Influence of Stik, Kinetin and Vitamin K_3 on Rooting, Regeneration and Polarity of Stem Cuttings of *Geranium* Sps.

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Abstract: The influence of Stik, Kinetin and Vit.K_3 at various concentrations was studied on the rooting and shoots growth behavior of *Geranium* stem cuttings. Treated cuttings have been transferred to polythene bags. Initially small red color of protuberances appeared from the buds in all cuttings after six days of potting. Lower concentration of all applied exogenous hormones was found more effective to enhance the sprouting, shoot growth and rooting behavior of treated cuttings in comparison to higher concentration. Poor results have been observed under the treatment of inverted cuttings for different parameters. Results revealed significant effects on different parameters. Highest sprouting percentage has been recorded under the treatment of Vit.K_3 200ppm in comparison to control. Stik100ppm stimulated the growth rate of lateral branches in all the potted cuttings. Higher concentration showed inhibitory effects for different parameters in comparison to control. Rooting behaviour of all potted cutting was found significant and maximum number of secondary roots was noticed under the treatment of inverted cuttings.

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Introduction

Vegetative propagation of plants by cuttings has been employed as an alternative means of multiplication particularly for the species which are in excessive demand especially for ornamental plants and underground crops since times immemorial (Nanda and Kochhar, 1987). Advantage of vegetative propagation include rapid multiplication, efficient maintenance of genetic uniformity and rapid attainment of size, convenience, ease of propagation, combination of genotypes and reduction of the juvenile period (Govinden-Soulange, J et al 2009). *Geranium* plant is easily propagated by stem cuttings. It is growing in dry, hot habitats, fissures of rocky outcrops, sandy soils and sand dunes (Dole, J.M. and H.F. Wilkins, 2005). Growing buds of *Geranium* stem cuttings promote rotting. In most species rooting decreases to a great extent with the removal of buds and leaves. Use of plant growth regulators plays a vital role in influencing the sprouting and survival of stem cuttings. Auxins, is one of the most important plant growth regulator are often called as phytohormones and play an essential role in coordination of growth and other vital activities in life cycle of plants (Delker et al., 2008). The exogenous application of IBA induces rooting in stem cuttings and in air layers due to their ability to achieve to active cambium regeneration, cell division and cell multiplication (Rymbai and Reddy 2010). Indole-3-Acetic Acid (IAA), α- Naphthalene Acetic Acid (NAA) and Indole-3-Butyric Acid (IBA) are typically the principal auxins which are available commercially and can be applied with liquid or powder formulation for rooting and sprouting of stem cuttings (Ercisli and Guleryuz, 1999; Hopkins, 1999). It is fact that success of rooting in cuttings depends on physiological age, time of rooting, environmental conditions, i.e. light, temperature and humidity and use of plant growth regulators (Elgimabi, M.E.N.E., 2009).

Keeping in view of the above facts the present study was conducted to find out the role of growth regulators i.e. Stik, Kinetin and Vit.K_3 on different parameters of *Geranium* stem cuttings.

Materials and methods

The material for present experiment i.e. *Geranium* sp. was taken from Bhagiratipuram locality of Tehri Garhwal in the month of second week March. The influence of various growth regulators such as Stik, Kinetin and Vit. K_3 at different concentration (100,200,500ppm) have been studied on stem cuttings of approximately identical size. From mother plant of *Geranium*, stem cuttings were made of about 12 cms. in length and having approximately equal diameter 2.0cm. In order to observe various morphological features, cuttings were grouped into 10 sets with three types i.e. cuttings without leaves, inverted cuttings and cuttings with leaves and each including 12

cuttings. In the normal set of control 5 upright cuttings was taken. A set for polarity effects was prepared with 4 inverted set of cuttings. Three types of cuttings i.e. cuttings without leaves, inverted cuttings and cuttings with leaves have been presoaked in distilled water for control and in different concentrations of Stik, Kinetin and Vit.K_3 for 72 hours at 22^0c. up to depth of 8 cms. These treated cuttings were then transferred to polythene bags and regularly watered with tap water. The upper ends of the cuttings were covered with moist cotton just to avoid drying. Throughout the entire duration of experiment, following parameters were recorded from time to time as influenced by different concentration of applied growth regulators.

Regeneration and shoot-growth: No. of buds sprouted per cutting, no. of lateral branch arisen/cutting, av. length of lateral branch/cutting, largest lateral branch formed in a treatment, no. of leaves formed/cuttings, length of petiole, size of leaf and width, growth rate of leaf area.

Rooting behavior: with a view to analyze the rooting pattern of cuttings, such samples which has been treated with different conc. of Stik, Kinetin and Vit. K3 have been uprooted intact carefully and washed smoothly with running water to remove soil and the following parameters have been recorded i.e. av. no. of cuttings rooted (%), av. no. of primary roots formed/ cutting, av. length of primary root (cm), av. no. of secondary roots formed/cutting, av. length of secondary root (cm) and rooting zone length in cuttings (cm). ANOVA was carried out on the data collected to compare significantly different means.

Results and discussion

The sprouting and subsequent growth of all three types of planted cuttings viz. without leaves, inverted and with leaves have influenced by different concentration of Stik, Kinetin and Vit.K_3 to variable degrees. All the lower concentration tested for Stik, Kinetin and Vit.K_3 in all cuttings proved better in sprouting percentage in comparison to control (81%). Vit.K_3 200ppm was recorded to show highest sprouting percentage (92%), but higher concentrations (500ppm) of Stik and Kinetin inhibited sprouting percentage after 24 days of potting (Fig.1). It was found that sprouting percentage of the cuttings was decreased with increase in the concentration of Stik, Kinetin and Vit.K_3. The concepts of inhibitors serving as regulators of dormancy grew stronger with the accumulation of evidence that the natural periods of dormancy are associated with high endogenous inhibitors contents. In this context Hemberg,s (1949b) had noted that the dormant buds of potato contain appreciable amounts of growth inhibitors and that the control of this inhibitors drops with treatments which tend to break dormancy, e.g. ethylene chlorohydrin. Sprouting increasing in buds might be due to better utilization of stored carbohydrates, nitrogen and other factors with the help of growth regulators (Sinha *et al* 2014). There was no sprouting have been observed under inverted cuttings in control set after 24 days. Weaver (1959) recorded that mostly in plants gibberllin application induced the dormancy of the buds. Table-2 shows the growth rate of lateral branches arising from the main cuttings after the treatments of different concentration of applied growth regulators. Stik100ppm was brought about highest branch length (4.8cm.) in without leaves cuttings which was lowest (0.3cm) under 100ppm of Vit.K_3 in inverted cuttings in comparison to control (1.2cm). For Vit.$K_3$100ppm as the concentration were augmented, the length of the lateral branch also increases although for Stik and Kinetin, this value increases only up to 200ppm beyond which it declines. Growth hormones causes enlargement of plant cells, cell division, laterals branching of shoots and roots, vascular differentiation and early embryonic development (Hobbie et al., 2000). Data presented in Table-3 indicates the growth behavior of the leaf as influenced by applied growth regulators. 100,200ppm concentrations of Stik and Kinetin resulted in increased leaf formation. Maximum average numbers of leaf formation (35) per cutting have been recorded at Kinetin 200ppm in without leaf potted cuttings followed by Stik 100ppm (30) in comparison to control (25). Inverted cuttings showed poor result in leaf formation and their subsequent growth. Brache *et al* (2005) reported the highest number of leaves with IBA 5000ppm. Increase in leaf number might be due to the vigorous rooting induced by the growth regulator enabling the cuttings to absorb more nutrients and thereby producing more leaves as reported by Stancato *et al* (2003). The growth patterns of petiole, leaf length and width have also been observed under the treatment of different concentration of tested growth regulators. Maximum length (3cm.) of petiole has been recorded under Vit.$K_3$100ppm in without leaves cuttings in comparison to control (2.3cm) with leaves cuttings. Vit.K_3 100ppm in without leaves and Stik 100ppm in with leaves potted cuttings enhanced the length of petiole, leaf and width. Inverted cuttings did not follow the same trend and it showed least response in this regard. Generally different concentration of tested growth regulators in inverted cuttings inhibited the lateral growth rate of cuttings. Vit. $K_3$100ppm enhanced the maximum leaf area (28.5cm^2) in with leaves potted cuttings followed by Stik 100 and 500ppm (25cm^2) in the same type of potted cuttings after 52 days of the potting (Fig.2). Similar findings have been reported by Kepinski and Leyser (2005) who found that increase in leaf area was due to the

auxin treatment.The potted cuttings up rooted after 80 days of potting and detailed rooting behavior have been observed under different treatments (Table-4). Different concentration of applied hormones stimulated the rooting percentage in all the treatments. 100% rooting percentage have been recording in without leaves and with leaves cuttings but majority of treatments showed 50% rooting percentage in inverted cuttings. Decrease in sprouting and rooting in inverted cuttings is due to the effects of its polarity. Higher concentration (500) of Vit.K_3 and Kinetin favored higher number of primary root formation (Fig.3). At Vit.K_3 500ppm, maximum number of primary root per cutting (45) have been observed in without leaves cuttings followed by Vit.K_3 100ppm (42). The primary roots length in with leaves cuttings was higher (27.0cm) at Kinetin 200ppm. Maximum inhibition for primary roots formation was noticed at inverted cuttings. Rooting is more likely to be related to the actual formation of root initials than to the mechanical restriction of a sclerenchymatous ring barring root emergence. In *Geranium* it looks that there are no performed root initials in the cuttings and the hormonal treatments also could not initiate their origin. The endogenous rooting inhibitors also play an important role in rooting of cuttings of certain difficult to root plants. This was found by Spiegel (1955) to be so with grapes, in which he noted that presence of two inhibitors associated with the rooting response. Moreover, Butola and Badola (2007) have recommended IAA and IBA as promising treatments to improve rooting, growth and biomass in *Angelica glauca* and *Heracleum candicans*. The possible explanation to this lies in better development of root system with good quality root and shoots parameters enabling the rooted cuttings to make better growth under field conditions after plantation and there by accounted the highest field survivability (Sharma *et al* 2009). Lower concentration of Stik and Kinetin and higher concentration of Vit.K_3 favored better secondary root formation in without leaves and with leaves potted cuttings but other lower and higher concentration of different applied growth regulators showed poor secondary root formation. Maximum (68) number of secondary root formation have been observed under inverted cuttings at Kinetin 500ppm in comparison to control (20) in all the tested concentration. Low auxin activity and its slow degradation by auxin destroying enzyme lead to the growth and vigour of roots. This might also be due to the reserved food in the cuttings (Singh *et al* 2013). Significant effects of applied growth regulators on stem cuttings have been observed (Table- 5,6&7).

Conclusion

It is clear from the above findings that Geranium cuttings have better growth potential and the use of growth regulators induces early growth vigour them. In this regards upright potted cuttings treated with optimum dose level of hormones showed superiority over the inverted potted cuttings. As the concentration of growth regulators increases, the sprouting percentage decreases. It can be concluded that cuttings treated with different concentration of Stik, Kinetin and Vit.K_3 was found to be the most efficacious in encouraging shoot and root growth behaviour in terms of sprouting percentage, number of lateral branch formation and length of primary and secondary roots per cutting. For large scale multiplication of *Geranium,* its cuttings should be treated with exogenous hormone.

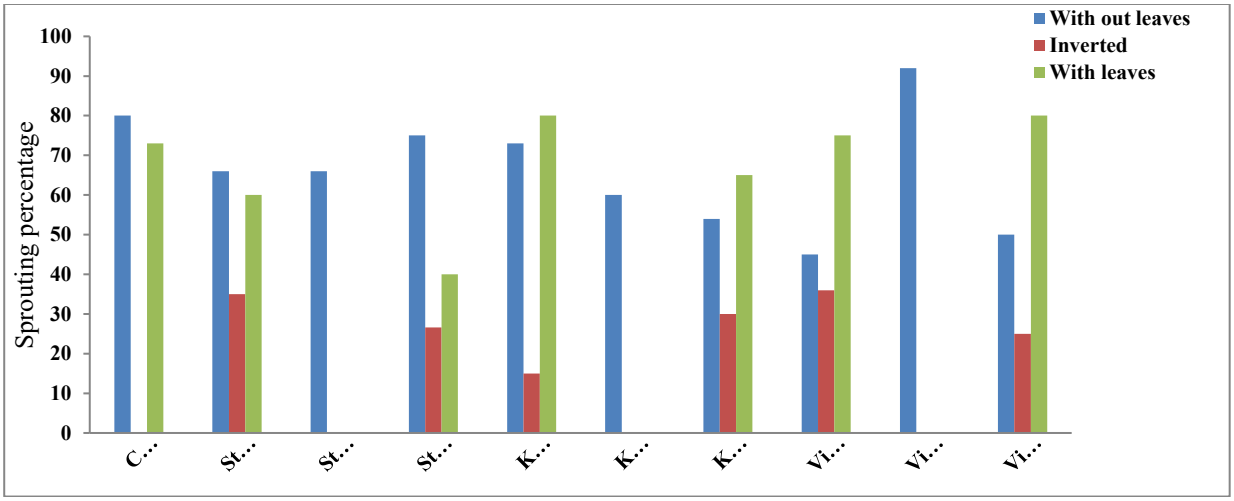


Fig. 1. Sprouting percentage of *Geranium* stem cuttings as influenced by different concentration of Stik, Kinetin and Vit. K_3 after 24 days.

Table-1. **(a)** No. of lateral branch arisen/cutting **(b)** Av. length of lateral branch/cutting **(c)** Largest lateral branch formed in a treatment as influenced by different concentration of Stik, Kinetin and Vit. K_3 after 24 days.

Cuttings without leaves					**Inverted cuttings**			**Cuttings with leaves**		
Treatments		**(a)**	**(b)**	**(c)**	**(a)**	**(b)**	**(c)**	**(a)**	**(b)**	**(c)**
Control		5±.06	1.2	1.6	1±.003	.5	2.2	2±.0067	3	3.4
Stik (ppm)	**100**	3±.02	4.8	5.2	2±.0067	4.5	4.6	5±.068	2.5	3
	200	1±.003	2	2	*	*	*	*	*	*
	500	4±.067	1.7	3	1±.003	.4	.4	1±.003	.3	.3
Kinetin (ppm)	**100**	4±.067	.6	.8	3±.0063	2	2	2±.0067	1.4	3.3
	200	4±.067	2.2	4	*	*	*	*	*	*
	500	1±.003	2	2	1±.003	.4	.4	Nil	Nil	Nil
Vit.K3 (ppm)	**100**	2±.009	.4	.5	1±.003	.3	.3	2±.0067	1.7	2.4
	200	1±.003	1	1	*	*	*	*	*	*
	500	3±.006	1.2	1.3	2±.0067	2.1	2.1	Nil	Nil	Nil

*Treatment not applied, ± SE of means

Table-2. Analysis of variance (ANOVA) for (a) length of lateral branch/cutting (b) length of largest lateral branch formed in a treatment.

a						**B**				
Source of variation	**ss**	**df**	**mss**	**c f**	**t f**	**ss**	**df**	**mss**	**c f**	**t f**
Dose	30.4	9	3.3	3.3	2.46	35.1	9	3.9	2.4	2.46
Days	4.3	2	2.1	2.1	3.55	5.6	2	2.8	1.7	3.55
Error	18.1	18	1	-	-	29.1	18	1.6	-	-

Table-3. **(a)** Av. no. of leaves formed/cutting **(b)** Av. length of petiole **(c)** Av. length of leaf and, **(d)** Av. width of of *Geranium* leaf as influenced by different concentration of Stik, Kinetin and Vit. K_3 after 24 days.

Cuttings without leaves						**Inverted cuttings**				**Cuttings with leaves**			
Treatments		**(a)**	**(b)**	**(c)**	**(d)**	**(a)**	**(b)**	**(c)**	**(d)**	**(a)**	**(b)**	**(c)**	**(d)**
Control		25 ±3.76	2.1	1.3	2.5	Nil	Nil	1.7	2.1	11 ±1.06	2.3	1.6	2.5
Stik (ppm)	**100**	30 ±3.97	1.5	1.4	2.2	11±1.01	1.6	1.2	1.4	25 ±3.76	3	2.1	3.4
	200	13 ±1.05	.8	1.05	1.7	*	*	*	*	*	*	*	*
	500	28 ±4.07	1.8	1.2	1.7	3 ±.07	.6	.6	1.05	5 ±.85	2.5	2.2	3
Kinetin (ppm)	**100**	23 ±3.21	1.3	1.3	1.8	3 ±.071	.07	1.4	1.8	24 ±3.23	1.4	1.25	2.1
	200	35 ±4.98	1.7	1.4	1.5	*	*	*	*	*	*	*	*
	500	17 ±2.23	1.35	1.35	1.6	5 ±.85	.65	1	1.2	21 ±2.76	.9	1.1	1.7
Vit.K_3 (ppm)	**100**	14 ±1.64	3	1.7	1.25	6 ±.89	.7	1.65	1.3	16 ±1.98	1.7	1.15	1.9
	200	15 ±1.95	1	1.3	1.9	*	*	*	*	*	*	*	*
	500	16 ±1.99	2	1.1	1.8	9 ±.99	2.8	1.2	2.9	11 ±1.01	1.3	1.05	1.7

*Treatment not applied, ± SE of means

Table-4. Analysis of variance (ANOVA) for (a) length of petiole (b) length of leaf and (c) width of of leaf.

A						**b**					**C**				
Source of variation	**Ss**	**df**	**mss**	**c f**	**t f**	**ss**	**df**	**mss**	**c f**	**t f**	**ss**	**df**	**mss**	**c f**	**t f**
Dose	12.4	9	1.3	2.6	2.46	9.3	9	1.0	3.0	2.46	14.6	9	1.6	3.7	2.46
Days	5.2	2	2.6	4.9	3.55	3.8	2	1.9	5.8	3.55	2.0	2	1.0	2.3	3.55
Error	9.5	18	.53	-		6.0	18	.33	-	-	7.8	18	.43	-	-

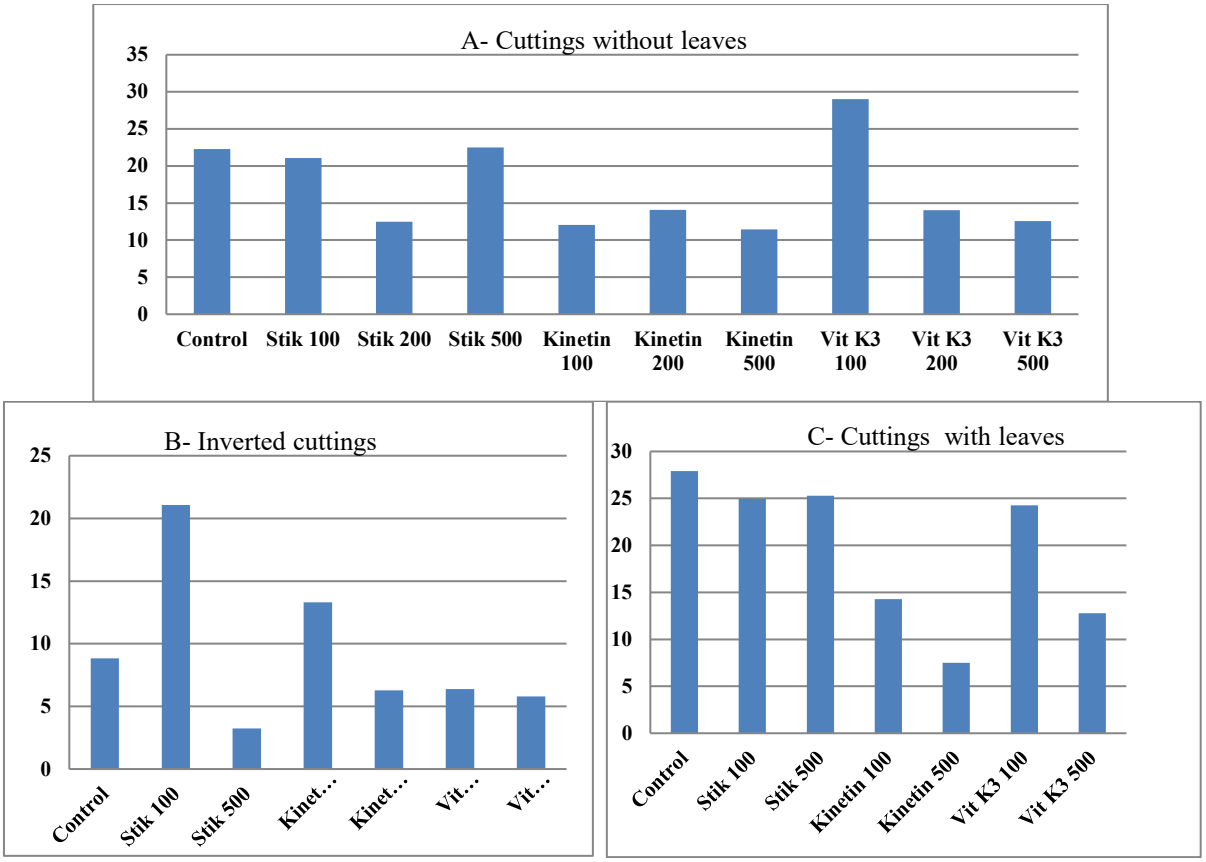


Fig. 2 (A,B,C) Growth rate of leaf area as influenced by different concentration of Stik, Kinetin and Vit. K_3 after 52 days.

Table-5. Rooting behaviour of *Geranium* stem cuttings without leaves as influenced by different concentration of Stik, Kinetin and Vit.K_3 after 80 days of potting.

Cuttings without leaves										
Parameters	**Control**	**Stik (ppm)**			**Kinetin (ppm)**			**Vit. K3(ppm)**		
		100	**200**	**500**	**100**	**200**	**500**	**100**	**200**	**500**
Av. no. of cuttings rooted (%)	50	100	100	100	100	100	100	100	100	100
Av. no. of primary roots formed/ cutting	6± .89	11± 1.06	12± 1.87	35± 4.98	12± 1.00	3± .02	12± 1.00	12± 1.00	27± 3.98	45± 4.12
Av. no. of secondary roots formed/cutting	10± 1.01	24± 3.03	10± .99	11± 1.01	37± 4.97	16± 2.12	32± 4.12	12± 1.00	16± 2.11	14± 1.64
Av. length of primary root (cm)	16	8	7.2	5.5	6.5	27	9.5	10	6.5	14
Av. length of secondary root (cm)	4	.9	1	4.2	1.4	14	4.2	5.1	2	9
Rooting zone length in cutting (cm)	1.2	3.02	3.4	.8	5	3.8	4.3	4.5	1.2	4.2

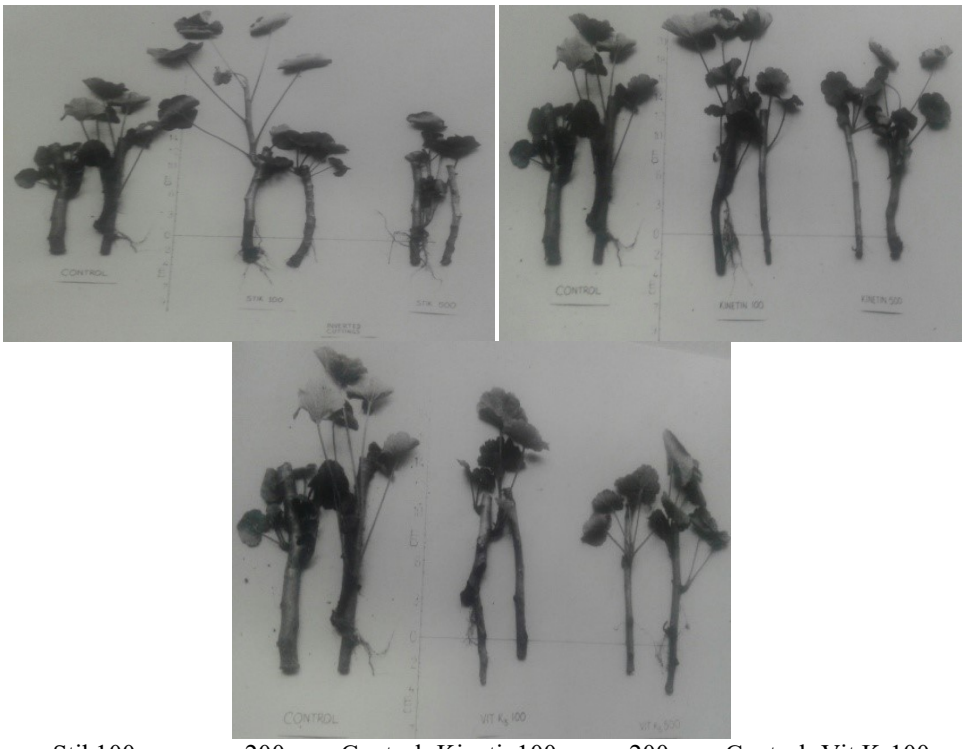
± SE of means

Table-6. Rooting behaviour of *Geranium* inverted cuttings and cuttings with leaves as influenced by different concentration of Stik, Kinetin and Vit.K_3 after 80 days of potting.

Treatments	**Inverted cuttings**							**Cuttings with leaves**						
Parameters	**Control**	**Stik**		**Kinetin**		**Vit. K_3**		**Control**	**Stik**		**Kinetin**		**Vit. K_3**	
		100	**500**	**100**	**500**	**100**	**500**		**100**	**500**	**100**	**500**	**100**	**500**
Av. no. of cuttings rooted (%)	25	100	50	50	50	50	75	75	100	100	100	100	100	100
Av. no. of primary roots formed/ cutting	10± .99	6± .80	4± .067	1±.003	4± .67	2± .006	2± .006	10± 1.11	29± 4.56	4± .67	38± 4.13	7± .87	42± 4.99	26± 3.88
Av. no. of secondary roots formed/ cutting	20± 1.87	22± 1.98	28± 4.07	68± 5.98	9± .99	12± 1.02	14± 1.64	20± 2.43	42± 4.87	8± 1.21	49± 5.02	14± 1.64	58± 5.01	7± .90
Av. length of primary root (cm)	8	5.5	11	9	4.6	4.2	4.6	3.2	6.5	7.8	9.5	4.5	2.4	5
Av. length of secondary root (cm)	3.5	6.5	1.4	4.5	.8	4.1	9	1.2	5.1	11	1.4	14	4.5	4.2
Rooting zone length in cutting (cm)	3.2	2.4	5.2	1.2	1.4	1.5	5	1.2	4.6	3.6	2.5	4.5	4.5	3.6

± SE of means

1- Control Stik100ppm, 200ppm, 500ppm Control Kinetin100 200ppm 500ppm Control Vit.$K_3$100, 200 500ppm



2- Control Stik100ppm 200ppm Control Kinetin100 200ppm Control Vit.$K_3$100, 200ppm

3- Control Stik100ppm 200ppm Control Kinetin100 200ppm Control Vit.$K_3$100, 200ppm

Fig.3. Rooting and shoot growth behavior of *Geranium* cuttings as influenced by different concentration of Stik, Kinetin and Vit.K_3 after 80 days of potting (1- Without leaves planted cuttings, 2- Inverted planted cuttings and 3- With leaves planted cuttings).

Table-7. Analysis of variance (ANOVA) for (a) length of primary roots (b) length of secondary roots (cm) (c) rooting zone length in cutting (cm).

a						**b**					**C**				
Source of variation	**ss**	**df**	**mss**	**c f**	**t f**	**ss**	**df**	**mss**	**c f**	**t f**	**ss**	**df**	**Mss**	**c f**	**t f**
Dose	139.4	9	15.4	.56	2.46	110.3	9	12.2	.64	2.46	49	9	5.4	2.2	2.46
Days	305.1	2	152.5	5.64	3.55	13.4	2	6.7	.35	3.55	7.3	2	3.6	1.5	3.55
Error	486.8	18	27	-	-	341.3	18	18.9	-	-	42.8	18	2.3	-	-

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