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Effects of certain plant growth hormone on the seed germination, survival & mortality of *Pisum sativum* and *Cicer arientinum* over the control

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Abstract
The present investigation is being carried out the effects of certain plant growth hormone cocentrations on the *Pisum sativum* and *Cicer arietinum* respectively. Pea *(Pisum sativum)* and Chick pea *(Cicer arietinum)* showed the hypogeal type of germination. The two selected species such as *Pisum sativum* and *Cicer arietinum* were investigated by the application of different concentrations of plant growth regulators such as GA_3 (10^{-2} M) to GA (10^{-7} M) in *Pisum sativum* and Kn (10^{-2} M) to Kn (10^{-7} M) in *Cicer arietinum* were applied on the seed germination, survival and mortality percentages and compared to the control condition. When these concentrations of the phytohormone were applied on the *Pisum sativum* & *Cicer arietinum*, the maximum germination, survival and less mortality percentage were recorded. But the best result was observed on the seed germination, survival and less mortality percentage were noted with the concentration of GA_3 (10^{-7} M) and Kn (10^{-3} M) on the *Pisum sativum* and GA_3 (10^{-6} M) and Kn (10^{-2} M) on the *Cicer arientinum* as compared to the control condition.

Keywords: *Pisum sativum*, germination, *Cicer arientinum*

Introduction
It is well well-known that the phytohormone, influence the growth and enlargement of plants, because these chemical substances are able to coordinate growth among different plant parts and chemical substances called as hormone. Plant growth regulators have been tried to improve growth and ultimately yield as was noted by the Patil *et al*., (1987) [19] and Kumar *et al*., (1996) [10], tried to various growth regulators to obtain better yield of good quality and obtained encouraging results. The maturity of the vegetable crops is hasten, due to the application of plant growth regulators as was observed by Buckovac & Wittwer, (1957) [1] & Chnonkar & Jha, (1963).
The importance of phytohormone also in the plant tissue culture is well known. The cytokinin is used for the stimulation of shoot growth, when added in the appropriate concentrations they may regulate cell elongation, tissue swelling and cell division, formation of adventitious roots and inhibition of adventitious and axillary shoot formation, callus initiation, growth & induction of embryogenesis, stimulate root formation, activate RNA synthesis and stimulate protein & enzyme activity. The gibberellins phytohormones promote stem elongation, flowering, breaking dormancy of seeds, buds & bulbs. There are approx 90 forms of the gibberellins hormone, in which GA_3 is the most commonly used. The plant growth hormone affect seed growth, time of flowering, sex of flowers and the senescence of leaves and fruits. Also, they affect the tissues that grow upward and downward, the formation of the leaf and the growth of the stem as were noted by Helgi-opik and Stephen *et al.,* (2005) [6]. The gibberellins hormone usually inhibits both adventitious root formation and adventitious shoot formation.
The role of cytokinin in the seed germination as was observed by Khan and Tao *et al.,* (1978). The overall growth of plant was improved by the phytohormone treatments, when it was compared to the control condition, because these phytohormone treatments increase all plant growth, than without treatments of plants. Improved and disease resistant crops could easily be made available to farmers, if the use of synthetic plant growth hormone for plantlet regeneration is vigorously pursued. The phytohormone could be made available at reduced cost to users for rapid multiplication of cultivated crops as was noted by Gana, A. S, (2010) [3].
The different studies on the effect of phytohormones on plants were noted by Mishra *et al.,* (1986) [14] & Reis *et al*., (2000) [21]. The positive effects of phytohormones on the different physiological charecters have been noted by different workers. Mahmud *et al.,* (1983) [13] observed that the effect of different phytohormone on the growth, development and yield of different varieties of oil-seed crops. The treatments of variousplant growth hormones have given encouraging results in seed germination in tomato, radish, lettuce, watermelon, brinjal, carrot and other vegetables have been reported by Swaminathan *et al.,* (1987) [23].
In *Brassica* species the enhanced siliqua and seed production was reported by Sharma *et al.,* (1997) [24], by the exogenous application of gibberellinand cytokinin respectively. The promotory effects of gibberellin and Indole

acetic acid in dwarf pea, which enhanced the internode elongation, when applied separatelyas was recorded by the Arney & Mancinelli *et al.,* (1967) and the function of gibberillic acid in the seed germination is also well recognized. Thus both oxygen and GA_3 enhance seed germination. The endogenous cytokinin is key factors in the initiation of the radicle growth. The exogenous application of cytokinins & gibberellins has been shown to substitute for the physiological influence of roots on the growth of de-rooted oat as was noted by the Jordan & Skoog *et al.,* (1971) [22] and in the soybean seedlings was observed by Holm & Key, (1969) [5]. The important increase in the content of the total chlorophyll by kinetin application as was also reported by Khalil & Mandurahi *et al.,* (1989) [9].

The more height of cytokinin treated plants also indicates the beneficial effects in general on plant growth and development. Mok (1994) [15] observed that a large number of plant developmental processes have been found to be influenced by the cytokinin effect on cell expansion, inhibition of leaf senescence, chloroplast development, root and shoot branching. Nagel *et al.,* (2001) [17] have been noted that cytokinin application plays a significant role in the flower production and exerted a positive effect on the yield of soybean, thus increasing the total seed production. Skoog and Miller *et al.,* (1959) [22] noted that the ratio of cytokinin in the nutrient media influences the morphogenesis of roots and shoots of the plants.

The gibberellins hormone strongly promoted transverse cell divisions and increased the internode length. The similar effects of GA_3 and IAA on the internode elongation have been noted by Phillips (1972) [18]. The gibberellin has the characteristics property to improve the yield, plant height and flower induction in the *chrysanthemum* as was noted by Mohariya *et. al.,* (2003) [16]. Pearce *et al*., (2004) observed that the application of different growth hormone increase the shoot length found to be in a poplar plants and the plant height was increased by gibberellins, while branch number per plant was increased by all plant phtohormones. Shah and Samiullah (2006) observed the effect of phytohormones on growth and yield of black cumin and observed that which more effective in promoting shoot length, dry weight & leaf number etc. The treatments with growth regulator probably antagonize the effect of growth inhibitory substances and also enhance the rate of metabolism during germination as was noted by Verma and Tondon *et al.,* (1988) [25].

There are also some reports available on cytokinin in combination with gibberellins enhanced germination and seedling growth in Chick pea (Kaur *et al.,* 1998) [11]. Therfore this study was aimed at comparing and characterizing the effects of GA_3 & Kn on the growth of the two species of *leguminous* species. The purpose of this study is to provide information to enhance the potential for successful production of *C*hick pea & Pea and its need in the National and International markets.

Aims and objective of present study

Present study was being proposed to evaluate the enhancement effects of certain phytohormone concentrations of Kn & GA_3 to noted the enhancement on seed germination, survival and less mortality proportion were recorded and compared to the control, during laboratory studies with the following major objectives:

1. Seed germination patterns

- Germination percentage of *Pisum sativum* & *Cicer arientinum*.
- Survival percentage of *Pisum sativum* & *Cicer arientinum.*
- Mortality percentage of *Pisum sativum* & *Cicer arientinum.*

Generel Experimental Design: During laboratory studies the following sets were taken into consideration:

Control: Seeds of both species of legumes were soaked for 24 hrs. in distilled water and placed on moistened filter paper in the Petridishes.

Growth Regulators: Test solution of Kn and GA_3 were prepared in concentrations viz. (10^{-7} to 10^{-2})treated in *Pisum sativum* & (10^{-7} M to 10^{-2} M) (molarities) in *Cicer arientinum (*Chick pea). The seeds of *both legumes* were soaked for 24 hrs. in different concentrations of plant growth regulators and soaked seeds were placed in paired Petridishes lined with moistened filter paper.

Table 1: General investigational design may be summarized as follows

Treatments	Control	**Cytokinin**						**Gibberellins**					
Hormones Concentrations		10^{-7} (M)	10^{-6} (M)	10^{-5} (M)	10^{-4} (M)	10^{-3} (M)	10^{-2} (M)	10^{-7} (M)	10^{-6} (M)	10^{-5} (M)	10^{-4} (M)	10^{-3} (M)	10^{-2} (M)

Study area

The study area of the present research work has been carried out in the laboratory of the R.C.U. Govt. P.G. College Uttarkashi. The district Uttarkashi is a Garhwal division of the Uttarakhand state in Northern India and its headquarters at Uttarkashi. The district contains the source of the Bhagirathi (traditionally considered the headstream of the Ganga) at Gangotri and Yamuna at Yamunotri, both of which are highly significant and popular pilgrimage sites. Uttarkashi town, which lies on the main road to Gangotri, is also considered an important Hindu pilgrimage centre, especially for Saivites. The district is bounded on the north by Kinnaur and Shimla districts of Himachal Pradesh, on the Northeast by Tibet, on the East by Chamoli

District, on the Southeast by Rudraprayag district, on the South by Tehri Garhwal district and on the west by Dehradun district (Uttrakhand), India.

Fig 1: Map of study area

Materials and methodology

Seed germination and seedling growth

For the studies of seed germination and seedling growth patterns, uniform seeds of *Pisum sativum* (Pea) and *Cicer arientinum* (Chick pea) were selected and surface sterilized by absolute ethyl alcohol and then 0.1% $HgCl_2$ for the one minute each thoroughly rinsed with distilled water. Total seeds of the both species of leguminous crops were divided into twenty six sets separately, i.e. twelve sets of each crop, except control. Two sets of both species were treated as control and placed in the incubator, without any treatment. Each species of leguminous crop viz. *Pisum sativum* & *Cicer arientinum* was treated with gibberellins & cytokinin hormone concentrations of (10^{-2} to 10^{-7}M) respectively.

Six sets of seeds of *Pisum sativum* were soaked in GA_3 solution of (10^{-2} to 10^{-7}M) concentrations as compared to control. Out of these six sets were also treated with Kn (10^{-2} to 10^{-7}M) concentrations with respective to the control.

Next six sets of seeds of *Cicer arientinum* were soaked in the GA_3 solution of (10^{-2} to 10^{-7} M) concentrations respectively, with respective to the control. Out of these, next six sets were also treated with Kn solution of (10^{-2} to 10^{-7} M) concentrations respectively, as compared to the control.

In the laboratory study, the all sets of Petridishes were supplied with appropriate concentrations of these plant growth regulators (PGRs) daily, except control. These sets were supplied with distilled water daily. The germination percentage was recorded on the basis of radicle emergence as 2 mm in length and it considered as germinated..

Germination percentage

After an interval of 24 hours seeds were counted and at the end of fifteen days the total numbers of seeds were added for the calculation of germination percentage as follows:

$$\mathbf{GP} = \frac{\textbf{Number of germinated seeds}}{\textbf{Total number of sown seed}} \times 100$$

Survival percentage

Survival is the struggle to remain alive and living. Survival rate is a part of survival analysis, indicated the percentage of seeds in a study group, who are alive for a given period of time after germination. The seeds for the calculation of survival percentage as follow:

$$\mathbf{SP} = \frac{\textbf{Number of Seed Survived}}{\textbf{Total Number of Germinated seed}} \times 100$$

Mortality percentage

Mortality is an estimated to account for seed that germinates, but fails to develop in to viable plants. These are many reasons for seedling mortality including disease, insects and excessive fertilizer into the seed row, improper seedling depth, light, temeperature, frost and drought due to seedling mortality can vary greatly.

$$MP = \frac{\text{Number of Dead Seed}}{\text{Total number of germinated seed}} \times 100$$

Result and observations

The experiment was conducted in the laboratory with and without treatment of different concentrations of certain phtohormones was treated daily on the germinating seeds of *Pisum sativum* & *Cicer arientinum* respectively. After two days, the germinating seeds were counted till fifteenth days and observed germination, survival & mortality percentages of both species.

In the present investigation, we have studies the seed germination, survival and mortality percentages were recorded with and without different concentrations of phtohormones such as GA_3 (10^{-2} to 10^{-7} M) and Kn (10^{-2} to10^{-7} M) concentrations in the *Pisum sativum* (Pea*)* and in the *Cicer arientinum* (Chick pea) respectively were observed during laboratory. The seed germination, survival and mortality percentage were recorded for different concentrations of these phtohormones, alone to assess the appropriate concentrations of this plant hormone and compared to the control and appropriate concentrations of plant hormones were applied for the further studies and data are presented in tables 1. & 2.

The results presented in table 1. & fig. 1. in control condition, indicate that the seed germination, survival and mortality percentages of *Pisum sativum* were recorded as ca. 86%, 80% and 20% respectively. When any one of these plant hormone was given to the germinating seeds, the significant germination, survival and less mortality percentage were amounted as ca. 71%, 86% and 14% for GA_3 (10^{-2} M) concentration; 88%, 84% and 16% for GA_3 (10^{-3} M) concentration; 70%, 82% and 18% for GA_3 (10^{-4} M) concentration; 89%, 79% and 21% for GA_3 (10^{-5} M) concentration; 72%, 88% and 12% for GA_3(10^{-6} M) concentration and maximum germination, survival and minimum mortality percentages were noted to be ca. 92%, 78% and 22% for GA_3 (10^{-7} M) concentrations respectively, as compared to the control.

The same result was also observed in other concentrations of Kn hormone on the seed germination, survival and mortality percentages of *Pisum sativum* respectively. Phytohormone was given to seedlings, the significant germination and survival percentages were increased and mortality percentage was reduced and amounted to be ca. 84%, 81% and 19 % for Kn (10^{-2} M) concentration; maximum germination, survival and minimum mortality percentage was noted to be ca. 95%, 82% and 18% for Kn (10^{-3} M) concentration; ca.72%, 78% and 22% for Kn (10^{-4} M) concentration; 70%, 73% and 27% for Kn (10^{-5} M) concentration; ca. 72%, 74% and 24% for Kn (10^{-6} M) concentration and ca. 86%, 83% and 17% for Kn (10^{-7} M) concentrations respectively, as compared to the control.

An observation of data presented in table 2 & fig. 2 indicated that the germination, survival and mortality percentages of *Cicer arientinum* were observed to be ca. 88%, 84% & 16% respectively, during control. When any one of phytohormone were supplied to the geminating seedlings, the significant promotion was observed in case of germination and survival percentages and mortality percentage was reduced to be ca. 94%, 89% and 10% for GA_3 (10^{-2} M); 95%, 88% & 12% for GA_3 (10^{-3} M); 89%, 81% & 19% for GA_3 (10^{-4} M); 91%, 86% & 14% for GA_3 (10^{-5} M); maximum germination, survival and minimum mortality percentage was recorded to be ca. 95%, 90% & 10% for GA_3 (10^{-6} M) and 93%, 89% & 11% for GA_3 (10^{-7} M) concentrations respectively, with respective to the control.

The same observation was also obtained with Kn concentrations i.e. from (10^{-2} M) to (10^{-7} M), on the germinating seedlings of *Cicer arientinum* respectively. The significant values of germination and survival percentage were increased with different plant hormone concentration and mortality percentage was reduced as compared to the control. The maximum values of seed germination, survival and minimum mortality percentages were observed to be ca. 90%, 82% and 18% for Kn (10^{-2} M) concentration. The significant enhancement of germination, survival and reduced mortality percentages were amounted to be ca. 89%, 81% and 19% for Kn (10^{-3} M); ca. 84%, 81% and 19% for Kn (10^{-4} M); ca. 90%, 79% and 21% for Kn (10^{-5} M); ca. 86%, 82% and 18% for Kn (10^{-6} M) and ca. 80%, 82% & 18% for Kn (10^{-7} M) concentrations respectively, as compared to the control petridish.

Table 2: Seed germination, survival and mortality percentage of *Pisum sativum* in control and treated by different concentrations of gibberellins & cytokinin hormone concentration, after 15 days of sowing.

Treatments	Germination percentage	Survival percentage	Mortality Percentage
Control	86	80	20
Gibberellin (10^{-7}) M	92	78	22
Gibberellin (10^{-6}) M	72	88	12
Gibberellin (10^{-5}) M	89	79	21
Gibberellin (10^{-4}) M	70	82	18
Gibberellin (10^{-3}) M	88	84	16
Gibberellin (10^{-2}) M	71	86	14
Cytokinin (10^{-7}) M	86	83	17
Cytokinin (10^{-6}) M	72	76	24
Cytokinin (10^{-5}) M	70	73	27
Cytokinin (10^{-4}) M	72	78	22

Cytokinin (10^{-3}) M	95	82	18
Cytokinin (10^{-2}) M	84	81	19

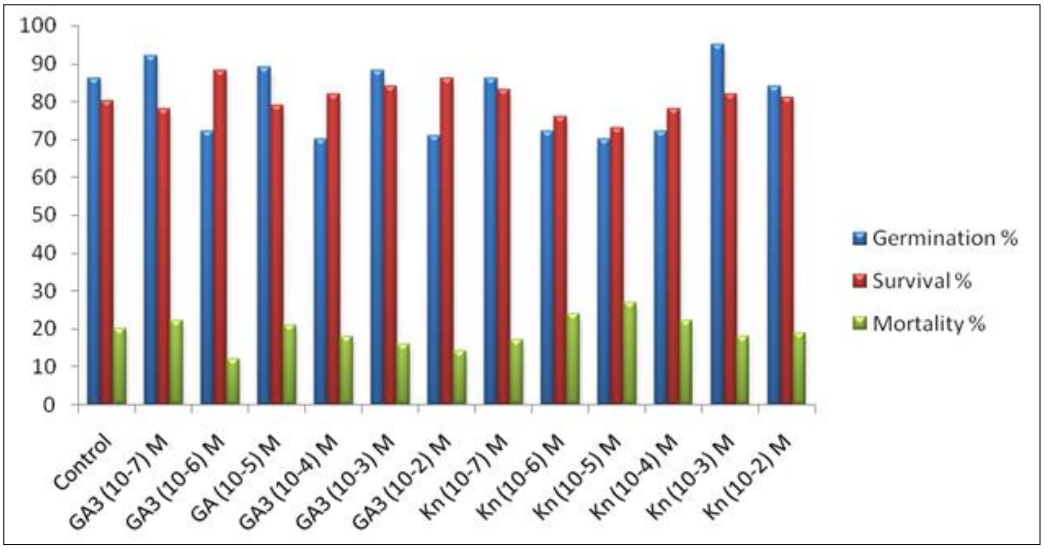

Fig 2: Seed germination, survival and mortality percentage of *Pisum sativum* in control and treated by different concentrations of GA_3 & Kn hormone concentration, after 15 days of sowing.

Table 3: Seed germination, survival and mortality percentage of *Cicer arientinum* (Chick pea) in control and treated by different concentrations of gibberellins & cytokinin hormone concentrations, after 15 days of sowing.

Treatments	Germination percentage	Survival percentage	Mortality percentage
Control	88	84	16
Gibberellin (10^{-7}) M	93	89	11
Gibberellin (10^{-6}) M	95	90	10
Gibberellin (10^{-5}) M	91	86	14
Gibberellin (10^{-4}) M	89	81	19
Gibberellin (10^{-3}) M	95	88	12
Gibberellin (10^{-2}) M	94	89	10
Cytokinin (10^{-7}) M	80	82	18
Cytokinin (10^{-6}) M	86	82	18
Cytokinin (10^{-5}) M	90	79	21
Cytokinin (10^{-4}) M	84	81	19
Cytokinin (10^{-3}) M	89	81	19
Cytokinin (10^{-2}) M	90	82	18

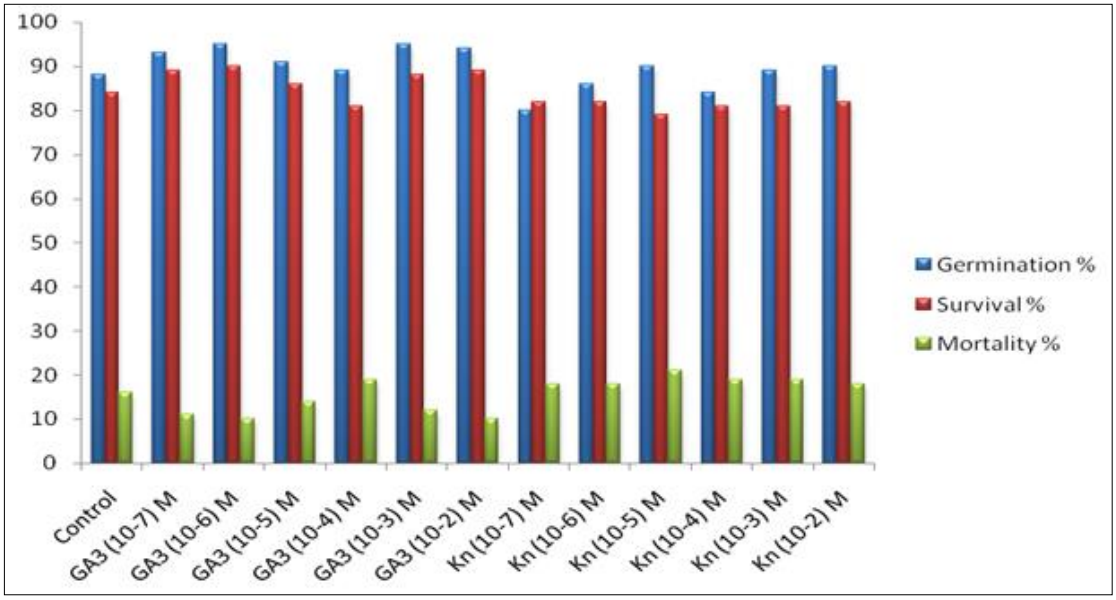

Fig 3: Seed germination, survival and mortality percentage of *Cicer arientinum* (Chick pea) in control and treated by different concentrations of gibberellins & cytokinin hormone concentrations, after 15 days of sowing.

Discussions

The present investigation has been carried out to study the enhancementry effects of various plant hormones, when applied on the some morphological aspects of *Pisum sativum* and *Cicer arientinum* respectively. It has been observed that, when plant hormones were applied to germinating seeds, they enhance the germination, survival and reduce the mortality percentage as compared to control. The same observation were also identified by Gupta, A. (2011) [4] and Kuriyal, S. (2011), Lal, S. and Russell *et al.* (1998) [20] observed that the enhancing impact of Kinetin in the *Phaseolus vulgaries*. They reported that the cytokinin enhanced the germination percentage of crop significantly as compared to the control. Control petridish was found to show minimum seed germination & survival and maximum mortality percentage of both the species of *leguminous* crops under investigation. When both the species of *leguminous* crops were treated with of various concentrations of gibberellins and cytokinin, the promotory effects were noted over control condition. The gibberellins and cytokinin found to be best effective on maximum germination and survival percentage and minimum mortality percentage in the concentration of GA_3 and Kn (10^{-7} M) & (10^{-3} M) and (10^{-6} M) & (10^{-2} M) in *Pisum sativum* & and *Cicer arientinum* respectively. The same concentration would show the maximum promotion over control on the *leguminous* species. There results are with the conformity of Kumari & Bharti *et al.*, (1992).

Conclusion

We concluded that, when germinating seeds were subjected to these plant hormones in its physiological range i.e. gibberellin (10^{-7} M) and cytokinin (10^{-3} M) concentration, the maximum values of germination and survival percentages were increased and mortality percentage was reduced in *Pisum sativum* respectively, as compared to control condition. In case of gibberellins (10^{-6} M) and cytokinin (10^{-2} M) concentration, the maximum values of germination and survival percentages were recorded and mortality percentage was also reduced in *Cicer arientinum* respectively, as compared to control condition. The maximum values of germination, survival and minimum mortality percentages were showed with gibberellins (10^{-7} M) and cytokinin (10^{-3} M) on the *Pisum sativum* and gibberellins (10^{-6} M) and cytokinin (10^{-2} M) on the *Cicer arientinum* respectively.

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