Improvement in Growth, Yield and Thymol Content of *Trachyspermum ammi* (L.) Sprague

**Introduction:**

At present botanicals and herbs are indicating their comeback all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human health and environment. Although herbs had been priced for their medicinal, flavoring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. But now the blind dependence on synthetics is over and today whole world is returning to the naturals with hope of safety and security.

The survey of literature indicated that scanty research has been done on improvement in growth, yield and physiology of medicinal plants in general and aromatic plants in particular. Amongst them Ajowan (*Trachyspermum ammi* L.) which is in great demand in domestic and world market is less attempted. Hence, in present investigation emphasis was given on improvement of its growth, yield and essential oil by using PGRs and micronutrients. At the same time its tolerance to salinity and drought stress was also tested in field conditions by focusing on the following objectives.

**Objectives:**

- To analyze the effect of different plant growth regulators, micronutrients, salt and water stress on seed germination, seedling growth and physiology of Ajowan.
- To evaluate the effects of PGRs, micronutrients, salt and water stress on physiological, biochemical and enzymological aspects during growth and development of field grown Ajowan.
- To determine the improvement in growth, yield and thymol content of Ajowan by using PGRs, micronutrients and induction of abiotic stress conditions.

**Material and Methods:**

The authentic seeds of *Trachyspermum ammi* (L.) var. NRCSS AA-2 were procured from National Research Centre for Seed Spices, Tabiji Farm, Ajmer.
(Rajasthan). Seed germination, seedling growth and physiology was studied using standard methods. The treatment details were as follows: NAA, GA$_3$ (10, 25 and 50 mg/L) and KIN (2, 5, 10 mg/L), MnSO$_4$, FeSO$_4$ and ZnSO$_4$ (25, 50 and 100 mg/L), NaCl (25, 50, 100 mM) and PEG 6000 (-0.5, -1.0, and -1.5 bars).

The field experiments were conducted at College of Agriculture, Shivajinagar, Pune (411005) M.S (India) during 2010-2013 using RBD in Triplicate and the plot size was 6M X 2.5 M. The treatment details were: NAA, GA$_3$ (25, 50, 100 mg/L) and KIN (5, 10, 20 mg/L), MnSO$_4$, FeSO$_4$ and ZnSO$_4$ (50, 100, 200 mg/L), NaCl (50, 100, 150 mM) and water stress was given by with-holding irrigation for 15, 18 and 21 days till harvesting.

The physiological, biochemical and enzymological changes in treated seedling and field grown plants were analyzed by using standard protocols. Similarly the growth and yield attributes, essential oil yield and its quality were determined using appropriate and standard methods at different phenological stages i.e. vegetative (60 DAS), bloom (90 DAS), full bloom (120 DAS) and harvesting (150-160 DAS) stages respectively.

**Significant findings and conclusion:**

The major findings and broad conclusion of present investigation are described in brief.

**Seed germination and seedling growth:**

The treatments of GA$_3$ had caused highest increase in seed germination and seedling growth which was followed by NAA and KIN. Application of FeSO$_4$ and ZnSO$_4$ (25 mg/L) showed maximum increase in seed germination over control. But NaCl and PEG 6000 treatments had strongly inhibited the seed germination and seedling growth in Ajowan.

**Seedling physiology:**

The treatments of GA$_3$ (50 mg/L) recorded significant enhancement in reducing and total sugars as well as starch contents while NAA (50 mg/L) showed maximum protein contents in the seedlings. The trend for enhancement in reducing and total sugars was FeSO$_4$ > ZnSO$_4$ > MnSO$_4$. The starch content was maximum at 25
mg/L ZnSO\(_4\). Highest protein content was noted in FeSO\(_4\) (100 mg/L). Whereas, NaCl and PEG 6000 treatments had caused very high accumulation of reducing sugars with subsequent decrease in total sugars, starch and protein contents.

**Antioxidants and ROS scavenging enzymes:**

The treatments of PGRs had caused least increase in proline, GB, phenols and TFAA and activities of POX, SOD and PPO over control. However, higher concentrations of micronutrients had caused comparatively more enhancement in all above parameters. The salinity and water stress both had shown maximum enhancement in antioxidants and antioxidant enzymes.

**Field experiments:**

**Physiological parameters:**

KIN at all the concentrations caused maximum increase in photosynthetic pigments, followed by NAA and GA\(_3\) at all the stages of growth such as vegetative, bloom and full bloom as compared to control. FeSO\(_4\) (200 mg/L) resulted into enhancement of photosynthetic pigments followed by ZnSO\(_4\) and MnSO\(_4\). The same trend was followed for photosynthetic rate, chlorophyll fluorescence and NR activity. The high concentration of salinity and prolonged water stress caused pronounced adverse effects on chlorophylls, RWC, photosynthetic rate, chlorophyll fluorescence and NR activity.

**Biochemical constituents:**

NAA (100 mg/L) showed maximum increase in reducing sugars, starch and protein over control. It was followed by KIN and GA\(_3\). ZnSO\(_4\) (200 mg/L) caused increase in reducing sugars by 36-57 % at all the stages of growth. It was followed by FeSO\(_4\) and MnSO\(_4\). Enhancement in protein content (27-34%) at all the stages was significant at highest concentration of FeSO\(_4\) (200 mg/L) which also caused increase in starch and total sugars. Under stress conditions, there was reduction in total sugars, starch and proteins but reducing sugars were increased.

**Antioxidants and ROS scavenging enzymes:**

The highest increase in antioxidants was recorded at (100 mg/L) NAA which was followed by GA\(_3\) and KIN. Proline and GB contents were highest in MnSO\(_4\).
followed by FeSO$_4$ and ZnSO$_4$ treatments. Whereas, for phenols and TFAA the trend of enhancement was ZnSO$_4$$\geq$MnSO$_4$$\geq$FeSO$_4$ at all the growth stages. The stimulation in antioxidant enzymes was in the order NAA$>$GA$_3$$>$KIN and for micronutrients it was MnSO$_4$$>$ZnSO$_4$$>$FeSO$_4$. Under water and salinity stress significant increase in proline, phenol, GB and TFAA was recorded along with enhanced activities of POX, SOD and PPO at all stages of growth.

**Mineral constituents:**

The contents of N, P, K, Ca, Mg, S, Cu, Fe, Mn and Zn in treated plants of Ajowan were highly enhanced by the treatments of PGRs and micronutrients, whereas reverse trend was noted for salt and water stress.

**Growth parameters:**

Application of NAA, GA$_3$ and KIN had shown positive influence on all the growth parameters and the trend was NAA$>$KIN$>$GA$_3$. Amongst micronutrients ZnSO$_4$ had induced maximum increase in plant height, number of branches and leaves per plant as well as leaf area, fresh and dry weight per plant of Ajowan at both the stages of growth. It was followed by FeSO$_4$ and MnSO$_4$ treatments. However, was significant reduction in growth attributes was recorded due to salinity and water stress.

**Yield attributes:**

The number of umbels, dry biomass and seed yield was significantly enhanced by the treatments of NAA, followed by KIN and GA$_3$. The 1000 seed weight of Ajowan was considerably increased due to application of GA$_3$ as compared to other PGRs and control. Amongst the treatment of micronutrients ZnSO$_4$ (200 mg/L) had enhanced the yield attributes, followed by the treatments of FeSO$_4$ and MnSO$_4$. Salinity and water stress had significantly reduced the yield parameters.

**Essential oil and Thymol content:**

Highest increase in essential oil percent was noted in the treatments of GA$_3$ (100 mg/L), followed by NAA and KIN. The increase in essential oil yield/plant was maximum due to NAA and it was followed by GA$_3$ and KIN treatments. Thymol content was enhanced due to application of 100 mg/L NAA (49.5%) and GA$_3$.
(25.2%) as well as 20 mg/L KIN (23.4%). Application of ZnSO₄ (200 mg/L) have increased both essential oil percent and yield/plant by 26.8% and 53.7% respectively. Similarly, ZnSO₄ and FeSO₄ (200 mg/L) had increased Thymol content by 42.6% and 41.2%. Very less increase (9.3% and 27.6%) in essential oil and Thymol was recorded at 100 mM NaCl but at high salinity level both were decreased. While moderate water stress caused an increase in essential oil content by 16.7% and Thymol content by 40.7% over control.

Conclusions:

GA₃ (50 mg/L) emerged as the best treatment for enhancing seed germination and seedling growth of Ajowan. While, KIN at all the concentrations was highly effective to improve the photosynthetic pigments, photosynthetic rate and chlorophyll fluorescence in field grown plants of Ajowan. NAA induced significant enhancement in basic metabolites such as reducing sugars, total sugars, starch and proteins. These treatments had positive impact on antioxidant and antioxidant enzymes as well as growth and yield attributes. Both GA₃ and NAA had shown significant improvement in EO percent and yield/plant. While, Thymol content was highly improved by the treatments of NAA.

Lower concentrations of all the micronutrients applied proved to be useful for improving seed germination, seedling growth and physiology. While, ZnSO₄ (200 mg/L) was most effective to enhance growth and yield attributes along with EO percent and yield/plant as well as Thymol content.

The abiotic stress conditions like salinity and water both had negative impact on seed germination, seedling growth, physiology and the growth as well as yield attributes. However, mild and moderate stresses had showed slight increase the EO and Thymol.

The field trials conducted indicated that Ajowan may be more productive if treated with foliar applications of PGRs and micronutrients. It may be a good candidate for cultivation, where mild/moderate saline and drought stress conditions exists.