

Impact of Bio-Priming on Seed Quality and Longevity in Rice (*Oryza sativa* L.)

S Ruth Olivya, Dijee Bastian, Rose Mary Francies, C R Rashmi, S Biju and K Vaisakh

College of Horticulture, Kerala Agricultural University, Thrissur, Kerala

ABSTRACT

Seed priming is mainly seed treatment before sowing. However, dehydration or re-drying is used to remove moisture in the seeds to a level can be stored for longer periods depending on the species and also to retain the beneficial effects of the seed. Primed seeds usually germinate faster and more evenly when soaked especially under adverse environmental conditions. Seeds treated with biological agents like *Pseudomonas fluorescens*, *Trichoderma viride* and organics like coconut water in various combinations recorded superior in seed quality with higher seedling vigour and lower electrical conductivity of seed leachates over other treatments and untreated control.

Keywords: Seed priming, Bio-priming, coconut water, longevity and seed quality

Rice is a major dietary staple for about 60 per cent of the world's population particularly in Asia where more than 90 per cent of rice is grown (Kumar and Prasanna, 2001). It is grown in humid, tropical and sub-tropical climates where regional cultivars are extremely favoured for their taste and agricultural suitability. But these ideal growing conditions are in contrary with rice storage conditions where warm, humid climates can lead to rapid seed deterioration (Emam, 2007). Seed senescence is an irreversible and inexorable process. Storage of seed till next sowing season is essential for sustenance of agriculture. In general, cereals are more susceptible to deterioration as they are rich in carbohydrates. The rapid deterioration of stored seed is a serious problem particularly in Kerala where high temperatures and relative humidity prevail leading to accelerated ageing. Due to environmental concerns, there is an urgent need to reduce the use of chemicals and search for alternative to chemicals are being sought. Bio-priming is one of the best eco-friendly methods which can be

safely used commercially as a substitute for traditional fungicide seed treatments for improving seed longevity (Iswariya *et al.*, 2019). Bio-priming involves the use of bioagents like *Pseudomonas fluorescens*, *Trichoderma viride* and naturally available ameliorants like leaf extracts, coconut water, cows' urine etc., (Shakuntala *et al.*, 2012). Coconut water is a rich source of enzymes and growth promoting substance especially cytokinin (Letham, 1974). These beneficial factors might contribute towards improvement in germination. Considering the above aspects present research work was formulated to investigate the effect of eco-friendly bio-priming approaches on seed vigour and longevity in rice.

MATERIALS AND METHODS

Seeds of rice cv. Jyothy with initial germination of 94% and 13.4% moisture were used for this study. Experiments were carried out in the Department of Seed Science and Technology, College of Agriculture, Vellanikkara (KAU). Seeds were soaked in water

(Hydropriming); Dry treatments of *Pseudomonas fluorescens* (10g/kg), *Trichoderma viride* (4g/kg), *P. fluorescens* + *T. viride* and wet treatments of coconut water (75%), *P. fluorescens* + coconut water, *T. viride* + coconut water, *P. fluorescens* + *T. viride* + coconut water the seeds were soaked in solution for 16 h, respectively. The seeds after priming and drying back to original moisture were packed in poly ethylene bags (700 gauge) and kept under ambient storage for a period of nine months. Unprimed seeds were used as control. The seed samples were drawn at monthly intervals up to nine months of storage and evaluated for the moisture content of the seed and germination were calculated and expressed as percentage (ISTA, 1999). Root length and shoot length were also measured at the end after 14 days. Vigour indices were calculated using the formula of Abdul-Baki and Anderson (1973) and Bewly and Black (1994). Speed of germination (Maguire, 1962), time to 50% germination (T_{50}) were calculated according to the formula of Coolbear *et al.*, (1984), Mean Germination Time (MGT) was calculated as formulated the equation of Ellis and Robert (1981), Electrical Conductivity (ISTA, 2010), Dehydrogenase enzyme activity (Kittock and Law, 1968), Super Oxide Dismutase enzyme activity (Dhindsa *et al.*, 1981) were calculated as per the procedure.

Statistical analysis

Analysis of the data on various seed quality parameters was performed following the Completely Randomized Design (CRD) with three replications using OPSTAT, a Statistical software package.

RESULTS AND DISCUSSION

Significant differences were observed among the treatments for germination per cent from the initial month of storage. The treatments had recorded gradual decrease in the mean germination per cent

with the advancement of storage period (Fig.1). Such gradual reduction in germination per cent with progress of seed ageing was earlier observed by Suganya (2015) in rice, Hussain *et al.*, (2015), Nithya and Geetha (2017) and Kalaivani (2010) in maize. At the end of storage, the combined treatment of *P. fluorescens*, *T. viride* and coconut water recorded higher germination per cent (83.69%) which was in concurrence with the findings of Abdel-Kader *et al.*, (2012) in vegetables, Rosna (2019) in okra. Increase in storage duration depressed the longevity of primed seeds in addition to delayed and reduced germination as opined by McDonald (1999). One of the objectives of priming is the quickest germination of seeds that is expressed lower MGT (5.08 days), T_{50} (4.42 days) which has been observed in *P. fluorescens* treatments with combination of *Trichoderma* spp., coconut water was in line with the findings of Athulya (2019) and Nagendra *et al.*, (2017) in oriental pickling melon, Jaiman *et al.* (2020) in vegetables, Ananthi *et al.* (2014) in chilli, Reddy *et al.*, (2011) in chick pea, Reshma (2018) in okra. Srivastava *et al.*, (2010) in tomato discovered that germination was expedited by 2.0 - 2.5 days which was explained that production of endogenous plant growth regulators such as gibberellins, cytokinin's, and/or indole-acetic acid, have increased the availability of minerals and other ions, and/or helped in the increase of water uptake. The combination of *P. fluorescens*, *T. viride* and coconut water gave the longest shoots (8.21 cm) and roots (11.44 cm). According to Somasundaram and Bhaskaran (2017) *Pseudomonas* spp. produces gibberellins, auxins, and cytokinin, such as isopentenyladenosine (IPA), dihydroxy zeatin riboside (DHZR), and zeatin riboside (ZR), which help in stimulating the plant growth. These results were also in line with the findings of Dezfuli *et al.*, (2008) in maize and Raj *et al.*, (2004) in pearl millet. Vigour index I and II found to be significantly higher in bio

Table 1. Impact of seed priming on seed quality parameters at the end of nine months of storage in rice

Treatments	G (%)	SPG	MGT	T ₅₀	M (%)	EC (μScm^{-1})
<i>Pseudomonas fluorescens</i>	83.23 ^{ab}	18.89 ^a	5.22 ^b	4.70 ^d	10.71 ^g	32.60 ^g
<i>Trichoderma viride</i>	82.89 ^b	17.61 ^{cd}	5.19 ^{bc}	4.56 ^e	10.91 ^f	36.28 ^c
Coconut water	81.84 ^c	18.3 ^b	5.15 ^d	4.9 ^c	11.02 ^e	33.94 ^e
<i>P.fluorescens</i> + <i>T. viride</i>	83.15 ^{ab}	19.02 ^a	5.13 ^d	4.42 ^f	10.61 ^h	37.85 ^b
<i>P.fluorescens</i> + coconut water	81.1 ^d	17.52 ^{cd}	5.08 ^e	4.54 ^e	11.22 ^d	30.83 ^h
<i>T. viride</i> + coconut water	79.08 ^e	16.49 ^d	5.19 ^{bc}	5.24 ^a	11.32 ^c	33.70 ^f
<i>P.fluorescens</i> + <i>T. viride</i> + coconut water	83.69 ^a	18.99 ^a	5.08 ^e	5.00 ^b	11.02 ^e	34.73 ^d
Hydro priming	77.85 ^f	15.80 ^e	5.18 ^{bc}	5.01 ^b	11.55 ^b	33.99 ^e
Control	77.62 ^e	17.84 ^c	5.36 ^a	5.29 ^a	11.62 ^a	38.86 ^a
C.D	0.702	0.38	0.044	0.058	0.027	0.144
SE(m)	0.234	0.127	0.015	0.019	0.009	0.048

Table 2. Impact of seed priming on seed quality parameters at the end of nine months of storage in rice

Treatments	SL (cm)	RL (cm)	DW (g)	VI-I	VI-II
T ₁ : <i>Pseudomonas fluorescens</i>	7.395 ^f	11.265 ^a	0.229 ^c	1553 ^b	1906 ^c
T ₂ : <i>Trichoderma viride</i>	7.905 ^d	10.650 ^b	0.240 ^{ab}	1537 ^b	1989 ^b
T ₃ : Coconut water	7.780 ^e	11.295 ^a	0.231 ^c	1561 ^b	1890 ^c
T ₄ : <i>P.fluorescens</i> + <i>T. viride</i>	7.380 ^f	10.715 ^b	0.230 ^c	1467 ^{cd}	1865 ^{cd}
T ₅ : <i>P.fluorescens</i> + coconut water	7.997 ^c	10.545 ^b	0.228 ^c	1541 ^b	1891 ^c
T ₆ : <i>T. viride</i> + coconut water	8.375 ^a	10.520 ^{bc}	0.232 ^c	1494 ^c	1834 ^d
T ₇ : <i>P.fluorescens</i> + <i>T. viride</i> + coconut water	8.215 ^b	11.445 ^a	0.242 ^a	1645 ^a	2029 ^a
T ₈ : Hydro priming	8.040 ^c	10.615 ^b	0.222 ^d	1452 ^d	1724 ^e
T ₉ : Control	7.730 ^e	10.295 ^c	0.223 ^d	1417 ^e	1867 ^{cd}
C.D	0.085	0.238	0.004	30.636	38.652
SE(m)	0.028	0.079	0.001	10.232	12.909

primed treatments of *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) (1645 and 2029 respectively) as development-promoting organisms like *Trichoderma* spp. and *Pseudomonas* spp. According to Diaz *et al.*, (2001), improved plant growth and biomass production by boosting nutrient absorption (e.g., N, P, K) and providing hormones in the rhizosphere will encourage plant growth which was in line with the results. Control recorded the least value (1417) similar results were also observed in findings

of Murungu *et al.*, (2004) in sorghum, Saglam *et al.* (2010) in lentil, Singh *et al.*, (2011) in cowpea. The meagre increase in moisture indicates the effectiveness of poly ethylene 700-gauge packaging and even under high humid conditions. The increase in the moisture content in wet treatment was slightly high when compared with dry treatments which was in concurrent with research findings of Yildirim *et al.*, (2021) in chilli, Mutai (2018) in soybean, Singh *et al.*, (2014) in okra, Nataraj *et al.* (2011) in sunflower. The

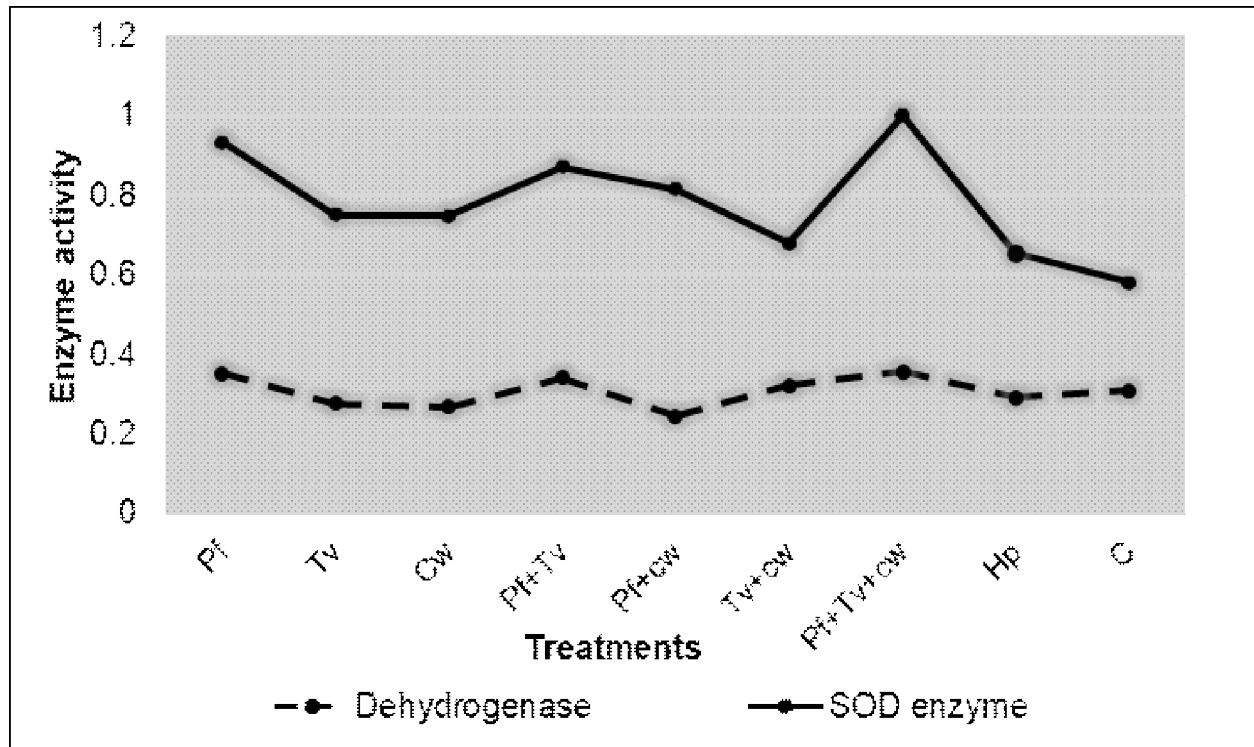


Fig1. Influence of seed priming on enzyme activity at the end of nine months of storage in rice cv. Jyothy

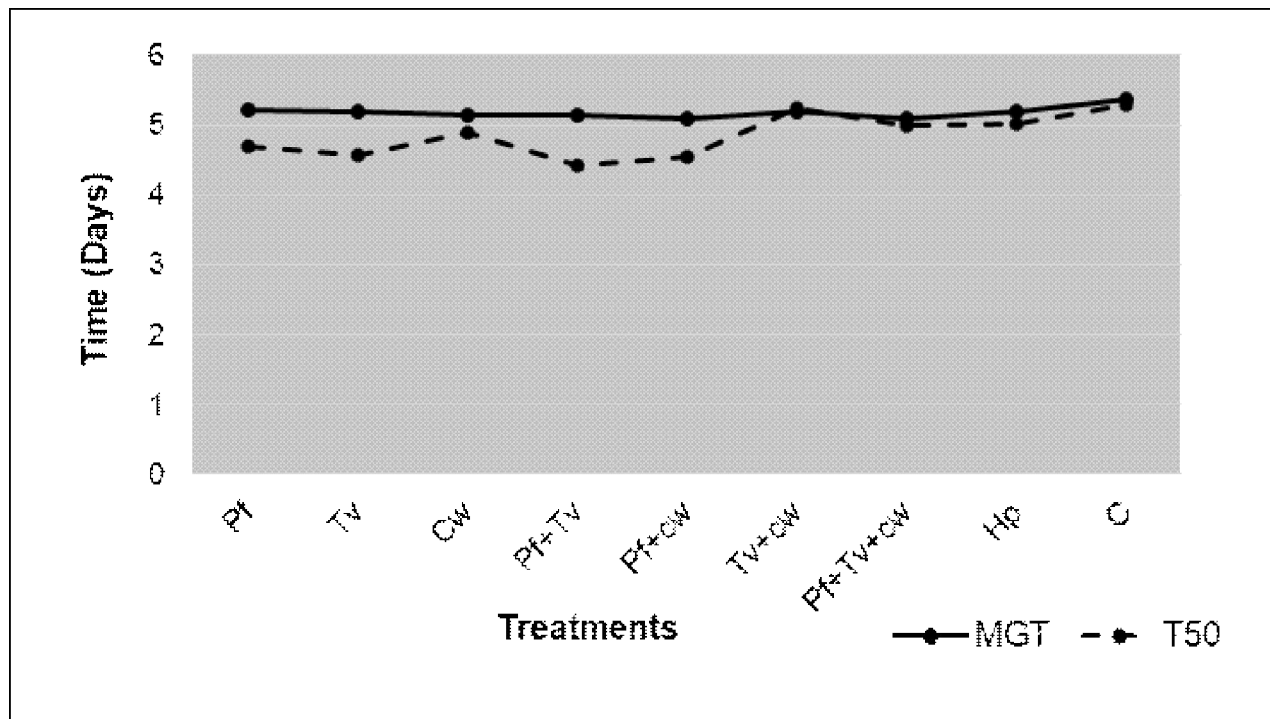


Fig2. Influence of seed priming on germination indicators (MGT, T₅₀) at the end of nine months of storage in rice cv. Jyothy

electrical conductivity (EC) of seed leachate is a measure of the seed's vitality and vigour. More chemicals escape into the media as the membrane integrity of damaged seeds deteriorates. *P. fluorescens* + coconut water recorded the least value ($30.83 \mu\text{Scm}^{-1}$) whereas, control recorded the highest electrical conductivity ($38.86 \mu\text{Scm}^{-1}$) which is in consistent with the findings of Rinku *et al.*, (2017), who found that biopriming had a lower electrical conductivity value than hydropriming and control. Bio-primed treatments showed superior performance with *P. fluorescens* + *T. viride* + coconut water with maximum dehydrogenase enzyme activity of 0.357 OD value followed by *Pseudomonas fluorescens* (10g/kg) (0.353 OD value) and *P. fluorescens* + *T. viride* (0.342 OD value) respectively. These results indicated dry dressed treatments of *P. fluorescens* gave highest enzyme activity. Similar findings were reported by Shakuntala *et al.*, (2012) in paddy and Reshma (2018) in okra. SOD is an enzymatic antioxidant defense mechanism in plants that catalyses H_2O_2 and reactive oxygen species (ROS). When plants are exposed to oxidative stress, these free radicals cause damage, which leads to a reduction in physiological function. These antioxidants and protective enzymes play a crucial role in protecting plants from oxidative damage. Bio-priming along with coconut water treatments *P. fluorescens* + *T. viride* + coconut water ($64.29 \text{ mg}^{-1}\text{protein}$) registered maximum SOD enzyme activity followed by *Pseudomonas fluorescens* (10g/kg) ($58.13 \text{ U/mg protein}$) and *P. fluorescens* + coconut water ($57.14 \text{ U/mg protein}$) respectively. Wattanakulpakin *et al.* (2012) in maize had shown similar results as that of present study.

CONCLUSION

It can be suggested that the combined application of hydrolytic priming and seed coating with

biological control agents would be a reasonable alternative to chemical seed treatment. The combination of *P. fluorescens* (10g/kg) + *T. viride* (4g/kg) + coconut water (75%) showed of natural ageing as they are farmer friendly with eco-friendly features without any side effects to the person who works with it. They are also cost friendly as talc powder of *P. fluorescens* and *T. viride* are cheap, coconut water is naturally obtained from plants and easily available to the farmers which help the farmers to make a move towards the organic farming without loss in productivity and also help in reducing the pollution caused by the excess use of agro chemicals.

LITERATURE CITED

- Abdel-Kader M M, El-Mougy N S, Aly M D E, and Lashin S M 2012** Long activity of stored formulated bio-agents against some soil-borne plant pathogenic fungi causing root rot of some vegetables. *Journal of Applied Science Research*, 8(4): 1882-1892.
- Abdul-Baki A A and Anderso J D 1973** Vigor determination in soybean seed by multiple criteria. *Crop Science*, 13(6): 630-633.
- Ananthi M, Selvaraju P and Sundaralingam K 2014** Effect of bio-priming using bio-control agents on seed germination and seedling vigour in chilli (*Capsicum annum L.*) 'PKM 1'. *Journal of Horticultural Sciences and Biotechnology*, 89(5): 564-568.
- Bewley J D and Black M 1994** Seeds: Physiology of Development and Germination (1st Ed.). Plenum Press, New York, 128p.
- Coolbear P, Francis A and Grierso D 1984** The effect of low temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. *Journal of Experimental Botany*. 35: 1609-1617.

- Dezfuli P M, Sharif-Zadeh F and Janmohammadi M 2008** Influence of priming techniques on seed germination behavior of maize inbred lines (*Zea mays* L.). *Journal of Agricultural Biological Sciences*, 3(3): 22-25.
- Dhindsa R S, Plumb-Dhindsa P and Thorpe T A 1981** Leaf Senescence: Correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *Journal of Experimental Botany*, 32(1): 93-101.
- Diaz P, Almaraz-Suarez F C and Alcantara J 2001** Inoculation of plant growth promoting bacteria in Lettuce. *International Journal of Biotech Trends and Technology*, 19:327-333.
- Ellis R A and Roberts E H 1981** The quantification of ageing and survival in orthodox seeds. *Seed Science and Technology*, 9: 373-409.
- Emam Y 2007** Cereal Production, 3rd ed. Shiraz, Iran: Shiraz University Press.
- Hussain S, Zheng M, Khan F, Khaliq A, Fahad S, Peng S, Huang J, Cuil K, and Niel L 2015** Benefits of rice seed priming are offset permanently by prolonged storage and the storage conditions. *Scientific Reports*, 29(5): 8101p.
- ISTA [International Seed Testing Association]. 2010** International rules for seed testing. *Seed Science and Technology*, 139: 23-31.
- ISTA [International Seed Testing Association]. 1999.** International rules for seed testing. *Seed Science and Technology*, 27: 1-340.
- Iswariya S, Sujatha K and Subhashini R 2019** Enhancement of seedling vigour through Bio-priming for Barnyard Millet Var. MDU1. *International Journal of Current Microbiology and Applied Sciences*, 8(4):2254-2259.
- Kittock P A and Law A G 1968** Relationship of seedling vigour to respiration and tetrazolium chloride reduction of germinating wheat seeds. *Journal of Agronomy*, 60: 286-288.
- Kumar G AY and Prasanna K P R 2001** Seed treatment to control seed borne fungi and to improve seed germination in hybrid rice. Proceedings of 2001-National seminar on Seed Science and Technology in the New Millenium –Vistas and Vision. University of Mysore, Mysore, Aug 6-8, 2002.105.
- Letham D S 1974** Regulators of cell division in plant tissues: The cytokinins of coconut milk. *Plant Physiology*, 32: 66-70.
- Maguire J D 1962** Speed of germination aid in selection and evaluation of seedling emergence and vigour. *Crop Science*, 2: 176-177.
- Murungu F S, Chiduza C, Nyamugafata P, Clark L J, Whalley W R and Finch-Savage W E 2004** Effects of “on-farm seed priming” on consecutive daily sowing occasions on the emergence and growth of maize in semiarid Zimbabwe. *Field Crops Research*, 89:49-57.
- McDonald M B 1999** Seed deterioration: physiology, repair and assessment. *Seed Science and Technology*, 27:177-237.
- Nagendra M S, Bastian D, Francies R M and Rajendra A A 2017** Effect of sowing time on fruit and seed yield in oriental pickling melon (*Cucumis melo* var. conomon). *International Journal of Chemical Studies*, 5(4): 1910-1912.
- Nataraj K, Balakrishna P, Ramegowda, Roopa A and Chandrashekar U S 2011** Influence of storage containers and seed treatment

- chemicals on quality of new sunflower hybrids during storage. *National Seed Congress*, 267-280.
- Nithya N and Geetha R 2017** Storability evaluation of primed seeds of Rice (*Oryza sativa*) cv. PMK-4. *Journal of Pharmacognosy Phytochemistry*, 1: 61-63.
- Raj S N, Shetty N P and Shetty H S 2004** Seed bio-priming with *Pseudomonas fluorescens* isolates enhances growth of pearl millet plants and induces resistance against downy mildew. *International Journal of Pest Management*, 50(1): 41-48.
- Reddy A R, Madhavi G B, Reddy K G, Yellareddygar S K and Reddy M S 2011** Effect of seed biopriming with *Trichoderma viride* and *Pseudomonas fluorescens* in chickpea (*Cicer arietinum*) in Andhra Pradesh. *Plant Growth-Promoting rhizobacteria (PGPR) for Sustainable Agriculture*, 324-429.
- Rinku V P, Krishna Y P, Jasrai R T and Nayana B 2017** Effect of hydropriming and biopriming on seed germination of brinjal and tomato seed. *Research Journal on Agricultural and Forestry Sciences*, 5(6):1-14.
- Shakuntala N M, Vasudevan S N, Patil S B, Vijaykumar A G, Mathad R C, Doddagoudar S R and Macha S I 2012** Organic Bio priming on Seed vigour inducing enzyme in paddy, an alternative to inorganics. University of agricultural sciences, Raichur, *Environmental Sciences*, 1:251- 257.
- Saglam S, Day S, Kaya G and Gurbuz A 2010** Hydro priming increases germination of lentil (*Lens culinaris* Medik) under water stress. *Natural Science and Biology*, 2(2):103-106.
- Singh A, Abubakar A H, Ahmed H G, Aliyu U, Sokoto M B, Alhassan J, Musa M and Singh R B 2011** Seed hydro priming effects on germination, emergence and growth of cowpea (*Vigna unguiculata* L. Walp.)". *Trends Advances in Science Engineering*, 1(3):37-42.
- Singh K, Kumar V, Sing S and Shukla V K 2014** *Trichoderma harzianum* isolates of Indo-Gangetic plains as antagonists and growth promotion agent for okra. *Journal of Biological Control*, 28(1): 48-52.
- Somasundaram G and Bhaskaran M 2017** Effect of seed priming on germination and vigour in low and high longevity rice genotypes. *International Journal of Agricultural Science and Research*, 7(2): 373-380.
- Srivastava R, Khalid A, Singh U S and Sharma A K 2010** Evaluation of arbuscular mycorrhizal fungus, fluorescent *Pseudomonas* and *Trichoderma harzianum* formulation against *Fusarium oxysporum* f. sp. *lycopersici* for the management of tomato wilt. *Biological Control*, 53(1) :24-31.
- Wattanakupakin P, Photchanachai S, Ratanakhanokchai K, Kyu K L, Ritthicha P and Miyagaw S 2012** Hydropriming effects on carbohydrate metabolism, antioxidant enzyme activity and seed vigor of maize (*Zea mays* L.). *African Journal of Biotechnology*, 11(15): 3537-3547.
- Yildirim K C, Orel D C, Okya H, Gursan M M and Demir I 2021** Quality of immature and mature Pepper (*Capsicum annum* L.) seeds in relation to bio-priming with endophytic *Pseudomonas* and *Bacillus* spp. *Horticulture*, 7(4): 75p.