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Research Article

Enhancement of Rice Seed Germination and Vigour by *Trichoderma* spp.

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Abstract: The present study was undertaken to examine the effectiveness of *Trichoderma* spp. to enhance rice germination and vigour. An *in vitro* experiment was carried out to assess the effect of seven isolates of *Trichoderma* spp. in enhancing rice germination and vigour. The results showed that all isolates of *Trichoderma* spp. significantly increased rice seedling growth, germination rate, vigour index and speed of germination with *Trichoderma* sp., SL2 showing the greatest increase in all the four parameters. *Trichoderma* sp., SL2 treated rice seeds attained values of 4.48 and 6.00 cm, 0.0084 and 0.0048 g and 1016.56 and 44.75 seeds/day for seedling shoot length seedling root length, shoot weight, root weight, vigour index and speed of germination respectively. We may conclude that *Trichoderma* spp. is able to enhance seed germination and vigour. The results of the study adds to the further understanding of the role of beneficial fungi in improving rice resistance to stress, yield and quality through seed invigoration.

Keywords: Germination, rice, *Trichoderma* spp., vigour

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major staple food in the world, especially in Asia, the region that consumes the most rice (FAO, 2012). Rice farming nowadays is facing many environmental problems caused by input of chemical fertilizers and pesticides. The use of chemical fertilizers and pesticides has led to soil a decline in fertility, ecosystem damage, elimination of soil biota and emergence of resistant pathogens. Therefore the use of eco-friendly bio-fertilizers should be encouraged. Beneficial microorganisms have been reported to be involved in maintaining agricultural production, protecting the ecosystem and decreasing the use of chemical fertilizers (Adesemoye and Kloepper, 2009).

Seed priming is a pre-germination seed treatment whereby seeds are placed in partially hydrated condition (Bradford, 1986). Seed priming is considered as a method to enhance seedling quality and vigour (Basra *et al*., 2004). Seed priming is also involved in enhancing rice resistance to environmental stress (Goswami *et al*., 2013), weed suppressive ability and rice yield (Juraimi *et al*., 2012). Previous studies have shown that rice seeds respond to seed priming in the early part of the germination stage and revealed that it can increase seed germination, vigour index and germination speed (Anwar *et al*., 2013; Zarei and Sinaki, 2012). Priming rice seed with plant growth regulators is one of the beneficial treatments that can be used to invigorate rice seed and improve rice seed quality (Farooq *et al*., 2010).

Plant Growth Promoting Fungi (PGPF) is considered an alternative way to enhance rice growth and yield. The mechanisms by which PGPF act to enhance rice growth and yield includes production of phytohormones, phosphate solubilization, cellulose degradation and siderophore production. PGPF are generally abundant in the soil system and roots. One of the well-known fungi species listed in the PGPF group is the *Trichoderma* spp. (Doni *et al*., 2013). Currently, *Trichoderma* spp. are widely used in industrial processes and agriculture due to their ability to produce enzymes and secondary metabolites (Jiang *et al*., 2011; Mukherjee *et al*., 2008). In bio-fertilizer production, *Trichoderma* spp. is well recognized because of its ability to establish mycorrhizal-like association with plants, control of root and foliar pathogens, change the micro-floral composition in roots, enhance nutrient uptake, enhance root development, increase root hair formation, aid the plant in acquiring systemic resistance, degrade cellulose, solubilize phosphate and produce siderophores (Saravanakumar *et al*., 2013;

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Saba *et al*., 2012; Harman *et al*., 2004; Harman, 2000; Yadidia *et al*., 1999).

Germination stage is reported to be the most critical phase in the plant life cycle, because during this stage, plants have a high vulnerability to injury, disease and environmental stress (Rajjou *at al*., 2012). Research on the role of *Trichoderma* spp. in enhancing rice seed germination is very important to be undertaken due to the potential for *Trichoderma* spp. to act as plant growth regulators. Previous research studies on the effect of *Trichoderma* spp. on the improvement of seed germination have been conducted for soybean (Tančić *et al*., 2013), mustard (Lalitha *et al*., 2012), maize and beans (Okoth *et al*., 2011) and chili (Asaduzzaman *et al*., 2010). However, very little information is available with regards to enhancement of rice seed germination and vigour by *Trichoderma* spp. to improve rice seed germination and vigour. This research was carried out to examine the effect of *Trichoderma* spp. on rice (*Oryza sativa* L.) seed germination and vigour.

MATERIALS AND METHODS

***Trichoderma* spp. spore preparation:** Isolates of *Trichoderma* spp. namely *Trichoderma* sp., SL1, *Trichoderma* sp., SL2, *Trichoderma* sp., SL3, *Trichoderma* sp., SL4, *Trichoderma* sp., SL5, *Trichoderma* sp., SL6 and *Trichoderma* sp., SL7 were obtained from the Fermentation Technology Laboratory, School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia. Each isolates were grown separately in potato dextrose agar and incubated for seven days at 30°C. After incubation, spores of the *Trichoderma* spp. were immediately transferred to Erlenmeyer flasks containing sterilized water.

Rice seed preparation: Organic rice seeds of the variety MRQ74 were surface sterilized with 70% ethanol, followed by 5% sodium hypochlorite and washed by sterilized distilled water. One hundred rice seed grains were selected for each treatment then soaked in the respective *Trichoderma* spp. in a flask containing 10^7 spores/mL spore suspension for 30 min. Rice seeds soaked in sterilized distilled water served as control. The treated seeds were incubated for 5 days in sterilized petri dishes at 28±2°C fitted with filter paper and each petri dish was irrigated with 10 mL sterilized water.

Seedling length and weight: The root and shoot length were measured by selecting five seeds randomly for each treatment. Shoot length was measured from the base of the primary leaf to the base of the hypocotyl and root length was measured from the tip of the primary root to the base of hypocotyl, with all measurements expressed in centimeters. Shoot and root weight were examined using digital weight scales and expressed in grams. All measurements were according to the method used in Dash (2012).

Seed germination rate: Germination rate is the average of number of seeds that germinate over the 5- and 10-day periods.). In this experiment data was calculated on day 5. Germination rate was computed using the formula proposed by IRRI (2011):

$$\text{Germination} = (\%)\,\frac{\text{Number of seeds that germinated}}{\text{Number of seeds on the tray}} \times 100$$

Seed vigour index: Seed vigour index was examined after five days of incubation using the formula given by Abul-Baki and Anderson (1973) based on the product of germination (%) and seedling length (cm):

$$\text{S.V.I} = \text{Germination (\%)} \times \text{Seedling Length}$$

Speed of germination: Numbers of seedlings emerging daily are counted from day 0 until day 5 of planting. Thereafter a Germination Index (G.I) is calculated by using the following formula as proposed by Gupta (1993):

$$\text{G.I} = \frac{\text{number of seedlings emerging on day}}{\text{day after planting}}$$

Statistical analyses: All the data were from triplicates and were represented as mean±standard deviation. Further, the variations in the results obtained with respect to different seedling length and seedling weight, as well as different seed germination rate, vigour index and speed of germination were statistically analyzed using one way Analysis of Variance (ANOVA) and tested for significance using Fisher's protected Least Significant Difference (LSD) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Seedling length and weight: The length of shoot and root seedling by *Trichoderma* spp. were significantly greater than the control (untreated). However, it was not significant on the weight of shoot and root basis (Table 1 and 2). The result revealed that *Trichoderma* sp., SL2 has the highest ability to stimulate seedling root elongation compared to other strains (Fig. 1). The role of *Trichoderma* spp. in the production of auxins and gibberellins is the key factor to enhance rice seedling length (Chowdappa *et al*., 2013; Martínez-Medina *et al*., 2011). Gibberellins were first identified as metabolites of *Gibberela fujikuroi* which causes bakanae fungal disease (Bomke and Tudzynski, 2009; Phinney, 1983). Auxins produced by *Trichoderma* spp. are able to stimulate root initiation and cell elongation, while gibberellins are involved in cell division. Production of auxins and gibberellins by *Trichoderma* spp. enhances rice seedling performance during seed germination (Fig. 1).

Trichoderma spp. which has the ability to promote rice seedling length can be an alternative way to be

Table 1: Comparison of length of shoot, length of root, weight of shoot and weight of root in different treatments

Treatment	Length of shoot (cm)	Length of root (cm)	Weight of shoot (g)	Weight of root (g)
Trichoderma sp. SL1	4.12 (0.43)	5.00 (1.04)	0.0056 (0.0009)	0.0011 (0.0004)
Trichoderma sp. SL2	4.48 (0.45)	6.00 (1.85)	0.0084 (0.0032)	0.0048 (0.0036)
Trichoderma sp. SL3	3.10 (0.48)	3.66 (1.21)	0.0050 (0.0007)	0.0022 (0.0017)
Trichoderma sp. SL4	3.04 (0.55)	4.36 (1.56)	0.0057 (0.0020)	0.0027 (0.0018)
Trichoderma sp. SL5	3.72 (0.30)	3.92 (0.74)	0.0066 (0.0016)	0.0024 (0.0012)
Trichoderma sp. SL6	4.16 (0.68)	3.76 (1.79)	0.0073 (0.0011)	0.0022 (0.0015)
Trichoderma sp. SL7	3.42 (0.42)	4.40 (0.75)	0.0064 (0.0013)	0.0021 (0.0009)
Control	2.62 (0.62)	3.30 (0.39)	0.0057 (0.0020)	0.0019 (0.0015)
$LSD_{0.05}$	8.15	2.33	ns	ns

ns: Not significant; Standard deviations are given in parentheses (n = 40)

Table 2: Comparison of length of shoot, length of root, weight of shoot and weight of root in different treatments (*Trichoderma* strains)

Treatment	Length of shoot (cm)	Length of root (cm)	Weight of shoot (g)	Weight of root (g)
Trichoderma sp. SL1	4.12 cdgh	5.00 b	0.0056 h	0.0011 b
Trichoderma sp. SL2	4.48 cdegh	6.00 acdh	0.0084 cdefh	0.0048 acefgh
Trichoderma sp. SL3	3.10 abf	3.66 bf	0.0050 b	0.0022 b
Trichoderma sp. SL4	3.04 abef	4.36 b	0.0057 b	0.0027 ns
Trichoderma sp. SL5	3.72 bdh	3.92 ns	0.0066 b	0.0024 b
Trichoderma sp. SL6	4.16 cdgh	3.76 c	0.0073 b	0.0022 b
Trichoderma sp. SL7	3.42 abfh	4.40 ns	0.0064 ns	0.0021 b
Control	2.62 abefg	3.30 b	0.0057 ab	0.0019 b

Means with different small letter was significantly different between treatments at $p<0.05$; Means with ''ns'' was not significantly different with all treatment at $p<0.05$

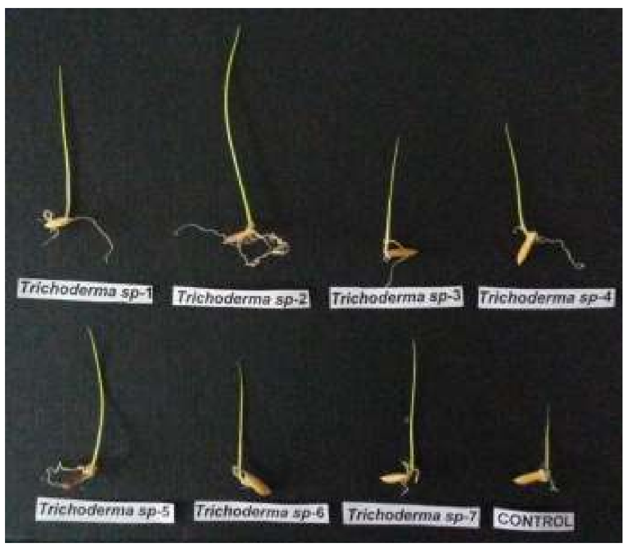

Fig. 1: *Trichoderma* strains enhance rice seedling growth compared to untreated control

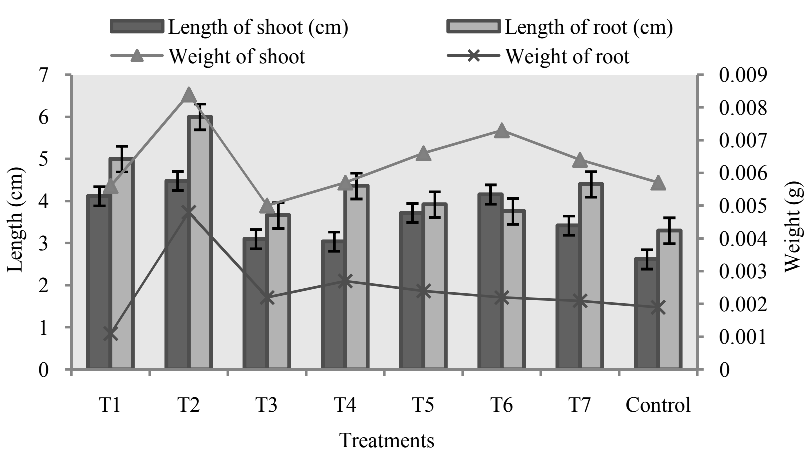

Fig. 2: Relationship between length of shoot and length of root with different treatments (*Trichoderma* strains)

Fig. 3: *Trichoderma* sp., SL2 caused better growth compared to control

Table 3: Rice seed germination rate, vigour index and speed of germination by different treatments (*Trichoderma* strains) during seed germination

Treatment	Germination (%)	Vigour index	Speed of germination (seeds/day)
Trichoderma sp. SL1	99	902.88	39.26
Trichoderma sp. SL2	97	1016.56	44.75
Trichoderma sp. SL3	97	655.73	40.33
Trichoderma sp. SL4	97	717.80	38.76
Trichoderma sp. SL5	98	748.08	36.85
Trichoderma sp. SL6	99	784.08	36.20
Trichoderma sp. SL7	96	750.72	36.40
Control	90	532.80	35.86

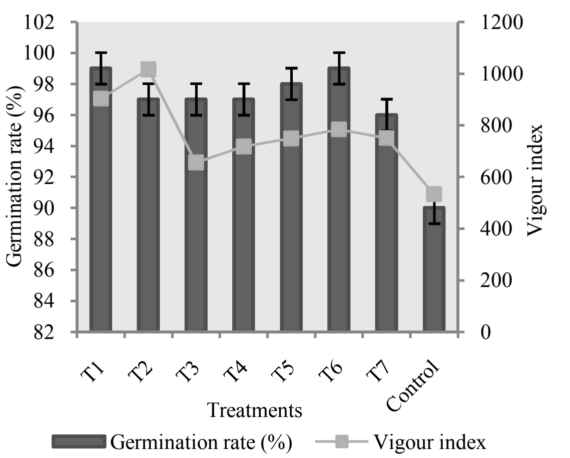

Fig. 4: Relationship between germination rate and vigour index with different treatments (*Trichoderma* strains) during seed germination

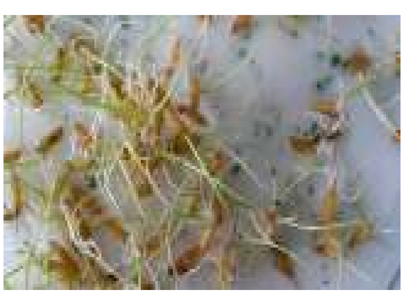

Fig. 5: *Trichoderma* spp. colonized rice seedling

used in sustainable crop production. Among the seven isolates of *Trichoderma* spp. studied, *Trichoderma* sp., SL2 showed the highest consistency in the ability to enhance rice seedling weight (Fig. 2). The results demonstrate that the length of shoot and root was significantly higher for *Trichoderma* sp., SL2 than other strains and control (Fig. 3). It is envisioned that rice seedlings infected with *Trichoderma* sp., SL2 strain having better root growth and higher physiological activity would be transporting larger amounts of cytokinin from roots to shoot, resulting in a lower rate of leaf senescence in the later stages of development (San-oh *et al*., 2006).

Rice seed germination rate, vigour index and speed of germination: The results showed that the inoculation of the rice seeds with *Trichoderma* spp. significantly increased rice seed germination rate, vigour index and speed of germination compared to untreated control (Table 3 and Fig. 4). Germination percentages were high in the rice seeds treated with *Trichoderma* spp. to the order of 96 to 99% compared to the untreated control seeds which registered 90% germination. The speed of germination was also high for the rice seeds treated with *Trichoderma* spp. compared to control. The range of germination speed in the rice seeds treated with *Trichoderma* spp. ranged from 36.2 to 44.75 seeds/day compared to the untreated control seeds which registered 35.86 seeds/day. The growth hormone which is produced by *Trichoderma* spp. enhances germination and speed. *Trichoderma* spp. also reduces the growth of pathogenic fungi by colonizing the rice seedlings as shown in Fig. 5. These results are in support of the findings of Jayalakshmi *et al*. (2009) who reported the biological control potential of *Trichoderma harzianum.* Vigour index was highest in the treatment of *Trichoderma* sp., SL2 followed by *Trichoderma* sp., SL1 *Trichoderma* sp., SL6 *Trichoderma* sp., SL7 *Trichoderma* sp., SL5, *Trichoderma* sp., SL4, *Trichoderma* sp., SL3 and the lowest recorded in the untreated control (Fig. 4). Zheng and Shetty (2000) reported that *Trichoderma* spp. induced phenolic compound production in the plant during seed germination and phenolic compounds produced by *Trichoderma* spp. led to enhancement of seed vigour. Further Cai *et al*. (2013) reported that a secondary metabolite namely harzianolide produced by *Trichoderma* spp. can influence the early stage of plant development through the enhancement of root length.

CONCLUSION

Seed priming has become fashionable among agricultural practitioners in the effort to improve crop stand under normal and adverse conditions. From this study, the higher seed vigour and germination rates of rice seeds treated with *Trichoderma* spp. in comparison to untreated seeds underscores the potential of beneficial fungal species such *Trichoderma* spp. as seed priming material to improve rice seed germination. This augurs well with the search for effective biological agents to enhance rice seed quality.

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REFERENCES

Abul-Baki, A.A. and J.D. Anderson, 1973. Vigour determination in soybean by multiple criteria. Crop Sci., 3: 630-637.

Adesemoye, A.O. and J.W. Kloepper, 2009. Plant-microbes interactions in enhanced fertilizer-use efficiency. Appl. Microbiol. Biot., 85: 1-12.

Anwar, S., M. Iqbal, S.H. Raza and N. Iqbal, 2013. Efficacy of seed preconditioning with salicylic and ascorbic acid in increasing vigor of rice (*Oryza sativa* L.) seedling. Pak. J. Bot., 45(1): 157-162.

Asaduzzaman, M., M.J. Alam and M.M. Islam, 2010. Effect of *Trichoderma* on seed germination and seedling parameters of chili. J. Sci. Found., 8(1/2): 141-150.

Basra, S.M.A., M. Ashraf, N. Iqbal, A. Khaliq and R. Ahmad, 2004. Physiological and biochemical aspects of pre-sowing heat strees on cotton seed. Seed Sci. Technol., 32: 365-774.

Bomke, C. and B. Tudzynski, 2009. Diversity, regulation and evolution of the gibberellin biosynthetic pathway in fungi compared to plants and bacteria. Phytochemistry, 70: 1876-1893.

Bradford, K.J., 1986. Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. HortScience, 21: 1105-1112.

Cai, F., G. Yu, P. Wang, Z. Wei, L. Fu, Q. Shen and W. Chen, 2013. Harzianolide, a novel plant growth regulator and systemic resistance elicitor from *Trichoderma harzianum*. Plant Physiol. Bioch., 73: 106-113.

Chowdappa, P., S.P.M. Kumar, M.J. Lakshmi and K.K. Upreti, 2013. Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3. Biol. Control, 65: 109-117.

Dash, A.K., 2012. Impact of domestic waste water on seed germination and physiological parameters of rice and wheat. Int. J. Res. Rev. Appl. Sci., 12(2): 280-286.

Doni, F., N.K.N. Al-Shorgani, E.M.M. Tibin, N.N. Abuelhassan, I. Anizan, C.M.Z. Che Radziah and W.Y. Wan Mohtar, 2013. Microbial involvement in growth of paddy. Curr. Res. J. Biol. Sci., 5(6): 285-290.

FAO, 2012. FAO Says Rice Production Outpacing Consumption. Retrieved from: http://www.fao.org/news/story/en/item/164713/icode/.

Farooq, M., S.M.A. Basra, A. Wahid, A. Khaliq and N. Kobayashi, 2010. Seed Invigoration: A Review. In: Lichtfouse, E. (Ed.), Organic Farming, Pest Control and Remediation of Soil. Springer, NY, pp: 137-175.

Goswami, A., R. Banerjee and S. Raha, 2013. Drought resistance in rice seedlings conferred by seed priming. Protoplasma, 250(5): 1115-1119.

Gupta, P.C., 1993. Seed Vigour Testing. In: Agarwal, P.K. (Ed.), Handbook of Seed Testing. National Seed Corporation, New Delhi, pp: 245-246.

Harman, G.E., 2000. Myths and dogmas of biocontrol changes in perception derived from research on *Trichoderma harzianum* T-22. Plant Dis., 84: 377-393.

Harman, G.E., R. Petzoldt, A. Comis and J. Chen. 2004. Interactions between *Trichoderma harzianum* strain T22 and maized inbred line Mo17 and effect of this interaction on diseases caused by *Pythium ultimum* and *Colletotrichum graminicola*. Phytopathology, 94: 147-153.

IRRI, 2011. Measuring Seed Germination. Post Harvest Fact Sheets. Retrieved from: http://www.knowledgebank.irri.org/factsheetsPDFs/CropEstablishment_Measuring%20Seed%20Germination.pdf.

Jayalakshmi, S.K., S. Raju, R. Usha, V.I. Benagi and K. Sreeramulu, 2009. *Trichoderma harzianum* L_1 as a potential source for lytic enzymes and elicitor of defense responses in chickpea (*Cicer arietinum* L.) against wilt disease caused by *Fusarium oxysporum* f. sp. ciceri. Aust. J. Crop Sci., 3(1): 44-52.

Jiang, X., A. Geng, N. He and Q. Li, 2011. New isolates *Trichoderma viride* strain for enhanced cellulolytic enzyme complex production. J. Biosci. Bioeng., 111(2): 121-127.

Juraimi, A.S., M.P. Anwar, A. Selamat, A. Puteh and A. Man. 2012. The influence of seed priming on weed supression in aerobic rice. Pak. J. Weed Sci. Res., 18: 257-264.

Lalitha, P., Srujana and K. Arunalakshmi, 2012. Effect of *Trichoderma viride* on germination of mustard and survival of mustard seedlings. Int. J. Life Sci. Biotechnol. Pharma Res., 1(1).

Martínez-Medina, A., A. Roldán, A. Albacete and J.A. Pascual, 2011. The interaction with arbuscular mycorrhizal fungi or *Trichoderma harzianum* alters the shoot hormonal profile in melon plants. Phytochemistry, 72: 223-229.

Mukherjee, P.K., C.S. Nautiyal and A.N. Mukhopadhyay, 2008. Molecular Mechanisms of Biocontrol by *Trichoderma spp*. In: Nautiyal, C.S. (Ed.), Molecular Mechanisms of Plant and Microbe Coexistence. Soil Biology 15, Springer-Verlag, Berlin, Heidelberg, DOI: 10.1007/978-3-540-75575-3.

Okoth, S.A., J.A. Otadoh and J.O. Ochanda, 2011. Improved seedling emergence and growth of maize and beans by *Trichoderma harziunum*. Trop. Subtrop. Agroecosyst., 13: 65-71.

Phinney, B.O., 1983. The History of Gibberellins. In: Crozier, A. (Ed.), The Biochemistry and Physiology of Gibberellins. Praeger Publishers, New York, pp: 19-52.

Rajjou, L., M. Duval, K. Gallardo, J. Catusse, J. Bally, C. Job and D. Job, 2012. Seed germination and vigor. Ann. Rev. Plant Biol., 63: 507-533.

Saba, H., D. Vibhash, M. Manisha, K.S. Prashant, H. Farham and A. Tauseff, 2012. *Trichoderma*-a promising plant growth stimulator and biocontrol agent. Mycosphere, DOI: 10.5943/mycosphere/3/4/14.

San-Oh, Y., T. Sugiyama, D. Yoshita, T. Ookawa and T. Hirasawa, 2006. The effect of planting pattern on the rate of photosynthesis and related processes during ripening in rice plants. Field Crop Res., 96: 113-124.

Saravanakumar, K., V. Shanmuga Arasu and K. Kathiresan, 2013. Effect of trichoderma on soil phosphate solubilization and growth improvement of *Avicennia marina*. Aquat. Bot., 104: 101-105.

Tančić, S., J. Skrobonja, M. Lalošević, R. Jevtić and M. Vidić, 2013. Impact of *Trichoderma* spp. on soybean seed germination and potential antagonistic effect on *Sclerotinia sclerotiorum*. Pestic. Phytomed., 28(3): 181-185.

Yadidia, I., N. Benhamou and I. Chet, 1999. Induction of defense responses in cucumbar plants (*Cucumis sativus* L.) by biocontrol agent *Trichoderma harzianum*. Appl. Environ. Microb., 65: 1061-1070.

Zarei, M. and M. Sinaki, 2012. Effect of priming on seed germination rice (*Oryza sativa* L.) in difficult environment conditions. Int. J. Agric. Res. Rev., 2(S): 1070-1078.

Zheng, Z. and K. Shetty, 2000. Enhancement of pea (*Pisum sativum*) seedling vigour and associated phenolic content by extracts of apple pomace fermented with *Trichoderma* spp. Process Biochem., 36: 79-84.