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EFFICACY OF ANTIMICROBIAL AGENTS ON *IN VITRO* MICROPROPAGATION POTENTIAL OF POTATO

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ABSTRACT: An experiment was conducted to find out the effect of different concentrations of antimicrobial agents *viz*., carbenicillin, streptocycline, cephotaxime and Plant Preservative Mixture (PPMTM; US patent no. 5,750,402) on *in vitro* growth and vigour of micro-plantlets of three potato varieties *viz*. Kufri Chandramukhi, Kufri Chipsona-3 and Kufri Sindhuri. The study revealed that antimicrobial agents, their different concentrations, potato varieties and their interaction had significant effect on different morphological characters *viz*., microplant height, inter-nodal length, number of leaves, nodes and roots, root length and fresh as well as dry weight. In general, carbenicillin upto 100 mg/l, cephotaxime upto 200 mg/l and PPM upto 0.15% had positive effect on all the morphological characters in all the three potato varieties except microplant root length in Kufri Chipsona-3 and Kufri Sindhuri as compared to control. Therefore, antimicrobial agents' like carbenicillin upto 100 mg/l, cephotaxime upto 200 mg/l and PPM upto 0.15% can be used for controlling the *in vitro* microbial contamination without affecting the growth and vigour of micro-plantlets.

KEYWORDS: Potato, antimicrobial agents, micropropagation, growth parameters

INTRODUCTION

Recent advances in tissue culture techniques especially micropropagation has facilitated the production, multiplication and maintenance of disease free potato clones. However, tissue culture media provides a rich mixture of nutrients which can also support the rapid growth of bacteria and fungi. Once these contaminants are established in the culture they usually grow fast, deplete the nutrients in the medium and also produce toxins that can affect the growth and ultimately kill the cultured plant tissue. As a consequence, almost all tissue culture facilities around the world propagate plants with minimum possible load of 'latent bacteria' (Kneifel and Leonhardt, 1992).

Sterile culture environment largely ensures eradication of contamination in culture media. However, in routine culture air borne contamination of medium with fungi and bacteria often occurs in spite of all precautions. *Pseudomonas, Flavobacterium* and *Blastobacter* have also been reported on the wet surfaces of air conditioning systems (Trudeau and Fernandez-Caldas, 1994). More than thirty microbes have been identified and characterized from ten different micropropagated plant cultivars (Odutayo *et al.,* 2004). Some of these microbes are harmless while the others are harmful to plants in normal *in vitro* culture conditions and may often get transferred to *in vivo* also.

The presence of these microbes in culture media usually results in plant mortality, variable growth, tissue necrosis, reduced shoot proliferation and reduced rooting (Odutayo *et al.,* 2004). Though surface sterilization eliminates the exophytic microbes (George, 1993) but, endophytic bacterial contamination

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can only be eradicated by antibiotic therapy (Jung, 2003).

Antimicrobial agents have been extensively tested for their ability to inhibit or prevent the growth of microbes in plant cultures. Antimicrobial agents are often phytotoxic or otherwise capable of altering the behavior of cultured plant tissues. However, stimulative effect of antimicrobial agents on plant growth was also observed in tobacco and carrot (Chang and Schimdt, 1985) and barley (Mathias and Mukasa, 1987). Phytotoxicity and stimulating effect of antimicrobial agent varies greatly with plant, explant type and even among different genotypes of the same plant species (Fiola *et al.,* 1990). But, till now, no information is available regarding incorporation of antimicrobial agents in potato micropropagation. Therefore, a study was carried out to investigate the effect of incorporating antimicrobial agents in the culture medium on growth and morphological characters of potato varieties having different maturity group. The information generated from this study will be useful in improving the rate of *in vitro* multiplication in potato.

MATERIAL AND METHODS

The experiment was conducted during 2010-2011 at Central Potato Research Institute, Shimla with three potato varieties *viz.,* Kufri Chandramukhi, Kufri Chipsona-3 and Kufri Sindhuri. Double-node cuttings essentially derived from middle portion of microplantlets were used as explant. The standard culture medium (Murashige and Skoog, 1962) was supplemented with four different antimicrobial agents at different concentrations *viz.,* carbenicillin (50, 75, 100 and 125 mg/l), cephotaxime (100, 150, 200 and 250 mg/l), streptocycline (100, 150, 200 and 250 mg/l) and PPM (0.10, 0.15, 0.20 and 0.25%). Required quantity of carbenicillin and streptocycline were added to the autoclaved medium under aseptic conditions after filter sterilization whereas, cephotaxime was added without filter sterilization, as it could not be passed through 0.22 µm syringe filter. The plant preservative mixture was added to the medium before adjusting the pH.

The explants (three double nodes per test tube) were cultured on above said media along with control (MS medium). Cultured tubes were incubated at 22±1˚C under 16h photoperiod (irradiance of 60 µmol/ m^2/s). After twenty-one days of culturing, observations were recorded on microplant height (cm); number of leaves, nodes and roots; inter-nodal and root length (cm) as well as the fresh and dry weight (mg) of each microplant of a test tube. In case of number of roots, only primary roots were counted, as there was secondary branching too. Root length was recorded for the longest root of each plant. Fresh and dry weight was taken for all the three plantlets along with root. For dry weight, micro-plants from each test tube were dried at 80˚C for 48h in the hot air oven and dry weight was recorded after bringing it to room temperature.

Experimental design and statistical analysis: The experiment was conducted in a factorial (3×4×5) completely randomized design. Each treatment comprised four replicates, each replicate consist of four test tubes having three plantlets. As the experiment was repeated once, data were pooled over individual experiments. The three-way analysis of variance was done using the software AGRES and means were separated according to the least significant differences at 0.05 level of probability.

RESULTS AND DISCUSSION

In Kufri Chandramukhi and Kufri Sindhuri all the concentrations of carbenicillin (25, 75, 100, 125 mg/l) and cephotaxime (100, 150, 200, 250 mg/l) and PPM upto 0.15%, (0.10,

0.15%) significantly increased the microplant height whereas, carbenicillin upto 100 mg/l, all the concentrations of cephotaxime and PPM upto 0.20% did so in Kufri Chipsona-3. Rest of concentration of carbenicillin (125 mg/l) and all the concentrations of streptocycline retarded the microplant height as compared to control. However, the effect of different concentrations of carbenicillin and cephotaxime was statistically at par (**Table 1**). In Kufri Chandramukhi, only different concentrations of cephotaxime significantly increased the number of leaves and nodes as compared to control but effect of different concentrations was found to be statistically at par with each other. In Kufri Chipsona-3, none of the antimicrobial agent had significant positive effect on number of leaves and nodes. In Kufri Sindhuri, carbenicillin 50 mg/l significantly increased the number of leaves and nodes, whereas, streptocycline 250 mg/l and PPM 0.25% significantly reduced them as compared to control. Antimicrobial agents except streptocycline in different concentrations significantly increased the inter-nodal length in different varieties as compared to control (**Tables 1 and 2**).

The positive effects of carbenicillin and cephotaxime upto certain concentrations may be due to plant growth promoting effect of these antimicrobial agents. Several earlier workers have also reported that antimicrobial agents promote morphogenetic response in different crops through their break down products formed by the metabolic activities of the cells during incubation period which mimic plant growth regulators (Borrelli *et al.*, 1992; Holford and Newbury, 1992; Nakona and Mii, 1993; Teng and Nicholson, 1997). Negative effects of higher concentrations of carbenicillin and cephotaxime on almost all the morphological characters as observed in the present study may be due to toxic effects of these antimicrobial agents and such effects have been reported in barley by Mathias and Mukasa, (1987). It was also reported by Zhang *et al.*, (1999) that at higher concentrations of antibiotic products so formed degrade the polyribosome, inhibit protein synthesis and disrupt the membrane permeability.

In Kufri Chandramukhi, cephotaxime upto 150 mg/l significantly increased the number of roots as compared to control, whereas, carbenicillin 125 mg/l had negative effect. In Kufri Chipsona-3, different concentrations of carbenicillin and cephotaxime and PPM upto 0.20% significantly increased the number of roots as compared to control. However, the concentration effect was at par to each other. In Kufri Sindhuri, carbenicillin upto 100 mg/l, PPM upto 0.15% and cephotaxime up to 200 mg/l significantly increased the number of roots as compared to control. All the concentrations of different antimicrobial agents were found to be at par.

In Kufri Chandramukhi, cephotaxime 100 mg/l and PPM 0.15% significantly increased the root length while carbenicillin had non significant effect. In Kufri Chipsona-3, different concentrations of cephotaxime significantly increased the root length while, effect of carbenicillin and PPM was non significant. Root length of Kufri Sindhuri was not influenced by antimicrobial agent and their concentration. Streptocycline, irrespective of concentration reduced the number of roots and root length in all the varieties (**Table 3**).

In Kufri Chandramukhi and Kufri Chipsona-3, carbenicillin upto 75 mg/l and all the concentrations of cephotaxime significantly increased the fresh weight of micro-plants as compared to control. PPM had no effect on fresh weight in comparison to control on Kufri Chandramukhi but, significantly increased the fresh weight upto 0.20% in Kufri Chipsona-3. Similar trend was observed in

Table 1. Effect of antimicrobial agent and their concentrations on microplant height and number of leaves of different potato varieties.

Variety	Antimicrobial agent	Microplant height (cm)						Number of leaves					
		C_1	C_2	C_3	C_4	C_5	**Mean**	C_1	C_2	C_3	C_4	C_5	**Mean**
Kufri Chandramukhi	Cephotaxime	7.5	10.3	10.4	10.7	10.9	10.0	4.4	6.2	6.0	5.8	5.2	5.5
	Carbenicillin	7.5	10.1	10.5	9.8	9.7	9.5	4.4	4.8	5.1	5.1	4.7	4.8
	PPM	7.5	10.1	8.5	7.4	5.5	7.8	4.4	4.8	4.5	4.1	3.8	4.3
	Streptocycline	7.5	4.9	3.2	2.3	2.1	4.0	4.4	4.4	4.4	3.8	3.4	4.1
	Mean	**7.5**	**8.9**	**8.2**	**7.6**	**7.1**	**7.8**	**4.4**	**5.0**	**4.9**	**4.6**	**4.4**	**4.7**
Kufri Chipsona-3	Cephotaxime	4.7	8.1	8.2	8.3	7.6	7.4	5.8	6.1	5.7	5.7	5.2	5.7
	Carbenicillin	4.7	7.0	6.9	6.5	5.7	6.2	5.8	6.2	6.2	6.0	5.9	6.0
	PP M	4.7	6.9	6.1	5.9	5.6	5.8	5.8	6.0	5.7	5.6	5.3	5.7
	Streptocycline	4.7	2.0	1.5	1.0	0.9	2.0	5.8	4.1	4.3	4.0	3.8	4.4
	Mean	**4.7**	**6.0**	**5.7**	**5.4**	**5.0**	**5.4**	**5.8**	**5.6**	**5.4**	**5.2**	**5.2**	**5.4**
Kufri Sindhuri	Cephotaxime	6.0	9.9	9.1	8.9	8.6	8.5	5.4	5.6	5.5	5.1	5.0	5.3
	Carbenicillin	6.0	8.9	8.0	7.7	7.6	7.6	5.4	6.3.	5.9	5.7	5.4	5.7
	PPM	6.0	8.2	8.2	6.3	5.7	6.9	5.4	6.0	6.1	5.7	4.8	5.6
	Streptocycline	6.0	3.8	3.2	2.9	1.3	3.4	5.4	5.3	5.2	4.9	3.7	4.9
	Mean	**6.0**	**7.7**	**7.1**	**6.5**	**5.8**	**6.6**	**5.4**	**5.8**	**5.5**	**5.3**	**5.0**	**5.4**
	Grand Mean	**6.1**	**7.5**	**7.0**	**6.5**	**5.9**		**5.2**	**5.5**	**5.2**	**5.1**	**4.9**	

	V	A	C	VA	AC	VC	VAC	V	A	C	VA	AC	VC	VAC
SEd	0.1	0.1	0.1	0.2	0.3	0.2	0.5	0.1	0.1	0.1	0.2	0.2	0.2	0.3
CD (0.05)	0.2	0.3	0.3	0.4	0.6	NS	1.0	0.1	0.2	0.2	0.3	0.4	0.3	0.7

V: Variety; A: Antimicrobial agent; C: Concentrations; Carbenicillin (C_1-Control, C_2-50 mg/ l , C_3-75 mg/ l , C_4-100 mg/ l and C_5-125 mg/ l); Streptocycline (C_1-Control, C_2-100 mg/ l , C_3-150 mg/ l , C_4-200 mg/ l and C_5-250 mg/ l); PPM: Plant preservative mixture (C_1-Control, C_2-0.1%, C_3-0.15%, C_4-0.20% and C_5-0.25%); Cephotaxime (C_1-Control, C_2-100 mg/ l , C_3-150 mg/ l , C_4-200 mg/ l and C_5-250 mg/ l).

Table 2. Effect of antimicrobial agent and their concentrations on number of nodes and inter-nodal length of different potato varieties.

Variety	Antimicrobial agent	Number of nodes						Inter nodal length (cm)					
		C_1	C_2	C_3	C_4	C_5	**Mean**	C_1	C_2	C_3	C_4	C_5	**Mean**
Kufri Chandramukhi	Cephotaxime	4.4	6.3	6.1	6.0	5.4	5.7	1.7	1.7	1.8	2.0	1.8	1.8
	Carbenicillin	4.4	4.9	5.2	5.2	4.8	4.9	1.7	2.2	2.0	2.0	1.9	1.9
	PPM	4.4	4.9	4.6	4.1	3.7	4.3	1.7	2.1	1.9	1.8	1.5	1.8
	Streptocycline	4.4	4.4	4.4	3.8	3.4	4.0	1.7	1.1	0.8	0.7	0.6	1.0
	Mean	**4.4**	**5.1**	**5.0**	**4.5**	**4.6**	**4.7**	**1.7**	**1.8**	**1.6**	**1.6**	**1.5**	**1.6**
Kufri Chipsona-3	Cephotaxime	6.0	6.2	5.9	5.7	5.3	5.8	0.8	1.5	1.5	1.4	1.3	1.3
	Carbenicillin	6.0	6.2	6.3	6.1	6.0	6.1	0.8	1.1	1.1	1.1	1.0	1.0
	PPM	6.0	6.1	5.8	5.6	5.3	5.8	0.8	1.2	1.1	1.1	1.0	1.0
	Streptocycline	6.0	4.1	4.2	4.0	3.8	4.4	0.8	0.5	0.4	0.3	0.2	0.4
	Mean	**6.0**	**5.7**	**5.5**	**5.3**	**5.2**	**5.5**	**0.8**	**1.0**	**1.0**	**1.0**	**0.9**	**0.9**
Kufri Sindhuri	Cephotaxime	5.6	5.7	5.5	5.2	5.1	5.4	1.1	1.8	1.8	1.7	1.6	1.6
	Carbenicillin	5.6	6.6	6.1	5.9	5.6	5.9	1.1	1.4	1.4	1.3	1.3	1.3
	PPM	5.6	6.2	6.3	6.1	4.7	5.8	1.1	1.4	1.4	1.3	1.0	1.2
	Streptocycline	5.6	5.3	5.2	4.9	3.7	5.0	1.1	0.7	0.7	0.6	0.3	0.7
	Mean	**5.6**	**5.9**	**5.4**	**5.6**	**5.0**	**5.5**	**1.1**	**1.3**	**1.3**	**1.2**	**1.1**	**1.2**
	Grand Mean	**5.3**	**5.6**	**5.3**	**5.2**	**4.9**		**1.2**	**1.4**	**1.3**	**1.2**	**1.2**	

	V	A	C	VA	AC	VC	VAC	V	A	C	VA	AC	VC	VAC
SEd	0.1	0.1	0.1	0.1	0.2	0.2	0.3	0.02	0.03	0.03	0.05	0.06	0.06	0.1
CD (0.05)	0.1	0.2	0.2	0.3	0.4	0.3	0.7	0.05	0.06	0.06	0.1	0.13	0.1	0.2

V: Variety; A: Antimicrobial agent; C: Concentrations; Carbenicillin (C_1-Control, C_2-50 mg/ l , C_3-75 mg/ l , C_4-100 mg/ l and C_5-125 mg/ l); Streptocycline (C_1-Control, C_2-100 mg/ l , C_3-150 mg/ l , C_4-200 mg/ l and C_5-250 mg/ l); PPM: Plant preservative mixture (C_1-Control, C_2-0.1%, C_3-0.15%, C_4-0.20% and C_5-0.25%); Cephotaxime (C_1-Control, C_2-100 mg/ l , C_3-150 mg/ l , C_4-200 mg/ l and C_5-250 mg/ l).

Table 3. Effect of antimicrobial agent and their concentrations on number of roots and root length of different potato varieties.

Variety	Antimicrobial agent	Number of roots						Root length (cm)					
		C_1	C_2	C_3	C_4	C_5	**Mean**	C_1	C_2	C_3	C_4	C_5	**Mean**
Kufri Chandramukhi	Cephotaxime	5.6	7.5	7.2	5.5	4.5	6.1	2.7	3.8	3.6	3.3	3.1	3.3
	Carbenicillin	5.6	6.2	5.4	5.1	3.9	5.2	2.7	2.4	2.9	2.6	2.2	2.5
	PPM	5.6	6.7	6.9	5.8	5	6.0	2.7	3.5	3.9	2.0	1.4	2.7
	Streptocycline	5.6	0.9	0.6	0.6	0.4	1.6	2.7	0.5	0.2	0.2	0.1	0.7
	Mean	**5.6**	**5.3**	**5.0**	**4.3**	**3.5**	**4.7**	**2.7**	**2.5**	**2.6**	**2.0**	**1.8**	**2.3**
Kufri Chipsona-3	Cephotaxime	4.5	7.8	7.3	6.9	6.8	6.7	6.7	8.1	8.0	7.8	7.7	7.7
	Carbenicillin	4.5	7.8	6.5	6.4	6.4	6.3	6.7	6.1	6.1	6.0	5.9	6.2
	PPM	4.5	6.6	6.2	6	5.8	5.8	6.7	7.2	6.6	5.7	5.1	6.3
	Streptocycline	4.5	0.7	0.6	0.5	0.4	1.3	6.7	0.3	0.2	0.2	0.1	1.5
	Mean	**4.5**	**5.7**	**4.9**	**4.9**	**5.1**	**5.0**	**6.7**	**5.4**	**5.2**	**4.8**	**4.9**	**5.4**
Kufri Sindhuri	Cephotaxime	4.5	7.2	6.9	6.0	5.1	5.9	7.5	7.7	7.5	7.3	7.2	7.4
	Carbenicillin	4.5	7.5	6.4	5.9	5.4	5.9	7.5	7.8	7.6	7.4	7.0	7.4
	PPM	4.5	6.4	5.9	4.4	4.3	5.1	7.5	7.0	6.7	5.9	4.7	6.3
	Streptocycline	4.5	2.0	1.7	1.3	1.1	2.1	7.5	0.6	0.5	0.3	0.2	1.8
	Mean	**4.5**	**5.7**	**4.9**	**4.0**	**4.7**	**4.8**	**7.5**	**5.5**	**5.3**	**6.0**	**5.0**	**5.8**
	Grand Mean	**4.9**	**5.4**	**4.6**	**4.5**	**4.8**		**5.6**	**4.5**	**4.3**	**4.1**	**3.9**	

	V	A	C	VA	AC	VC	VAC	V	A	C	VA	AC	VC	VAC
SEd	0.2	0.2	0.2	0.3	0.4	0.3	0.7	0.1	0.1	0.1	0.2	0.3	0.2	0.5
CD (0.05)	NS	0.3	0.4	0.6	0.8	0.7	1.3	0.2	0.2	0.3	0.4	0.5	0.5	0.9

V: Variety; A: Antimicrobial agent; C: Concentrations; Carbenicillin (C_1-Control, C_2-50 mg/ l , C_3-75 mg/ l , C_4-100 mg/ l and C_5-125 mg/ l); Streptocycline (C_1-Control, C_2-100 mg/ l , C_3-150 mg/ l , C_4-200 mg/ l and C_5-250 mg/ l); PPM: Plant preservative mixture (C_1-Control, C_2-0.1%, C_3-0.15%, C_4-0.20% and C_5-0.25%); Cephotaxime (C_1-Control, C_2-100 mg/ l , C_3-150 mg/ l , C_4-200 mg/ l and C_5-250 mg/ l).

Kufri Sindhuri with other antimicrobial agents except carbenicillin. Rest of the concentrations of different antimicrobial agents were found to be at par with control. Cephotaxime @100 mg/l was the best antibiotic for obtaining maximum fresh and dry weight of all the test varieties (**Table 4**).

In Kufri Chandramukhi, cephotaxime upto 150 mg/l significantly increased the dry weight while PPM reduced the dry weight at all the concentrations. In Kufri Chipsona-3 carbenicillin 50 mg/l, PPM 0.10% and all the concentrations of cephotaxime significantly increased the dry weight while streptocycline has decreased the same. In Kufri Sindhuri, PPM 0.10% and all the concentrations of cephotaxime significantly increased the dry weight as compared to control. In general, streptocycline irrespective of concentration neither had positive effect nor negative effect on fresh and dry weight in different varieties while cephotaxime had maximum positive effect in promoting the fresh and dry weight (**Table 4**).

Plant preservative mixture (PPM) is a patented thermo-stable, broad-spectrum biocide that reduces microbial contamination in plant tissue cultures. PPM has been shown to inhibit growth of microorganism while having a minimal effect on *in vitro* seed germination of most plant species tested at concentrations ranging between 0.05-0.4% (Guri and Patel, 1998). However, it has not been used in potato yet. In the present study, PPM up to 0.15% only had positive effect in different morphological characters while at higher concentration it had negative effect in different varieties. Similar inhibitory effect of PPM on petunia leaf explant-s (above 0.2%) of Solanaceae family, melon explants (up to 0.5%) of Cucurbitaceae family and insensitive nature of tobacco explants (up to 1.0%) of Solanaceae family was also reported earlier (Compton and Koch, 2001).

This study clearly indicates that microplant sensitivity as well as its tolerance limit to different microbial agent is conferred by genotype. Therefore, both anti-microbial agent choice and optimization of its concentration is prerequisite for different genotypes.

CONCLUSIONS

From the study, it can be concluded that antimicrobial agents like carbenicillin, cephotaxime and PPM can be incorporated in the micropropagation medium of potato to minimize microbial contaminants as well as for improving the vigour of microplantlets. In general, irrespective of variety, carbenicillin up to 100 mg/ l, cephotaxime upto 150 mg/ l and plant preservative mixture (PPM) upto 0.15% are the optimum dose and can be used as growth stimulants safely in potato micro-propagation medium. To reduce the cost of antimicrobial agents' lower concentration of carbenicillin 50-100 mg/l, cephotaxime 100-200 mg/l and PPM 0.10-0.15% can be used. Further studies on the synergistic/stimulatory effect of these agents on growth and productivity in different compatible combinations and concentrations both in micropropagation and micro-tuber production may still generate better information to reduce the cost of chemical/production as well as on reduction of microbial contaminations. The inter alia comparative means of all concentrations of antimicrobial agents reveal that cephotaxime has shown better positive incremental responses than others for almost all the morphological parameters of all three varieties studied (**Fig. 1-3**). It was best to achieve the highest length of microplants and inter nodal segments in all varieties by cephotaxime followed by carbenicillin and or PPM.

In general, all the concentrations of streptocycline had negative effects on almost all the morphological characters studied except for fresh and dry weight and this may be

Table 4. Effect of antimicrobial agent and their concentrations on fresh and dry weight of different potato varieties.

Variety	Antimicrobial agent	Fresh weight (mg)						Dry weight (mg)					
		C_1	C_2	C_3	C_4	C_5	**Mean**	C_1	C_2	C_3	C_4	C_5	**Mean**
Kufri Chandramukhi	Cephotaxime	153.6	238.8	249.3	217.5	194.9	210.8	13.9	18.4	19.4	16.6	14.3	16.5
	Carbenicillin	153.6	198.2	190.7	180.3	178.3	180.2	13.9	16.4	16.6	14.5	13.6	15.0
	PPM	153.6	175.4	156.3	112.4	90.3	137.6	13.9	13.2	11.5	9.2	8.9	11.3
	Streptocycline	153.6	138.9	125.0	112.7	95.6	125.1	13.9	14.0	13.8	13.1	11.8	13.3
	Mean	153.6	187.8	169.3	150.1	156.5	163.4	13.9	15.5	13.9	12.7	14.1	14.0
Kufri Chipsona-3	Cephotaxime	133.5	259.7	265.9	277.7	250.9	237.5	11.5	19.8	19.7	19.3	23.7	18.8
	Carbenicillin	133.5	187.9	167.0	136.9	130.5	151.1	11.5	14.5	12.4	9.9	8.6	11.4
	PPM	133.5	225.0	171.3	166.5	155.1	170.3	11.5	17.1	12.8	12.1	11.8	13.1
	Streptocycline	133.5	88.9	83.2	81.5	78.2	93.1	11.5	9.1	9.4	8.8	8.7	9.5
	Mean	133.5	190.4	171.8	164.0	155.3	163.0	11.5	15.1	13.4	12.4	13.5	13.2
Kufri Sindhuri	Cephotaxime	136.4	285.9	250.8	238.0	228.5	227.9	12.1	22.3	19.5	18.6	17.8	18.0
	Carbenicillin	136.4	157.5	152.2	144.2	139.3	145.9	12.1	13.9	12.1	11.5	11.4	12.2
	PPM	136.4	189.4	167.2	117.6	95.7	141.2	12.1	15.1	14.0	9.9	8.3	11.9
	Streptocycline	136.4	68.5	62.2	62.4	41.7	74.2	12.1	7.0	6.4	6.2	4.0	7.1
	Mean	136.4	175.3	155.0	140.2	130.0	147.3	12.1	14.4	12.8	11.7	10.6	12.3
	Grand Mean	141.1	184.5	165.3	151.4	147.2		12.5	15.0	13.3	12.3	12.7	

	V	A	C	VA	AC	VC	VAC	V	A	C	VA	AC	VC	VAC
SEd	3.2	3.7	4.2	6.5	8.3	7.2	14.4	0.3	0.4	0.4	0.6	0.8	0.7	1.4
CD (0.05)	6.4	7.4	8.2	12.7	16.5	14.3	28.5	0.6	0.7	0.8	1.3	1.6	1.4	2.8

V: Variety; A: Antimicrobial agent; C: Concentrations; Carbenicillin (C_1-Control, C_2-50 mg/ l , C_3-75 mg/ l , C_4-100 mg/ l and C_5-125 mg/ l); Streptocycline (C_1-Control, C_2-100 mg/ l , C_3-150 mg/ l , C_4-200 mg/ l and C_5-250 mg/ l); PPM: Plant preservative mixture (C_1-Control, C_2-0.1%, C_3-0.15%, C_4-0.20% and C_5-0.25%); Cephotaxime (C_1-Control, C_2-100 mg/ l , C_3-150 mg/ l , C_4-200 mg/ l and C_5-250 mg/ l).

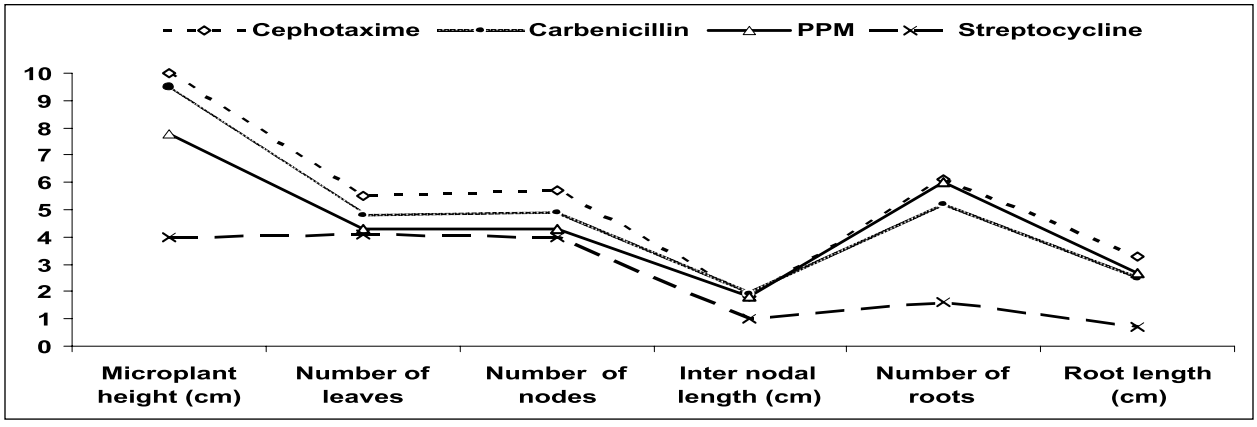

Fig. 1. Effect of different antimicrobial agents on morphological characters of Kufri Chandramukhi.

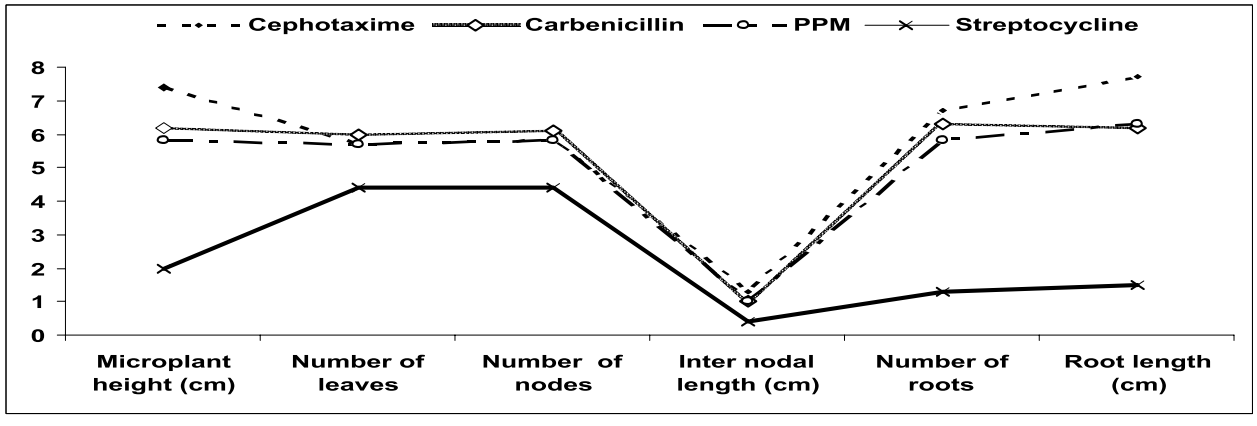

Fig. 2. Effect of different antimicrobial agents on morphological characters of Kufri Chipsona-3.

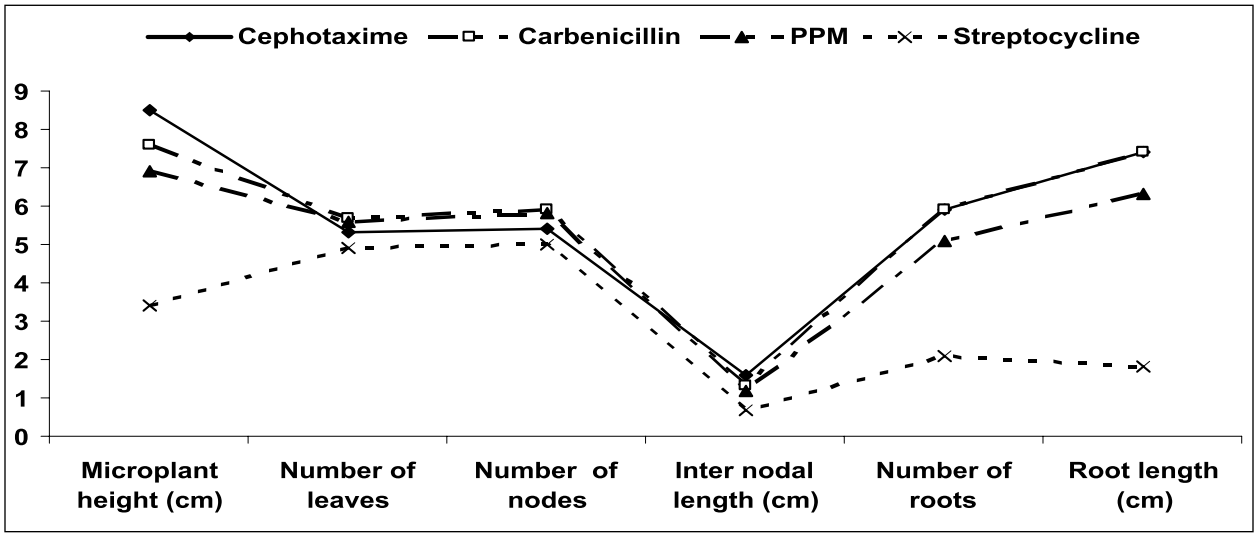

Fig. 3. Effect of different antimicrobial agents on morphological characters of Kufri Sindhuri.

due to toxic effect of the concentrations used and it needs further investigation with lower concentrations.

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