SAARC J. Agric., 21(1): 79-87 (2023) *DOI: https://doi.org/10.3329/sja.v21i1.65590*

POTENTIAL USES OF UNDERUTILIZED PLANT SPECIES FOR THE MASS PRODUCTION OF *Trichoderma harzianum* L.

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ABSTRACT

Several plant diseases can be controlled biologically using *Trichoderma* spp. However, the mass production and storage of *Trichoderma* spp. is highly expensive. Therefore, this research was carried out at Fruit Crop and Development Center, Horana, Sri Lanka to screen the effects of leaves of four different crops and another 13 underutilized wild plant species on their suitability in the mass production of *Trichoderma harzianum* L. Seventeen different media were prepared using green leaves (5g) of individual plant species comprised with glucose (5g/L) and distilled water (50ml). Treatments were arranged in Complete Randomized Design (CRD) with five replications. Spore counts of fungus were recorded using hemocytometer at 1st, 2nd, 3rd and 4th week after inoculation. Different treatments showed significant variations in spore counting of *T. harzianum* after 1st, 2nd, 3rd and 4th week of inoculation ($p<0.05$). Castor ($9.8x10^7$ CFU/ml), *gadapana* ($9.64x10^7$ CFU/ml) and *erabadu* ($9.64x10^7$ CFU/ml) had significantly higher spore count at the 1st week while *kappettiya* ($25.31x10^7$ CFU/ml) and *habarala* ($25.21x10^7$ CFU/ml) had the highest values at 2nd week. Significantly increased spore count of $61.5x10^7$ CFU/ml and $61.2x10^7$ CFU/ml were resulted from castor and *kappettiya* during 3rd week after inoculation. However, a sharp increase in spore count was found at 4th week, particularly in *wal sooriya kantha* ($157.17x10^7$ CFU/ml) while in contrary, the lemon, *rambutan, bovitiya,* jack and mango leaves showed poor performances in the mass production of *T. harzianum.* Based on these results, *wal sooriya kantha* can be successfully used as growing media for *T. harzianum*.

Keywords: Biological control, Castor, *Kappettiya*, *Trichoderma harzianum* L. *Wal sooriya kantha*

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Received: 12.04.2023 *Accepted: 17.05.2023*

INTRODUCTION

The extensively researched bio-control agent *Trichoderma* spp. has been used to combat a variety of soil-borne pathogens, particularly fungi (Mukherjee et al., 2012). *Trichoderma* species have been shown to have an antagonistic effect on phytopathogenic microbes that are particularly destructive to agriculture, including those belonging to the genera *Fusarium*, *Phytophthora*, *Sclerotinia*, *Rhizoctonia*, and *Pythium* (Jayasooriya et al., 2022: Rini and Sulochana, 2007). By secreting hydrolytic enzymes like chitinase and glucanase, which dissolve cell walls, these fungi act as mycoparasites (Kubicek et al., 2001). Additionally, *Trichoderma* spp. produces antibiotic substances that affect the biocontrol potential (Howell, 2003). They can directly compete with phytopathogens for resources and space to their rapid development (Irfeey et al., 2018), while they can also prevent infection by encouraging plant growth and triggering acquired resistance mechanisms in the plant. This biological management technique is thought to be environmentally safe and it eliminates the dangers that chemical pesticides present (Kumar et al., 2017).

Culture media is a term used to describe nutritional material used for the development of microorganisms in a laboratory. A variety of fungi, including *Trichoderma spp*., can be grown on potato dextrose agar (PDA), and its components are well known; Agar, dextrose, and potato extract (Asiandu et al., 2023). Instead of potatoes, maize and rice can be used as fungus-growing media, and they are frequently employed to spread *Trichoderma* spp. However, high price of these media and the lack of information have discouraged farmers, particularly in underdeveloped nations, from utilizing them on their fields (Gusnawaty et al., 2017). Large quantities of propagation media are needed for the large-scale production of *Trichoderma* spp.

Previous studies have been reported several alternatives to PDA media that can be used to grow *Trichoderma* spp. Different organic media like neem cake, coir pith, farm yard manure and decomposed coffee pulp have been suggested for *Trichoderma* spp. multiplication (Saju et al., 2002). The most prevalent and reasonably priced organic resources to be found in nearby farm fields include compost, cow dung, paddy husk, paddy straw, coir dust, and *Gliricidia sepium* (Irfeey et al., 2018). After optimizing the conditions, these materials can be used to quick collect a sizable population of *Trichoderma* spp. from farmer's fields. Tofu liquid waste and rice washing waste (Asiandu et al., 2023), chick pea (Uthayasooriyan et al., 2016) and sago medium (Tharmila et al., 2011) have also given positive results. Hence, the present study was aimed in replacing the nutrient source by various locally available different underutilized plant species for the mass production of *Trichoderma harzianum* L.

MATERIALS AND METHODS

Preparation of initial inoculate

The culture of *T. harzianum*, already available in Plant Pathology Laboratory, Fruit Crop and Development Center, Department of Agriculture, Horana was cultured on PDA medium. Under sterilized environment the petri dishes having PDA media were incubated at 26℃ ± 2℃ and the growth of the fungus was observed periodically. Initial inocula were made by scraping cultures that had been growing on the surface of PDA plates for seven days and were fully sporulated.

Preparation of organic media and inoculation of *T. harzianum*

Seventeen different types of plant species namely Gandapana (*Camara aculeata* L. Kuntze), Castor (*Ricinus communis* L.), Wal Suriyakantha (*Tithonia diversifolia*), Gliricidia (*Gliricidia sepium*), Habarala (*Alocasia cordifolia (Bory)* Cordem, Pota wel (*Pothos scandens* L.), Erabadu (*Erythrina variegata*), Kudzu (*Pueraria montana var. lobata*), Keppetiya (*Aleurites laccifer* L.), Mango (*Mangifera indica*), Dry banana leaves (*Musa acuminata*), Jack fruit (*Artocarpus heterophyllus*), Rambutan *(Nephelium lappaceum)*, Heen Bovitiya (*Osbeckia octandra*), Coconut (*Cocos nucifera*), Pani thora (*Cassia caroliniana walter)* and Lemon (*Citrus limon*) were selected for this study.

Fully expanded green leaves from each plant species were collected and washed three times by using distilled water. Leaves were cut into small pieces to prepare the growing media. Liquid growth media comprising 5g of green leaves of individual plant species, 5g of glucose and 50 ml of distilled water was prepared in a conical flask (250 ml) and sterilized at 121℃ for 30 minutes under the pressure of 1.09kg/cm^2. Then *T. harzianum* was inoculated in each and every media and incubated for one month on the shaker (10 rpm). Treatments (17 different media) were arranged in Complete Randomized Design (CRD) with five replications.

Spore counting and data collection

The growth of *T. harzianum* was counted in terms of number of spores per ml for each treatment after dilution in laboratory. One ml of culture was added into test tube containing nine milliliters of distilled water. The light microscope was set up such that the grids of the hemocytometer, which was kept on stage, could be seen clearly. On either side of the hemocytometer's grid, a drop of suspension was added, then the region was covered with a cover slip. Through a 40 times magnification, spores were counted. Spore counts were recorded in 1st, 2nd, 3rd and 4th week after inoculation. The following formula was used to calculate the spore count of *T. harzianum.*

Spore count = Total spore counting × 50000 × Dilution factor

Data analysis

Statistical analysis was done using SPSS software (version 25). Treatments were compared using the analysis of variance (ANOVA). To determine whether there was

a significant difference between the treatment means at 0.05 probability levels, the Tukey's post-hoc test was performed.

RESULTS AND DISCUSSION

The counting of colony forming units (CFU) generated on selective media, as well as chemical, biological, and immunological assays, are traditional methods for monitoring fungi (Lievens et al., 2006). CFUs were measured in the current study to examine *T. harzianum* growth in various green manures for its mass production.

Growth of *T. harzianum* after 1st week of inoculation

Different treatments showed significant variations in spore counting of *T. harzianum* after one week of inoculation ($p<0.05$). Castor ($9.8x10^7$ CFU/ml), Gadapana ($9.64x10^7$ CFU/ml) and Erabadu ($9.64x10^7$ CFU/ml) had significantly higher CFU while the lowest values were recorded in Pani Thora, Jack, *Pota Wal*, Lemon, Heen bovitiya, Rambutan and Mango where the CFU ranged from $0.011x10^7$ CFU/ml (Mango) to $1.159x10^7$ CFU/ml (Pani Thora) (Table 1).

Castor, a non-food plant in the Euphorbiaceous family, is a key ingredient in the manufacturing of biodiesel and biofuel. Approximately, 1.3% of N, 8.12% of crude protein, 0.3% of P, 0.36% of K, and 0.43% of S are found in castor leaves (Nahar, 2013). In earlier studies, Castor was shown to be a superb medium for *T. harzianum* mass production. Rao et al. (1998) evaluated aqueous extracts of Neem (*Azadiracta indica*), Castor, and Pongamia (*Pongamia pinnata*) cakes as substrates for the mass production of *T. harzianum* and came to the conclusion that Castor extract at 10 % resulted in the maximum growth of mycelial mat and spore production ($9.74x10^3$ spores/ml of extract). According to Reddy, et al. (1996), *T. harzianum* combined with oil cakes of Neem, Castor, and Karanj (*Pongamia pinnata* Merr.) was successful in lowering the citrus nematode and boosting the growth of acid lime seedlings.

The tropical ornamental weed known as Gandapana (*Lantana camera* L.), which is a member of the Verbenaceae family, develops into an upright shrub. Triterpenoids, proteins, sugars, lactones, furfural, and flavonoids are among the main and secondary metabolites found in Gandapana leaves. According to the study by Kapil and Kapoor (2012), Wheat bran, *Gandapana*, and farm yard manure were the next most common places to find mass proliferation of *Trichoderma* spp. Feyisa et al. (2016) used Gandapana in conjunction with *T. harzianum* successfully control the root-knot nematode in tomato.

Growth of *T. harzianum* after 2nd week of inoculation

After 2nd week of fungal inoculation, significant differences were observed in the spore count of *T. harzianum* ($p<0.05$). Significantly higher spore counts were obtained in Kappettiya ($25.31x10^7$ CFU/ml) and Habarala ($25.21x10^7$ CFU/ml) while lower spore counts were recorded in Lemon ($0.075x10^7$ CFU/ml), Rambutan ($0.037x10^7$ CFU/ml), Heen Bovitiya ($0.05x10^7$ CFU/ml), Jack ($0.078x10^7$ CFU/ml)

and Mango ($0.027x10^7$ CFU/ml) (Table 1). Though the spore count of *T. harzianum* were higher in Castor and *Erabadu* treatments during 1st week, it remained almost same during 2nd week after inoculation.

The Euphorbiaceae family contains the genus Kappettiya (*Croton aromaticus*), which is common in tropical areas. It has been discovered that the genus Croton is abundant in bioactive substances like diterpenes and alkaloids (Salatino et al., 2007). In Sri Lanka, the leaves of the Kappettiya plant are used to fertilize paddy fields because several soil-borne pests and diseases may be prevented by them (Jayaweera and Senaratna, 2006).

Growth of *T. harzianum* after 3rd week of inoculation

Significant differences were observed in the spore count of *T. harzianum* after 3rd week of inoculation ($p<0.05$). Higher spore count of $61.5x10^7$ CFU/ml and $61.2x10^7$ CFU/ml were resulted from Castor and Kappettiya respectively. Lemon (0.076×10^7 CFU/ml), Rambutan (0.05×10^7 CFU/ml), Heen Bovitiya ($0.242x10^7$ CFU/ml), dry Coconut leaves ($1.623x10^7$ CFU/ml), Jack (0.102×10^7 CFU/ml) and Mango ($0.034x10^7$ CFU/ml) resulted the lower spore counts (Table 1). A decrease in spore count was observed in Habarala and dry Coconut leaves 3rd week after inoculation.

Growth of *T. harzianum* after 4th week of inoculation

Different treatments had significant effect on spore count of *T. harzianum* after 4th week of fungal inoculation ($p<0.05$). Wal Sooriya Kantha had the highest spore count ($157.1x10^7$ CFU/ml) compared to other treatments in contrast lower spore counts were recorded in Gadapana, Lemon, Rambutan, Heen Bovitiya, dry Coconut leaves, Pani Thora, Erabadu, Jack and Mango where the CFU ranged from $0.035x10^7$ CFU/ml (Mango) to $16.27x10^7$ CFU/ml (*Erabadu*) (Table 1). A decrease in spore count was observed in Gadapana and dry Coconut leaves 4th week after inoculation. Though the multiplication of *T. harzianum* in Wal Sooriya Kantha treatment was lower during 1st, 2nd. and 3rd week after inoculation, the multiplication was higher during 4th week after inoculation compared to other treatments.

The Mexican sunflower, also known as *Tithonia diversifolia* (*Wal sooriya kantha*), is a species of flowering plant in the Asteraceae family. Even though it is invasive, the plant has been used as an organic fertilizer to boost the output of maize and vegetable crops (Sangakkara et al., 2004). *T. diversifolia* can be used as a component in fertilizer production due to its high N and K content. *T. diversifolia*'s ability to bio-fertilize crops means that it is crucial for crop nutrition and also in pest control (Kandungu et al., 2013). According to Hewavitharana and Kannangara (2019), compost containing *T. diversifolia* and *Trichoderma* improved Brinjal plant growth and disease tolerance. In Strawberry, *T. diversifolia* and *Trichoderma asperellum* boosted plant growth and diminished disease severity (Wambui, 2021). As a biological control, *Trichoderma asperellum* causes plants to take in more nutrients, which promotes plant development and has a disease-suppressing effect.

Table 1. Effect of spore count of *T. harzianum* on different treatments after 1st, 2nd, 3rd and 4th week of inoculation

Treatment	Spore count at 1st week (CFU/ml × 10^7)	Spore count at 2nd week (CFU/ml × 10^7)	Spore count at 3rd week (CFU/ml × 10^7)	Spore count at 4th week (CFU/ml x 10^7)
Kappettiya	8.72 ± 0.37^{ab}	25.31 ± 0.55^{a}	61.2 ± 3.54^{a}	123.4 ± 3.14^{b}
Kudzu	6.47 ± 0.30^{b}	$15.6\ 1\pm 0.52^{c}$	55.0 ± 3.54^{b}	123.8 ± 9.84^{b}
Habarala	6.77 ± 0.20^{b}	25.21 ± 0.22^{a}	18.51 ± 0.25^{c}	36.29 ± 1.15^{c}
Wal sooriya kantha	8.71 ± 0.26^{ab}	9.19 ± 0.09^{d}	19.01 ± 0.25^{c}	157.1 ± 5.63^{a}
Gadapana	9.64 ± 1.75^{a}	17.21 ± 0.43^{b}	19.52 ± 0.25^{c}	15.84 ± 0.26^{d}
Gliricidia	8.49 ± 0.34^{ab}	9.87 ± 0.38^{d}	9.97 ± 0.18^{d}	114.0 ± 3.40^{b}
Dry banana leaves	3.73 ± 0.14^{c}	6.5 ± 0.09^{e}	4.82 ± 0.47^{de}	35.9 ± 23.60^{b}
Lemon	0.057 ± 0.006^{d}	0.075 ± 0.09^{h}	0.076 ± 0.001^{e}	0.09 ± 0.002^{d}
Rambutan	0.0307 ± 0.007^{d}	0.037 ± 0.09^{h}	0.05 ± 0.002^{e}	0.065 ± 0.003^{d}
Heen bovitiya	0.05 ± 0^{d}	0.05 ± 0.01^{h}	0.242 ± 0.01^{e}	0.509 ± 0.022^{d}
Dry coconut leaves	1.4778 ± 0.32^{cd}	1.81 ± 0.09^{g}	1.623 ± 0.02^{e}	1.562 ± 0.01^{d}
Pota wal	0.06 ± 0.004^{d}	4.14 ± 0.09^{f}	20.4 ± 0.02^{c}	114.7 ± 2.58^{b}
Pani thora	1.159 ± 0.014^{d}	1.16 ± 0.01^{gh}	5.584 ± 0.08^{de}	10.59 ± 0.11^{d}
Castor	9.8 ± 0.44^{a}	9.8 ± 0.01^{d}	61.5 ± 1.38^{a}	117.9 ± 2.73^{b}
Erabadu	9.64 ± 0.15^{a}	9.64 ± 0.15^{d}	16.65 ± 0.63^{c}	16.27 ± 0.3^{d}
Jack	0.078 ± 0.003^{d}	0.078 ± 0.003^{h}	0.102 ± 0.004^{e}	0.116 ± 0.01^{d}
Mango	0.011 ± 0.001^{d}	$0.027 \pm\ 0.001^{h}$	0.034 ± 0.001^{e}	0.035 ± 0.01^{d}
Mean	4.41	7.98	17.31	55.88
F	77.301	1006.396	386.492	325.688
P	0.001	0.002	0.001	0.003

Values are mean ± SE. Values having same superscripts are not significant at $p<0.05$.

In earlier research, Kudzu and Glyricidia worked admirably in the mass generation of *Trichoderma* spp. In comparison to Velvet bean and bark additions, Kudzu-amended soil encouraged the growth of *Trichoderma koningii* (2.9 log CFU/g soil) (Blum and Rodrguez-Kábana, 2006). In a different experiment, 10% Gliricidia leaf extract resulted in mean spore counts/ml of $0.5x10^7$ (7 days after inoculation) and 1.6×10^7 (14 days after inoculation) (Emerson and Mikunthan, 2015). However, according to Shilmy and Salgadoe (2016), after the second and third weeks of inoculation, Gliricidia showed a decline in *Trichodrema* spp. multiplication.

CONCLUSION

In the present study, Castor, Gadapana and Erabadu treatments produced significantly higher *T. harzianum* after 1st week of inoculation. However, among those treatments, a sharp increase in the growth of *T. harzianum* spores were observed in Wal Sooriya Kantha, Kappetiya and Kudzu exhibited the latent potential of producing substantially increased spore count after 4th week of inoculation. Hence, these were important findings and the first step in developing mass production and commercial application of *Tricoderma* as a biocontrol agent in agriculture, as most of the underutilized indigenous plant species studied here are easily available in tropical Asian countries, by using these may contribute to develop a simple and cost-effective culture medium for *T. harzianum*. Future research is needed to focus on the chemical composition analysis of these identified promising underutilized plant species. Efforts should be focus on the plant extracts to promote the growth and development of the bio-control agent.

ACKNOWLEDGEMENT

Authors would like to thank the research assistants from Plant Pathology Laboratory, Fruit Crop and Development Center, Department of Agriculture, Horana for supporting this research.

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