

Original Research Article

In vitro Sterilization of *Alstroemeria*hybridacv. Pluto

ABSTRACT: The present study was designed to standardize protocol for asepsis of different explants of *Alstroemeria*hybrida cv. Pluto (rhizome tips, rhizome sections, shoot tips and shoot nodal segments). Carbendazim 200 ppm for 30 minutes + mercuric chloride (0.1 %) dip for 10 minutes and final treatment with ethyl alcohol (70 %) for one minute proved best for culture asepsis of rhizome tips and rhizome sections at 2 and 4 weeks of culture., whereas Carbendazim 200 ppm for 15 minutes followed by mercuric chloride 0.1 % dip for 3 minutes proved better for culture asepsis of shoot tip and shoot nodal segments at 2 and 4 weeks of culture. Also Carbendazim 200 ppm for 30 minutes + mercuric chloride (0.1 %) dip for 10 minutes and final treatment with ethyl alcohol (70 %) for one minute proved best for culture survival of rhizome tips and rhizome sections at 4 weeks of culture., whereas Carbendazim 200 ppm for 15 minutes followed by mercuric chloride 0.1 % dip for 3 minutes proved better for culture survival of shoot tip and shoot nodal segments at 4 weeks of culture. In this study protocol for sterilization of different explants leads to the standardization of the explant for tissue culture in *Alstroemeria*hybrida cv. Pluto.

Key words: Sterilization, rhizome tip, rhizome section, shoot tip and shoot nodal segment.

1. INTRODUCTION: *Alstroemeria* hybridais a rhizomatous monocot belonging to the family *Alstroemeriaceae*. *Alstroemeria* hybrids are counted among top ten cut flowers globally owing to diversity of colours, low energy cultivation and long vase life. *Alstroemeria*hybrida is a relatively recent introduction into the world's floriculture scene and has become a major cut flower. In India crop was introduced in 2001 by Ministry of Agriculture, GOI, under Food and Agriculture Organization programme at three Model Floriculture Centers Ooty (Tamil Nadu), Chial (Himachal Pradesh) and Srinagar (Jammu & Kashmir). The crop was introduced in SKUAST-Kashmir, Shalimar in 2005-06 under ICAR sponsored Horticulture Mini Mission-I. Legal planting material imported from foreign breeder companies is still very costly ranging from Rs. 500-600/plant. Cultivars introduced in late nineties in India are currently out of the patent regime. However, planting material is not easily available due to constraints imposed on multiplication rate under conventional propagation method of rhizome division. This has been the single major stumbling block that has prevented *Alstroemeria*hybrida from becoming widely adopted by cut flower growers in Kashmir and Himachal Pradesh. *Alstroemeria*hybrida is generally propagated by rhizome division of three year old plants. However, multiplication rate is low. Propagation through seed is not commonly practiced due to variability in hybrids and

long and difficult germination. Attempts have been made by few workers to multiply *Alstroemeria* *in vitro* through rhizome tips (Hussey *et al.*, 1979; Gabryszewska and Hempel, 1985; Lin and Monette, 1987; Chiari and Bridgen, 2000) but contamination was the major bottleneck to start cultures. *In vitro* multiplication of elite genotypes of *Alstroemeria* is widely used in commercial multiplication and offers immense opportunities to multiply more number of disease free plants in shortest possible time (Lin *et al.*, 2000). *Alstroemeria* can be started with apical shoot tips but there is also a strong dominance effect suppressing the growth of axillary buds (Bond and Alderson, 1993). To promote cultivation of *Alstroemeria* there is a need to establish *in vitro* propagation protocols for the cultivars already available with research institutes and Government departments. This shall not only ensure availability of quality planting material for growers but also open up avenues for cultivar improvement through *in vitro* mutagenesis. Therefore current study involved standardization of protocol for sterilization of various explants of *Alstroemeria* cv. Pluto.

2. MATERIALS AND METHODS

The experiment was conducted in the Division of Floriculture and Landscape Architecture during the year 2012-2013 at SKUAST Kashmir. Rhizome tips, rhizome sections, shoot tips and shoot nodal segments of an orange flowered cultivar Pluto were cut from freshly dugout plants and thoroughly washed with tween-20 followed by 200 or 400 ppm Carbendazim treatment for 30 minutes. Further treatment with mercuric chloride 0.1% for 5 or 10 minutes followed by 70% ethanol dip for 30 seconds or one minute was done under laminar air flow. Explants were given final 3-4 washings with sterile water before placing on the medium. In all experiments 12 cultures per treatment were maintained each replicated four times. Data recorded was subject to statistical analysis using ANOVA.

2.1. Sterilization of culture media

Culture medium (MS solid and liquid) in test tubes/flasks was sterilized by autoclaving in a horizontal autoclave at 121°C and 1.05 kg cm⁻² (15 psi) for 20 minutes. This high temperature not only kills bacteria and fungi, but also their heat-resistant spores. Flasks and test tubes were placed in autoclave when it had developed the required pressure. Lid of the autoclave was closed and vacuum valve opened to remove air from the autoclave chamber. Vacuum valve was closed and steam inlet valve opened. Pressure indicator lamp (green lamp) switches off when required pressure (15 psi) is developed inside the autoclave chamber. Time for autoclaving was counted from the switching off of the pressure indicator lamp of the autoclave. After autoclaving the test tubes and flasks for the required time, electric supply of the autoclave was switched off and

59 exhaust valve opened to release the pressure slowly. Autoclaved medium was allowed to cool overnight at room
60 temperature and used when required but within 10 days.

61 **2.1.1.Culture Environment**

62 All the aseptic manipulations like surface sterilization, preparation and inoculation of explants and subsequent
63 sub-culturing were carried out in the laboratory using laminar air flow cabinet where pure air is forced out
64 continuously so that no contaminant is allowed to enter the cabinet during working time. Pure air is generated by
65 forcing the air to pass through a series of fine filters which remove dust and other potential contaminants. The
66 working table of laminar air flow chamber was first surface sterilized with absolute alcohol and then by
67 ultraviolet light for 30 minutes. The forceps, scalpel and Petri-dishes were first steam sterilized in an autoclave
68 at 121°C for 20 minutes. These instruments were further sterilized at the start of inoculation by pouring some
69 alcohol over them in the laminar hood followed by flaming. During inoculation forceps and blade were flame
70 sterilized several times. Hands were cleaned and wiped with Hi-Care disinfectant gel before working.

71 **2.1.2. Culture incubation**

72 The cultures were generally incubated at 24±1°C in an air conditioned culture room with a 16/8 hour light/dark
73 regime and a light intensity of 3500 lux.

74 **2.1.3. Collection of explants**

75 Different types of explants that were tried during the course of present investigation were collected from plants
76 maintained at green houses in Floriculture fields, Sher-e-Kashmir University of Agricultural Sciences and
77 Technology of Kashmir, Shalimar. The details regarding collection of different explants are as follows.

78 **2.1.4. Rhizome tips and rhizome sections**

79 Healthy plants of the *Alstroemeriaceae*. “Pluto” were dug out from the field with the help of spade and disease
80 free rhizome tips and rhizome portions were placed in clean flasks containing distilled water and brought to the
81 laboratory for further processing.

82 **2.1.5. Shoot tips and shoot nodal segments**

83 Disease free shoot tips and shoot nodal segments were separated from the mother plant and cut into smaller
84 pieces which were then placed in clean flasks containing distilled water and brought to the laboratory for further
85 processing.

86 3. Experimental parameters

87 A variety of experiments were conducted during the present investigation aimed at the development of a
88 protocol for *in vitro* propagation of *Alstroemeria* cv. "Pluto". Details regarding the methodologies adopted are
89 given in the sections that follow.

90 3.1. Standardisation of disinfection protocol for various explants in *Alstroemeria*

91 Explant surface sterilization is the first vital step for the initiation of cultures *in vitro*. Final steps in
92 surface sterilization were performed under the aseptic conditions of laminar flow hood. Knives and forceps were
93 flame sterilized before use. While making incisions, knives and forceps were flame sterilized as and when
94 thought necessary to avoid spread of contamination between different parts of the explant. Details of surface
95 sterilization procedures for different explants are given in the following sections.

96 3.1.1. Rhizome tips and rhizome sections

97 Explants on arrival in the laboratory were washed with running tap water to remove any adhering dust and
98 debris and cut into manageable size pieces (centimetres). Afterwards the explants were put in a Tween-20
99 surfactant solution (few drops) along with required quantity of fungicides and given a vigorous shake for 30
100 minutes to ensure thorough cleaning. There after the surfactant along with the fungicides was washed off under
101 running tap water followed by a final washing with single distilled water. The specimens were later brought to
102 laminar flow hood for treatment with mercuric chloride 0.1%, ethyl alcohol 70% and aseptic inoculation. The
103 details of composition of different sterilant formulations and duration of application are given in Table 1.

104 3.1.2. Shoot tips and nodal segments

105 Shoot tips and nodal segments were procured from greenhouse grown plants. These are separated with a sharp
106 scalpel. Explants on arrival in the laboratory were washed with running tap water and thereafter given a
107 vigorous shake for 15 minutes in Tween- 20 surfactant solution fortified with appropriate fungicide and
108 antibiotic concentration. The surfactant and fungicide/antibiotic solution was later removed by placing the
109 explant under running tap water and a final 2-3 rinses with single distilled water. The details regarding the
110 composition of various sterilant formulations and duration of application are given in the Table 2.

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114 **Table 1:** Composition of sterilant formulations and duration of application for surface
 115 sterilization of rhizome tips and rhizome portions in *Alstroemeriacv. Pluto*

Details of the Treatment

S ₁	Mercuric chloride 0.1 % dip for 5 minutes
S ₂	Mercuric chloride 0.1 % dip for 10 minutes
S ₃	Ethyl alcohol dip (70%) for 30 seconds
S ₄	Ethyl alcohol dip (70%) for 1 minute
S ₅	Carbendazim 200 ppm for 30 minutes followed by S ₁ and S ₃
S ₆	Carbendazim 200 ppm for 30 minutes followed by S ₂ and S ₄
S ₇	Carbendazim 400 ppm for 30 minutes followed by S ₁ and S ₃
S ₈	Carbendazim 400 ppm for 30 minutes followed by S ₂ and S ₄

116
 117 **Table 2.** Composition of sterilant formulations and duration of application for surface sterilization of
 118 shoot tips
 119 and shoot nodal segments in *Alstroemeriacv. Pluto*

S ₁	Mercuric chloride 0.1% for 3 minutes
S ₂	Mercuric chloride 0.1% for 5 minutes
S ₃	Carbendazim 200 ppm for 15 minutes + Mercuric chloride 0.1% for 3 minutes
S ₄	Carbendazim 200 ppm for 15 minutes + Mercuric chloride 0.1% for 5 minutes
S ₅	Carbendazim 400 ppm for 15 minutes + Mercuric chloride 0.1% for 3 minutes
S ₆	Carbendazim 400 ppm for 15 minutes + Mercuric chloride 0.1% for 5 minutes

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121 **4. RESULTS AND DISCUSSION**

122 **Culture asepsis and survival of rhizome tips, rhizome sections, shoot tips and shoot nodal**
 123 **segments**

124 In *Alstroemeria*, rhizome is the main explant an underground structure and carries a significant microbial load.
 125 Therefore, achieving culture asepsis and subsequent survival of explants is a significant challenge in
 126 *Alstroemeria* .In the current study, rhizome tips and rhizome sections were surface sterilized for 5.0 and 10
 127 minutes in 0.1% mercuric chloride after a 30 minute surfactant treatment fortified with 200 and 400 ppm
 128 Carbendazim and final treatment with ethyl alcohol (70 %) for one minute. Significant differences among means

in terms of per cent asepsis and subsequent survival were observed for both explants and sterilant combinations in rhizome tips and rhizome sections (Table 3 and 4). Highest culture asepsis was achieved in rhizome tips. Maximum asepsis in rhizome tips may be attributed to its lesser surface area exposed as a result of single cut at the distal end of the rhizome and hence less area for entry of potential infections. This is in comparison to rhizome sections where two cuts are made at the proximal and distal ends and hence more exposed tissue. Further rhizome tips are compact structures with relatively fewer subtending organs like roots and tubercles and hence have less quantity of extraneous material like dirt, soil etc. adhering which may have contributed to higher asepsis in comparison to rhizome sections.

Among the sterilant combinations, a 10 minute mercuric chloride (0.1 %) solution dip significantly improved culture asepsis in comparison to 5 minute dip. Ethyl alcohol 70 % treatment alone for one minute proved better (S₄) than a 30 seconds treatment with ethyl alcohol 70 % (S₃). However one minute ethyl alcohol treatment at the end of sterilization procedure involving Carbendazim 200 ppm for 30 minutes followed by mercuric chloride (0.1%) for 10 minutes under laminar flow hood significantly increased culture asepsis (79.20%) at different stages of propagation in *Alstroemeria* (Fig 1 A-D). The results indicate passing explants through fungicidal treatment followed by mercuric chloride and final treatment with ethyl alcohol is indispensable for culture asepsis in rhizome explants of *Alstroemeria*. Concentrated ethanol is a powerful disinfectant that kills majority of fungal and bacterial spores through instant dehydration. However increasing concentration of Carbendazim from 200 to 400 ppm did not improve the final level of culture asepsis any further. The difference between the two sets of treatments involving 5.0 and 10 minute mercuric chloride dip and 30 seconds and one minute ethyl alcohol (70 %) treatment underlines the importance of minimally adequate time duration of final mercuric chloride (0.1 %) for 10 minutes and ethyl alcohol (70 %) for one minute surface sterilization procedure under the laminar flow hood.

In the current study, shoot tips and shoot nodal segments were surface sterilized for 3 and 5 minute in 0.1% mercuric chloride after a 15 minute surfactant treatment fortified with 200 and 400 ppm Carbendazim. Significant differences among means were observed for both explants. Highest culture asepsis at 2 and 4 weeks of culture was achieved in shoot nodal segments rather than shoot tips (Table 5 and 6) with Carbendazim 200 ppm for 15 minutes followed by mercuric chloride (0.1%) for 3 minutes. Lower asepsis of shoot tips may be due to bud morphology that allows accumulation of extraneous material among loosely placed leaves which are also sticky. However, survival was relatively better in shoot tips than shoot nodal segments with Carbendazim 200 ppm for 15 minutes followed by mercuric chloride (0.1%) for 3 minutes (Table 8). There was less elusion of

exudates from shoot tips in comparison to shoot nodal segments. Less exudates in shoot tips may be due to more proximal position in shoots as most of the exudates flow down and accumulate in the lower portion of flowering shoots which translated into more exudate outflow *in vitro* in the latter and hence less survival. Data also point to the importance of use of carbendazim at 200 and 400 ppm in improving asepsis and survival of the explants. Shoot tips and shoot nodal segments are tender structures and hence use of ethyl alcohol is not advocated. Seyyedyousefiet al. (2013) also advocated fragments of stem containing node and internode were washed thoroughly under running tap water for 20 minutes and disinfected

Surface sterilization of rhizome tips and rhizome sections with carbendazim 200 ppm for 30 minutes followed by mercuric chloride (0.1 %) dip for 10 minutes and ethyl alcohol 70% for one minute resulted in highest per cent aseptic cultures (68.08 per cent in case of rhizome tips and 61.63 per cent in case of rhizome sections at 4 weeks of culture respectively), which is at par with asepsis attained by 5 minute mercuric chloride 0.1 % treatment followed by a 30 second 70% ethyl alcohol dip (Table 3 and 4). Rhizome tips proved to be better for starting *in vitro* cultures. Higher asepsis in rhizome tips may be attributed to its lesser surface area exposed as a result of single cut at the distal end of the rhizome and hence less area for entry of potential infections. At the end of four weeks of culture, 68.08 per cent rhizome tips survived under carbendazim 200 ppm for 30 minutes followed by mercuric chloride (0.1 %) dip for 10 minutes and ethyl alcohol 70 % for 1 minute (Table 7). Rhizome tips survived better than the rhizome sections which may be due to less injured area in the former and hence lesser quantity of exudates. In the current experiment elusion of exudates from explants was a major cause of physiological mortality *in vitro* (Fig 2 A-B). Data reveal the importance of ethyl alcohol in the eventual survival of the aseptic explants recorded at the end of 4 weeks of culture. Use of carbendazim followed by ethyl alcohol dip significantly improved culture survival both in rhizome sections and rhizome tips. Carbendazim decreased mortality due to fungal infections whereas ethyl alcohol may have contributed in survival by not only through sterilization but also due to mitigation of harmful effects of mercuric chloride. Ethyl alcohol being a dehydrant may have played a role in drawing out excess Hg^{2+} ions out of the explant tissues along with the exudates which may have significantly contributed to higher survival in explants under S_6 and S_8 sterilant treatment. Mohanty et al. (2005) advocated use of final wash in 1% hypertonic KCl solution to draw out any traces of Hg^{2+} from the explant tissue. Carbendazim, mercuric chloride and ethyl alcohol have been used by several workers to achieve the desired level of culture asepsis and survival *in vitro*. Hakkart and Versluijs (1988) initially rinsed the rhizomes of *Alstroemeria* cv's Rosario, Toledo, and Jubilee with tap water and later dipped for few seconds in 70% ethyl alcohol. Final sterilization was done in a laminar flow hood with

5 % Ca Hypochlorite (CaOCl) followed by rinsing 3 times with sterile water. In *Alstroemeria*, Pieriket *al.* (1988) reported use of 70 % ethyl alcohol for 2-3 seconds followed by 20 minute dip in 1.5 % NaOCl (with few drops of Tween 20). Jyothiet *al.* (2008) reported use of 1.0 % mercuric chloride for 8 minutes in ginger which is a plant with an underground rhizome similar in architecture to *Alstroemeria*. Sathyagowri and Seran (2011) also reported reasonable level of culture asepsis and survival with Carbendazim (0.3%) + Doxycycline (0.2%) for 10 minutes followed by 70% ethanol for 1 minute in ginger rhizome explants with buds. In the present study 10 minute dip in mercuric chloride (0.1 %) appears to be appropriate for a reasonable level of culture asepsis and to avoid mortality of explants.

Conclusion

Rhizome tips taken during the vegetative growth survived better than the other explants used during study and are considered suitable explant for starting the culture in *Alstroemeria* cv. Pluto. Maximum aseptic growing cultures were obtained with Carbendazim 200 ppm for 30 minutes followed by mercuric chloride (0.1%) treatment for 10 minutes and final treatment with ethyl alcohol (70%) dip for one minute. Thus the [present](#) study resulted in [standardized-standardizing a suitable](#) protocol for *in vitro* propagation [in-of Alstroemeria](#).

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233 **Table 3.** Influence of sterilants on culture asepsis (%) of rhizome tip explant of *Alstroemeria* cv. Pluto

Sterilants		Percent asepsis of rhizome tips at 2 weeks	Percent asepsis of rhizome tips at 4 weeks
S ₁	Mercuric chloride 0.1 % dip for 5 minutes	27.0875 (27.929)	20.84 (24.064)
S ₂	Mercuric chloride 0.1 % dip for 10 minutes	41.6625 (40.165)	33.33 (35.181)
S ₃	Ethyl alcohol (70%) dip for 30 seconds	6.2725 (8.244)	4.1875 (6.767)
S ₄	Ethyl alcohol (70%) dip for 1 minute	37.4975 (37.616)	29.1625 (32.388)
S ₅	Carbendazim 200 ppm for 30 minutes followed by S ₁ and S ₃	62.4975 (52.382)	54.1625 (47.432)
S ₆	Carbendazim 200 ppm for 30 minutes followed by S ₂ and S ₄	93.7275 (79.207)	85.4125 (68.083)

S ₇	Carbendazim 400 ppm for 30 minutes followed by S1 and S3	81.245 (64.938)	70.83 (57.608)
S ₈	Carbendazim 400 ppm for 30 minutes followed by S2 and S4	91.6525 (75.297)	83.33 (66.255)
C.D (P=0.05)		15.66	12.62

Data in parenthesis are arcsine transformed values of the percentage values

Table 4: Influence of sterilant on culture asepsis (%) of rhizome portion explant of *Alstroemeria* cv. Pluto

Sterilants		Percent asepsis of rhizome portions at 2 weeks	Percent asepsis of rhizome portions at 4 weeks
S ₁	Mercuric chloride 0.1 % dip for 5 minutes	16.68 (18.127)	12.515 (15.496)
S ₂	Mercuric chloride 0.1 % dip for 10 minutes	27.0875 (27.929)	22.9225 (25.379)
S ₃	Ethyl alcohol (70 %) dip for 30 seconds	6.2725 (8.244)	4.1875 (6.767)
S ₄	Ethyl alcohol (70 %) dip for 1 minute	29.165 (32.631)	27.08 (31.154)
S ₅	Carbendazim 200 ppm for 30 minutes followed by S1 and S3	62.4975 (52.382)	54.1625 (47.432)
S ₆	Carbendazim 200 ppm for 30 minutes followed by S2 and S4	79.1625 (63.462)	77.08 (61.634)
S ₇	Carbendazim 400 ppm for 30 minutes followed by S1 and S3	60.4125 (51.065)	52.08 (46.197)
S ₈	Carbendazim 400 ppm for 30 minutes followed by S2 and S4	74.9975 (60.159)	72.9125 (58.842)
C.D (P=0.05)		17.00	14.86

Data in parenthesis are arcsine transformed values of the percentage values

245 Table 5: Influence of sterilant on culture asepsis (%) of shoot tip explant of *Alstroemeria* cv. Pluto

Sterilants		Percent asepsis of shoot tips at 2 weeks	Percent asepsis of shoot tips at 4 weeks
S ₁	Mercuric chloride dip for 3 minutes	74.9975 (60.159)	60.4125 (51.065)
S ₂	Mercuric chloride dip for 5 minutes	62.495 (52.264)	52.08 (46.197)
S ₃	Carbendazim 200 ppm for 15 minutes followed by S ₁	83.33 (66.255)	74.9975 (60.159)
S ₄	Carbendazim 200 ppm for 15 minutes followed by S ₂	79.1625 (63.462)	66.6625 (54.815)
S ₅	Carbendazim 400 ppm for 15 minutes followed by S ₁	74.9975 (60.159)	62.4975 (52.382)
S ₆	Carbendazim 400 ppm for 15 minutes followed by S ₂	64.58 (53.581)	54.1625 (47.432)
C.D (P= 0.05)		7.81	7.71

246 Data in parenthesis are arcsine transformed values of the percentage values

247
248 Table 6 Influence of sterilants on culture asepsis (%) of shoot nodal segment explant of *Alstroemeria* cv. Pluto

Sterilant		Percent asepsis of shoot nodal segments after 2 weeks	Percent asepsis of shoot nodal segments after 4 weeks
S ₁	Mercuric chloride dip for 3 minutes	79.1625 (63.462)	64.58 (53.581)
S ₂	Mercuric chloride dip for 5 minutes	64.58 (53.581)	47.9125 (43.798)
S ₃	Carbendazim 200 ppm for 15 minutes followed by S ₁	87.485 (71.959)	79.1625 (63.462)
S ₄	Carbendazim 200 ppm for 15 minutes followed by S ₂	79.1625 (63.462)	70.83 (57.608)
S ₅	Carbendazim 400 ppm for 15 minutes followed by S ₁	74.9975 (60.159)	66.6625 (54.815)
S ₆	Carbendazim 400 ppm for 15 minutes followed by S ₂	64.58 (53.581)	47.9125 (43.798)
C.D (P=0.05)		11.32	8.45

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250 Data in parenthesis are arcsine transformed values of the percentage values

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254 **Table 7 :** Influence of sterilant treatments on survival of rhizome tip and rhizome section explants
 255 of *Alstroemeria* cv. Pluto

Sterilants		Rhizome tip Survival *	Rhizome section Survival*
S ₁	Mercuric chloride dip (0.1%) for 5 minutes	20.84 (24.06)	6.27 (8.24)
S ₂	Mercuric chloride dip (0.1%) for 10 minutes	33.33 (35.18)	16.68 (18.12)
S ₃	Ethyl alcohol (70%) dip for 30 seconds	4.18 (6.76)	4.18 (6.76)
S ₄	Ethyl alcohol (70%) dip for 1 minute	29.16 (32.38)	24.99 (29.83)
S ₅	Carbendazim 200 ppm for 30 minutes followed by S ₁ and S ₃	54.16 (47.43)	49.99 (44.99)
S ₆	Carbendazim 200 ppm for 30 minutes followed by S ₂ and S ₄	85.41 (68.08)	74.99 (60.15)
S ₇	Carbendazim 400 ppm for 30 minutes followed by S ₁ and S ₃	70.83 (57.60)	49.99 (44.99)
S ₈	Carbendazim 400 ppm for 30 minutes followed by S ₂ and S ₄	83.33 (66.25)	68.74 (56.13)
C.D_(p≤0.05)		14.31	14.87

256 Values in parenthesis are arcsine transformed data in per cent

257 *Data recorded at 4 weeks of culture

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259 **Table 8 :** Influence of sterilants on survival percent of shoot tip and shoot nodal segments of
 260 *Alstroemeria* cv. pluto
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Sterilants		Shoot tips survival (%)	Shoot nodal segments survival (%)
S ₁	Mercuric chloride 0.1% dip for 3 minutes	41.22 (39.77)	42.56 (40.58)
S ₂	Mercuric chloride 0.1% dip for 5 minutes	39.78 (32.11)	40.49 (35.87)
S ₃	Carbendazim 200 ppm for 15 minutes followed by T ₁	68.7475 (56.13)	62.4975 (52.38)

S ₄	Carbendazim 200 ppm for 15 minutes followed by T ₂	50.23 (44.63)	54.1625 (47.43)
S ₅	Carbendazim 400 ppm for 15 minutes followed by T ₁	54.1625 (47.43)	58.33 (49.83)
S ₆	Carbendazim 400 ppm for 15 minutes followed by T ₂	45.83 (42.56)	47.58 (39.37)
C.D		9.33	9.06

Data in parenthesis are arcsine transformed values of the percentage values

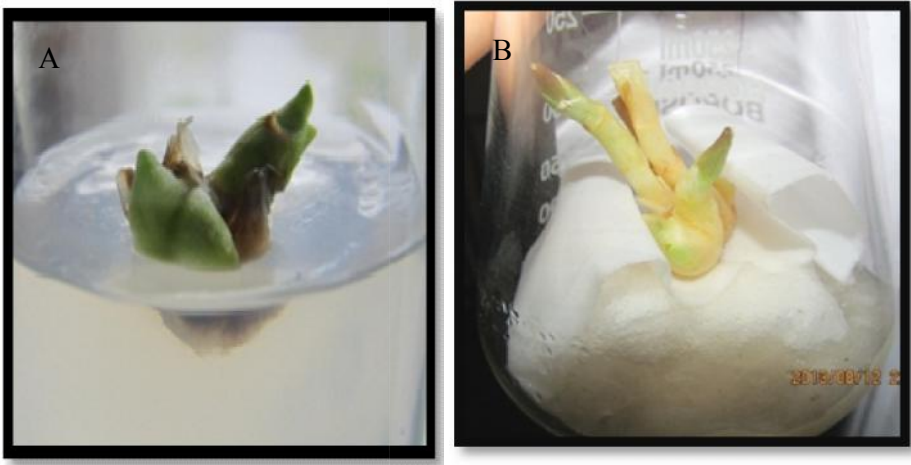


Fig 1A*: Aseptic Establishment of rhizome tips in solid media Fig 1B*: Aseptic Establishment of rhizome tips in liquid media

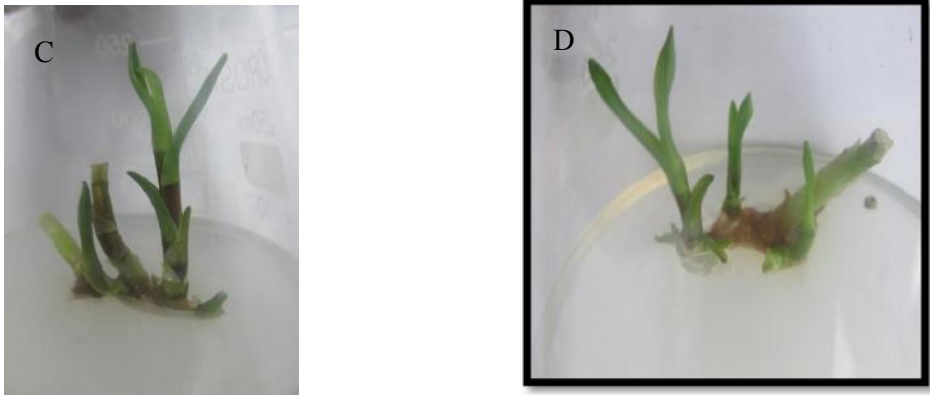


Fig 1C*: Aseptic proliferation of rhizome tips in solid media Fig 1D*: Aseptic proliferation of rhizome tips in liquid media

Fig 1. A-D) Aseptic cultures at different stages of propagation: A) Establishment of rhizome tips in solid media, B) Establishment of rhizome tips in liquid media. C) and D) Proliferation of rhizome tips in solid media

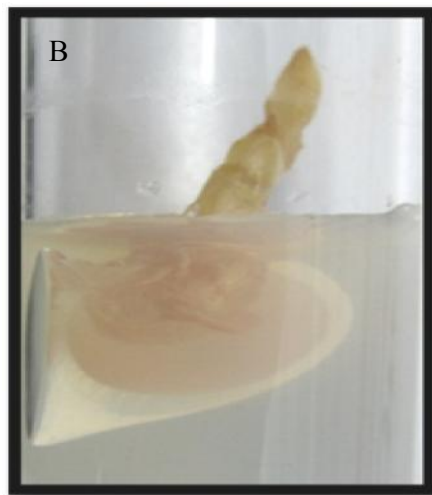
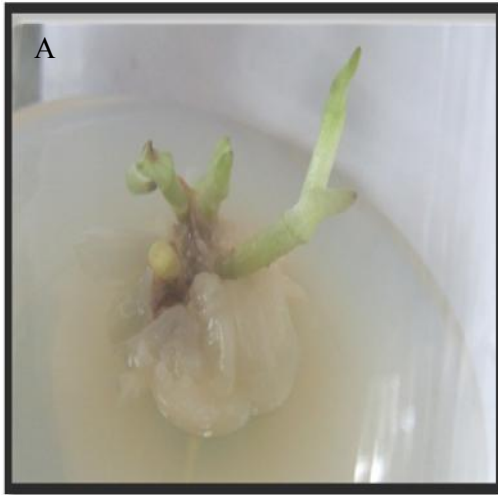


Fig 2. A- Exudate production from rhizome tips in solid media Fig 2. B- Exudate production from shoot tips in solid media

Fig 2. A-B) Exudate production from explants in solid media: A) Rhizome tips and B) shoot tips