

# ORGANIC SEED TREATMENT WITH SEAWEED NANO POWDERS ON PHYSIOLOGICAL QUALITY AND ENZYME ACTIVITIES IN AGED SEEDS OF PIGEON PEA

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## KEYWORDS

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## ABSTRACT

In the present study the organic seaweed nano powders treatment were imposed to elucidate their effects as pre-sowing treatments. In another experiment, the same treatments were imposed on the seeds and the treated seeds were subjected to accelerated ageing for three days (treated-aged) to assess their performance as pre-storage treatment. The seaweed nano powders treatments were also given to aged seeds (aged-treated) to identify the efficacy as mid-storage treatments. The results of the present investigation revealed that, the seeds treated with *Sargassum myricocystum* (1%- 3 h) performed better than other treatments and control. The per cent increase over control for higher speed of germination, germination, seedling length, dry matter production, vigour index,  $\alpha$ -amylase and peroxidase in fresh (20, 10, 18, 16.7, 26.5, 21.3 and 20.7, respectively), treated aged (32.8, 12.5, 29.3, 19.4, 38.1, 21.4 and 24.5, respectively) and aged treated seeds (29.1, 6.6, 24.4, 22.4, 29.5, 17.9 and 13.5, respectively).

## INTRODUCTION

Pigeon pea (*Cajanus cajan* (L.) Mills.) is one of the major grain legume crops grown in the tropics and sub-tropics and accounts for about 5% of world legume production. It is the fifth prominent pulse crop in the world and second most important pulse crop after chickpea in India. India is ranking first having about 90% of the world area and 85% of production.

Organic farming in recent years gaining impetus due to realization of inherent advantages it confers in sustaining crop production under aberrant rain fed farming situations and also maintaining dynamic soil nutrient status and safe environment. In organic farming it is essential to use organically treated seeds as a source material for ensuring quality of organic product from unit area of land. Organic seed treatment is an effective method of seed enhancement technique using organically available resources. It speeds up seed germination, promotes faster growth and produces higher yield potential. Vigor tests have potential of assessing the seed physiological quality and among them; the accelerated ageing test has proved to be one of the most sensitive and efficient. It has been proven that the deteriorative effect of seed ageing is mainly due to production of free radicals (Bailly *et al.*, 2008) and use of antioxidants can quench the free radicals and retain seed vigour during germination (Sattler *et al.*, 2006). Recently, nano particles have been attempted to quench the reactive oxygen species and reduce the oxidative damage to improve the vigour and viability of seeds. Nano particles act as

antioxidants through which they quench the superoxide anion radical ( $O_2^-$ ) and consequently generates  $O_2$  like catalase (Kajita *et al.*, 2007).

Nano particles are an effective scavenger of free radicals and suppress the propagation of lipid peroxidation (Watanabe *et al.*, 2009). Among the several organics available, it has been proved that seaweeds possess natural antioxidant property, which can be attempted for seed treatments for vigour and viability maintenance either in commercial form or reducing the particles to near nano size. Seaweed extract is a new generation of natural organic fertilizers containing highly effective nutritious and promotes faster germination of seed and increase the yield and resistant ability of many crops. Seaweeds contain various trace elements, vitamins, amino acids and plant growth hormones which cause many beneficial effects on plant growth and development (Spinelli *et al.*, 2010 and Abdel-Mawgoud *et al.*, 2010). Application of seaweed extracts enhances seed germination, seedling vigour and yield (Economou *et al.*, 2007; Dwivedi *et al.*, 2014 and Lodhi *et al.*, 2015). Therefore, the present experiment was to identify the effect of pre sowing, pre and mid storage treatment of seaweed nano powder in the fresh and aged seeds of pigeon pea.

## MATERIAL AND METHODS

The seaweeds *Sargassum myricocystum* (Brown algae), *Caulerpa racemosa* (Green algae) and *Gracilaria edulis* (Red algae) collected from South East of Mandapam coast, Tamil

Nadu were washed with seawater initially to remove macroscopic epiphytes and sand particles finally with fresh water to remove adhering salt then shade dried for two weeks followed by oven drying at 40°C for 24 h and powdered. The powdered seaweeds were milled using ball mill for 5 h to bring out into nano size. Seaweed extracts were prepared as described by Bhosle *et al.* (1975) with minor modifications. A total quantity of 100 ml of acetone and 100 ml of methanol were added to 100 g each of the dried powder of *Sargassum myricocystum*, *Caulerpa racemosa* and *Gracilaria edulis* and kept for overnight with intermittent stirring and extracted through rotary evaporator at 40°C and 45 rpm. The liquid fertilizer was collected and stored in air tight container (stock solution). 0.1, 0.25, 0.50, 0.75 and 1.0 % were prepared from stock solution. Under laboratory through pilot studies, the duration of ageing (40 ± 1°C and 95 ± 5% RH) required to reach a germination percentage around 75% (Prescribed Indian Minimum Seed Certification Standard for pulses) was identified as 3 days for pigeon pea. The organic seaweed nano powders were imposed to the fresh seeds as pre-sowing treatment and the treated seeds subjected to accelerated ageing for three days (treated-aged) to assess their performance as pre-storage treatment. The seaweed nano powder was treated with aged seeds (aged-treated) to identify the efficacy as mid-storage treatments. The speed of germination was calculated according to Maguire (1962). After 7 days, germinating seeds which showed normal development of root and shoot alone were counted and expressed as germination percentage (ISTA, 2013). The seedlings were measured from the tip of the root to tip of the growing meristem of the shoot and mean seedlings length was expressed in cm. The seedlings used for growth measurement were shade dried for 24 h (after removing the cotyledons and seed coat) and dried again in hot air oven maintained at 85 ± 2°C for 24 h and cooled in desiccator filled with silica gel for 30 min. The dry weight of seedlings was recorded using an electronic balance and expressed as

as g seedlings<sup>-10</sup>. Vigour index was calculated by using the formula of Abdul Baki and Anderson (1973), Vigour index = Germination (%) × Seedling length (cm),  $\alpha$ -amylase by Simpson and Naylor (1962) and peroxidase by Malik and Singh (1980). The experiment was arranged in completely randomized design with four replications and 100 seeds per replication. Result data (in per cent) were transformed to arc sine values before statistical analysis in order to unify the variance of the data (Ansari *et al.*, 2012). The data were statistically analyzed using analysis of variance and treatment means were compared using LSD test at 0.05 level of probability, when the F- values were significant (Steel and Torrie, 1984).

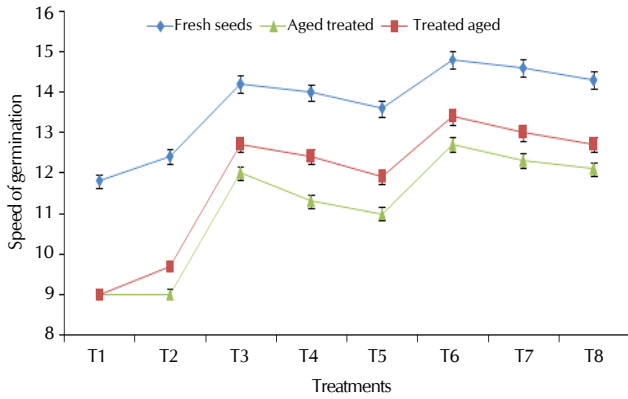
## RESULTS AND DISCUSSION

In this experiment, the fresh treated seeds revealed that among the treatments, *S. myricocystum* (1%-3 h) was superior to other treatments *viz.*, control, water soaking, *S. myricocystum* (3 g/kg-3 h), *G. edulis* (3g/kg-3 h), *C. racemosa* (3 g/kg-3 h), *G. edulis* (1%-3 h) and *C. racemosa* (1%-3 h). Per cent of improvement over control for speed of germination (Fig. 1), seedling length, vigour index, dry matter production,  $\alpha$ -amylase activity and peroxidase activity was 20, 18, 27, 16, 21 and 20, respectively.

In treated aged seeds and aged treated seeds, *S. myricocystum* (1%-3 h) recorded maximum germination (80 and 75%, respectively). The other treatments were better than control (70%) (Fig. 2). Among the treatments seedling length was also more in *S. myricocystum* (1%-3 h) treated seeds for both treated aged (35.8 cm) and aged treated seeds (33.5 cm) (Fig. 3). This was closely followed by *G. edulis* (1%-3 h). The other seaweed treatments were better than control (25.3%) for treated aged and aged treated seeds. Among the treatments *S. myricocystum* (1%-3 h) registered higher dry matter production. The percentage increase of dry matter production for treated aged recorded was 19.4 and 22.4, respectively

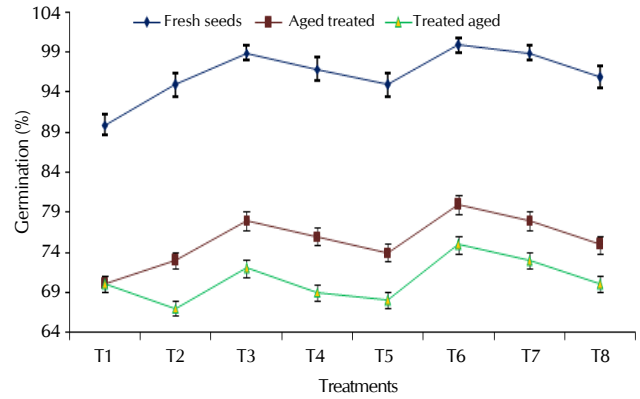
**Table 1: Statistical significance of organic seaweed nano powders on pigeon pea seed germination and biochemical parameters was given in Fig 1, Fig 2, Fig 3, Fig 4, Fig 5, Fig 6 and Fig 7.**

| Parameters  | Fresh seeds | Treated aged | Aged treated |
|---|-------------|--------------|--------------|
| Speed of germination                                |             |              |              |
| SEd   | 0.2862      | 0.2487       | 0.2343       |
| LSD (P=0.05)  | 0.5907      | 0.5132       | 0.4836       |
| Germination (%)                                     |             |              |              |
| SEd   | 2.0072      | 1.5653       | 1.4584       |
| LSD (P=0.05)  | 4.1426      | 3.2305       | 3.0100       |
| Seedling length (cm)                                |             |              |              |
| SEd   | 0.8825      | 0.6637       | 0.6259       |
| LSD (P=0.05)  | 1.8213      | 1.3698       | 1.2918       |
| Dry matter production (g seedlings <sup>-10</sup> ) |             |              |              |
| SEd   | 0.0108      | 0.0095       | 0.0092       |
| LSD (P=0.05)  | 0.0223      | 0.0196       | 0.0190       |
| Vigour index  |             |              |              |
| SEd   | 85.3802     | 50.4835      | 44.3503      |
| LSD (P=0.05)  | 176.2165    | 104.1932     | 91.5347      |
| $\alpha$ -amylase (mm)                              |             |              |              |
| SEd   | 0.2697      | 0.2595       | 0.2504       |
| LSD (P=0.05)  | 0.5566      | 0.5355       | 0.5167       |
| Peroxidase (mg g <sup>-1</sup> min <sup>-1</sup> )  |             |              |              |
| SEd   | 0.0050      | 0.0039       | 0.0036       |
| LSD (P=0.05)  | 0.0102      | 0.0080       | 0.0074       |

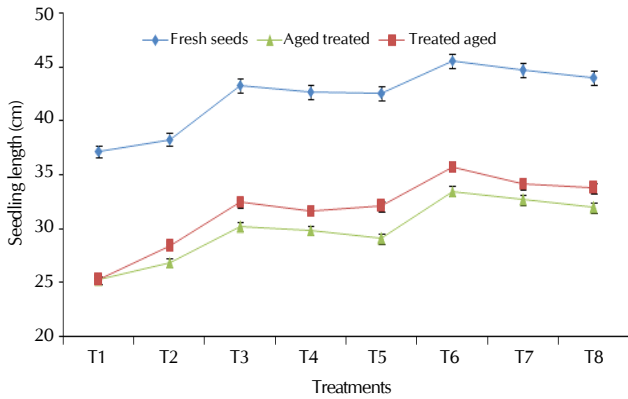


T<sub>1</sub> Control; T<sub>2</sub> Water soaking; T<sub>3</sub> *S. myricocystum* (3 g/kg<sup>3</sup> h); T<sub>4</sub> *G. edulis* (3g/kg<sup>3</sup> h); T<sub>5</sub> *C. racemosa* (3 g/kg<sup>3</sup> h); T<sub>6</sub> *S. myricocystum* (1%<sup>-3</sup> h); T<sub>7</sub> *G. edulis* (1%<sup>-3</sup> h); T<sub>8</sub> *C. racemosa* (1%<sup>-3</sup> h)

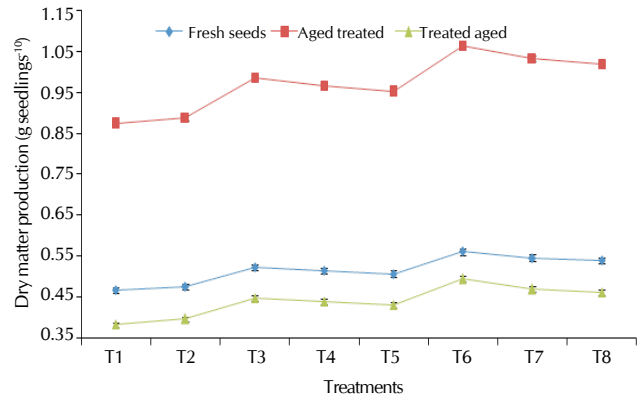
**Figure 1: Effect of organic seaweed nano powders on speed of germination in pigeon pea**



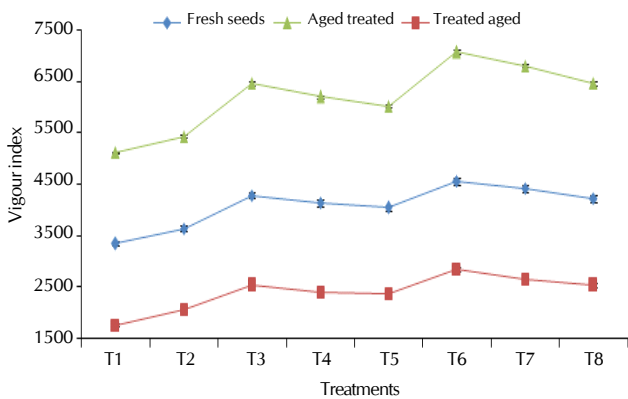
**Figure 2: Effect of organic seaweed nano powders on germination (%) in pigeon pea**



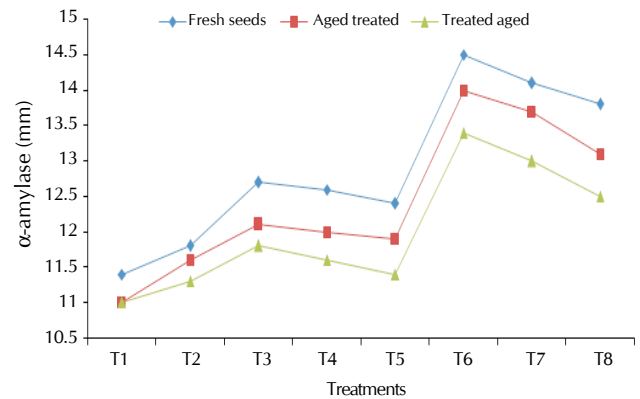
**Figure 3: Effect of organic seaweed nano powders on seedling length (cm) in pigeon pea**



**Figure 4: Effect of organic seaweed nano powders on Dry matter production (g seedlings<sup>-10</sup>) in pigeon pea**



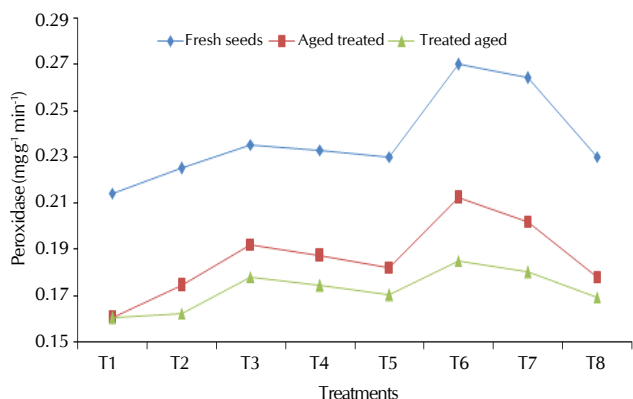
**Figure 5: Effect of organic seaweed nano powders on vigour index in pigeon pea**



**Figure 6: Effect of organic seaweed nano powders on α-amylase (mm) in pigeon pea**

over control (Fig. 4). Vigour index also higher in *S. myricocystum* (1%-3 h) for fresh, treated aged and aged treated seeds. The per cent increase over control was 38 and 30, respectively for treated aged and aged treated (Fig. 5). Among the treatments, water soaking was the only treatment which proved to be detrimental to seeds, in terms of speed of germination, germination, seedling length, dry matter

production as well as vigour index. The results clearly suggested that water soaking was not suitable and also seeds were subjected to *C. racemosa* (3 g/kg-3 h) was found to be detrimental to seed quality. Among the treatments, *S. myricocystum* (1%-3 h) recorded the highest improvement in terms of speed of germination, germination per cent, seedling length and dry matter production in fresh as well as both



**Figure 7: Effect of organic seaweed nano powders on peroxidase ( $\text{mg g}^{-1} \text{min}^{-1}$ ) in pigeon pea**

treated aged and aged treated seeds. It was evident that soaking of seeds with organic seaweed extracts triggers all pre-germination process which persist in the seed even after re-drying leading to quick re-imbibition and rapid revival of germination metabolism. The observations on biochemical parameters made in this study revealed that, *S. myricocystum* (1%-3 h), water soaking, *S. myricocystum* (3 g/kg-3 h), *G. edulis* (3g/kg-3 h), *C. racemosa* (3 g/kg-3 h), *G. edulis* (1%-3 h) and *C. racemosa* (1%-3 h) recorded superiority over control seeds in fresh, treated aged seeds as well as in aged treated seeds. Among the treatments, *S. myricocystum* (1%-3 h) recorded highest  $\alpha$ -amylase (mm) and peroxidase activity ( $\text{mg g}^{-1} \text{min}^{-1}$ ) in all the seeds compared to control and other treatments. With respect to  $\alpha$ -amylase activity also, highest values was recorded by *S. myricocystum* (1%-3 h) in fresh, treated aged and aged treated seeds by recording 21, 21 and 20 per cent increase over control, respectively (Fig. 6). Among the treatments, *S. myricocystum* (1%-3 h) registered highest peroxidase activity of  $0.270 \text{ mg g}^{-1} \text{min}^{-1}$  in fresh seeds, treated aged ( $0.212 \text{ mg g}^{-1} \text{min}^{-1}$ ) and aged treated seeds ( $0.185 \text{ mg g}^{-1} \text{min}^{-1}$ ) (Fig. 7). The data stands evidence for the enhanced metabolic potential of the treated seeds. It reveals that superiority of seed treatment with organic seaweed nano powders is capable of improving the vigour potential of fresh, treated aged and aged treated seeds in pigeon pea.

Faster germination, uniform seedling emergence, increased seedling length and dry matter production in seeds treated with *S. myricocystum* (1%) for 3 h might be due to the induction of above metabolic activities in the seed embryo. Among the treatments, *S. myricocystum* (1%) for 3 h recorded highest improvement in terms of seed germination, speed of germination, dry matter production, seedling length and seedling vigour as well as biochemical parameters in pigeon pea viz.,  $\alpha$ -amylase (mm) and peroxidase activity ( $\text{mg g}^{-1} \text{min}^{-1}$ ). Similar results have been reported by Thirumalai Kannan (2013) in sesame, Suganthi (2014) in sunflower, Paul and Yuvaraj (2014) in *Vigna radiata* and *Pennisetum Glaucums* and Estefania *et al.* (2014) in *Pachyrhizus erosus*. Amylase and peroxidase are important enzymes which have great role in initial growth and development of embryo; and increase in their activity leads to rapid initial growth of the embryo (Abnavi and Ghobadi, 2012).  $\alpha$ -amylase is an important pre-requisite

enzyme in germinating seeds which degrades the complex starch, maltose and release energy in the form of ATP, which is utilized by emerging seedlings (Bewley and Black, 1978). The increase in  $\alpha$ -amylase was recorded in seaweeds treated seeds. The increase in  $\alpha$ -amylase activity might be due to the presence of growth promoting substances like gibberellins. During seed germination, gibberellic acid induces the synthesis and secretion of  $\alpha$ -amylase that break down the starch reserves in the endosperm into simple sugars and the enzyme is largely responsible for hydrolysis in the endosperm (Jacobsen *et al.*, 1995). Similar results were reported by Asir Selin Kumar *et al.* (2005) in paddy; Ramamoorthy *et al.* (2006) in cowpea; Gireesh *et al.* (2011) in cowpea and Kalaivanan and Venkatesalu (2012) in blackgram seeds. The beneficial effect of seaweed might be due to the presence of antioxidants, growth promoting substances (Sylvia *et al.*, 2005) such as IAA, kinetin, zeatin and gibberellins (Zodape *et al.*, 2010) auxins and cytokinins (Zhang and Ervin, 2004); metabolic enhancers (Zhang and Schmidt, 1997); macro and micro elements (Strik *et al.*, 2003). Peroxidase act as hydrolytic enzyme involved in starch degradation. Antioxidant enzymes were increased in treated seeds due to counteraction of lipid peroxidation, reduction of free radical reaction and repair of damage to vital organelles. This antioxidant enzymes activity was low in aged treated seeds compared to treated aged seeds irrespective of the treatments. The antioxidant property of the seaweeds might have slowed down the process of seed deterioration with higher peroxidase enzyme activity. Patra *et al.* (2008) reported that methanol extract of *Sargassum* sp. protecting cells against ROS by act as antioxidant. Results were conformity with Asir Selin Kumar *et al.* (2005) in paddy; Ramamoorthy *et al.* (2006) in cowpea; Kalaivanan and Venkatesalu, (2012) in blackgram; Sujatha *et al.* (2013) in blackgram and Kumareswari and Rani, (2015) in amaranthus. Water soaking leads to initiation of germination process, DNA cloning, RNA activity and consequently protein synthesis, which leads to improvement in seed germination and seedling growth (Bailly *et al.*, 2000 and Ashraf and Foolad, 2005).

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