

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

Comment [NR Ranwa1]:

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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 the Effect of different seed treatment on shoot growth of Ashwagandha *Withania somnifera* (L.) Dunal Seeds seeds were 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 were 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.

Comment [NR Ranwa2]: herbal plant grows throughout the world and used in Ayurvedic system of medicine.

The treatment (M₃) Media-media combination of Red soil + Vermicompost + FYM (1:1:1) which was treatment (M₃) gave significantly highest results 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

Ashwagandha, *Withania somnifera* (L.) Dunal also known as Ashwagandha or Indian ginseng or prince of herb with have chromosome complementation is 2n=48. It is one of the most important herbs plant of medicinal importance and widely grown in various parts of the world and used 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 its 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 Rheedee 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 ~~Prepre-sowing Treatment-treatments~~ viz., GA₃, Kinetin, NaHClO₃ and KNO₃ along with control were applied in different concentrations to study their effects on growth of ~~Withania-W.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 ~~before sowing, were along with control to gave pre-sowing treatment on seed~~. Good ~~quality-fertile~~ Red-red earth was used ~~for grow the plants~~ 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-leaf~~ area (cm²) was estimated by Image J software (Abramoff *et al.*, 2004).

RESULTS AND DISCUSSION

SHOOT CHARACTERS:

1. Germination Percentage (%)

~~The data presented in table 1 showed that maximum germination percentage (66.11 %) were observed in the pre sowing seed treatment of (S₁) [GA₃- (250 ppm) - 24 hrs] followed by 64.44 per cent in (S₅) [KNO₃- (250 ppm)-1 min.] and both were remained at par to each other. Pre-sowing treatments applied (S₄) [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 findings of one obtained result by Niyaz and Siddiqui (2014) in *Withania somnifera* L. and Krishna (2014) in *Withania somnifera* L., KNO₃ who also reported that gave good germination percentage after GA₃.~~

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

The interaction effect of ~~Prepre-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.)

Comment [NR Ranwa3]: Clear the findings and rewrite.

sowed in red soil + vermicompost + FYM (1:1:1) and at par with treatment combination of S₃M₃ (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~~ were 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 findings of~~ Tzortzakis (2009) in *Cichoriumendivia* and *Cichoriumintybus* L., ~~Also~~ Shukla and Shukla (2012) in *Withania somnifera* L.

The effect of different media ~~on have significant effect~~ on plant height was also found significant as compared to control (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 *Withania somnifera* and Vishnoiet *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 *Cichoriumendivia* and *Cichoriumintybus* L., ~~Also~~ Shukla and Shukla (2012) in *Withania somnifera* 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 also~~ obtained by Manohar *et al.* (2012) in *Withania somnifera* and Vishnoiet *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 also~~ reported by Tzortzakis (2009) in *Cichoriumendivia* and *Cichoriumintybus* L., ~~Also~~ Shukla and Shukla (2012) in *Withania somnifera* 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 the observations of Manohar *et al.* (2012) in *Withania somnifera* 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 data shows that 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 *Withania somnifera* L.

Maximum total leaf area (32.08 cm²) was recorded (Table 4) with in the treatment of (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 results was—were found by Tzortzakis (2009) in *Cichorium endivia* and *Cichorium intybus* L., Shukla and Shukla (2012) in *Withania somnifera* L.

The highest ~~Shoot-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 *Withania somnifera* 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— are also reported by Tzortzakis (2009) in *Cichorium endivia* and *Cichorium intybus* L., Shukla and Shukla (2012) in *Withania somnifera* 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 *Withania somnifera* 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 *Withania somnifera* 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].

UNDER PEER REVIEW

Table 1: Effect of different seed treatments and media on germination % and plant height (cm) in ashwagandha (<i>Withania somnifera</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

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

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>Withania somnifera</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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Comment [NR Ranwa4]: Follow the same pattern in all reference as per journal format.

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