

EFFECT OF DIFFERENT SEED TREATMENT AND MEDIA ON SHOOT GROWTH OF ASHWAGANDHA *Withaniasomnifera(L.)Dunal*

ABSTRACT

Indian Ginseng or Ashwagandha, is one of the most important herbs in Ayurvedic system of medicine and one of the most widely used Indian medicinal plant throughout the world. Under the present study Effect of different seed treatment on shoot growth of Ashwagandha - *Withaniasomnifera* (L.) *Dunal* Seeds soaked with treatment (S₁) GA₃ (250 ppm - 24 h.) recorded maximum germination percentage (66.11 %) and maximum plant height at 15 (3.00), 30 (8.38), 45 (15.98), 60 (25.20), 75 (34.05), 90 (42.98), 105 (52.07) and 120 (59.02) days, respectively. Maximum no. of branches/plant at 15 (0.0), 30 (0.22), 45 (0.71), 60 (1.73), 75 (1.76), 90 (2.80), 105 (3.0) and 120 (3.84) and no. of leaves/plant were also recorded higher in S₁ at 15 (3.42), 30 (7.67), 45 (20.42), 60 (33.11), 75 (41.62), 90 (55.98), 105 (67.24) and 120 (75.87) DAS respectively. In the Similar treatment, no. of leaves was also found superior over other treatments with maximum total leaf area (33.57 cm²), shoot fresh weight (55.06 g), shoot dry biomass (38.34 g), plant fresh weight (60.10 g) and plant dry weight (18.77 g) as compared to other treatments applied.

Media combination Red soil + Vermicompost + FYM (1:1:1) which was treatment (M₃) gave significantly highest result in context to seed germination percentage (71.94 %), total leaf area (32.08 cm²), shoot fresh weight (58.70 g), shoot dry biomass (40.91 g), plant fresh weight (64.05 g) and plant dry weight (19.82 g), respectively. In same treatment, highest plant height at 15 (3.29), 30 (9.78), 45 (18.14), 60 (26.85), 75 (35.54), 90 (44.13), 105 (55.03) and 120 (60.17), no. of branches/plant at 15 (0.0), 30 (0.27), 45 (0.68), 60 (1.89), 75 (1.97), 90 (2.87), 105 (2.90), 120 (3.83) and no. of leaves/plant at 15 (3.72), 30 (8.46), 45 (22.46), 60 (35.23), 75 (43.91), 90 (55.87), 105 (67.47) and 120 (75.92) respectively were recorded.

Interaction effect between pre-sowing treatment and different media was observed significant in seed germination percentage and maximum (80 %) were noted in the treatment S₁M₃.

Keywords:Seed treatment, Media, Ashwagandha and GA₃

INTRODUCTION

Withaniasomnifera also known as Ashwagandha or Indian ginseng with chromosome complementation is 2n=48. It is one of the most important herbs in Ayurvedic system of medicine and one of the most widely used Indian medicinal plant throughout the world. Ayurvedic physicians and traditional healers have used it extremely in wide range of therapeutic indications. It derives its name “Ashwagandha” because of two reasons. First reason is that fresh root smells like horse urine and other reason is that it vitalizes body to provide strength just like horse. Ashwagandha also popularly known as “prince of herb”because it has wide therapeutic uses. The plant was first mentioned in English language

text by Van Rheede in 1868. (Desai and Dumbre, 2003). The use of Ashwagandha in ayurvedic medicines extends back over 3000 to 4000 years to the teaching of an esteemed Rishi (sage) Punarvasu Atriya. It has been described in the sacred texts of Ayurveda, including the Charaka and Sushruta Samhitas where it is widely extolled as a tonic, especially for fecundation in people of all ages including babies, enhancing the reproductive function of both men and women. (Kurian *et al.*, 2007).

MATERIALS AND METHODS

The experiment was laid out at Model Nursery on Medicinal and Aromatic Plants, NAU, Navsari. In the present experiment Pre-sowing Treatment *viz.*, GA₃, Kinetin, NaHClO₃ and KNO₃ along with control were applied in different concentrations to study their effects on growth of *Withania somnifera*. Seeds of Ashwagandha plants were treated with GA₃ @ 250 ppm for 24 h, Kinetin (0.2 %) for 24 h, KNO₃ (250 ppm) for 1 min., NaHClO₃ 4 % for 5 min and 10 min respectively along with control to give pre-sowing treatment on seed. Good quality Red earth was used and it was filled in to 7' × 9' (15 x 20 cms) sized perforated polythene bags. Polythene bags were kept under shade in a greenhouse for 4 months and irrigated by water-cane daily. Observations were taken for days required for germination percentage (10 DAS), plant height (cm) (at 15, 30, 45, 60, 75, 90, 105 and 120 DAP), branches /plant (at 15, 30, 45, 60, 75, 90, 105 and 120 DAP), leaves / branch (at 15, 30, 45, 60, 75, 90, 105 and 120 DAP), shoot fresh weight, shoot dry biomass, fresh weight of plant (g), dry weight of plant (g), Leaf area (cm²) was estimated by Image J software (Abramoff *et al.*, 2004).

RESULTS AND DISCUSSION

SHOOT CHARACTERS:

1. Germination Percentage (%)

Pre-sowing treatments applied (S₁) [GA₃- (250 ppm) - 24 hrs] gave higher germination percentage (66.11 %), which was at par with treatment (S₅) [KNO₃- (250 ppm)-1 min.] value of 64.44 % (Table 1). This result was similar with the one obtained by Niyaz and Siddiqui (2014) in *Withania somnifera* L. and Krishna (2014) in *Withania somnifera* L., KNO₃ also gave good germination percentage after GA₃.

The germination percentage (Table 1) was also influenced considerably by the different media applied where highest germination percentage (71.94 %) was observed in treatment (M₃) [Red soil + Vermicompost + FYM- 1:1:1]. This result is similar with the earlier findings by Manohar *et al.* (2012) in *Withania somnifera* and Vishnoi *et al.* (2011) in *Terminalia arjuna*.

The interaction effect of Pre-sowing treatments and different media was significant on germination percentage (%). Higher germination percentage (80 %) was recorded (Table 1) in the treatment S₁M₃ which was seed treated with GA₃ (250 ppm - 24 h.) sowed in red soil + vermicompost + FYM (1:1:1) and at par with treatment combination of S₅M₃ (75 %).

2. Plant height (cm) at 15, 30, 45, 60, 75, 90 105 and 120 DAP

The effect of different pre-sowing treatments on plant height (Table 1) was recorded at 15 days interval. The maximum plant height was recorded in treatment (S₁) [GA₃- (250

ppm)- 24 hrs] at 15 (3.00), 30 (8.38), 45 (15.98), 60 (25.20), 75 (34.05), 90 (42.98), 105 (52.07) and 120 (59.02) DAS, it was at par with treatment (S₅) [KNO₃- (250 ppm)- 1 min.]. These observations are similar with the one obtained result by Tzortzakis (2009) in *Cichorium endivia* and *Cichorium intybus* L., Also Shukla and Shukla (2012) in *Withaniasomnifera* L.

The effect of different media on have significant effect on plant height (Table 1). The highest plant height was recorded in treatment (M₃) [Red soil + Vermicompost + FYM- 1:1:1] at 15 (3.29), 30 (9.78), 45 (18.14), 60 (26.85), 75 (35.54), 90 (44.13), 105 (55.03) and 120 (60.17) DAS respectively. Similar findings as reported by Manohar *et al.* (2012) in *Withaniasomnifera* and Vishnoi *et al.* (2011) in *Terminalia arjuna*.

3. No. of Branches/plant at 15, 30, 45, 60, 75, 90 105 and 120 DAP

The data pertaining to the number of branches per plant as affected by different pre-sowing treatments are shown in Table 2. The maximum no. of branches per plant was recorded in the treatment (S₁) [GA₃- (250 ppm)- 24 hrs] at 15 (0.0), 30 (0.22), 45 (0.71), 60 (1.73), 75 (1.76), 90 (2.80), 105 (3.0) and 120 (3.84) DAS respectively. The obtained data might be ascribed to GA₃ have important physiological property to regulate shoot apical dominance in plants and it stimulates the lateral buds so ultimately it increases the number of branches per plant. These results are in harmony with Tzortzakis (2009) in *Cichorium endivia* and *Cichorium intybus* L., Also Shukla and Shukla (2012) in *Withaniasomnifera* L.

The effect on production of lateral shoots plant was also modified by different media. The highest no. of branches per plant was recorded in treatment (M₃) [Red soil + Vermicompost + FYM- 1:1:1] at 15 (0.0), 30 (0.27), 45 (0.68), 60 (1.89), 75 (1.97), 90 (2.87), 105 (2.90) and 120 (3.83) DAS, respectively. Similar results were noticed by Manohar *et al.* (2012) in *Withaniasomnifera* and Vishnoi *et al.* (2011) in *Terminalia arjuna*.

4. No. of Leaves/plant at 15, 30, 45, 60, 75, 90 105 and 120 DAP

The maximum no. of leaves per plant was significantly recorded (Table 3) higher in treatment (S₁) [GA₃- (250 ppm)- 24 hrs] at 15 (3.42), 30 (7.67), 45 (20.42), 60 (33.11), 75 (41.62), 90 (55.98), 105 (67.24) and 120 (75.87) DAS, respectively. This might be due to GA₃ have important physiological property to regulate shoot apical dominance in plants and it stimulates the lateral buds so ultimately it increases the no. of leaves per plant. Similar findings as reported by Tzortzakis (2009) in *Cichorium endivia* and *Cichorium intybus* L., Also Shukla and Shukla (2012) in *Withaniasomnifera* L.

The seed sown in treatment (M₃) [Red soil + Vermicompost + FYM- 1:1:1] recorded (Table 3) significantly highest no. of leaves at 15 (3.72), 30 (8.46), 45 (22.46), 60 (35.23), 75 (43.91), 90 (55.87), 105 (67.47) and 120 (75.92) DAS, respectively. These results are in harmony with Manohar *et al.* (2012) in *Withaniasomnifera* and Vishnoi *et al.* (2011) in *Terminalia arjuna*.

5. Total leaf area (cm²)

The total leaf area as affected by various pre-sowing treatments of ashwagandha seeds applied are presented in Table 4. The total leaf area recorded significantly highest (33.57 cm²) in treatment (S₁) [GA₃- (250 ppm)- 24 hrs]. These observations are also in

accordance with the results obtained by Tzortzakis (2009) in *Cichorium endivia* and *Cichorium intybus* L., Shukla and Shukla (2012) in *Withaniasomnifera* L.

Maximum total leaf area (32.08 cm²) was recorded (Table 4) with treatment (M₃) [Red soil + Vermicompost + FYM- 1:1:1].

6. Shoot fresh weight (At final stage)

The highest fresh weight of shoot (55.06 g) was recorded (Table 4) significantly higher in treatment (S₁) [GA₃ (250 ppm)- 24 hrs]. which was statistically at par with treatment (S₅) [KNO₃ (250 ppm)- 1 min.] and (S₂) [Kinetin (0.2 %)– 24 hrs] with the values of 53.88 g and 53.31 g. Similar result was found by Tzortzakis (2009) in *Cichorium endivia* and *Cichorium intybus* L., Shukla and Shukla (2012) in *Withaniasomnifera* L.

The highest Shoot fresh weight (58.70 g) was registered (Table 4) in treatment (M₃) which was Red soil + Vermicompost + FYM- (1:1:1). These results are in harmony with Manohar *et al.* (2012) in *Withaniasomnifera* and Vishnoi *et al.* (2011) in *Terminalia arjuna*.

7. Shoot dry biomass (At final stage)

The maximum shoot dry biomass (38.34 g) was reported (Table 4) in the treatment (S₁) [GA₃ (250 ppm)- 24 hrs], which was at par with the treatment (S₅) [KNO₃ (250 ppm)- 1 min.] with a value of 38 g. Similar findings as reported by Tzortzakis (2009) in *Cichorium endivia* and *Cichorium intybus* L., Shukla and Shukla (2012) in *Withaniasomnifera* L.

Shoot dry biomass (Table 4) was highest (40.91 g) in treatment (M₃) [Red soil + Vermicompost + FYM- 1:1:1]. Similar result was found by Manohar *et al.* (2012) in *Withaniasomnifera* and Vishnoi *et al.* (2011) in *Terminalia arjuna*.

8. Fresh weight of plant (At final stage)

The plant fresh weight recorded (Table 4) was higher (60.10 g) in treatment (S₁) [GA₃ (250 ppm)- 24 hrs] which was at par with treatment (S₅) [KNO₃ (250 ppm)- 1 min.] with the values of 58.91 g. It might be due to GA₃ have important physiological property to regulate shoot apical dominance in plants and it stimulates the lateral buds so ultimately it increases the plant fresh weight. Similar finding as reported by Tzortzakis (2009) in *Cichorium endivia* and *Cichorium intybus* L., Shukla and Shukla (2012) in *Withaniasomnifera* L.

It is also further seen from Table 4 that the various media also have significant effect on the plant fresh weight. The highest plant fresh weight (64.05 g) was registered in treatment (M₃) [Red soil + Vermicompost + FYM- 1:1:1].

Table 1: Effect of different seed treatments and media ongermination % andplant height (cm)in ashwagandha (<i>Withaniasomnifera</i>)									
Treatment	Germination %	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	105 DAS	120 DAS
SEED TREATMENT (S)									
S ₁ : GA ₃ (250 ppm - 24 h.)	66.11	3.00	8.38	15.98	25.20	34.05	42.98	52.07	59.02
S ₂ : Kinetin (0.2% - 24 h.)	62.22	2.68	7.55	13.93	23.40	31.96	41.39	50.03	56.80
S ₃ : NaHClO ₃ (4% - 5 min.)	59.44	2.30	6.69	13.79	23.33	31.77	39.44	47.87	54.82
S ₄ : NaHClO ₃ (4% - 10 min.)	60.56	2.55	6.97	13.82	23.50	31.56	40.67	48.87	55.71
S ₅ : KNO ₃ (250 ppm- 1 min.)	64.44	2.82	8.19	15.80	24.89	33.51	42.24	51.22	58.06
S ₆ : Control	61.11	2.62	7.34	14.81	24.01	33.11	41.09	49.96	56.76
S. Em. ±	1.57	0.14	0.26	0.33	0.35	0.63	0.78	0.85	0.86
C.D. at 5 %	3.19	0.29	0.52	0.66	0.71	1.27	1.57	1.73	1.75
MEDIA (M)									
M ₁ : Red Soil	51.94	1.93	5.86	11.64	21.12	29.66	38.50	46.93	53.55
M ₂ : Red Soil + Vermicompost (1:1)	63.06	2.76	6.92	14.29	24.19	32.94	41.27	50.03	56.87
M ₃ : Red Soil + Vermicompost + FYM (1:1:1)	71.94	3.29	9.78	18.14	26.85	35.54	44.13	53.05	60.17
S. Em. ±	1.11	0.10	0.18	0.23	0.25	0.44	0.55	0.60	0.61
C.D. at 5 %	3.19	0.29	0.52	0.66	0.71	1.27	1.57	1.73	1.75
Mean of S x M									
S ₁ M ₁	51.67	-	-	-	-	-	-	-	-
S ₁ M ₂	66.67	-	-	-	-	-	-	-	-
S ₁ M ₃	80.00	-	-	-	-	-	-	-	-
S ₂ M ₁	56.67	-	-	-	-	-	-	-	-
S ₂ M ₂	63.33	-	-	-	-	-	-	-	-
S ₂ M ₃	70.00	-	-	-	-	-	-	-	-
S ₃ M ₁	46.67	-	-	-	-	-	-	-	-
S ₃ M ₂	55.00	-	-	-	-	-	-	-	-
S ₃ M ₃	65.00	-	-	-	-	-	-	-	-
S ₄ M ₁	50.00	-	-	-	-	-	-	-	-
S ₄ M ₂	58.33	-	-	-	-	-	-	-	-
S ₄ M ₃	68.33	-	-	-	-	-	-	-	-
S ₅ M ₁	53.33	-	-	-	-	-	-	-	-
S ₅ M ₂	66.67	-	-	-	-	-	-	-	-
S ₅ M ₃	75.00	-	-	-	-	-	-	-	-
S ₆ M ₁	55.00	-	-	-	-	-	-	-	-
S ₆ M ₂	61.67	-	-	-	-	-	-	-	-
S ₆ M ₃	65.00	-	-	-	-	-	-	-	-
S.Em.± (D x N)	2.72	0.24	0.44	0.56	0.60	1.08	1.34	1.47	1.49
C.D. at 5 % (D x N)	7.81	NS	NS	NS	NS	NS	NS	NS	NS
C.V. %	7.56	15.89	10.22	6.66	4.35	5.74	5.64	5.10	4.54

Table 2: Effect of different seed treatments and media on no. of branches in ashwagandha (<i>Withaniasomnifera</i>)								
Treatment	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	105 DAS	120 DAS
SEED TREATMENT (S)								
S ₁ : GA ₃ (250 ppm - 24 h.)	0	0.22	0.71	1.73	1.76	2.80	3.0	3.84
S ₂ : Kinetin (0.2% - 24 h.)	0	0.11	0.33	1.16	1.22	2.09	2.18	3.51
S ₃ : NaHClO ₃ (4% - 5 min.)	0	0.07	0.33	1.11	1.20	2.16	2.16	3.02
S ₄ : NaHClO ₃ (4% - 10 min.)	0	0.07	0.42	1.27	1.38	2.38	2.40	3.49
S ₅ : KNO ₃ (250 ppm- 1 min.)	0	0.16	0.51	1.64	1.71	2.51	2.56	3.56
S ₆ : Control	0	0.09	0.40	1.40	1.56	2.40	2.47	3.24
S. Em. ±	0	0.04	0.07	0.10	0.08	0.09	0.09	0.12
C.D. at 5 %	0	0.08	0.14	0.20	0.17	0.19	0.18	0.24
MEDIA (M)								
M ₁ : Red Soil	0	0.03	0.32	0.97	1.11	2.08	2.10	3.11
M ₂ : Red Soil + Vermicompost (1:1)	0	0.06	0.36	1.30	1.33	2.21	2.27	3.39
M ₃ : Red Soil + Vermicompost + FYM (1:1:1)	0	0.27	0.68	1.89	1.97	2.87	2.90	3.83
S. Em. ±	0	0.03	0.05	0.07	0.06	0.07	0.06	0.08
C.D. at 5 %	0	0.08	0.14	0.20	0.17	0.19	0.18	0.24

Table 3: Effect of different seed treatments and media on no. of leaves/plant in ashwagandha (<i>Withaniasomnifera</i>)								
Treatment	15 DAS	30 DAS	45 DAS	60 DAS	75 DAS	90 DAS	105 DAS	120 DAS
SEED TREATMENT (S)								
S ₁ : GA ₃ (250 ppm - 24 h.)	3.42	7.67	20.42	33.11	41.62	55.98	67.24	75.87
S ₂ : Kinetin (0.2% - 24 h.)	2.76	6.49	17.13	30.24	39.58	51.04	60.73	73.53
S ₃ : NaHClO ₃ (4% - 5 min.)	2.43	6.20	16.60	29.20	39.13	50.36	61.16	70.56
S ₄ : NaHClO ₃ (4% - 10 min.)	2.64	6.38	17.24	30.07	39.80	52.11	62.76	71.89
S ₅ : KNO ₃ (250 ppm- 1 min.)	3.11	7.36	19.62	33.22	42.71	55.27	64.62	73.64
S ₆ : Control	2.84	6.64	18.50	31.63	41.07	52.67	64.20	71.00
S. Em. ±	0.14	0.20	0.40	0.54	0.73	0.90	1.10	1.24
C.D. at 5 %	0.28	0.40	0.81	1.09	1.48	1.84	2.22	2.51
MEDIA (M)								
M ₁ : Red Soil	2.16	5.51	13.57	26.60	37.38	49.57	60.64	70.08
M ₂ : Red Soil + Vermicompost (1:1)	2.73	6.40	18.73	31.91	40.67	53.12	62.24	72.24

M ₃ : Red Soil + Vermicompost + FYM (1:1:1)	3.72	8.46	22.46	35.23	43.91	55.87	67.47	75.92
S. Em. ±	0.10	0.14	0.28	0.38	0.51	0.67	0.77	0.87
C.D. at 5 %	0.28	0.40	0.81	1.09	1.48	1.91	2.22	2.51

Table 4: Effect of different seed treatments and media on ashwagandha (<i>Withaniasomnifera</i>)					
Treatment	Total leaf area (cm²)	Shoot fresh weight (g)	Shoot dry biomass	Fresh weight of plant	Dry weight of plant (g)
SEED TREATMENT (S)					
S ₁ : GA ₃ (250 ppm - 24 h.)	33.57	55.06	38.34	60.10	18.77
S ₂ : Kinetin (0.2% - 24 h.)	30.27	53.31	36.53	57.93	18.14
S ₃ : NaHClO ₃ (4% - 5 min.)	27.95	50.91	35.46	55.22	16.77
S ₄ : NaHClO ₃ (4% - 10 min.)	29.82	51.71	35.68	55.84	17.47
S ₅ : KNO ₃ (250 ppm- 1 min.)	31.22	53.88	38.00	58.91	18.72
S ₆ : Control	30.23	52.35	36.11	56.97	17.18
S. Em. ±	0.58	0.89	0.76	0.97	0.48
C.D. at 5 %	1.17	1.80	1.55	1.96	0.98
MEDIA (M)					
M ₁ : Red Soil	28.45	45.99	31.33	50.0	16.25
M ₂ : Red Soil + Vermicompost (1:1)	30.99	53.92	37.82	58.44	17.87
M ₃ : Red Soil + Vermicompost + FYM (1:1:1)	32.08	58.70	40.91	64.05	19.82
S. Em. ±	0.41	0.63	0.54	0.68	0.34
C.D. at 5 %	1.17	1.80	1.55	1.96	0.98

9. Dry weight of plant (g)

The present data (Table 4) on plant dry weight reveals that the higher (18.77 g) plant dry weight was recorded in treatment (S₁) [GA₃ (250 ppm)- 24 hrs], which was at par with the treatment (S₅) [KNO₃(250 ppm)- 1 min.] and (S₂) [Kinetin (0.2 %)- 24 hrs] with the values of 18.72 g and 18.14 g, respectively.

It is also observed from Table 4 that the various media also have significant effect on the plant dry weight. The highest plant dry weight (19.82 g) was registered in treatment (M₃) [Red soil + Vermicompost + FYM- 1:1:1].

CONCLUSION

Based on the investigative findings, it can be inferred that treatment S₁, involving the application of GA₃ at a concentration of 250 ppm for a 24-hour duration, as well as treatment M₃ (consisting of a mixture of Red Soil, Vermicompost, and FYM in a 1:1:1 ratio), exhibited superior performance across various parameters including germination percentage, plant height, number of branches per plant, number of leaves per plant, total leaf area, fresh weight of shoots, and both fresh and dry weights of the plant, outperforming the other applied treatments.

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