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Standardization of seed priming technology to enhance seed yield and quality in chickpea

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Abstract
The ever-increasing global population necessitates more food production while dealing with limited land resources and climate change. More oversupply of quality seeds to meet ever-increasing food demand is a difficult task. Low vigour seeds limit crop growth and yield in a variety of field conditions. As a result, an experiment was carried out in order to develop a technique for the rapid and homogeneous growth of BGD-103 chickpea variety seeds. The priming treatments evaluated in the present study enhanced the various seed quality parameters of BGD-103 chickpea significantly when compared to control. The results of the experiment that among all the treatments, KNO_3 (@ 0.3%) solution and drying showed significant performance for Seed quality parameter like germination (97.67%), seedling length (27.67 cm), seedling vigour index (2700), dehydrogenase activity (0.923 OD value), test weight (33.87 gm) and protein content (20.72%) compared to control and lowest was observed was electrical conductivity (0.854) and moisture content (9.21%) compare to control. Seed pre-sowing seed treatment is a simple method for overcoming problems associated with poor germination and seedling establishment and it aids in the sustainability of agriculture by using cost-effective, non-toxic and eco-friendly sources.

Keywords: BGD-103 chickpea variety, priming, seed quality enhancement

Introduction
Chickpea (*Cicer arietinum* L.) is one of the most important grain legumes as it ranks third in the world after dry bean and field pea. Two diverse market types *viz*. desi and kabuli prevail in chickpea (Pundir *et al.,* 1985) [26]. Bengal gram is another name for it. It is a cool-season legume crop that is grown as a nutritional crop in many countries around the world. Bengal gramme is another name for it. It is a cool-season legume crop that is grown as a nutritional crop in many countries around the world. Chickpeas are high in protein (22-28%), fat (4.8-5.5%), carbohydrates (40-65%), ash (48%), moisture (4.9-15.59), and essential vitamins such as vitamin A, B1, B2, and B3, vitamin C, vitamin D, vitamin E, vitamin K, Folate, and Pantothemic acid (Zohary and Hopf, 2000) [33]. Chickpeas also contain a variety of minerals, including calcium, iron, molybdenum, potassium, manganese, copper, and zinc. Chickpeas also contain dietary fibres, water, and other nutrients. Chickpea yields are low (700-800 kg ha^{-1}) and have been relatively stable for the last two decades. Chickpea yield potential is hampered by a variety of biotic and abiotic factors. The decline in chickpea production and productivity in India can be attributed to Abstract The ever-increasing global population necessitates more food production under limited land resources, which is exacerbated by climate change, resulting in (i) a shift in crop area from favorable to marginal environments; (ii) a slow varietal replacement rate and other production technologies; and (iii) chickpea cultivation in poor soils under inconsistent rainfall conditions (Parthasarathy *et al.,* 2010) [24].
China has the highest productivity of 3759 kg/ha, followed by Israel, the Republic of Moldova, and Bosnia and Herzegovina. The productivity in India was 995 kg/ha. (FAO Stat. 2021). Chickpea (*Cicer arietinum* L.) is known by various names in different countries, including gram, chana, bengal gram, kadle and so on. Chickpea is a major Rabi season legume with a wide geographical distribution. After beans and peas, chickpeas are the world's third most important pulse crop. It is grown on an area of 12 million hectares and produces 8.9 million tonnes per year. Chickpea contributes significantly to soil fertility by fixing atmospheric nitrogen via root nodules (Anabessa *et al.,* 2009) [2]. The chickpea is indigenous to Turkey and Syria.
The aforementioned issues pose a serious threat to the cultivation of chickpea varieties. As a result, it is necessary to develop appropriate technology to improve seed viability and vigour.

Seed priming is one of the potential techniques for improving seed and seedling performance, beginning with uniform and faster seed germination, early seedling establishment, better final stand establishment that allows the initial germination process, and limiting the final phase of seed germination, *i.e.*, radicle emergence. Heydecker coined the term priming in 1973 to describe the soaking drying seed treatments. Theophrastus (372-287 BC) proposed soaking cucumber seeds in milk or water to help them germinate faster and more vigorously (Michael Evanari, 1984) [20]. Heydecker (1973) [15] successfully used seed priming to improve germination and seedling emergence under a variety of stress conditions. The seed priming treatments activate metabolic processes that are activated during phase II of germination and are then temporarily stopped before desiccation loss occurs. Seed priming promotes the repair of cell organelles, the metabolism required for germination, and various antioxidant activities within the seed (Paparella *et al.*, 2015) [23].

Hydro-priming is a very traditional pre-sowing seed treatment that involves soaking seeds in tap water for an appropriate amount of time and then dehydrating them for further sowing. In hydro-priming, the seed absorbs enough water to start the germination process while avoiding radicle and plumule protrusion. Seeds are hydrated for a set period of time, which causes metabolic changes within the seed, but dehydration after priming prevents germination from completing. This seed absorbs moisture from the substrate during sowing, resulting in rapid and synchronised emergence. Hydro-priming reduces the magnitude of germination failure while also increasing the vigour of weak seeds. This traditional method significantly increases the activity of hydrolytic enzymes, which breaks down food material in the endosperm and delivers energy to the living embryo for further growth. It involves imbibitions of seeds in water under controlled conditions up to the point of radical emergence followed by drying the seed back to the initial moisture content of the seeds. The different methods of seed priming such as hydro priming and halo priming can be applied as pre-sowing treatments under various conditions. Although priming improves germination and seedling early growth predominantly under undesirable conditions.

Several researchers reported that seed priming treatments improved seed vigour and viability in a variety of environments. Seed priming with organic and inorganic substituents, bioagents and phytohormones has been found to be superior for improving seed performance. Umesha *et al.* (2014) [31]. The overall result of seed priming is increased seed vigour, which is defined as the entire set of properties that condition seed lot performance in a wide range of environments (Zhang *et al.*, 2015) [32]. There have been few studies on the development of seed priming technology in kabuli chickpea. Keeping this in mind, the current study sought to identify suitable priming treatments for fresh and aged seeds in order to improve seed viability, vigour, and seed quality.

Materials and Methods

The experiment was conducted at Seed Unit, University of Agricultural Sciences, Raichur, during the year 2020 and 2021. The variety taken namely were BGD-103, this variety belong to the desi. Treatments details like T_1 Control (untreated), T_2 Thiram (2 g/kg), T_3 Hydropriming – Soaking in water for 6h (at 20 °C) and air-drying at 25 °C for 72 hr, T_4 Seed coating (on hydro primed seeds) with BioNPK, T_5 Seed coating (on hydro primed seeds)) with Drought Alleviating Bacteria + BioNPK, T_6 Seed coating (on hydro primed seeds) with *T. harzianum* (15 g in 50 ml of water and applied on 1 kg of seed uniformly. Later seeds were shade dried for 20 – 30 minutes before sowing), T_7 Halopriming- Soaking in KNO_3 (@ 0.3%) solution and drying, T_8 Halopriming- Soaking in $ZnSO_4$ (@ 0.3%) solution and drying and T_9 Halopriming-Soaking in KH_2PO_4 (@0.5%) solution and drying.

Standard germination (%) was determined by 'between paper' method (BP) as described by International Seed Testing Association (ISTA) rules (ISTA, 1999) [16]. The first and final germination counts were recorded on 4th and 14th day of germination test, respectively. On eighth day of germination test, ten normal seedlings were taken random from four replications of each treatment and measured from the tip of primary root to the tip of apical shoot. The average of ten seedlings from each replication was calculated and expressed as mean seedling length in centimetres. Based on the above parameters, the seedling vigour indices [Seedling vigour index I (SVI-I) = Germination (%) x Seedling length (cm) (Abdul-Baki and Anderson, 1973) [1] were determined and expressed as whole number.

Electrical conductivity was measured according to procedure described by Presley, 1958. Four replications of five grams of seeds from each treatment were taken. Then the seeds were soaked in 25 ml of distilled water and incubated at constant temperature of 25±1 °C for 24hours. The electrical conductivity of the seed leachate was measured by the digital conductivity meter and expressed in dS cm^{-1}.

Dehydrogenase enzyme activity was estimated according to Kittock and Law. Ten seeds from each treatment were soaked in water for 16 hours. After removing the testa, the seeds then dipped in 0.5% solution of tetrazolium for 3 hours at room temperature (25±2 °C). Then the seeds were dipped in 10 ml of methyl cellosolve for 2 hours. After removing the seeds from methyl cellosolve the optical density of solution were measured at 480 nm wave length with the help of spectrophotometer. The data was expressed as OD/10 seeds.

Protein content was estimated by micro-Kjeldhal method of nitrogen estimation (Miller and Houghton, 1945) [21]. Nitrogen is one of the major elements found in living organism being an essential constituent of amino acids, protein, nucleic acid, amides, vitamins, coenzymes, hormones etc. In most proteins, nitrogen constitutes nearly 16 percent of the total composition and hence, the total nitrogen content of the sample is multiplied by 6.25 to calculate the protein content. The sample was digested with concentrated H_2SO_4 in the presence of a catalyst to convert the nitrogen in protein or any other organic material to ammonium sulphate. By steam distillation of this salt in the presence of a strong alkali, ammonia is liberated and collected in boric acid solution as ammonium borate which was estimated against a standard acid by titration. On an average most proteins have 16 per cent nitrogen in their composition i.e., 1 mg nitrogen equals 6.25 mg protein. Thus, by finding out the amount of ammonia formed from a known amount of sample, the amount of protein present in the sample was calculated. a) Preparation of reagents weight about 0.2 g of finely powdered homogenate sample into the digestion flask. b) Add a pinch of digestion mixture (0.1 g K_2SO_4 and 10 mg Hgo) and 10 ml of concentrated H_2SO_4 and mix well. Keep the sample overnight for pre-digestion c) Add 10 ml of water along the sides of the

digestion tube for the reaction to stop and transfer to the dictation apparatus with successive rinsing water. d) Place a 100 ml conical flask containing 20 ml of boric acid solution with a few drops of mixed indicator in such a way that the tip of the condenser dipping inside the solution. e) Distil and collect the ammonia in boric acid. The colour change from pink to green is an indication of ammonia absorbed. f) Collect the distillate and titrate against the standard HCl or H_2SO_4 (0.1 N) until the appearance of original pink colour as the end point. The nitrogen and protein content of the sample were obtained by the following equations,

Percent N content (%) = Titre value x Normality of acid x 0.014 x 100/Weight of sample

Percent protein content = Percent N content × 6.25

The moisture content of seeds was determined by using low constant temperature oven method Five grams of seed material was taken from each of the treatments in three replications for moisture content analysis. The moisture content of the seed sample was determined by quantitative / gravimetric method by using low constant temperature oven method (103 ± 1 °C for 17 ±1 h) as per ISTA rules (Anon., 2014) and was expressed in percentage on weight basis by using the following formula.

$$\text{Moisture content (\%)} = \frac{(M_2 - M_3)}{(M_2 - M_1)} \times 100$$

Where, M_1: Weight of the metal dish and its lid (g), M_2: Weight of the metal dish, its lid and seed before drying (g) and M_3: Weight of the metal dish, its lid and seed after drying (g).

Results and Discussions

Various priming treatments improved germination and other seed quality parameters of a chickpea variety in the laboratory between 2021 and 2022. When primed with T_7 - Halopriming - Soaking in KNO3 (@ 0.3%) solution followed by Seed coating (on hydro primed seeds) with Drought Alleviating Bacteria + Bio NPK showed significant increase in seed quality parameters on pooled basis data compared to control of both years except electrical conductivity and moisture content were found to be significantly lower than control. Chickpea seeds treated with Seed coating with T_7 - Halopriming - Soaking in KNO_3 (@ 0.3%) solution and drying pool data was recorded significantly higher germination percentage (97.67%), seedling length (27.64 cm), seedling vigour index (2700), dehydrogenase activity (0.923 OD value) and Protein content (20.72%) followed by T_5 Seed coating (on hydro primed seeds) with Drought Alleviating Bacteria + BioNPK has percentage (97.22%), seedling length (27.21 cm), seedling vigour index (2674), dehydrogenase activity (0.910 OD value) and Protein content (20.68%) compared to T_1 -control (Germination percentage (93.61%), seedling length (26.24 cm), seedling vigour index (2457), dehydrogenase activity (0.855 OD value) and Protein content (19.07%). The lowest was observed was electrical conductivity (0.854) and moisture content (9.21%) was observed in seed coating with T_7 -Halopriming - Soaking in KNO_3 (@ 0.3%) solution and drying compare to T_1 -control (Table 1 and Table 2) and (Fig. 1 and Fig. 2).

The process of hydration that takes place during seed priming makes it possible for the earliest physiological stages of germination to reach their conclusion. This, along with the possibility of the physiological repair of membranes and organelles that were damaged during seed storage, ultimately results in a more rapid and uniform emergence of the seedlings (Copeland and Mc Donald, 1995) [9]. Priming causes an increase in germination, which may be associated with a change in the biosynthesis and signaling of plant hormones. It is possible that this change is associated with the priming-induced increase. Priming has been shown to increase the ratio of gibberellins (GA) to abscisic acid (ABA) (El-Arab *et al*., 2006) [10], and this may be a direct consequence of the priming impact on the pattern of gene expression. In addition, priming has been shown to decrease the ratio of abscisic acid (ABA) to gibberellins (GA) (El-Arab *et al.*, 2006 [10] (Schwember *et al.*, 2010) [27]. In addition, ethylene has a direct influence on both the rate of germination and the percentage of seeds that actually germinate. An increase in the production of ethylene during priming may promote endomannase activity, which in turn facilitates endosperm weakening and germination that takes place after priming (Chen and Arora, 2013) [7]. Hydropriming was described as a straightforward and low-cost approach to seed priming in Fujikura *et al* (1993) [13] article. The characteristics of primed seeds include an increased germination rate, improved germination uniformity, and in some cases a higher seed germination percentage (Basra *et al.*, 2005) [4]. Primed seeds also have improved germination rates even when the environmental conditions are unfavourable (Lin and Sung, 2001) [19]. Improved germination rate and uniformity have been attributed to metabolic repair during the first phase of seed germination, which is called imbibition (Bray *et al*., 1989) [34], accumulation of germination augmenting metabolites (Basra *et al.*, 2005) [4], and osmotic modification (Bradford, 1986) [5]. Additionally, for seeds that are not subjected to drying back to their original moisture content after treatment, a simple reduction in imbibition lag time has been (Bradford, 1986) [5].

Priming treatments can have a beneficial effect in uniform germination, which can lead to an increase in seedling length. This effect can be attributed to an intensified hydrolytic process and better water uptake by the seedlings. According to Nascimento *et al.* (2004) [22], priming increased the germination of seed with low vigour, and the researchers found that the response varied depending on the cultivar. Canola seeds with high and low vigour were subjected to hydration (with distilled water) and pre-osmotic treatments (with -1.5MPa of osmotic potential in manitol solution for four periods of treatment lasting 12, 24, 48, and 72 hours) at a temperature of 10 degrees Celsius. The researchers evaluated the effects of these treatments on the quality of the canola seeds. They came to the conclusion that pre-osmotic conditioning with manitol solution was not an effective method for increasing the germination rate of rape seeds or their overall vigour. However, hydro-priming was an appropriate priming method for canola seeds, and it was successful with the low vigour lot. According to Christos *et al.* (2019) [8] findings, hydropriming faba bean seeds resulted in the production of seedlings with a higher vigour index than did not priming the seeds.

It is possible for the quality of the seed, and more specifically

its viability and vigour, to have a significant influence on the establishment of a crop as well as its yield. Even in unfavourable conditions, healthy plants that have well-developed root systems have a greater chance of surviving, and research has linked the early establishment of vigorous seedlings with higher crop yields (Harris *et al.*, 2000) [14]. The findings of this study are in agreement with those of Shantha Nagarajan and Panditha (2001) [29], who also observed that osmopriming of tomato seeds leads to increased germination and seedling dry weight of aged seeds to a greater extent than hydropriming does; however, hydropriming of tomato seeds leads to increased germination and seedling dry weight of aged seeds to a lesser extent. Additionally, Surekha (2002) [30] reported that the hydration dehydration treatment had a favourable effect on the seed quality of onion cultivars. In order to achieve better plant establishment, plant growth, and ultimately a higher yield, it is necessary to have vigorous seedling growth, which can be observed by observing a greater seedling length and seedling dry weight. In their study, Shaila *et al.* (2019) came to the conclusion that hydropriming bitter gourd seeds improved seed germination, sped up seedling growth by increasing shoot length, and ultimately increased yield.

Casenave and Toselli (2007) demonstrated that hydropriming increased the germination significantly compared to control seeds, decreased the thermal time required for radicles to emerge, and improved seed vigour in cotton. Additionally, they found that hydropriming reduced the thermal time required for radicles to emerge. Hydropriming is a form of seed priming that is extremely uncomplicated, cost-effective, and friendly to the environment (Jamil *et al.*, 2016). Hydropriming has been shown in multiple studies to improve germination rates in a variety of crop species, leading to higher values for germination, root growth, shoot growth, and seedling vigour index. According to Shaila *et al.* (2019) [28], seed germination, seedling growth, and other growth parameters were all improved when bitter gourd seeds were hydroprimed. When compared with non-primed seeds, Christos *et al.* (2019) [8] discovered that the hydro-priming of faba bean seeds increased the germination speed by 16.2%, the germination synchrony by 20.7%, and the seedling vigour index by 13.4%. However, the hydro-priming had no significant impact on the final germination percentage or the mean daily germination rate. According to the findings of our research, seedlings grown from primed seeds emerge more quickly and have greater vigour than those grown from non-primed seeds. This is demonstrated by higher values of speed of emergence and seedling vigour indices in hydroprimed seeds than in non-primed seeds. Increased seed vigour was observed in this experiment due to seed priming, which was consistent with the findings of Shaila *et al.* (2019) [28] in bittergourd. Higher seedling growth parameters were observed as a result of the priming process.

Table 1: Effect of seed priming on germination, total seedling length, seedling vigor index and electrical conductivity in Chickpea

Treatments details	Germination (%)			Total Seedling Length (Cm)			SVI - I			EC (dSm-1)		
	2020	2021	Pool	2020	2021	Pool	2020	2021	Pool	2020	2021	Pool
T_1	93.11	94.11	93.61	25.87	26.61	26.24	2409	2505	2457	1.031	1.112	1.072
T_2	94.56	94.67	94.61	26.07	26.81	26.44	2465	2538	2502	0.909	0.863	0.886
T_3	95.67	95.33	95.50	26.53	27.28	26.91	2538	2601	2570	0.897	0.937	0.917
T_4	95.00	96.00	95.50	26.30	27.41	26.86	2499	2632	2565	0.903	0.943	0.923
T_5	97.11	97.33	97.22	27.27	27.75	27.51	2648	2700	2674	0.846	0.856	0.851
T_6	96.00	95.67	95.83	26.67	27.30	26.98	2560	2612	2586	0.899	0.939	0.919
T_7	96.67	98.67	97.67	27.00	28.28	27.64	2610	2790	2700	0.862	0.845	0.854
T_8	96.24	97.28	96.76	26.73	27.54	27.14	2573	2679	2626	0.891	0.874	0.883
T_9	94.78	94.78	94.78	26.55	27.05	26.80	2517	2563	2540	0.928	0.969	0.949
S.Em.±	0.75	0.69	0.65	0.20	0.20	0.17	28.45	23.08	22.54	0.022	0.019	0.013
C.D @ 1%	3.05	2.80	2.63	0.80	0.83	0.70	115.80	93.95	91.74	0.088	0.076	0.052

Legend

T_1 Control (untreated)
T_2 Thiram (2 g/kg)
T_3 Hydropriming – Soaking in water for 6h (at 20 °C) and air-drying at 25 °C for 72 hr
T_4 Seed coating (on hydro primed seeds) with BioNPK
T_5 Seed coating (on hydro primed seeds)) with Drought Alleviating Bacteria + BioNPK
T_6 Seed coating (on hydro primed seeds) with *T. harzianum* (15 g in 50 ml of water and applied on 1 kg of seed uniformly. Later seeds were shade dried for 20 – 30 minutes before sowing)
T_7 Halo priming- Soaking in KNO_3 (@ 0.3%) solution and drying,
T_8 Halo priming- Soaking in $ZnSO_4$ (@ 0.3%) solution and drying
T_9 Halo priming- Soaking in KH_2PO_4 (@ 0.5%) solution and drying.

Table 2: Effect of seed priming on OD value, protein content and moisture content in Chickpea

Treatments details	OD Value			Protein Content (%)			Moisture Content (%)		
	2020	2021	Pool	2020	2021	Pool	2020	2021	Pool
T_1	0.858	0.852	0.855	19.10	19.04	19.07	9.36	9.57	9.47
T_2	0.870	0.880	0.875	19.25	19.46	19.36	9.51	9.67	9.59
T_3	0.872	0.887	0.880	19.62	19.99	19.80	9.93	9.88	9.90
T_4	0.880	0.871	0.875	20.28	20.64	20.46	9.39	9.60	9.50
T_5	0.926	0.894	0.910	20.62	20.75	20.68	9.10	2.90	9.22
T_6	0.883	0.893	0.888	20.25	20.61	20.43	9.43	9.48	9.46
T_7	0.903	0.942	0.923	20.45	20.98	20.72	9.25	9.17	9.21
T_8	0.888	0.893	0.890	20.36	20.73	20.55	9.31	9.56	9.43

T_9	0.875	0.882	0.879	20.34	20.71	20.53	9.40	9.51	9.46
S.Em.±	0.011	0.010	0.008	0.26	0.32	0.27	0.11	0.10	0.064
C.D @ 1%	0.044	0.042	0.034	1.06	1.31	1.10	0.46	0.41	0.262

Legend

T_1 Control (untreated)
T_2 Thiram (2 g/kg)
T_3 Hydropriming – Soaking in water for 6h (at 20 °C) and air-drying at 25 °C for 72 hr
T_4 Seed coating (on hydro primed seeds) with BioNPK
T_5 Seed coating (on hydro primed seeds)) with Drought Alleviating Bacteria + BioNPK
T_6 Seed coating (on hydro primed seeds) with *T. harzianum* (15g in 50 ml of water and applied on 1 kg of seed uniformly. Later seeds were shade dried for 20 – 30 minutes before sowing)
T_7 Halo priming- Soaking in KNO_3 (@ 0.3%) solution and drying,
T_8 Halo priming- Soaking in $ZnSO_4$ (@ 0.3%) solution and drying
T_9 Halo priming- Soaking in KH_2PO_4 (@ 0.5%) solution and dry

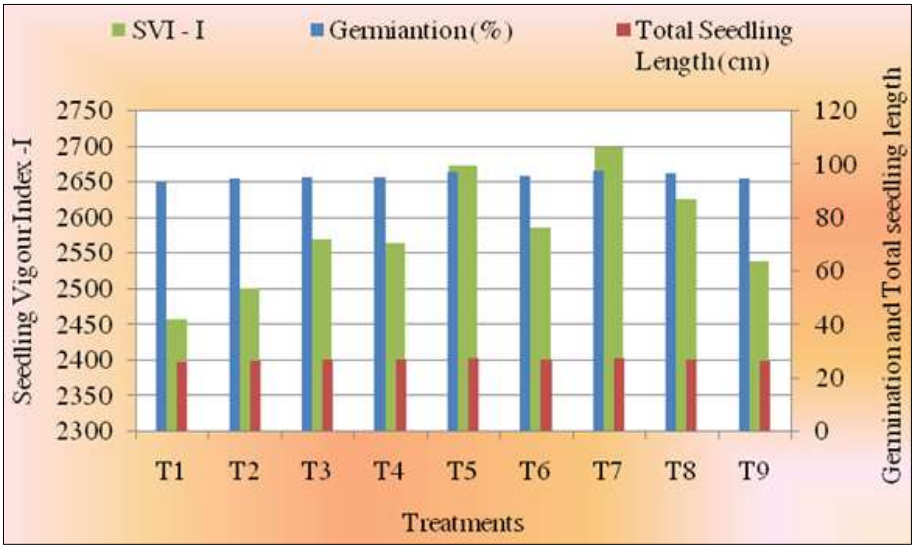

Fig 1: Effect of seed priming on germination, total seedling length and seedling vigor index in chickpea

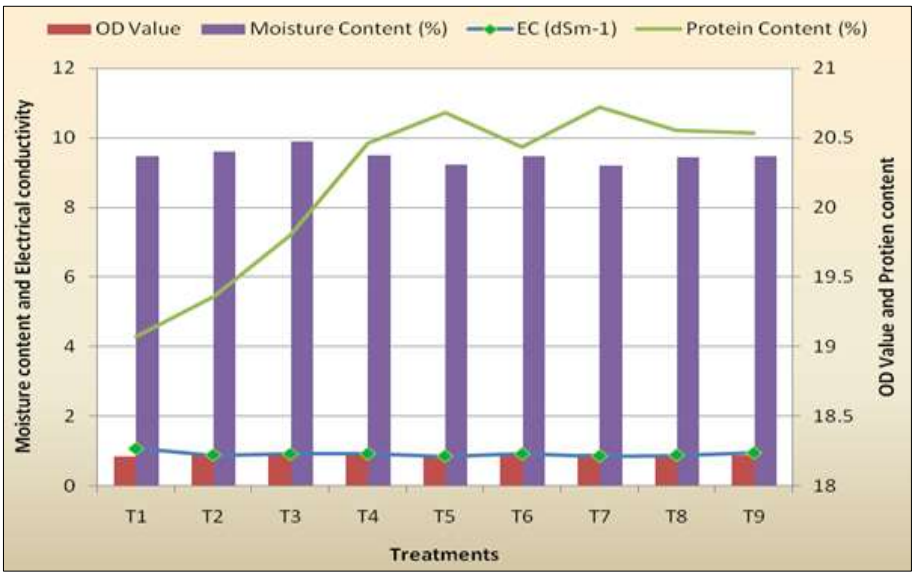

Fig 2: Effect of seed priming on OD value, protein content, moisture content and electrical conductivity in chickpea

Conclusion

It is concluded from the results of the experiment that among all the treatments, KNO_3 (@ 0.3%) solution and drying showed significant performance for Seed quality parameter like Germination (97.67%), seedling length (27.67cm), seedling vigour index (2700), dehydrogenase activity (0.923 OD value), Test weight (33.87 gm) and Protein content (20.72%) compared to control and lowest was observed was electrical conductivity (0.854) and Moisture content (9.21%) compare to control.

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