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Studies on bio and solid matrix priming in rice (*Oryza sativa* L.)

Purushottam and RDS Yadav

Abstract
An experiment comprising bio priming with *Trichoderma viride* @ 5g/kg seed, *Pseudomonas fluorescence* @ 5g/kg seed and solid matrix with sand was conducted in 3 genetically diverse and promising cultivars *viz.*, NDR-2064, Shusk Samrat and Shabha Sub-1of rice for 12 and 16 hrs during *Kharif* 2017 and 2018 in order to explore their possibilities for seed enhancement. All the priming treatments showed significant enhancement in seed germination (%), speed of germination, root length (cm), shoot length (cm), seedling length (cm) and vigour index in all the varieties in comparison to their respective control. The bio priming was appeared superior as compared to solid matrix. Thus, the seed priming with either *Pseudomonas fluorescence or Trichoderma viride* @ 5g/kg seed and solid matrix with sand for 12/16 hrs depending upon the genetic architecture of the trait/ seed could be exploited as cost effective/economic, non-toxic and eco-friendly sources for enhancing the seed quality parameters in rice.

Keywords: *Trichoderma viride, pseudomonas fluorescence,* solid matrix, seed enhancement, rice

Introduction
Rice is such an important food in some countries that "to eat" means "to eat rice". It is an indispensable source of calories for nearly half of the people in the world (Webmd.com). More than 90 per cent of the world, rice is produced and consumed in Asia. It is cultivated in all continents except Antarctica, occupying an area of 167.13 million ha, and producing 782.00 million ton paddy (Anon. 20178-19) [1]. Improved production and access to this vital food crop is very important as it feeds more than half the world's population while providing income for millions of rice producers, processors and traders. The protein content of milled rice is about 6-7 per cent however, compares favourably with other cereals in amino acid content. The biological value of protein is high, the fat content of rice is low (2.0-2.5%) and much of the fat is lost during milling. Rice grain contains as much B group vitamin as wheat. Milled rice losses valuable proteins, vitamins and minerals in the milling process during which embryo and aleuronic layer are removed and much of the loss of nutrients can avoided through parboiling process. The by-products of rice milling are used for a variety of purposes. Rice bran is used as cattle and poultry feed. Rice hull can be used in manufacture of insulation materials, cement and cardboard as well as a litter in poultry keeping. Rice straw can be used as cattle feed as well as litter during winter.

In India, rice is grown over an area of about 43.79 million hectares which produces 118.42 Million metric tonnes with an average productivity of 2688 kilogram/hectare (Anon. 2018-19) [1]. In Uttar Pradesh, it is grown in an area of about 5.86 million hectares with production of 13.27 million tonnes and productivity of 2283 kilogram/hectares (Anon. 2018-19) [1]. To meet the demand of increasing population and maintain self-sufficiency, the present production level needs to be increased up to 14 million tonnes by 2025 which can be achieved only by increasing the rice production by over 2 million tone/year in coming decade (Subaiah 2006) [23].

The term "seed priming" was coined /reported by Heydecker (1974) [9] to describe a treatment to improve germination at low temperature and his co-workers have extensively used the "priming" to describe an osmotic seed treatment to enhance seed germination processes. Seed priming is defined as the technique associated with uptake of water by the seed to initiate the early event of germination but not sufficient to permit radical protrusion followed by drying (Basu 1994) [3]. Seed priming has now been emphasized to exploit as an easy, eco-friendly and cost-effective technique to improve seed quality parameters leading to increase seed yield in most of the crops (Kumar *et al*. 2019, Srivastava *et al.* 2011, 2008, Yadav *et al.* 2019) [12, 21, 22, 26].

Beneficial microbes are included in the priming process, either as a technique for colonizing seeds and/or to control pathogen proliferation during priming compatibility with existing crop protection seed treatment.

In the recent years the low-input agricultural systems have gained increasing importance in many industrialized countries, for reduction of environmental degradation. Cropping system component of integrated farming systems with reduced inputs have been developed. It is under these conditions that plants are expected to be particularly dependent on beneficial rhizosphere microorganisms. Key component of soil micro biota is to form symbiotic relationships with the roots of most terrestrial plants, improving the nutritional status of their host and protecting it against several soil-borne plant pathogens. The incidence and the effect of root colonization vary depending on the plants species.

Trichoderma spp. is a common component of rhizosphere soil and has been reported to suppress a great number of plant diseases. Some strains have also been reported to colonize the root surface, enhancing root growth and development, crop productivity, resistance to abiotic stresses, and the uptake and use of nutrients (Martínez-Medina *et al.* 2011) [14]. *Trichoderma* genus has so far been mainly registered as effective antagonists of soil-borne pathogens such as *Sclerotinia sclerotiorum, Sclerotinia rolfsii, Macrophomin aphaseolina, Rhizoctonia solani, Pythium spp. Phytophthora spp.* and *Fusarium spp.* causing root rot of several agriculturally important crops. In addition to their effects against pests, entophytes might contribute to the overall plant improvement. *Trichoderma* spp. is cosmopolitan fungi found in agricultural, forest, and desert soils. Also, they colonize roots of various plants found in different ecosystems. They have been defined as pre-plant symbiont opportunistic avirulent organism, able to colonize plant roots and to produce compounds that stimulate growth and plant defence mechanisms under suboptimal conditions (Harman *et al*.2004) [7]. For the past many years, *Trichoderma* spp. has been mostly used as bio control agents. However, in the recent years, they have become popular as plant growth promoter (Hermosa *et al*. 2012) [8]. Studies show that, *Trichoderma* spp. form a symbiotic relationship with the host plant as early as the third phase of seed germination and this association lasts till the plant matures. The use of *Trichoderma* fungi in agriculture can provide numerous advantages, Improvement of the plant health by promoting plant growth, and stimulation of root growth (Abd, el-rehim *et al*. 2004) [2]. In addition, certain *Trichoderma* strains have produced a growth-regulating factor which increased the rate of seed germination and dry weight of shoots and stems (Windham *et al.* 1986) [24]. Further, when seed were treated with *Pseudomonas fluorescence* strain PfALR2, Mishra and Sinha (2000) [16] got increased seed germination of rice from 26.3 to 52.6 per cent. Two *P.fluorescence* strains, viz., PF1 and PF7, inhibited the mycelial growth of sheath blight and increased the seedling vigour of rice plant and yield under green house and field conditions. Ramakrishnan *et al*. (1998) [19] also observed that seed treatment of rice seed with *P.fluorescence* enhanced 13 per cent yield Solid matrix priming (SMP) involves the use of a wet organic or inorganic material (Parera et al. 1993) [18], which simulates the natural imbibition processes taking place in the soil (McDonald 2000) [15]. The substrate must possess given characteristics: low matric potential; high seed safety; high specific surface (ie., high surface to volume ratio); negligible water solubility; high adhesiveness to seed surface and high capacity to retain water (Khan 1992) [11]. The seed is placed on or mixed with the hydrating substrate which gradually moisturizes the seed (McDonald 2000) [15]. It is the incubation of seeds in a solid, insoluble matrix with a limited amount of water. This method confers a slow imbibition. Matrix carriers are Calcinated clay, Vermiculite, Peat Moss, Sand, Micro-Gel, etc.

Materials and Methods

The experiment was conducted in Post Graduate Laboratory of Seed Science and Technology, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya-224229. U.P. during *kharif* 2017 and 2018 in three rice cvs. NDR-2064, Shusk Samrat and Shabha Sub-1. The treatments used at different concentrations for priming were, Thiram @ 2.5 g/kg, *Trichoderma viride* @ 5g/kg, *Pseudomonas fluorescence* @ 5g/kg and Solid matrix sand for 12 hrs and 16 hrs separately. Unprimed seed of each cultivar was treated as respective control. Primed and unprimed seed were placed in completely randomized design (CRD) with 4 replications following towel paper method for germination in laboratory under controlled condition. The observations on the seed quality parameters *viz*., germination (%) (ISTA 1999) [10], speed of germination, seed moisture content (%), root length (cm), shoot length (cm), seedling length (cm) and vigour index (Baki and Anderson 1973) [3] were recorded. . The experimental data recorded were subjected to statistical analysis for calculating analysis of variance, range, mean, critical difference and coefficient of variation (Fisher 1936) [6].

Results and Discussion

Seed germination

Effect of seed priming treatments under study were found to be the most effective for significant increasing the seed germination (%) in all three varieties *viz*., NDR-2064, Shusk Samrat and Shabha Sub-1 during both the duration. The most effective treatment was found to be the Thiram @ 2.5 g/kg hydration for 16 hrs (94). The lowest germination was noticed with the control 12 hrs (87) in NDR 2064. In the case of Shusk Samrat, the maximum germination was observed with Solid matrix priming for 16 hrs (94) followed by Thiram @ 2.5 g/kg hydration for 16 hrs. the control again revealed the lowest germination. Seed priming with *Pseudomonas fluorescence* @ 5g/kg showed the highest germination in Shabha Sub-1.It is reported that the earlier and better-synchronized germination is associated with increased metabolic activities in the soaked seed (Basra *et aL*. 2003) [4].

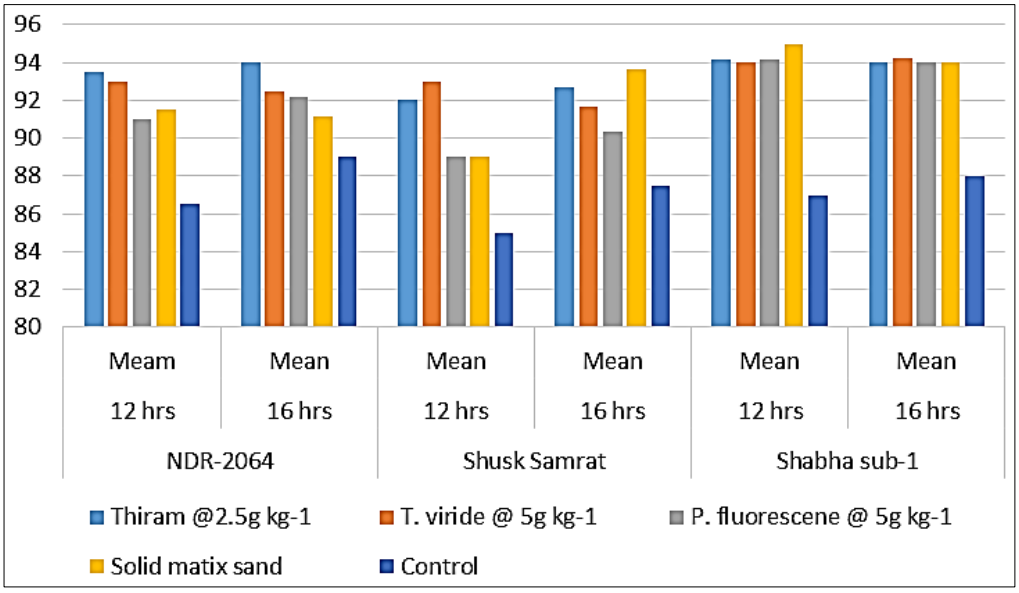

Fig 1: Effect of seed priming treatments on seed germination (%) in three cultivars of rice

Speed of germination

Performance of different bio and solid matrix priming treatments on speed of germination in three genetically diverse cultivars is presented in Fig. 2. In general, all the priming treatments increased significantly speed of germination in all cultivars during both duration of priming. The maximum speed of germination was noticed by Thiram @ 2.5 g/kg during 12 hrs of priming followed by *Trichoderma viride* @ 5g/kg although for 16 hrs of priming in NDR2064. Similarly, the maximum speed of germination was observed by priming with *Trichoderma viride* @ 5g/k for 12 hrs of priming in Shusk Samrat. The next best priming treatment was appeared for *Pseudomonas fluorescence* @ 5g/kg during same duration of priming in Shusk Samrat. In case of Shabha Sub-1, the maximum speed of germination was appeared in priming with *Pseudomonas fluorescence* @ 5g/kg followed by Thiram @ 2.5 g/kg during 16 hrs of priming. Thus, it could be advocated that effect of seed priming treatment under study were found to be varied depending upon the genetic architecture of the seed.

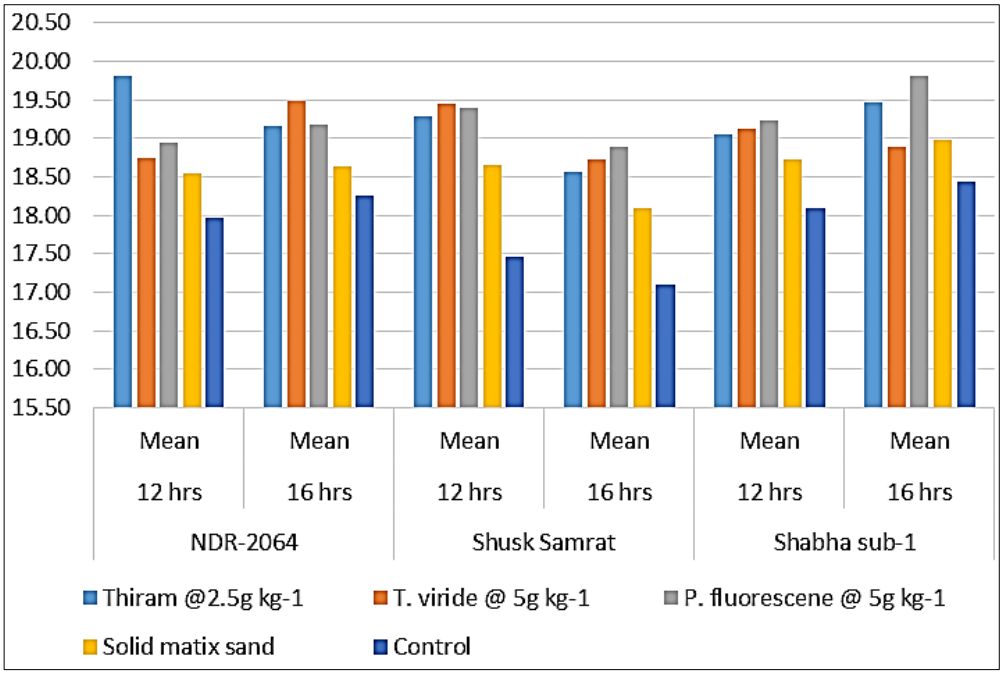

Fig 2: Effect of seed priming treatments on speed of germination in three cultivars of rice

Seed moisture content (%)

Effect of seed priming treatments on seed moisture content (%) in three cultivars of rice are depicted in Fig.3. after priming, the seed moisture content was increased drastically during imbibition but after drying back, the moisture was remained at par to original moisture content. Principally, it is required during the seed priming technique.

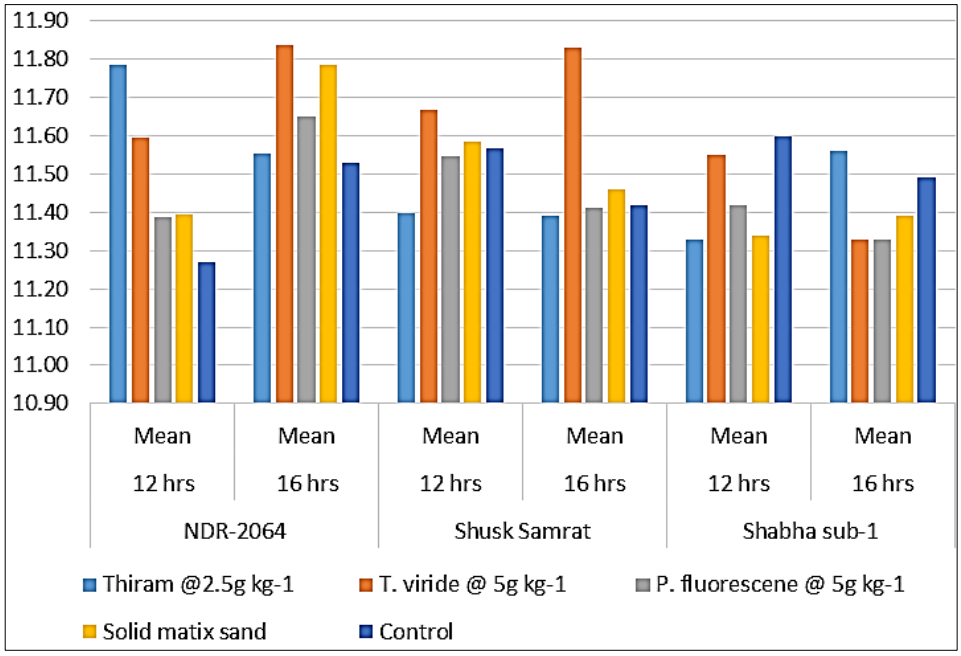

Fig 3: Effect of seed priming treatments on seed moisture content (%) in three cultivars of rice

Root length (cm)

Effect of seed priming treatments were found to be most effective for significant increasing the root length (cm) in all three cultivars *viz.;* NDR-2064, Shusk Samrat and Shabha Sub-1 during both the duration under study (Fig., 4). The most efficient seed priming treatment was *Pseudomonas fluorescence* @ 5g/kg for 16 hrs (11.57) in NDR 2064 and Shabha Sub-1, whereas in Shusk Samrat, the maximum root length was expressed with solid matrix priming.

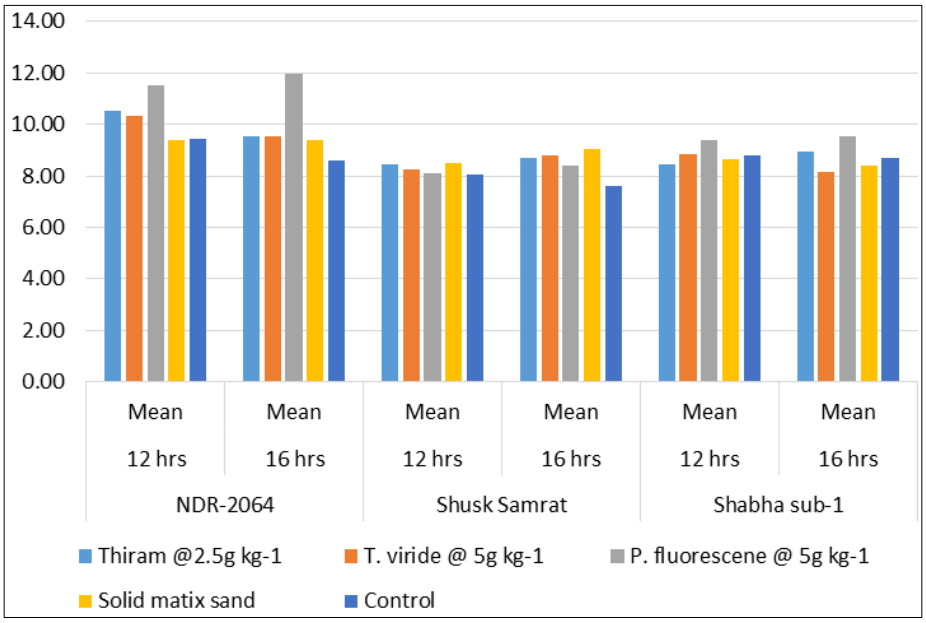

Fig 4: Effect of seed priming treatments on root length (cm) in three cultivars of rice

Shoot length (cm)

Performance of seed priming treatments under study were found to be most effective for significant increasing the shoot length (cm) in all three cultivars *viz.*, NDR-2064, Shusk Samrat and Shabha Sub-1 during both the duration (Fig.,5). The effect of priming with Thiram @ 2.5 g/kg, *Trichoderma viride* @ 5g/kg and *Pseudomonas fluorescence* @ 5g/kg was almost at par for 12 hrs in NDR 2064. In the case of Shusk Samrat, the solid matrix priming was appeared as the most effective. The priming with Thiram @ 2.5 g/kg during 12 hrs and or 16 hrs were found to enhance maximum shoot length in Shabha Sub-1.

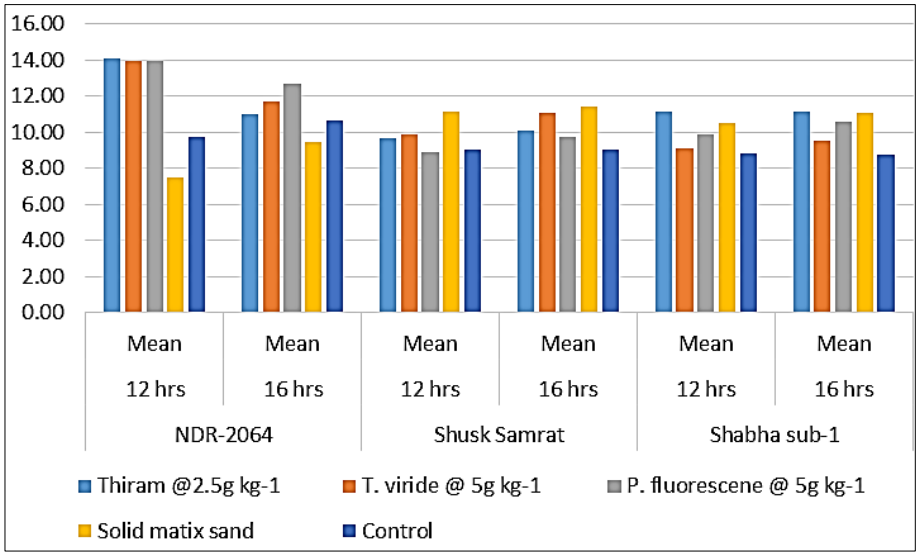

Fig 5: Effect of seed priming treatments on shoot length (cm) in three cultivars of rice

Seedling length (cm)

Effects of seed priming treatments on seedling length during 12 and 16 hrs in three cultivars viz., NDR-2064, Shusk Samrat and Shabha Sub-1 of rice are presented in Fig., 6. The seed priming done with *Pseudomonas fluorescence* @ 5g/kg ether 12 hrs or 16 hrs were found most effective for maximum increasing the seedling length in NDR 2064 and Shabha Sub-1. In the case of Shusk Samrat, the most effective priming was observed with solid matrix during both duration in comparison to other as well as control.

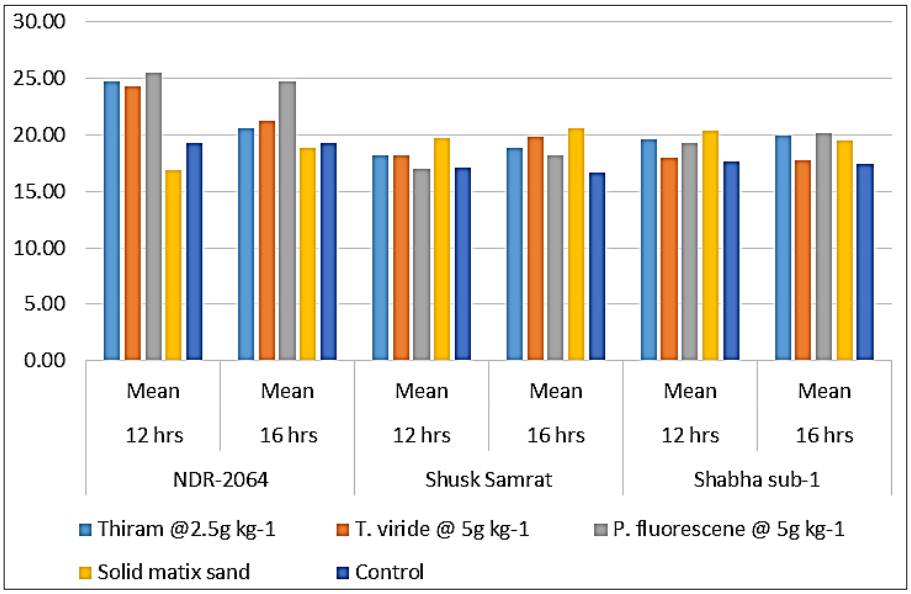

Fig 6: Effect of seed priming treatments on seedling length (cm) in three cultivars of rice

Vigour index

Vigour index is a comprised value of germination (%) and seedling length (cm). Since these both parameters have already responded significantly to seed priming treatments. Accordingly, effect of seed priming treatments under study were found to be most effective for significant increasing the seedling vigour index in all three varieties *viz.*, NDR-2064, Shusk Samrat, and Shabha Sub-1 during both the duration. The most efficient seed priming treatment was *Pseudomonas fluorescence* @ 5g/kg in NDR 2064 and Shabha Sub-1 for 12 /16 hrs. In the case of Shusk Samrat, priming processed with solid matrix was found superior in comparison to other treatments (Fig., 7).

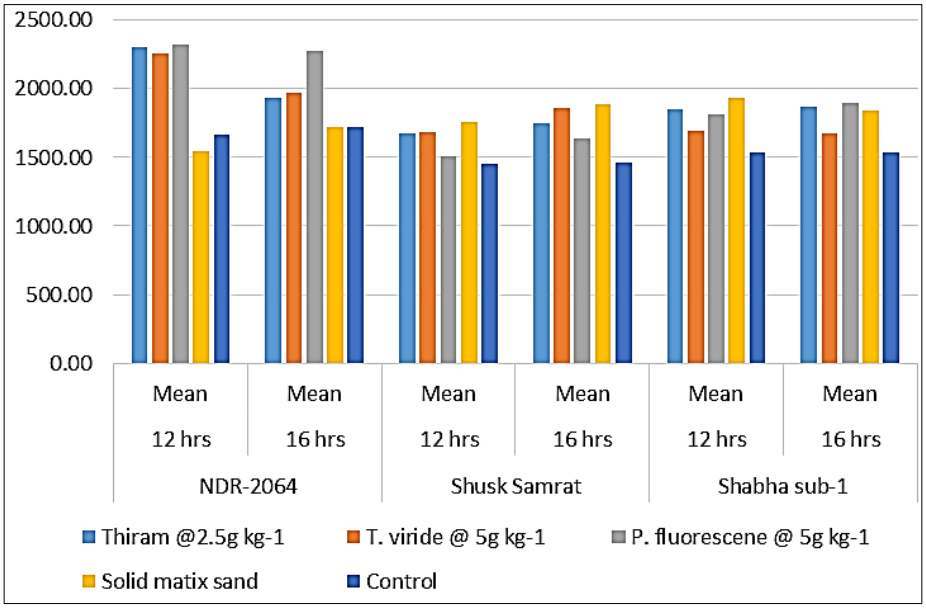

Fig 7: Effect of seed priming treatments on vigour index in three cultivars of rice

The beneficial effects of *Trichoderma viride* in the trems of increased seed germination rates, seedling vigour and reduced incidence of seed-borne fungal pathogens compared to control habe well been elaborated by Mukhtar *et al.* (2008) [17]. The *Pseudomonas fluorescence* ,being a major Rhizobacteria, encouraged the plant growth through producing yellowish green fluorescent siderophore involve in high affinity transport of iron into the cell. The study showed significantly higher increase in root length over control plants. It has been shown that these bacteria competively colonize plant roots and cause the plant statistically significant root and shoot increases by stimulating plant growth and reduce the incidence of plant disease (Mandal and Kotasthane 2014) [13]. Further, the studies have revealed that bio-priming with Phosphobacteria 20 % concentration for 24 hrs expressed high values for all the parameters namely speed of germination, germination (%), root length (cm), shoot length (cm), dry matter production (g/seedlings-5) and vigour index which accounted for 36, 14, 12, 24, 30 and 28%, respectively increase over non-primed seed (Sivakumar *et al.* 2016 [20] . Besides, the present studies are in accordance to other earlier reports also (Basra et al. 2003, Mishra and Sinha 2000, Yadav 2018) [4] [16] [25]. However, the effect of seed priming treatment under study were found to be varied depending upon the genetic architecture of the seed.

Conclusion

On the basis present studies, it could be concluded that bio-priming with *Pseudomonas fluorescence, Trichoderma viridi* @ 5g/kg and sand solid matrix could be employed eco-friendly either 12 hrs or 16 hrs depending upon the genetic architecture of seed for improving seed quality parameters *viz*., germination, speed of germination, root length, shoot length, seedling length and vigour length in rice.

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