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RESEARCH ARTICLE

Bio-Priming of African Marigold (Tagetes erecta) cv. Pusa Narangi Gainda Seeds

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Abstract

This investigation was done to determine the effect of biopriming on African marigold (*Tagetes erecta*) cv. Pusa Narangi Gainda. Biopriming is a process of invigoration seeds with a solution containing beneficial micro-organisms. Randomized block design with seven treatments was used and replicated thrice, in which biopriming agents, namely phosphate-solubilizing bacteria (PSB), *Trichoderma harzianum*, microbial consortia, *Psuedomonas fluorescens*, jeevamrutha, and indigenous effective micro-organisms (IEM) were used, and water was used as a control to study their influence on seed germination, seedling growth and development parameters. Parameters like germination percentage (83.67%), speed of germination (19.89), mortality (5.33%), and germination rate (18.74) were observed to be maximum with the seeds primed with *Trichoderma harzianum*, which was on par with microbial consortia and the observations from PSB were on par with *Pseudomonas fluorescens*. These parameters were observed to be lowest in control, followed by IEM and Jeevamrutha. Parameters for seedling, such as shoot length (12.97 cm), root length (14.33 cm), seedling length (27.30 cm), seedling vigour index (2283.27), seedling dry weight (6.96 g), were also maximum in the seeds primed with *T. harzianum*, followed by consortia. In contrast, control showed minimum seedling growth and development.

Keywords: Biopriming, *Trichoderma harzianum*, microbial consortia, Seed germination, African marigold.

Introduction

African Marigold (*Tagetes erecta*), a flowering plant belonging to the family Asteraceae, is a hardy, annual, erect and branched plant. It is one of the most commonly grown loose flowers and is extensively used in religious and social functions. Compounds like carotenoids and lutein are rich in the florets of marigolds, which are used as food colour (INS number E161b) for foods such as pasta, vegetable oil, margarine, mayonnaise, salad dressing, baked foods, confectionery, dairy products, ice cream, yogurt, citrus juice, and mustard. In poultry feed, powders and extracts are used as colorants. Marigold, also known as "Gainda," is a very important loose flower crop grown commercially in southern India and West Bengal. Propagation is generally done by seeds and stem cuttings. The seeds are long and black. 1.0 to 1.5 kg of seed is needed for planting a one-hectare area, whereas 250 g/ha is sufficient in the case of F_1 hybrids. The marigold flower industry in India stands next to the chrysanthemum among all annuals. The major problems to produce marigolds are damping off, collar rot, leaf spot, and root rot and among insect pests, leaf miners, thrips, flower feeders and flower borers are major. Another major drawback in the production of marigolds is poor seed germination, resulting in low yield. Priming treatment can be done with agrochemicals; however, for the better health of soil and environment, one can use biological agents, microbial agents, and organic solvents. Excessive and unscientific use of chemicals is one of the main reasons for the degradation of soil health and the environment. Toxic chemicals can also kill non-targeted species such as predators, parasites, beneficial microbes, and the residues of chemicals also affect the health of humans and animals. Under the stress conditions, seed priming can improve the emergence of crops (Pill *et al*., 2009; Rakshit *et al*., 2014), which has presented surprise results for flower crops like pansy, china aster, sunflower and many other crops like

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field crops, vegetables, and grasses. Information on the effectiveness of using different priming agents on flower seeds, especially on marigolds, is scanty. Hence, there is a critical need to develop some strategies and techniques to enhance the seed germination, vigour, quality, and yield of marigolds. Moreover, it will be an alternative to chemical seed treatment for better soil and plant health. By emphasizing these points, the investigation was conducted to find out the efficacy of biopriming agents on germination of seed and seedling development of African marigolds in the hill region of Nagaland.

Materials And Methods

The investigation was done in the instructional farm of SAS, Nagaland University, Medziphema campus during 2021-22. Randomized block design with seven treatments was used and replicated thrice. Bio-priming agents, namely *Trichoderma harzianum* (T_7), *Pseudomonas fluorescens* (T_6), PSB (T_2), microbial consortia containing *Psuedomonas taiwanensis*, *Bacillus aryabhatai*, *Azotobacter tropicalis* (T_4) were obtained from UAS, Bengaluru. At the same time, Indigenous effective micro-organisms (IEM-T_5) were made in the department by following a standard procedure. Jeevamrutha (T_3) was prepared by following the standard procedure, where five litres of cow urine was mixed with five kg of local cow dung, 1 kg local jaggery, one kg pulse flour and a handful of virgin soil and finally the hundred litres volume was made up. The drum was kept in shade by covering with a wet gunny bag and the mixture was stirred clockwise thrice a day and incubated. These priming agents were diluted with water at a 1:5 ratio and used for seed-bio priming and water (T_1) was used as a control. About 100 seeds were bio-primed and sown in portrays in which coco-peat was used as the medium. These seeds were regularly hydrated and observed from first seed germination to the final count of germination (10th day) and root and shoot length till the 20th day.

Parameters like days taken for the first germination, speed of germination, germination percentage, mean germination time (days), mortality (%) were observed and germination rate, germination index were calculated based on observations. Lengths of shoot and root were observed, length of seedling and seedling vigor index were recorded using the formulae.

Percentage of germination (%)

The total number of healthy seedlings in each treatment replication was computed to find the percentage of germination.

Percentage of Germination = No. of seeds germinated x100Total no. of seeds

Germination speed (Nos per day)

Germination test was conducted, and daily germination counts were calculated. Germination was counted daily till no germination was observed for 12 days. The speed of germination index was then computed by adding the quotients of the daily counts divided by the number of germination days.

Germination speed = Σ (n/t)

Where, n = newly germinating seeds number at time ‘t’
t = number of days from sowing

Mean germination time (days)

The germinated seeds were counted every day till the 10th day, i.e., the final count. Mean germination time was computed by using the formula.

Mean germination time = ∑nt / ∑n

Where, ∑n= n1+n2+n3+…+nt

n= no. of seeds germinated on day t and t was the number of days.

t= tth day computed from the start of the germination test.

Days taken for the first germination

The no. of days taken for the germination of first seed in each treatment was observed from each replication and the average was taken to find the days taken for the first germination from all treatments.

Mortality (%)

The Percentage death of seedlings in each replication of treatment was recorded and the mean was taken.

Seedling length (cm)

10 normal seedlings in each replication of a treatment were selected randomly for measuring seedling length on the final count of germination.

Germination index

The rate and percentage, along with the vigour index, were computed by using the equations given below.

Germination index (GI) = Σ (Gt/Tt)

Gt is the seeds germinated on day t in numbers and
It is the no. of days from the start of the germination test.

Seedling dry weight (mg/10 seedlings)

Good seedlings from every replication were picked, put in a butter paper bag and the dry weight was recorded after drying for 24 hours.

Shoot length (cm)

Five normal seedlings were randomly selected. Shoot length was measured from the base of the shoot to the tip of the leaves.

Root length (cm)

Five normal seedlings were chosen randomly. The length of the root was estimated from the root base to the rootlet tip.

Seedling vigour index

Seedling vigour index was calculated by using the formula.
∑ (length of shoot +length of root) / number of seedlings taken X Germination percentage

Results And Discussion

Previous works carried out showed that combining seed priming with the application of biocontrol agents led to improvement in crops, where different methods were utilized for bio-priming (Harman and Taylor, 1988; Callan *et al.*, 1991; Jensen *et al.*, 2004). *Trichoderma spp.*, which is commonly found in the rhizosphere, apart from stimulating plant growth, acts as a mycoparasite pathogenic fungi (Rakshit *et al.*, 2015; Meena *et al.*, 2016; Singh *et al.*, 2013). *Trichoderma spp.* is used for bio-priming of seed as it is a popular biocontrol agent (Pill *et al.*, 2009; Harman and Taylor, 1988).

Seed Germination

Data pertaining to seed germination are presented in Table 1. It is clear from this table that there was a significant difference among the treatments in all the parameters except for the days taken for seed germination. Germination percentage was maximum (83.87%) in T_7, which was on par with T_4 (83.33%) while it was minimum in T_1 (75.76%) i.e., control. *Trichoderma harzianum* boosted the germination parameters like germination rate (18.74), germination speed (19.89) and less mortality (5.33%), which are at par with microbial consortia. The positive outcome in germination parameters of African marigold seeds primed with *T. harzianum* and microbial consortia might be because of *Trichoderma harzianum* is a fungal antagonist and microbial consortia contains Pseudomonas which suppresses pathogens thereby reducing mortality and increasing the germination percentage the results are in line with the work of Kavitha *et al.*, (2005) who found the highest germination in chilli and Bhargava *et al.*, (2014) in Antirrhinum majus and also The extensive network of hyphae from microbes like *Trichoderma harzianum* and consortia helped in nourishment of seeds through nutrient uptake and moisture conservation near seeds which increases the speed of germination. These treatments could have exerted significant changes in the seeds, their metabolism, biochemical changes like the synthesis of proteins (cruciferin and globulins), apart from enhancing the integrity of the membrane as compared to the control. These findings are in line with the results of Bhargava *et al.* (2014) in snapdragon, who recorded the highest germination percentage, shoot length, root length, seedling development with *Trichoderma harzianum* (1×10^5cfu/ml) and Kavitha *et al.* (2007) who also observed the highest seed germination by using *Trichoderma viride* @8 g per kg in chili seeds and lowest under control.

Seedling growth and development

The data about the parameters of seedling growth and development are presented in Table 2. It is apparent that a significant difference was there among the treatments in all the parameters. Maximum shoot length (13.23 cm), seedling length (28.10cm), root length (14.87 cm), seedling vigour index (2339.47) and seedling dry weight (6.96g) were observed in T_4 (microbial consortia) As shown in the Figure 1, which were on par with T_7 (*T. harzianum*) As shown in the Figure 2 while the minimum value was observed in all parameters in T_1 (control) As shown in the Figure 3. The increase in the seedling length, root length, and shoot length is due to the progressive mineralization of nutrients and supplementation of balanced nutrition for seedling growth, owing to quick and greater availability of nutrients, which in turn leads to an increased rate of meristematic activity. Simultaneously, cytokinin and IAA released by *T. harzianum* and microbial consortia might have resulted in the speedy growth of the shoot and root. These results conform to the findings of Krishna *et al.* (2019) in China aster, Latt *et al.* (2017) in China aster, Karishma *et al.* (2011) in Chrysanthemum, Kumari *et al.* (2013) in gladiolus. Plants treated with the *Trichoderma* isolates showed enhanced length of root and shoot, along with the dry weight of plants, with a remarkable

Table 1: Effect of bio-priming agents on seed germination of African marigold cv. Pusa Narangi Gainda

Treatment	*Germination percentage (%)*	*Speed of germination (number/day)*	*Mean germination time (days)*	*Mortality (%)*
T_{1-}Control	75.76	16.84	4.60	8.67
T_{2-} PSB	81.33	18.41	4.69	7.67
T_{3-}Jeevamrutha	78.67	18.00	4.51	7.33
T_{4-}Microbial consortia	83.33	19.48	4.19	6.33
T^{5-}IEM	76.33	17.50	4.55	6.33
T^{6-}*Pseudomonas fluorescens*	79.33	18.15	4.35	6.67
T^{7-}*Trichoderma harzianum*	83.67	19.89	4.44	5.33
F test	*	*	*	*
S.Em ±	1.303	0.338	0.086	0.868
CD (5%)	4.016	1.040	0.265	2.676

NS: Non-significant* : Significant

Table 2: Effect of bio-priming on seedling growth and development.

Treatments	*Shoot length (cm)*	*Root length (cm)*	*Seedling length (cm)*	*Seedling vigour index*	*Seedling dry weight (g)*
T_{1-}Control	08.93	2.97	11.90	0899.93	5.59
T_{2-} PSB	12.70	5.93	18.63	1515.23	6.55
T_{3-}Jeevamrutha	12.37	3.97	16.23	1284.13	6.39
T_{4-}Microbial consortia	13.23	14.87	28.10	2339.47	6.96
T_{5-}IEM	12.67	3.47	16.13	1230.03	5.84
T_{6-} *Pseudomonas fluorescens*	12.87	3.27	16.13	1279.97	6.77
T_{7-}*Trichoderma harzianum*	12.97	14.33	27.30	2283.27	6.96
F test	*	*	*	*	*
S.Em ±	0.258	0.640	0.711	49.266	0.131
CD (5%)	0.794	1.971	2.191	2.996	0.403

*** :** Significant.

Figure 1: Seedlings from T4 (Microbial consortia)

Figure 2: Seedlings from T7 (*Trichoderma harzianum*)

Figure 3: Seedlings from T1 (Control)_9(cC

increase in the number of leaves (Saxena *et al*., 2015). The results can be ascribed to the production of plant growth-promoting substances by these microbes, which enhanced cell division and root development. Early germination of seeds, production of healthy and quality seedlings were due to increased nitrogen fixation by nitrogen-fixing bacteria, zinc and phosphorous solubilization by the zinc-solubilizing bacterium and phosphorous solubilizing bacterium, respectively. The results are in line with the findings of (Shenoy and Kalagudi, 2002), Jayashree *et al*. (2018) and Saravanan *et al*. (2012) in *Casuarina equisetifolia* seedlings. From this study, it was observed that a microbial consortium that comprises different microbial strains helps in inducing early germination, better seed germination, seedling growth, and development. Treatment with *Pseudomonas fluorescens* and PSB also resulted in better germination parameters than the control. These microbes colonize the roots of plants and assist in the growth of plants by increasing solubilization and uptake of nutrients or by producing phytohormones (Arshad and Frankenberger, 1991; Kloepper *et al*., 1989).

Conclusion

Based on the results of this investigation, inference can be drawn that among different biopriming agents, *Trichoderma harzianum* and microbial consortia can be recommended for seed treatment in marigold as they significantly increased the percentage of germination, germination speed, rate of mean germination, germination rate, length of seedling, shoot and root length, seedling vigour index. Moreover, bio-priming is an eco-friendly technique, economical and an alternative to chemical seed treatment.

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