of plant tonic at all rates (2, 3, and 4 mL/L) could significantly inhibit the mycelial growth and conidial germination of fungus with the highest inhibition (83.63% and 95.15%, respectively) recorded by the rate of 4 mL. Plant tonic treatment (3 and 4 mL) was more effective than fungicide treatment (propiconazole 25% EC (0.1%); 250 ppm) to inhibit mycelial growth and conidial germination of *M. oryzae*. Under in vivo conditions, plant tonic application (4 mL) was also the most effective treatment and resulted in a significant reduction (57.12%) of the area under the disease progress curve (AUDPC) value as compared with the control. Application of plant tonic also caused increased accumulation of phenolic compounds and higher activity of peroxidase and polyphenol oxidase enzymes than the control. The maximum amount of phenolic compounds (0.49 mg Gallic acid equivalent/g leaf fresh weight) and the highest activity of the enzymes (1.24 and 7.85 Units/mL for peroxidase and polyphenol oxidase, respectively) were observed in plants treated with plant tonic (4 mL) and challenged with *M. oryzae* as compared with other treatments. No phytotoxicity was observed in plant tonic treated rice plants when compared with the control. Results of the present study confirmed the beneficial effects of plant tonic in controlling rice blast disease. Therefore, its application may help to develop appropriate management strategies and provide with the opportunity to have cleaner and safer environment for agriculture.

1. Introduction

Rice (*Oryza sativa* L.) is a globally staple food providing 15% of world human per capita protein and 21% of per capita energy (IRRI, 2013). For Malaysians, rice is considered as a staple foodstuff in their everyday diet symbolizing of traditional Malay culture. Rice blast disease caused by the fungus *Magnaporthe oryzae* (Couch and Kohn, 2002) (asexual morph, *Pyricularia oryzae* Cavara) is considered as one of the main rice yield limiting factors worldwide (Abed-Ashtiani et al., 2012) having the potential of being extremely disastrous under conducive environmental conditions (Scardaci et al., 1997). The disease may cause up to 60–100% yield losses (Kihoro et al., 2013).

The serious challenge in disease control is to develop and expand sustainable management techniques in order to lessen the ability of the pathogen to be a threatening factor in the future. Fungicide application and planting resistant varieties are considered as two main methods to control blast disease (Kato, 2001). Although both aforementioned practices have provided interim relief, the disease still remains as a problem in rice-growing tracts of Malaysia (Personal communication with Department of Agriculture (DOA), Malaysia). This can be explained through the fact that repeated use of the same or similar fungicide may create resistance to that fungicide (García et al., 2003). In addition, rice blast is a fast spreading disease and the use of resistant varieties cannot be considered as a long term remedy (Abed-Ashtiani

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et al., 2012). Hence an integrated management program is needed to successfully control rice blast disease (Pooja and Katoch, 2014; Scardaci et al., 1997).

In modern agricultural practices, it is very important to manage pests and diseases using highly efficient methods with minimum damage and harm to the environment. In this issue, the concept of environmentally friendly methods of control is noticeable. Recently due to the concerns relating to the safety of synthetic fungicides, there is a growing tendency for researchers to find out the possibility and benefits of the application of natural products such as plant extracts, plant-based essential oils, and vegetable oils to control plant pathogenic fungi, since these products can greatly inspire modern agro-chemical research and their usage is an alternative strategy to the currently applied chemical fungicides (Bajpai and Kang, 2012).

Medicinal and antimicrobial properties of plant extracts have been known since ancient times (Lalitha et al., 2010). Potential antifungal activities of some plant extracts and essential oils against plant pathogenic fungi have been reported in previous studies (Al-Reza et al., 2010; Veloz-García et al., 2010). Plant extracts and natural chemicals are formulated using botanical and vegetable oils extracted from some trees such as palm and neem. They are easily biodegradable, readily available and cost-effective. Therefore application of these products can be considered as a perfect alternative to synthetic pesticides (Bajpai and Kang, 2012) especially for resource-poor farmers in developing countries where synthetic fungicides are scarce and expensive (Mossini et al., 2004).

Plant Tonic9 (EOX-SOV; Sovereign Innovations Sdn Bhd, Malaysia in collaboration with EOX International b.v. Netherlands), is an environmentally friendly, anti-fungus, anti-bacteria and anti-viral bio-Nano surfactant made through a biotechnological process with the following characteristics as stated by the manufacturer: pH = 7.1–7.4; physical state = liquid; chemical entity = lipid ester; surface tension = 27 millinewtons per meter ($mN\ m^{-1}$); solubility in water = -soluble; and micelle diameter = 4–7 nm. The equivalent acknowledgement was also produced by the Pesticides Control Division, Department of Agriculture of Malaysia which cited the Sovereign Plant Tonic Liquid requires no registration from Pesticide Board, since it does not contain any chemical active ingredient (as listed in the First Schedule of the Pesticides Act 1974). As stated by the manufacturer, plant tonic contains palm oil and palm kernel oil with lauric acid.

Earlier studies reported that lauric acid has antifungal properties against plant pathogenic fungi (Liu et al., 2008; Walters et al., 2003). Antifungal activities of other fatty acids on plant pathogenic fungi have also been reported in previous studies (Walters et al., 2004). However, to the best of our knowledge, the efficacy of application of plant tonic9 (EOX-SOV) against *M. oryzae* has not previously been published. Therefore the objective of this study was to evaluate the in vitro and in vivo antifungal activities of plant tonic on *M. oryzae* in Malaysia.

2. Materials and methods

2.1. In vitro assessment of antifungal activity of plant tonic

2.1.1. Fungal material

A virulent isolate of *M. oryzae* (isolate PO-FA35; ITS acc. no. KM249971, Actin acc. no. KM250006, β-tubulin acc. no. KM250041, and Calmodulin acc. no. KM250076), obtained from the Laboratory of Plant Pathology, Department of Plant Protection, Universiti Putra Malaysia (UPM) was used as source of fungal material in this study. The fungal isolate was maintained on potato dextrose agar (PDA, Difco, USA) culture medium and incubated at 27 ± 1 °C for 10 days under 12/12 h fluorescent light/darkness regime for experimental usage. For long term maintenance the isolate was plated on PDA slants and kept at 5 °C.

2.1.2. Mycelial growth assay

Proper amount of plant tonic (original concentration 100%, solubility in water > 95%) was separately added to the molten sterilized PDA (50 °C) to prepare desired concentrations (2, 3, and 4 mL plant tonic/L PDA; based on the manufacturer recommendations). Conventional fungicide propiconazole 25% EC (0.1%; 250 ppm) was included as positive control (Naik et al., 2012). Non-treated PDA served as negative control. The mycelial discs taken from 10-day-old culture of fungus using a sterile 5 mm diameter cork borer were aseptically located in the center of petri dishes containing solidified treated and non-treated PDA. Prior to experiment the petri dishes were marked with two perpendicular lines at the bottom to indicate their center. Plates were incubated at 27 ± 1 °C and after 10 days of incubation, the observations on the colony diameter of tested fungus were recorded. The inhibition percent in mycelial growth of fungus in each treatment was calculated according to the given equation (Nisa et al., 2011) after slight modification:

$$\text{Mycelial growth inhibition (\%)} = [(dc - dt)/(dc - 5)] \times 100$$

Where dc is fungal colony diameter of the control, dt is fungal colony diameter of treatment group, and 5 is the diameter of the inoculum disk.

2.1.3. Conidial germination assay

To prepare conidial suspension, the surface of 10-day-old fungal culture plates were softly scratched by spatula and plates were kept under wet muslin cloth for two days with continued light to impel sporulation. Then the surface of the plates were washed with 5–10 mL sterilized distilled water. A haemocytometer was used to adjust the concentration of conidia to 3×10^5 conidia/mL. Desired concentrations of plant tonic (2, 3, and 4 mL plant tonic/L water; based on the manufacturer recommendations) were prepared. The amount of 0.1 mL of different concentrations of plant tonic was pipetted into cavity glass slides. Conidial suspension (0.1 mL) was placed in cavity glass slides containing different concentrations of plant tonic. Slides were placed in petri dishes laden with moist filter papers and were incubated at 24 ± 2 °C (Nisa et al., 2011). After 7 h (Madani et al., 2014) a calibrated microscope (Olympus, Japan) at magnification of 100 × was used to observe the germination of 100 conidia in 10 randomly chosen microscopic fields to calculate germination percent. Conidial germination of fungus in propiconazole 25% EC (0.1%; 250 ppm) and in distilled water served as positive and negative control respectively. Conidia were considered germinated when the length of germ tube was equal or longer than the conidia (Ogbebor and Adekunle, 2005). The conidial germination percent was calculated according to the equation below (Kiraly et al., 1974):

$$\text{Conidial germination (\%)} = \frac{\text{Number of conidia germinated}}{\text{Total number of conidia examined}} \times 100$$

In vitro experiments were carried out in a completely randomized design with four replications maintained for each concentration. The experiments were repeated twice.

2.2. in vivo assessment of antifungal activity of plant tonic

2.2.1. Rice variety and cultivation

Rice seeds of MR219 variety obtained in Field 10, University Agriculture Park at UPM were used as source of plant materials. Rice variety MR219 is the most popular variety planted in 72% of all rice plantation areas in Malaysia (Noor, 2011). This variety was developed by the Malaysian Agricultural Research and Development Institute (MARDI) in 2001 as a blast-resistant variety. However, currently due to the emergence of new patho-types of blast fungus it has become blast-susceptible (Tanweer et al., 2015). Therefore, this variety was chosen to work with in this experiment. An Ultisol with the following chemical characteristics: pH 4.60; K, P, Ca, Fe, Mg, Na = 439.00, 24.00, 1330.00,