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Comparison of the effect of different concentrations of brassinolide, potassium nitrate and thiourea on germination of pea (*Pisum sativum* L.)

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Abstract
Pea (*Pisum sativum* L.) is one of the important pulses in the world and in our country. A number of reports inform that reduced germination rate is one of the reason for the low production and productivity. There are some natural and synthetic chemicals which have shown well in the field of germination improvement. Among those we had chosen three chemicals for the current study, i.e., brassinolide, potassium nitrate (KNO_3) and thiourea in different concentrations along with distilled water as control. Both the concentrations of Brassinolide have shown remarkable results as compared to others. Furthermore, this experiment calls for a careful and systematic study on these chemicals on plant health, which can cause improvement in the field of agriculture.

Keywords: Comparison, effect, different, concentrations, potassium nitrate, (*Pisum sativum* L.)

1. Introduction

Pea is the third most important pulse crop at global level, after dry bean and chickpea and third most popular *Rabi* pulse of India after chickpea and lentil. It provides a variety of vegetarian diet hence liked throughout the world. The mature seeds are used as whole or split into dal and put to use in various ways for human consumption. Beside vegetable purposes, it is also grown as a forage crop for cattle and cover crop to prevent soil erosion but mainly for matured seed for human consumption. Highly nutritive not only having protein 22-25%, Calcium 64 mg/100g, fat 0.8- 1%, iron 4.8 mg/100g, dietary fiber 13.4%, moisture 11%, carbohydrate 62.1% but also provides agronomic significance that being leguminous crop it leaves 25-30kg N/ha to the succeeding crops. Canada rank first in area (21%) and production (35%) at Global level. China stands second position in area (13.70%) followed by Russian Fed. (12.94%). India occupy forth position in area (10.53%) and 5th position in production (5.36%). Highest productivity is recorded in Ireland (5000 kg/ha) followed by Netherland (4766 kg/ha), and Denmark (4048 kg/ha), while India's productivity is only 822 kg/ha. Due to the drought situation especially in Bundelkhand region of UP, farmers are discouraged to cultivate field peas. Therefore, the overall acreage for field peas has shortfall by 4%. Due to unsupportive weather the yield rate of the crop has fallen down and as a result there is a major shortfall of 13.6% in Field pea / matar production in UP. Seed is the most basic need of farmers for their livelihood. Seed germination is the first action performed by the seed, which gives us an idea about the whole crop life, although other factors are also responsible for the status of the crop. Hence if we are improving seed germination and controlling seedling mortality, then we can control 30-35% crop loss.

Brassinosteroids are chemically similar to animal steroids involve in growth promoting natural products, generally found at low intensity in pollen, seeds and young vegetative tissues all through the plant kingdom. The brassinosteroids are having prominent growth promoting activities. This group of bio-chemicals has remarkable and distinctive biological activities when applied to plant tissue at very minute concentration, i.e., nano or micro molar levels. As a result of extensive investigation, brassinosteroids were found to show quality physiological activities on the growth and development of plants in micro quantities. Thus, brassinosteroids are regarded as a latest class of plant hormone, in addition to auxin, gibberellin, cytokinin, abscisic acid and ethylene (Fujioka and Sakurai, 1997; Clouse and Sasse, 1998) [7, 4]. Primarily brassinosteroids were identified based on their growth promoting activities; however, subsequent physiological and genetic studies revealed additional functions of Brassinosteroids in regulating a wide range of processes, including source/sink relationships, seed germination, photosynthesis, senescence, photomorphogenesis, flowering and responses to different abiotic and biotic stresses (Deng *et al.*, 2007) [6]. photosynthesis, senescence, photomorphogenesis, flowering and responses to different abiotic and biotic stresses (Deng *et al.*, 2007) [6].

Nitrogenous chemicals are well known for their role in germination activity. Here, there are evidences for the above statement by a number of scientists on various crops. Beal seeds taken by Hore and Sen (1985) and soaked in KNO_3 1% for 24 hrs, they found maximum germination percentage (95.00%). Increased germination of Malta and Hill lemon seeds was reported by Srivastava and Singh (1985) [16], when soaked in KNO_3 @2000 ppm for 6 hours over control. The germination percentage was found appreciably superior over control, when Kagzi lime seeds, treated with KNO_3 1500 ppm (Uaghel and Sanaik, 1986) [17]. Ghosh and Sen (1988) [8] reported that germination percentage was highest when seeds treated with KNO_3, 1%, as compare to GA_3 (100 ppm), after 30 days of storage, whereas, utmost germination percentage were found, when seeds treated with 1% KNO_3 over Thiourea and control treatments, at seeds stored for 203 days in Ber. Further, Singh et al. (1989) studied pre-sowing seed treatment with GA, IBA and KNO_3 on Jambhiri and Karna khatta and found greater germination percentage of Jambhiri seeds with KNO_3 750 ppm, over control. Bose (1992) [2] found that treating the coconuts for 48hrs with 0.01 or 0.02M KNO_3 and Na_2CO_3 solution significantly reduced the germination period and gave higher percentage of germination. Padma and Reddy (1998) [14] observed that, germination started earlier and recorded the highest germination, when the Mango stones were soaked in 0.5% KNO_3 for 24 hrs. They further stated that kernels soaked in 0.5% water for 24 hrs gave the next best result.

Not only KNO_3, but also another nitrogenous chemical which has prominent role in seed germination is the thiourea. Below, there are some relevant evidence for the above statement. Centinbas and Koyuncu (2006) [3] proved through their experiment that, the thiourea treatment enhanced the germination of *Prunus avium* L. seeds and the effect of thiourea on germination was significant. The highest germination rate was observed with 120 days stratification + 10,000 ppm thiourea with seed coat and 100 days stratification +10,000 ppm thiourea without seed coat. Khanderkar *et al*. (2007) [13] revealed the following effects of pre sowing treatment of thiourea in Nutmeg. The mean germination of 88.88% was noted in thiourea (500 ppm) treatment, which was notably better to the rest of the growth regulators treatments. The least amount germination (77.09%) was observed in thiourea (1000 ppm), which was poorer than control.

The current study is dealing with the comparison of the above three chemicals, i.e., 24, epi-brassinolide, KNO_3 and thiourea with different concentrations (which are decided after thorough study of research papers), with the objective to check and asses the actions of chemicals on parameters like germination percentage, vigour index, speed of germination and germination rate index.

2. Materials and Methods

Different concentrations of 24-epibrassinoide (eBL) @0.01 mM eBL and 0.05 mM, KNO_3 @2%, 4% and 6% and two concentrations of thiourea were taken @200 ppm and 500ppm along with control. The seeds of pea were washed and sterilized. This process was followed by rinsing of seeds twice with distilled water and now the seeds were placed on filter paper kept in Petri-dishes of 15 cm diameter and 2 cm depth. For each replication, 8 Petri-dishes, each containing 10 seeds were allowed to germinate separately, at various concentrations against control. This pattern was replicated thrice.

Percentage of germination, seedling length etc. were measured at regular time interval. Germination parameters were determined according to following formulae at final days of observation:

A. Germinability (%G) =Total No. of Seeds Germinated X 100/Total No. of Seeds Sown

B. Standard germination test: It was calculated as germination rate index (GRI) and Speed of Germination (SpG) (Maguire, 1962).

B1. Germination rate index (GRI) = No. of normal seedlings of days x/Days X

X = No. of days from seed soaking

B2. Speed of Germination index (SpG) = G1/t1+G2/T2+G3/T3….Gn/Tn

Where G1= Germination on day of 1st count, T1= time or days to 1st count after soaking.

C. Vigour index (VI) = Germination (%) x seedling length (Abdul-Baki and Anderson, 1973) [1].

Data collected using the above procedures were analyzed using suitable statistical tool.

3. Result and Discussion

Present investigation revealed (Table: 1) overall increase in germination and associated parameters using these chemicals over control. Starting with germination percentage, it was higher in eBL (93.3%, 90%) than thiourea followed by KNO_3. Among the 2 concentrations of eBL, 0.01 mM (93.3%) was the better in the case of %G. In case of KNO_3, 4% concentration performed its best followed by 6%; 2% KNO_3has shown the lowest germination (63.3%), even lower than the control (70%). Thiourea treated seeds @200ppm (86.67%) was better than control, but @500 ppm (70%) it was equivalent to control. Yamaguchi *et al.* (1987) reported that brassinosteroids improved germination of aged rice (Oryza sativa L.) seeds and seedlings emergence. Srivastava *et al.* (2011) [15] described a positive effect of brassinosteroids on germination of moong bean (*Phaseolus aureus* Rox.).

In the second case i.e. GRI almost same pattern has been maintained by seeds. Among all the treatments 0.01mM eBL (1.17) was the best and was followed by 0.05mM eBL. As reported by Ramzan *et al.* (2009), in gladiolus (*Gladiolus alatus*) most excellent germination rate of 92% was achieved in control i.e. distilled water, followed by 80% in 1% KNO_3 and 70% in 2% KNO_3. Minimum time required for 50% germination i.e., 8 days was obtained with distilled water and in the same way shortest mean germination time required by seeds was in distilled water. Maximum weight and diameter of bulb gained was with 3%. Such experimental data support the present investigation, because among KNO_3 and control, control has always performed better than 2% KNO_3. In case of another parameter, i.e., vigour index both 2% and 6% have performed inferior than distilled water.

Moving towards the third parameter i.e. SpG, there was deviation in the previous pattern. Here 0.05mM eBL (4.21) performed best, which was followed by both 0.01mM eBL and 200ppm thiourea i.e. 4.17. The lowest amount was found in case of 2% KNO_3 (2.32). The vigour index was observed to be highest in 0.01mM eBL (468.33) followed by 4% KNO_3 (434.67). In this case, controlled condition (294) performed better than 2% (258.33) and 6% (269.33) of KNO_3 and two concentrations of thiourea (273 and 288.67). The result obtained in current studies is also supported by studies of Dabhi (2000) [5] on aonla (*Emblica officinalis*). The germination parameters such as survival percentage of seedling, germination percentage and fresh weight of seedling percentage was found improved @250 ppm thiourea as compared to 500ppm. The increase in fresh weight of seedlings might be due to cell multiplication and more biomass production. This result is in agreement with the finding of Hore *et al.* (1988) [12] in case of onion seedlings. According to the research data of Khandekar (2005), the maximum germination was 88.28% with thiourea treated @500ppm as compared to @1000ppm and control. Maximum shoot length was also obtained in same case. Role of thiourea in cell division might have resulted in faster growth of seedlings, as reported by Hartman and Kester (1997). 24-epibrassinoide (eBL) has to its credit, a host of roles in general plant growth and development. Brassinosteroids can activate the cell cycle during seed germination (Zadvornova *et al.*, 2005) [19] and control progression of cell cycle (González-Garcia *et al.*, 2011) [9].

Table 1: Effect of various concentrations of 24-epibrassinolide, KNO_3 and thiourea on different seed germination parameters in pea. All the data is an average of triplicates.

Treatments		%G	GRI	SpG	VI
	control	**70**	**0.87**	**2.74**	**294**
eBL	0.01mM	93.3*	1.17*	4.17	468.33*
	0.05mM	90	1.13	4.21*	315
KNO_3	2%	63.3	0.8	2.32	258.33
	4%	80	1	3.53	434.67
	6%	73.3	0.92	3.08	269.33
Thiourea	200ppm	86.67	1.08	4.17	273
	500ppm	70	0.88	3.13	288.67
CD at 5%		14.25	0.18	0.85	136.98

4. Conclusion

From the above study, it is concluded that among the three chemicals, 24-epibrassinolide showed best results at theshold and economic concentration of 0.01mM. But there is a difference in the performance of both (0.01mM and0.05mM) concentrations, which needs a thorough and comprehensive work for critical observations.

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