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Original Research Article

Enhancement of Seed Quality of Black Cumin (*Nigella sativa* L.) through Seed Priming

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Keywords

Black cumin, hydropriming, osmopriming, GA_3, KNO_3

A B S T R A C T

Black cumin (*Nigella sativa* L.) is a multipurpose *rabi* crop cultivated for its seeds. The present investigation was carried out at Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi in the year 2011-12 aiming to investigate the effects of hydropriming and osmopriming on germination, seedling growth and vigour index I and II of three seed lots of Black cumin (*Nigella sativa* L.). Based on imbibitional curve studies, hydropriming and osmopriming with GA_3 (500 ppm) and KNO_3 (0.2%) was given on 3 seed lots of NRCSS AN 1 variety. The results showed that priming significantly enhanced different seed quality parameters in one seed lot having lower germination. All three priming treatments significantly enhanced germination and vigour of poor quality lot (Lot 2). Mean germination % of seed lot 2 was significantly enhanced from 80.00% to 90.00%, 89.13% and 88.69% by hydro, GA_3 and KNO_3 priming treatments respectively.

Introduction

Nigella L. (Family *Ranunculacece*) includes about 20 species among which most commonly cultivated species are *Nigella sativa*, *Nigella damascene* and *Nigella arvensis* (Hegnauer 1973; Bown 2002). *Nigella sativa* L. (Black seed/black cumin) is emerging as a miracle herb with a rich historical and religious background and several researches revealed its wide spectrum of pharmacological potential. The seeds of *N. sativa* and their oil have been widely used for centuries in the treatment of various ailments throughout the world. It is also used as an important drug in the Indian traditional system of medicine like Unani and Ayurveda. Black seed has identified as a valuable source of edible oil (Piras *et al.*, 2013) with 24.8–29.2% saturated and 69.7–73.5% unsaturated fatty acids (Atta, 2003).

The physiological potential of black cumin seeds is adversely affected by several stresses (low temperature, high moisture and aphid attack) due to which rapid and

uniform field emergence of seed doesn't occur. The enhancement of seed vigour is generally done through seed pre-treatment technique which involves uptake of water by the seed to initiate the early events of germination up to the point where radicle emergence is not allowed followed by drying (Mc Donald, 2000). Rapid germination and field emergence is an important determinant of successful stand establishment (Heydecker *et al.,* 1973, 1977). Harris *et al.,* (1999) reported seed priming as one of the most important developments to help rapid and uniform germination and emergence of seeds and to increase seed tolerance to adverse environmental conditions.

Several researchers have studied the effect of seed treatment in different crops viz; in tomato by Earlpuls and Lambeth (1974), cotton by Sharma *et al.,* (1984), chilli by Vishwanath *et al.,* (2006), maize by Rehman *et al.,* (2014), balck cumin by Ahmadin *et al.,* (2014) etc. Vyakaranahal *et al.,* (1998) suggested the technique of hydration-dehydration to improve germinability and vigour of 8 months old sunflower seeds. The major aim of the present study was to determine effect of various priming treatments in Indian *Nigella sativa* cultivar with the possibilities to improve its productivity.

Materials and Methods

Three seed lots (Lot 1, Lot 2 and Lot 3) of *Nigella sativa* (Var. NRCSS AN-1) were used for the experiment. Seed Lot 1 and 2 were harvested in year 2010 and Lot 3 was harvested in year 2011 from experimental plot of Division of Seed Science and Technology, Indian Agricultural Research Institute, New Delhi. Prior to priming studies, seed quality was measured by assessing seed germination at four different incubation temperature (15^0, 20^0, 25^0 and 30^0C) by Between Paper (BP) as well as Top of Paper (TP) methods, in order to find out the optimum temperature for germination, germination time (first count and final count) and to find out the requirement of dormancy break down, if any. This study revealed that no significant difference in germination was observed between 20°C and 25°C of incubation temperature. First and final counts were taken after 7 and 14 days respectively. Germination in seed lot 1 and 3 was significantly high i.e. more than 90 %, compared to lot 2 where minimum germination (80%) was noticed. No germination was observed at 15°C and thermo inhibition of germination was observed at 30°C. In the subsequent germination studies (after hydropriming and osmopriming of 3 seed lots), BP method at 25°C was selected so that seedling measurement is easier and accurate (compared to TP).

Before selecting priming treatments imbibitional curve of *Nigella sativa* was constructed by soaking 100 seeds (in 3 replicates of 3 seed lots) at 20°C in water and weighed at an interval of 1 hr so that we could know the duration of imbibition in *Nigella sativa*.All the experiments were carried out in triplicate. There was an increase in seed weight due to water uptake up to 4-5 hours and after this no further increase in weight was observed. On the basis of this study 1 hr, 2 hrs, 3 hrs, 4 hrs and 5 hrs priming treatments were imposed by soaking seeds at 20°C. After imposing priming treatments primed seeds were dried at room temperature for 3-4 days when moisture content of seeds reached to initial moisture content of the seed lot.

Seed enhancement studies were conducted by giving two types of priming treatments, hydro-priming and osmopriming [500 ppm

gibberrelic acid (GA_3) and 0.2 % KNO_3] for suitable time period based on imbibitional curve at 20°C on three different seed lots (Lot 1, Lot 2 and Lot 3). After imposing priming treatments primed seeds were dried at room temperature for 3-4 days till moisture content of seeds were reached to initial moisture content of the seed lot. After priming treatments seeds were evaluated for germination percentage, Seedling length and Vigour index I & II.

Results and Discussion

Various priming treatments has been found to enhance the seed quality viz. germination and vigour especially under slightly unfavourable or stress environment (Varier *et al.,* 2010). Priming of seed results in uniform and synchronizes germination which gives us a uniform and desired plant stand which enhances the production and productivity of the crop in a unit area.

Effect of priming treatments on germination

Germination is one of the most important seed quality parameters which ensure good crop stand. Hydropriming in 3 seed lots of *Nigella sativa* having different germination percentage and vigour which reveals that it enhanced germination and vigour in one lot where initial germination was low (80.00 %), compared to two seed lots where germination was high.

Perusal of data in Fig. 1 reveals that maximum mean germination of 93.46 % was observed in seed lot 1 followed by seed lot 3 (92.92 %) and minimum mean germination was observed in seed lot 2. As regards to different hydropriming treatments, significant difference in mean germination % was observed in different treatments as compared to control. Maximum mean germination (93.67 %) was recorded in 4 hrs hydropriming treatments. In seed lot 1 and 3 germination in control was 93.25 and 92.50 % respectively and it was not significantly enhanced due to priming treatments. Gibberrelic acid (GA_3) has been known to promote germination in a great variety of species and is play important role in controlling the germination in nature. In present study, GA_3 enhanced mean germination percentage of lot 2 from 80.00% to 89.13 % significantly. Fig. 2 shows that mean germination percentage of lot 1 and lot 3 were at par with each other and there was no significant increase in mean germination percentage from control. There was significant increase in mean germination percentage in different priming treatment hours over control; however, there was no significant difference between different priming periods. The observed improvements in germination percent of primed seed may be attributed to priming that induces quantitative changes in biochemical content of the seed and improves membrane integrity and enhances physiological activities at seed germination (Sung & Chang, 1993). The beneficial effects of priming have also been demonstrated for many field crops such as wheat, sugar beet, maize, soybean and sunflower (Parera & Cantliffe, 1994; Singh, 1995; Sadeghian & Yavari, 2004).

Potassium Nitrate (KNO_3) is the most widely used chemical for promoting seed germination (Mc Donald, 2000). Seeds which are sensitive to GA_3 also sensitive to KNO_3.

Simple inorganic forms of nitrogen such as nitrate, nitrite and ammonium are potent promoters of germination, but the biochemical mechanism underlying this phenomenon remains unclear despite decade of research (Bethke *et al.*, 2007).

Table.1 Effect of three different priming treatments (hydro, GA_3 and KNO_3) on germination % of three different seed lots of *Nigella Sativa*

Seed Lots	Control	Hydropriming					GA_3 (500 ppm)			KNO_3 (0.2%)			Mean A
		H1	H2	H3	H4	H5	G1	G2	G3	K1	K2	K3	
L1	93.25	93.50	93.50	93.50	93.75	93.25	93.50	93.75	94.25	93.5	93.75	94.00	93.63
	(75.00)	(75.25)	(75.25)	(75.25)	(75.52)	(75.05)	75.41)	(75.66)	(76.20)	(75.53)	(75.60)	(75.81)	(75.46)
L2	80.00	88.25	91.25	93.25	93.75	93.50	88.50	93.00	95.00	88.25	92.50	94.00	90.94
	(63.43)	(69.95)	(72.82)	(75.00)	(75.54)	(75.47)	70.17)	(74.78)	(77.21)	(69.93)	(74.12)	(75.86)	(72.86)
L3	92.50	92.75	93.00	93.25	93.50	93.50	92.75	93.00	94.00	92.25	93.25	93.50	93.02
	(74.12)	(74.38)	(74.72)	(74.96)	(75.49)	(74.33)	74.67)	(74.96)	(75.99)	(73.98)	(75.04)	(75.25)	(74.82)
Mean B	88.58	91.50	95.58	93.33	93.67	93.08	91.58	93.25	94.42	91.33	93.17	93.83	
	(70.85)	(73.19)	(74.27)	(75.07)	(75.52)	(74.95)	73.42)	(75.13)	(76.47)	(73.15)	(74.92)	(75.64)	
Factors	L	PT	L*PT										
C.D.	0.93	1.85	3.21										

Table.2 Effect of three different priming treatments (hydro, GA_3 and KNO_3) on seedling length (cm) of three different seed lots of *Nigella Sativa*

Seed Lots	Control	Hydropriming					GA_3 (500 ppm)			KNO_3 (0.2%)			Mean A
		H1	H2	H3	H4	H5	G1	G2	G3	K1	K2	K3	
L1	7.12	7.18	7.35	7.43	7.51	7.49	7.28	7.37	7.53	7.25	7.35	7.41	7.36
L2	6.87	7.19	7.47	8.18	8.27	8.31	7.25	7.64	8.36	6.91	7.14	8.02	7.63
L3	7.59	7.59	7.69	7.74	7.84	7.81	7.66	7.79	7.86	7.69	7.73	7.83	7.73
Mean B	7.19	7.32	7.51	7.78	7.87	7.87	7.40	7.60	7.92	7.28	7.41	7.75	
Factors	L	PT	L*PT										
C.D.	0.196	0.391	NS										

Table.3 Effect of three different priming treatments (hydro, GA_3 and KNO_3) on Vigour Index I of three different seed lots of *Nigella Sativa*

Seed Lots	Control	Hydropriming					GA_3 (500 ppm)			KNO_3 (0.2%)			Mean A
		H1	H2	H3	H4	H5	G1	G2	G3	K1	K2	K3	
L1	663.59	671.31	687.36	693.85	703.88	697.92	680.62	690.63	709.47	677.60	689.69	696.35	688.52
L2	548.94	634.00	681.85	762.77	775.33	777.66	641.31	710.22	795.38	609.30	659.67	753.84	695.85
L3	702.26	703.52	715.32	721.65	731.88	721.08	708.81	723.90	738.85	708.86	720.72	731.63	719.04
Mean B	638.26	669.61	694.84	726.09	737.03	732.22	676.91	708.25	747.90	665.25	690.03	727.27	
Factors	L	PT	L*PT										
C.D.	18.27	36.54	63.29										

Table.4 Effect of three different priming treatments (hydro, GA_3 and KNO_3) on Vigour Index II of three different seed lots of *Nigella Sativa*

Seed Lots	Control	Hydropriming					GA_3 (500 ppm)			KNO_3 (0.2%)			Mean A
		H1	H2	H3	H4	H5	G1	G2	G3	K1	K2	K3	
L1	0.65	0.64	0.64	0.66	0.66	0.65	0.72	0.72	0.73	0.74	0.76	0.80	0.70
L2	0.43	0.54	0.58	0.67	0.68	0.64	0.60	0.66	0.70	0.59	0.71	0.75	0.63
L3	0.58	0.61	0.64	0.66	0.70	0.68	0.72	0.77	0.79	0.74	0.76	0.78	0.70
Mean B	0.55	0.60	0.62	0.66	0.68	0.65	0.68	0.72	0.74	0.69	0.74	0.78	
Factors	L	PT	L*PT										
C.D.	0.02	0.04	0.07										

Control (No priming treatment); H1, H2, H3, H4 and H5 (Hydropriming treatments for 1, 2, 3, 4 and 5 hours respectively); G1, G2 and G3 (GA_3 treatments for 1, 2 and 3 hours respectively); K1, K2 and K3 ($KMNO_4$ treatments for 1, 2 and 3 hours respectively); PT (Priming treatment); NS (Not significant)

Fig.1 Effect of hydropriming treatments on germination (%) of three seed lots of *Nigella sativa*

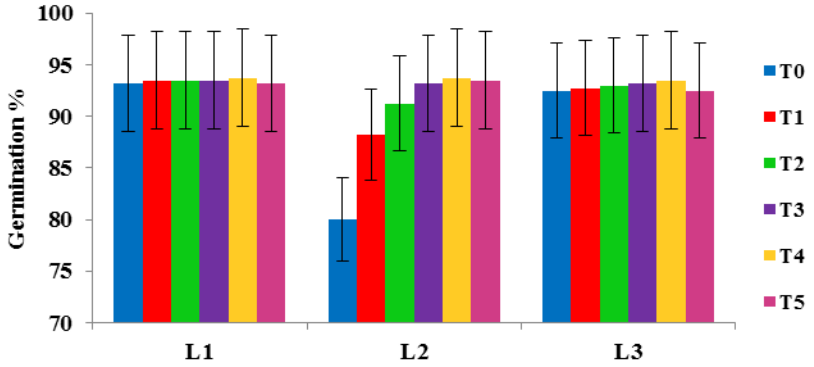


Fig.2 Effect of GA_3 treatments (500 ppm) on germination (%) of three seed lots of *Nigella sativa*

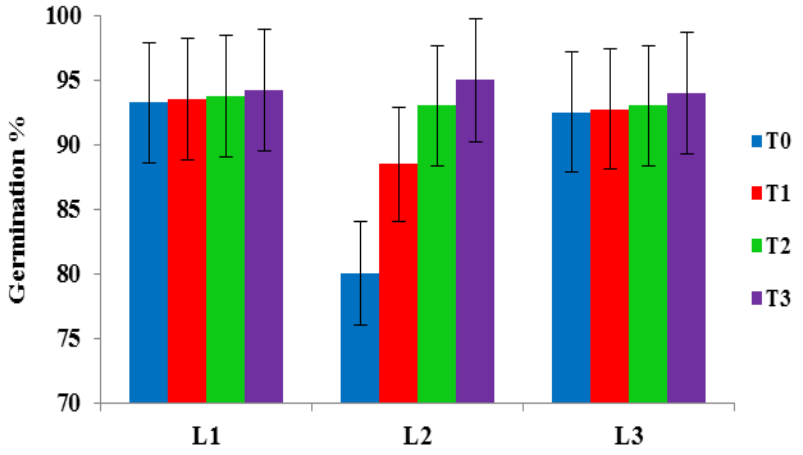
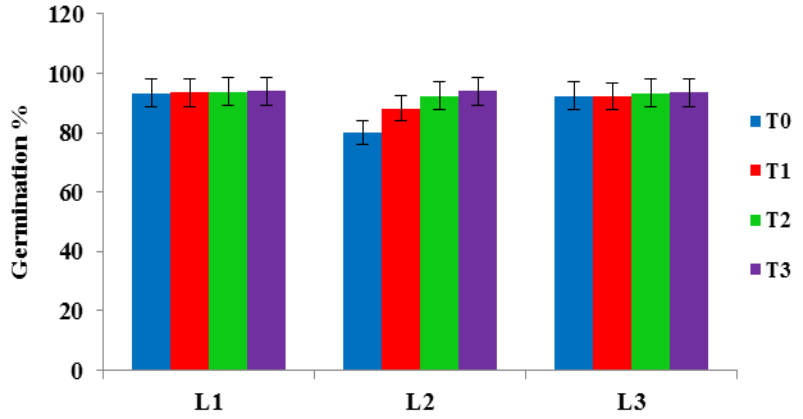


Fig.3 Effect of KNO_3 (0.2%) treatments on germination (%) of three seed lots of *Nigella sativa*



L_1 (Lot 1), L_2 (Lot 2), L_3 (Lot 3), T_0 (No treatment), T_1 (1 hour priming treatment), T_2 (2 hours priming treatment), T_3 (3 hours priming treatment), T_4 (4 hours priming treatment), T_5 (5 hours priming treatment)

Fig.4 Effect of hydropriming treatments on Vigour Index I of three seed lots of *Nigella sativa*

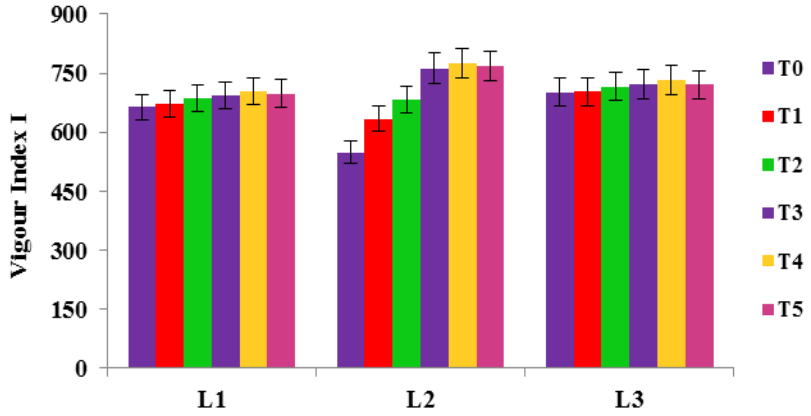


Fig.5 Effect of GA_3 treatments (500 ppm) on Vigour Index I of three seed lots of *Nigella sativa*

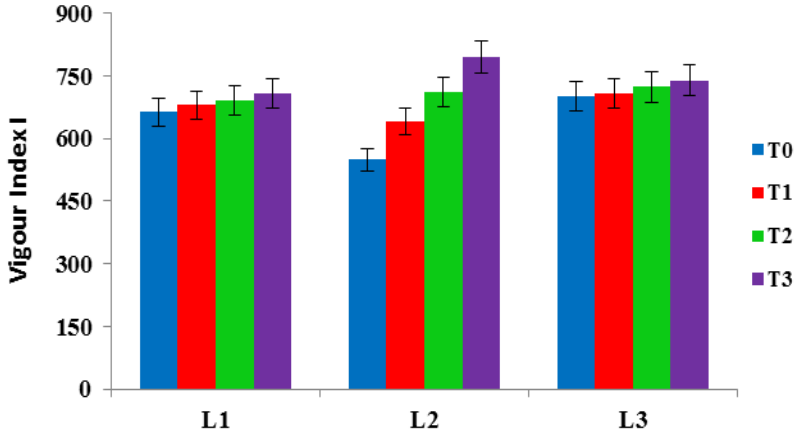
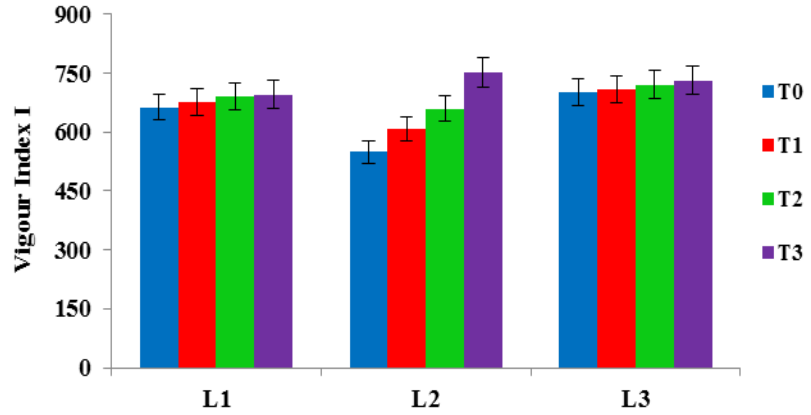


Fig.6 Effect of KNO_3 treatments (0.2%) on Vigour Index I of three seed lots of *Nigella sativa*



L_1 (Lot 1), L_2 (Lot 2), L_3 (Lot 3), T_0 (No treatment), T_1 (1 hour priming treatment), T_2 (2 hours priming treatment), T_3 (3 hours priming treatment), T_4 (4 hours priming treatment), T_5 (5 hours priming treatment)

Fig.7 Effect of hydropriming treatments on Vigour Index II of three seed lots of *Nigella sativa*

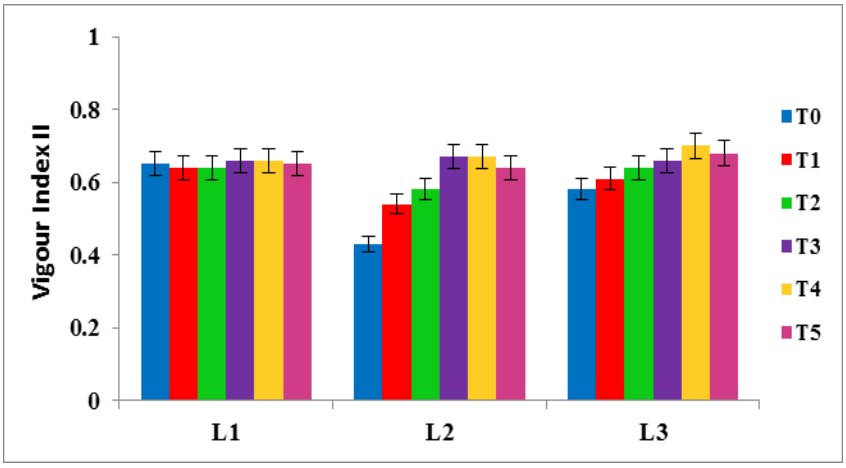


Fig.8 Effect of GA_3 treatments (500 ppm) on Vigour Index II of three seed lots of *Nigella sativa*

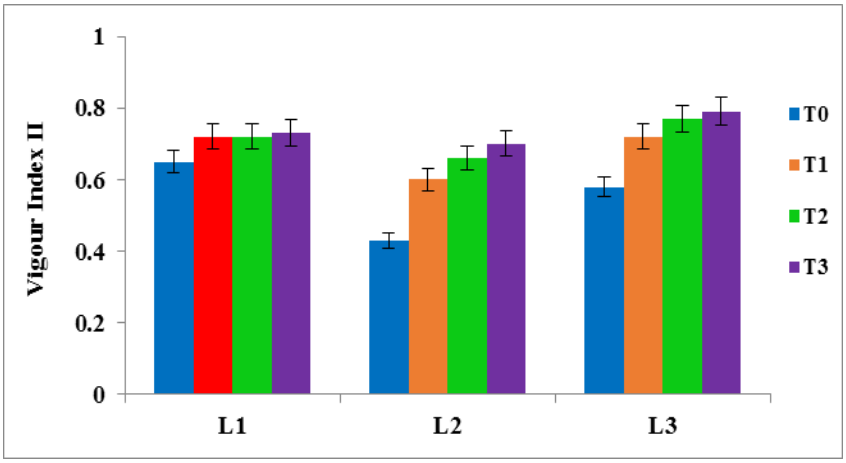
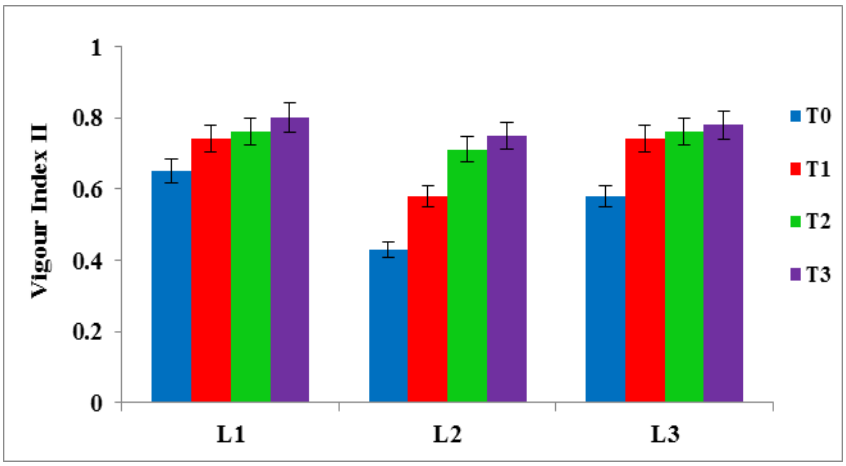


Fig.9 Effect of KNO_3 treatments (0.2%) on Vigour Index II of three seed lots of *Nigella sativa*



L_1 (Lot 1), L_2 (Lot 2), L_3 (Lot 3), T_0 (No treatment), T_1 (1 hour priming treatment), T_2 (2 hours priming treatment), T_3 (3 hours priming treatment), T_4 (4 hours priming treatment), T_5 (5 hours priming treatment)

The highest germination percentage enhancement was observed in lot 2 with all the priming treatments while lot 1 and lot 3 showed very less enhancement (Fig. 1-3). In lot 2 the germination percentage was enhanced by 13.5 %, 15 % and 14 % over the control value i.e. 80 % by hydropriming, GA_3 and KNO_3 respectively. The increase in duration of priming treatments led to increase in germination percentage and highest increase was recorded for 4 hour treatments with hydropriming and 3 hours treatments in case of both GA_3 and KNO_3 in all the three lots.

Effect of priming treatments on Vigour Index I

Seed vigour is an important quality parameter which needs to be assessed to supplement germination and viability tests to gain insight into the performance of a seed lot in the field or in storage. The seed lot showing the higher seed vigour index is considered to be more vigorous (Abdul-Baki and Anderson, 1973).

The experimental results revealed that there was significant increase in Vigour Index I by all the three priming treatments (Fig. 4-6). Among all the three seed lots, seed lots 2 showed highest increase in Vigour Index I in case of hydropriming treatments there was no significant difference in mean vigour index I among three seed lots. Among all three seed lots lot 2 showed highest increases in vigour index I in all three priming treatments. GA_3 treated seed showed increase in Vigour Index I by 45.88, 246.44, 36.59 and 109.64 from control value of 663.59, 548.94, 702.26 and 638.26 for seed lot 1, 2 and 3 respectively and highest value was recorded for 3 hour treatment. Hydropriming and KNO_3 showed highest increase in Vigour Index I for 4 and 3 hours treatments respectively.

Effect of priming treatments on Vigour Index II

Vigour Index II is the other quality parameters which reflect the overall performance of seed lot in field stand establishment of seedling and subsequent productivity of crop. Vigour Index II reflects the increase in dry mass of seedlings. The perusal of data (Fig. 7-9) reveals that all three priming treatments were significantly increased the Vigour Index of seed lot 2 & 3 whereas no enhancement was observed in lot 1 which was of high vigour. GA_3 priming was significantly effective in improving the vigour of all three lots. There was also positive effect of KNO_3 priming treatments on mean Vigour Index II of three seed lots.

Comparison among three priming treatments

An analysis was analysed to compare three priming treatments *viz.* hydro, GA_3 and KNO_3 (Table 1-4). The perusal of data in Table 1 reveals that the mean germination was significantly enhanced due to three priming treatments. Although both hydropriming & KNO_3 enhanced germination percentage of seed lot 2. But the enhancement obtained was lower than the enhancement obtained from GA_3 priming treatment. The maximum germination was enhanced in seed lot 2 over control with hydropriming (4hrs), KNO_3 (3hrs) & GA_3 (3hrs) treatment was 93.75 %, 94 % & 95 % respectively. Thus, GA_3 was most promising than others in enhancing germination percentage.

The improvement in emergence of primed seed may be due to the fact that priming induces a range of biochemical changes in the seed that are required to initiate the germination process i.e. breaking of dormancy, hydrolysis or metabolism of

inhibitors, imbibition and enzymes activation (Ajouri *et al.*, 2004). Asgedom & Becker (2001) reported that some or all processes that precede the germination are triggered by priming and persist following the re-desiccation of the seed. Thus upon sowing, primed seed can rapidly imbibe and revive the seed metabolism, resulting in a higher germination rate and a reduction in the inherent physiological heterogeneity in germination (Rowse, 1995).The probable reason for early emergence of the primed seed may be due to the completion of pre-germinative metabolic activities making the seed ready for radical protrusion and the primed seed germinated soon after planting compared with untreated dry seed (Heydecker & Coolbear, 1978).

In our experiment, seeds primed with GA_3 (500 ppm) for 3 hours recorded higher germination and germination % of lot 2 increases from 80.00 % to % significantly. It might be attributed to a stimulation of hydrolytic enzyme activity or synthesis (Jhorar *et al.*, 1982). GA_3 seed priming with increases in sugars, protein hydrolysis and RNA (Earl Plus and Lambeth, 1974), on the contrary, GA_3 was neither effective in promoting nor hastening the process of emergence of okra seed (Suryanarayana and Rao, 1984). Changes in ribonucleic acids through increased enzymatic activity enhanced the germination and emergence in various crops (Kumar *et al.*, 1996; Vijayaraghavan, 1999; Singh *et al.*, 2004; Kumar *et al.*, 2005).

On the basis of above observations, it may be concluded that *Nigella sativa* L. seeds responded to different priming treatments and performance of seed lots were improved due to priming treatments especially where seeds were deteriorating. Osmopriming performed better than hydropriming treatments. GA_3 priming was preferable over KNO_3 priming in enhancing seed quality parameters *viz.* germination percentage, seedling length, Vigour Index I and Vigour Index II. However, all the priming treatments were significantly effective for enhancing the poor quality seed lot i.e. seed lot 2 by 13.5%, 15% and 14% over the control. While the germination of seed lot 1 & 3 were not enhanced significantly where initial mean germination percentage was high i.e. 93.25% and 92.5% respectively.

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