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RESEARCH ARTICLE

SOWING SMART: AJWAIN (*TRACHYSPERMUM AMMI* SPRAGUE) GERMINATION UNDER THE LENS OF DEPTH AND SOIL VARIABILITY

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ABSTRACT

Seed germination and seedling establishment are critical phases in the life cycle of *Trachyspermum ammi* (Ajwain), a medicinally and economically important plant. While sowing depth and soil type are known to influence these stages, their combined effects on Ajwain remain understudied, particularly in diverse agroecosystems. This study investigated the impact of four sowing depths (0, 1, 2, and 3 cm) and four soil types (red, black, sandy, and alluvial) on germination indices and seedling growth parameters. The experiment was conducted in controlled conditions using plastic bags, with germination percentage (GP), mean germination time (MGT), germination speed (GS), and seedling morphological traits monitored over six weeks. Data were analyzed using non-parametric tests and multivariate techniques, including Principal Component Analysis (PCA). Results revealed that shallow sowing (0 cm) in alluvial soil yielded the highest GP (60%), GS (3.00), and GI (5.00), while deeper sowing (2–3 cm) significantly reduced germination performance, particularly in sandy soil. Seedling growth was optimal at 1 cm depth, with sandy and alluvial soils promoting superior stem diameter and leaf development. PCA highlighted a trade-off between germination vigor (PC1) and seedling establishment (PC2), with 0 cm favouring germination and 1–2 cm enhancing later growth. Soil type played a secondary but notable role, with alluvial soil exhibiting resilience across depths. These findings emphasize the importance of shallow to moderate sowing depths (0–1 cm) for Ajwain cultivation, especially in well-drained soils like alluvial or sandy types. Farmers can leverage these insights to improve crop establishment and uniformity. Limitations include the study's-controlled environment and focus on early growth stages. Future research should explore field-scale trials, long-term yield effects, and interactions with abiotic stresses to refine agronomic practices for *T. ammi* and similar small-seeded medicinal plants. This study contributes to optimizing cultivation strategies while highlighting the need for further ecophysiological investigations.

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INTRODUCTION

Seed germination and seedling establishment are critical phases in the life cycle of plants, significantly influencing crop productivity and yield (Finch-Savage & Bassel, 2016). For medicinal and aromatic plants like *Trachyspermum ammi* (Ajwain), optimal germination conditions are particularly important due to their economic and pharmacological value (Prasad, 2024). Sowing depth is a key agronomic factor that affects seed-soil contact, moisture availability, and seedling emergence, with shallow depths often promoting faster germination but deeper sowing potentially enhancing root anchorage and stress resilience (Benvenuti & Mazzoncini, 2019; Dürr et al., 2015). Soil type further modulates these effects, as physical properties like texture and porosity influence water retention, aeration, and mechanical resistance to seedling emergence (Bengough et al., 2011; Weil & Brady, 2016).

Despite these known interactions, studies specifically addressing the combined effects of sowing depth and soil type on *T. ammi* remain limited, particularly in the context of diverse Indian agroecosystems. Previous research has highlighted the sensitivity of small-seeded species like *T. ammi* to sowing depth, with excessive burial often impairing germination due to hypoxia or energy depletion during hypocotyl elongation (Milberg *et al.*, 2000; Bewley *et al.*, 2013). However, most studies focus on cereal or legume crops, leaving gaps in understanding for aromatic herbs. Additionally, while soil type is recognized as a determinant of seedling growth (Gardner *et al.*, 1999), its interaction with sowing depth in *T. ammi* cultivation has not been systematically explored. Existing work also lacks consensus on optimal depths, with some advocating surface sowing (Al-Mudaris, 1998) and others suggesting slight burial (Colombi & Keller, 2019), underscoring the need for species-specific investigations. This study aimed to evaluate the effects of different sowing depths

(0, 1, 2, and 3 cm) and soil types (red, black, sandy, and alluvial) on seed germination and seedling growth of *T. ammi*. The objectives were to quantify germination indices across treatments, assess seedling growth parameters, and identify optimal sowing conditions for maximizing establishment. By addressing the research gap on soil-depth interactions in *T. ammi*, this work provides actionable insights for farmers cultivating the species in variable soil environments, while contributing to broader knowledge on the ecophysiology of small-seeded medicinal plants.

MATERIALS AND METHODS

Soil sample collection: Soil samples were taken from four different zones of Porbandar, one representing a principal soil type prevalent in the area, and one hectare from each zone was chosen. The chosen sites were Red Soil of Sakhpur Village (Latitude 21°48'10.2"N, Longitude 69°28'17.5"E), Black Soil of Advana Village (Latitude 21°56'20.2"N, Longitude 69°35'50.1"E), Sandy Soil of Kuchhdi Village (Latitude 21°40'57.8"N, Longitude 69°32'23.7"E), and Alluvial Soil of Bagvadar (Latitude 21°47'05"N, Longitude 69°36'08"E). These areas were selected so that the prevailing soil types in Porbandar could be represented, and there could be a thorough study of their properties. Soil samples were taken employing the zigzag method of sampling, which is suggested by FAO (2004). Soils at each location were excavated from 8–10 points using a trowel to a depth of 0–150 cm. All sampling points' gathered soil was completely mixed in a bucket to arrive at a homogenous mixture. The composite sample was then set in labelled polythene bags for further use.

Source of seeds: The seeds used in the study, variety GA 3 (Gujarat Ajwain 3), were obtained from a certified agro-input supplier. They were stored in a cool, dry place until sowing.

Experimental setup: The experiment was carried out in the Botany Department of Maharshi Dayanand Science College, Porbandar. Seeds of Ajwain(variety GA 3) were planted in disposable plastic bags at five soil depths: 0, 1, 2 and 3 cm. Four soils (red, black, sandy, and alluvial) were used, with two plastic bags per soil type and depth combination. Five seeds per bag, 10 seeds for each soil combination of depths, were used with each bag. The depths used for sowing were measured with a ruler and marked HB pencil to ensure accuracy.

Following sowing, seeds were coated with soil of the same composition to obtain the desired depth. Water was applied regularly to ensure the best moisture content. The experimental arrangement facilitated a systematic analysis of the impact of sowing depth and type of soil on seed germination and plant growth. Important observations, such as the number of days to the emergence of the first seedling and the completion of germination, were made for all treatments.

Data collection: Data was collected over a period of six consecutive weeks. The primary variable observed was germination, and subsequent measurements included seedling height, stem width, canopy area, and the number of leaves. Germination was measured as the number of seeds that germinated successfully. In case of growing seedlings, stem height was the distance from ground level to the tip of the tallest leaf and stem girth was measured from the plant base with the aid of a vernier calliper the leaves counted every week. These parameters were observed to evaluate the seedling growth and development under various soil conditions and depths of sowing.

Germination Indices

Germination Percentage (GP): Germination percentage was calculated to determine the proportion of seeds that successfully germinated over the total number of seeds sown. GP was determined using the formula (Al-Mudaris, 1998):

$$GP\ (\%) = \left(\frac{\mathrm{Ng}}{\mathrm{Nt}}\right) X\ 100$$

Where, N_g=Number of germinated seeds, N_t=Total number of seeds sown

Mean Germination Time (MGT): MGT was calculated to assess the average time required for seed germination, using the equation (Prasad, 2024):

$$MGT = \frac{\sum(\mathrm{n}_i \mathrm{\ X\ } t_i)}{\sum t_i}$$

Where, n_i=Number of seeds germinated on day t_i, t_i=Time in days from sowing, $\sum n_i$ =Total number of germinated seeds

Germination Speed (GS): The germination speed was determined using the Germination Index (GI), calculated as follows (Evetts& Burnside, 1972):

$$GS = \frac{GI}{FGP}$$

Where, GI= Germination Index and FGP=Final germination percentage.

Germination Index (GI): The germination index was determined based on the weighted germination counts over time (Prasad, 2024):

$$GI = \sum \frac{G_t}{T_t}$$

Where, G_t = Number of seeds germinated on day T_t, T_t = Time in days

Peak Value (PV): Peak value was calculated as the highest mean daily germination(Czabator, 1962):

$$PV = \frac{N_{max}}{D}$$

Where, N_{max} = Maximum number of germinated seeds recorded on any day, D = Number of days taken to reach the peak germination

Coefficient of Velocity of Germination (CVG): CVG was used to measure the speed of germination in percentage terms and was calculated as (Al-Mudaris, 1998):

$$CVG\ (\%) = \left(\frac{\sum G}{\sum(G\ \ X\ T)}\right) X\ 100$$

Where, G = Number of seeds germinated each day, T = Time in days

Data Analysis: The collected data were analysed using non-parametric and multivariate statistical methods to evaluate the effects of sowing depth and soil type on seed germination and seedling growth of Ajwain. The Kruskal-Wallis test was employed to assess the significance of differences among treatments for germination indices and growth parameters. This test was chosen due to the non-normal distribution of the data and the presence of multiple independent groups. Principal Component Analysis (PCA) was performed to reduce the dimensionality of the dataset and uncover underlying patterns in the relationships between sowing depths, soil types, and growth parameters. Correlation analysis was conducted to examine the relationships among germination and growth parameters.

RESULTS AND DISCUSSION

The data reveal significant impacts of sowing depth and soil type on cumin germination (Table 1). Shallow sowing (0 cm) consistently promoted higher germination percentage (GP), with the best performance in alluvial soil (GP: 60%), followed by red and black soils (60% and 50%, respectively). Germination speed (GS) and germination index (GI) were also highest at 0 cm, particularly in alluvial soil (GS: 3.00, GI: 5.00), indicating faster and more uniform seedling emergence. Conversely, deeper sowing (2–3 cm) drastically reduced GP (10–30%) and germination vigor (GI ≤ 0.95), with some treatments failing completely (e.g., sandy soil at 3 cm).Mean germination time (MGT) was shortest at 0 cm (1.33–3.17 days) and increased with depth (up to 7.33 days at 2 cm), while the coefficient of velocity of germination (CVG) showed an inverse trend, peaking in alluvial soil at 0 cm (75%) and declining to ≤22.22% at deeper depths. Peak value (PV), representing germination synchrony, was highest in alluvial soil at 0 cm (4.00) and lowest at deeper depths (≤0.33).

The non-parametric Kruskal-Wallis tests revealed significant effects of sowing depth on germination speed (GS; $p = 0.014$), peak value (PV; $p = 0.008$), and coefficient of velocity of germination (CVG; $p = 0.027$), while soil type had no statistically significant impact on any germination indices ($p > 0.05$). Post-hoc Mann-Whitney U tests with Bonferroni correction, however, showed no pairwise differences between individual depths for these indices ($p > 0.05$ after adjustment), suggesting that while depth influences germination dynamics overall. Notably, germination index (GI) and germination percentage (GP) were unaffected by either factor ($p > 0.05$), contrasting with earlier observations that shallow sowing (0 cm) promoted higher GP and GI in raw data.

The number of leaves in Ajwain seedlings varied significantly across different sowing depths and soil types over six weeks (Table 2). At 0 cm sowing depth, black soil exhibited the highest initial leaf count (2.2 ± 0.44) in Week 1, while red soil had the lowest (1.5 ± 0.54). By Week 6, sandy soil showed the highest mean leaf number (5 ± 1.41), followed by red (4.83 ± 1.16) and alluvial soils (4.42 ± 1.27), whereas black soil plateaued at 4.4 ± 0.54.When seeds were sown at 1 cm depth, leaf production increased markedly across all soil types. Red soil demonstrated the most pronounced growth, reaching 7.5 ± 0.70 leaves by Week 6, followed by sandy (7 ± 1.41) and alluvial soils (6.66 ± 0.57). Black soil, despite a slower start, also showed substantial late-stage growth (6.66 ± 0.51).At 2 cm depth, alluvial soil exhibited erratic growth, peaking at 9 ± 0 leaves in Week 6. Sandy and red soils maintained steady increases, while black soil showed moderate but consistent leaf development (6 ± 1).The 3 cm depth resulted in reduced leaf counts compared to shallower depths, except in alluvial soil, which again showed an unusually high final count (9 ± 0). Notably, sandy soil at this depth failed to germinate, indicating possible depth-induced inhibition. The data suggest that sowing depth critically influences leaf emergence in Ajwain, with 1 cm depth emerging as optimal for most soil types. The decline in growth at 3 cm depth—particularly the complete failure in sandy soil may reflect hypoxia or mechanical resistance impairing seedling emergence (Bewley *et al.*, 2013; Benvenuti, 2003; Colmer & Voesenek, 2009). Soil type also played a significant role. Black soil initially supported vigorous growth, but its advantage diminished by Week 6, possibly due to compaction or nutrient depletion(Bengough *et al.*, 2011; Weil &Brady, 2016). Sandy soil, despite lower initial counts, often surpassed other soils in later stages, likely due to better drainage and root aeration (Kramer & Boyer, 1995; Lynch, 2019).

The Kruskal-Wallis test revealed varying effects of sowing depth and soil type on the number of leaves in Ajwain seedlings over six weeks. In Week 1, neither sowing depth ($H = 4.71$, $p = 0.194$) nor soil type ($H = 4.25$, $p = 0.236$) had a significant effect, suggesting that initial leaf emergence was not strongly influenced by these factors. However, by Week 3, sowing depth began to exhibit a statistically significant impact ($H = 8.68$, $p = 0.034$), which persisted through subsequent weeks (Week 4: $H = 8.04$, $p = 0.045$; Week 5: $H = 8.44$, $p = 0.038$; Week 6: $H = 8.74$, $p = 0.033$). This trend indicates that deeper sowing may have initially delayed leaf development, but its effect became more pronounced as seedlings matured.In contrast, soil type did not significantly influence leaf count at any stage ($p > 0.05$ for all weeks), suggesting that soil conditions may play a secondary role compared to sowing depth in leaf production. The interaction effect between depth and soil type was also non-significant ($p = 0.450$ across all weeks), implying that the effect of sowing depth remained consistent regardless of soil variation.

These findings align with previous studies demonstrating that deeper sowing can impede early seedling growth due to limited access to light and increased energy expenditure during emergence (López-Castañeda *et al.*, 1996). However, the sustained significance of depth over time suggests that *T. ammi* may exhibit compensatory growth once seedlings overcome initial establishment challenges (Poorter et al. 2012). Further research could explore whether optimal sowing depth balances early growth suppression with long-term vigor (Freschet*et al.*, 2018; Colombi *et al.*, 2017).

The data on seedling height in Ajwain revealed significant variations based on sowing depth and soil type over six weeks (Table 3). At 0 cm depth, red soil produced the tallest seedlings (3.13 ± 0.64 cm by Week 6), while alluvial soil showed the slowest initial growth but caught up by Week 6 (2.71 ± 0.86 cm). Black and sandy soils exhibited intermediate growth, though sandy soil displayed greater variability (±0.62 cm).At 1 cm depth, sandy and alluvial soils demonstrated remarkable late-stage growth, with sandy soil seedlings reaching 7.3 ± 2.96 cm by Week 6—more than double their initial height. Black soil showed an unusual growth pattern, with a sudden height increase in Week 5 (3.26

Table 1. Germination Parameters of Ajwain Seeds Under Different Soil Types and Sowing Depths

Sowing Depth	Soil	GP (%)	MGT (days)	GI	GS	PV	CVG (%)
0 cm	Red	60	3.17	3.02	1.81	2.00	28.57
	Black	50	3.00	1.67	0.83	1.67	33.33
	Sandy	40	1.75	2.83	1.13	2.00	57.14
	Alluvial	60	1.33	5.00	3.00	4.00	75.00
1 cm	Red	20	3.00	0.75	0.15	0.50	33.33
	Black	60	7.17	1.55	0.93	1.00	20.69
	Sandy	40	4.00	1.03	0.41	0.50	25.00
	Alluvial	30	3.67	0.95	0.28	0.50	27.27
2 cm	Red	20	5.00	0.42	0.08	0.25	20.00
	Black	30	7.33	0.50	0.15	0.33	13.64
	Sandy	20	3.50	0.14	0.14	0.50	28.57
	Alluvial	10	5.00	0.20	0.02	0.20	20.00
3 cm	Red	30	6.00	0.46	0.14	0.25	16.67
	Black	40	4.50	0.95	0.38	0.33	22.22
	Sandy	-	-	-	-	-	-
	Alluvial	10	5.00	0.20	0.02	0.20	20.00

Germination Percentage (GP), Mean Germination Time (MGT), Germination Speed (GS), Germination Index (GI), Peak Value (PV), Coefficient of Velocity of Germination (CVG)

Table 2. Number of Leaves in Ajwain Plants Under Different Soil Types and Sowing Depths

Sowing Depth	Soil	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
0 cm	Red	1.5±0.54	2±0.63	2.83±0.75	3.33±1.03	4±1.09	4.83±1.16
	Black	2.2±0.44	3±0	3.4±0.54	4±0.70	4.4±0.89	4.4±0.54
	Sandy	2.25±0.5	2.5±0.57	3.25±0.5	3.5±0.57	4±0.81	5±1.41
	Alluvial	1.83±0.75	2.66±0.81	3.16±0.75	3.66±1.03	4.66±1.36	4.42±1.27
1 cm	Red	2.5±0.70	3.5±0.70	5±0	5.5±0.70	6±0	7.5±0.70
	Black	2.33±0.51	3.6±0.54	3.83±0.40	4.16±0.75	4.66±0.51	6.66±0.51
	Sandy	2.2±0.44	3.75±0.5	4.25±0.5	4.75±0.5	5.75±0.5	7±1.41
	Alluvial	2.33±0.57	3.33±0.57	3.66±0.57	4.66±0.57	5.66±0.57	6.66±0.57
2 cm	Red	2±0	2.5±0.70	3.5±0.70	4.5±0.70	5±0	6.5±0.70
	Black	2.5±0.70	3±1	3.33±1.15	4±1	5.33±0.57	6±1
	Sandy	2.5±0.70	3.5±0.70	4±0	5±0	6.5±0.70	6.5±0.70
	Alluvial	2±0	4±0	4±0	6±0	7±0	4±0
3 cm	Red	2±1	3.33±1.15	3.66±0.57	4.33±0.57	4.66±0.57	5.33±0.57
	Black	2.25±0.5	3.5±0.57	4±0.81	4.25±0.5	5±0.81	6±0.81
	Sandy	-	-	-	-	-	-
	Alluvial	2±0	4±0	4±0	5±0	6±0	9±0

Table 3. Seedling Height in Ajwain Plants Under Different Soil Types and Sowing Depths

Sowing Depth	Soil	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
0 cm	Red	2.13±0.50	2.25±0.48	2.36±0.45	2.45±0.48	2.68±0.54	3.13±0.64
	Black	1.84±0.31	2±0.37	2.14±0.43	2.16±0.49	2.22±0.48	2.36±0.34
	Sandy	1.62±0.83	1.75±0.52	1.8±0.47	1.75±0.51	2.3±0.58	2.62±0.62
	Alluvial	1.48±0.64	1.66±0.31	1.83±0.46	1.86±0.27	2.08±0.34	2.71±0.86
1 cm	Red	1.9±0.56	2±0.70	2.4±0.84	2.5±0.56	2.65±0.49	3.25±0.77
	Black	1.52±0.79	1.66±0.68	1.61±0.53	1.55±0.50	3.26±1.43	3.45±1.27
	Sandy	1.57±0.06	1.67±0.53	1.57±0.44	2.77±1.53	5.2±1.84	7.3±2.96
	Alluvial	1.96±0.92	2.2±0.81	1.7±0.75	2.8±1.47	5.3±3.00	6.1±3.25
2 cm	Red	0.9±0.14	1.3±0.28	1.5±0.28	1.35±0.21	1.55±0.35	1.9±0.28
	Black	0.75±0.35	0.66±0.28	0.86±0.35	1.23±0.45	1.56±0.64	1.9±0.52
	Sandy	1.65±0.91	2.2±0.42	2.45±0.63	5.35±1.20	6.7±2.12	9.15±3.18
	Alluvial	2±0	2.5±0	2.8±0	5.7±0	5.9±0	6.9±0
3 cm	Red	1.5±0.2	1.66±0.23	1.8±0.26	1.9±0.26	2.06±0.15	1.96±0.63
	Black	1±0.84	1.12±0.80	1.27±0.76	1.45±0.75	2.25±0.74	3.3±2.2
	Sandy	-	-	-	-	-	-
	Alluvial	2.5±0	2.7±0	2.8±0	3±0	8.5±0	10.8±0

Table 4. Seedling stem diameter in Ajwain Plants Under Different Soil Types and Sowing Depths

Sowing Depth	Soil	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
0 cm	Red	0.02±0.01	0.02±0.008	0.03±0.01	0.03±0.01	0.04±0.01	0.04±0.01
	Black	0.028±0.008	0.03±0.005	0.04±0.008	0.05±0.007	0.05±0.007	0.05±0.008
	Sandy	0.03±0.008	0.03±0.009	0.04±0.009	0.04±0.005	0.05±0.005	0.05±0.005
	Alluvial	0.03±0.01	0.03±0.01	0.04±0.01	0.04±0.01	0.04±0.01	0.05±0.007
1 cm	Red	0.05±0.007	0.05±0.007	0.07±0.007	0.08±0.01	0.08±0.02	0.09±0.02
	Black	0.04±0.01	0.04±0.007	0.04±0.004	0.07±0.007	0.07±0.01	0.08±0.01
	Sandy	0.04±0.008	0.04±0.01	0.05±0.005	0.08±0.008	0.09±0.008	0.09±0.02
	Alluvial	0.03±0.005	0.04±0.005	0.05±0.005	0.06±0.005	0.07±0.01	0.07±0.01
2 cm	Red	0.04±0.007	0.05±0.007	0.06±0.007	0.07±0.007	0.08±0	0.09±0.01
	Black	0.03±0.01	0.04±0.01	0.04±0.005	0.06±0.01	0.06±0.01	0.1±0.01
	Sandy	0.04±0.014	0.05±0	0.05±0	0.08±0.007	0.09±0.007	0.1±0.01
	Alluvial	0.04±0	0.05±0	0.06±0	0.07±0	0.08±0	0.1±0
3 cm	Red	0.02±0	0.04±0.03	0.04±0.01	0.05±0.01	0.05±0.005	0.05±0.005
	Black	0.03±0.008	0.05±0.01	0.06±0.02	0.06±0.01	0.07±0.01	0.07±0.01
	Sandy	-	-	-	-	-	-
	Alluvial	0.03±0	0.05±0	0.07±0	0.06±0	0.07±0	0.09±0

± 1.43 cm), possibly due to delayed root establishment. Red soil maintained steady but moderate growth (3.25 ± 0.77 cm).The 2 cm depth produced contrasting results: sandy and alluvial soils again exhibited rapid growth, with sandy soil seedlings reaching 9.15 ± 3.18 cm by Week 6. However, red and black soils at this depth showed stunted growth (≤1.9 cm), suggesting that some soil types may hinder seedling emergence at greater depths.At 3 cm depth, seedling growth was generally poor, except in alluvial soil, which showed an anomalous spike to 10.8 ± 0 cm by Week 6. Black soil exhibited erratic growth (3.3 ± 2.2 cm), while red soil seedlings declined slightly in Week 6 (1.96 ± 0.63 cm). Sandy soil failed to germinate entirely at this depth, reinforcing the inhibitory effect of deep sowing.

The results highlight that sowing depth strongly influences seedling height, with 1 cm and 2 cm depths promoting optimal growth in sandy and alluvial soils, while deeper sowing (3 cm) generally suppressed development (Benvenuti & Mazzoncini, 2019). The superior performance of sandy soil at 1–2 cm depths align with its loose structure, which facilitates root penetration and oxygen availability (Håkansson & Lipiec, 2000). The sudden growth spurts in black soil (Week 5 at 1 cm) and alluvial soil (Week 6 at 3 cm) may indicate compensatory growth after initial stress (Pierik & Testerink, 2014). The poor performance at 3 cm depth (especially in sandy soil, where no germination occurred) supports the hypothesis that small-seeded species like Ajwain struggle with deep sowing due to limited energy reserves for hypocotyl elongation (Milberg *et al.*, 2000; Dürr*et al.*, 2015). The exceptional height in alluvial soil at 3 cm (10.8 cm) contradicts this trend and warrants further investigation—possibly reflecting unique nutrient properties or measurement anomalies (Colombi & Keller, 2019).

The Kruskal-Wallis test results for seedling height over six weeks indicate that sowing depth had no significant effect ($p > 0.05$ in all weeks) on Ajwain growth. In Week 1, neither depth ($H = 1.03$, $p = 0.794$) nor soil type ($H = 3.60$, $p = 0.308$) influenced seedling height, suggesting that initial growth was independent of these factors. This trend continued in subsequent weeks, with depth remaining non-significant (Week 2: $H = 0.31$, $p = 0.957$; Week 3: $H = 0.70$, $p = 0.874$; Week 4: $H = 0.53$, $p = 0.912$; Week 5: $H = 1.68$, $p = 0.643$; Week 6: $H = 2.77$, $p = 0.429$).Similarly, soil type did not significantly affect seedling height at any stage ($p > 0.05$ for all weeks), though a marginally non-significant trend appeared in Week 4 ($H = 6.45$, $p = 0.092$), suggesting a possible but inconclusive influence. The interaction between depth and soil type was also non-significant ($p = 0.450$ across all weeks), reinforcing that these factors operated independently without compounding effects. These findings contrast with the significant impact of sowing depth on leaf number, indicating that height growth may be less sensitive to planting depth than foliar development (Weiner *et al.,* 2009). This could be due to seedlings prioritizing vertical growth to access light, regardless of initial sowing conditions (Gommers*et al.,* 2013). The lack of soil effects suggests that *T. ammi* may be adaptable to varying soil types, at least in the early growth stages (Poorter*et al.,* 2012). Further studies could explore whether these trends persist in later growth phases or under stress conditions (Garnier & Navas, 2012).

The stem diameter measurements of Ajwain seedlings showed distinct developmental patterns across different sowing depths and soil types over six weeks (Table 4). At the 0 cm sowing depth, black soil consistently produced seedlings with the largest stem diameters (0.05±0.008 cm by Week 6), followed closely by sandy soil (0.05±0.005 cm). Red soil showed the slowest stem thickening at this depth (0.04±0.01 cm), suggesting potential limitations in nutrient availability or root development in this soil type. The 1 cm sowing depth demonstrated superior stem development across all soil types. Red soil exhibited the most vigorous growth, reaching 0.09±0.02 cm by Week 6, with sandy soil showing comparable results (0.09±0.02 cm). Black and alluvial soils at this depth displayed slightly slower but consistent thickening patterns. Notably, the stem diameters at 1 cm depth were approximately twice those observed at 0 cm depth by Week 6, highlighting the importance of optimal sowing depth for stem development. At 2 cm depth, stem diameters showed accelerated growth in later weeks, particularly in sandy and alluvial soils, both reaching 0.1 cm by Week 6.Black soil exhibited a sudden increase in stem diameter in Week 6 (0.1±0.01 cm), potentially reflecting a late-stage growth spurt. The 3 cm depth produced mixed results, with black soil showing steady growth (0.07±0.01 cm by Week 6). Red soil at 3 cm depth showed limited development (0.05±0.005 cm), significantly less than at shallower depths.

The stem diameter data reveal several important trends in Ajwain development. The consistent superiority of 1 cm sowing depth across all soil types suggests this depth optimally balances soil contact and emergence energy for stem development (Finch-Savage & Bassel, 2016; Dürr *et al.*, 2015). The doubling of stem diameters at 1 cm compared to surface sowing (0 cm) strongly supports the importance of slight burial for proper seedling establishment. The exceptional performance of red and sandy soils at intermediate depths (1-2 cm) may relate to their physical properties. Sandy soil's loose structure likely facilitated root expansion and nutrient uptake (Bengough *et al.,* 2011), while red soil's mineral composition might have provided essential trace elements for vascular development (Ryan *et al.,* 2001). The complete failure of sandy soil at 3 cm depth further emphasizes the species' sensitivity to deep sowing (Gardner *et al.,* 1999). The analysis of stem diameter in Ajwain seedlings revealed a distinct pattern of response to sowing depth, while showing remarkable consistency in its lack of response to soil type. The Kruskal-Wallis tests demonstrated that sowing depth had a significant effect on stem diameter in most weeks of observation (Week 1: $H = 8.65$, $p = 0.034$; Week 2: $H = 10.17$, $p = 0.017$; Week 4: $H = 10.78$, $p = 0.013$; Week 5: $H = 9.61$, $p = 0.022$; Week 6: $H = 11.37$, $p = 0.010$), with only Week 3 showing non-significant results ($H = 5.79$, $p = 0.122$). This consistent pattern suggests that deeper sowing generally promoted thicker stem development, possibly as a morphological adaptation to provide mechanical support for emerging seedlings. In contrast, soil type showed no significant influence on stem diameter throughout the six-week observation period ($p > 0.05$ for all weeks), indicating that this growth parameter was largely unaffected by soil conditions. The interaction between depth and soil type was also non-significant ($p = 0.450$ across all weeks), suggesting that the effect of sowing depth on stem diameter was independent of soil variation. The observed pattern of stem diameter response differs notably from the trends seen in seedling height (which showed no treatment effects) and leaf number (which showed late-emerging depth effects). This differential response among growth parameters suggests that *T. ammi* seedlings may allocate resources differently to various growth components under varying sowing conditions (Poorter *et al.*, 2012). The consistent enhancement of stem diameter with deeper sowing may represent an adaptive strategy, where seedlings invest more in structural support when emerging from greater depths, potentially as a response to the mechanical challenges of soil penetration (Lynch & Brown, 2012; Colombi & Keller, 2019). These findings align with the ecological principle of phenotypic plasticity, where plants modify their morphology in response to environmental conditions during establishment (Sultan, 2000; Nicotra *et al.*, 2010).

Principal Component Analysis (PCA) revealed the underlying structure of relationships between sowing depths, soil types, and growth parameters. The scree plot (Fig. 1) indicated that the first two principal components (PC1 and PC2) together explained 80.4% of the total variance (PC1: 64.7%, PC2: 15.7%), capturing the majority of variability in the dataset. PC1 was strongly associated with germination dynamics, showing high positive loadings from Germination Index (GI: 0.40), Germination Speed (GS: 0.39), Peak Value (PV: 0.40), and Coefficient of Velocity of Germination (CVG: 0.35). In contrast, PC2 represented seedling establishment, driven primarily by Week 6 seedling height (SH Week 6: 0.61) and CVG (0.36), with negative contributions from Mean Germination Time (MGT: -0.50).

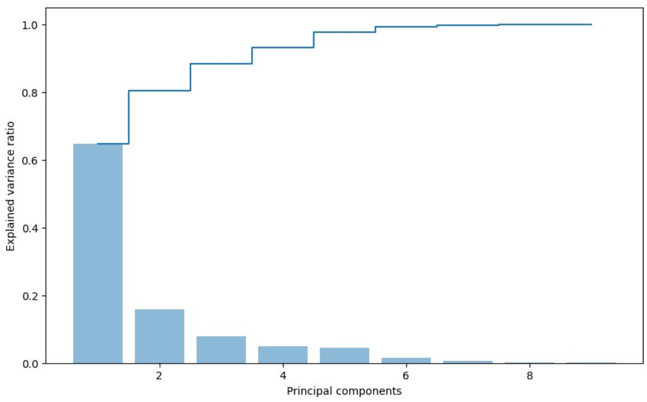

Fig. 1. PCA Screen Plot

The biplot (Fig. 2) revealed three distinct clustering patterns corresponding to sowing depths. Surface-sown treatments (0 cm), particularly in red and alluvial soils, clustered in the high-PC1 region, demonstrating superior germination performance. This was exemplified by alluvial soil at 0 cm depth, which showed the highest Germination Index (GI: 5.00) and Peak Value (PV: 4.00) among all treatments. Intermediate sowing depths (1-2 cm) occupied a transitional space with moderate PC1 but elevated PC2 values, indicating a biological trade-off between initial germination vigor and subsequent seedling growth. For instance, sandy soil at these depths exhibited remarkable height gain by Week 6 (7.3-9.15 cm) despite relatively lower Germination Speed (GS) values. In contrast, deep-sown treatments (3 cm) predominantly clustered in the low-PC1/PC2 quadrant, with the notable exception of alluvial soil that appeared as a clear outlier due to its anomalous height development (10.8 cm). The complete absence of sandy soil at this depth in the biplot reinforced its particular sensitivity to deep sowing conditions. The analysis further highlighted important soil-specific responses. Alluvial soil consistently appeared in high-performance regions regardless of sowing depth, likely due to its favorable physical structure and nutrient retention capacity. Sandy soil displayed polarized behavior, showing excellent performance at intermediate depths (1-2 cm) but complete germination failure at 3 cm, emphasizing its strict dependence on optimal sowing depth. Black and red soils followed intermediate trajectories, with red soil preferentially enhancing germination parameters (PC1) while black soil supported more sustained growth patterns (PC2).

These multivariate findings complement and extend the univariate results by demonstrating that: (1) germination efficiency (PC1) and seedling establishment (PC2) represent partially independent physiological processes, optimally achieved at different depths (0 cm versus 1-2 cm respectively); (2) soil type significantly modulates depth effects, with alluvial soil showing particular resilience to depth-related stress; and (3) the exceptional performance of alluvial soil at 3 cm depth suggests unique soil-depth interactions that merit targeted investigation in future studies.

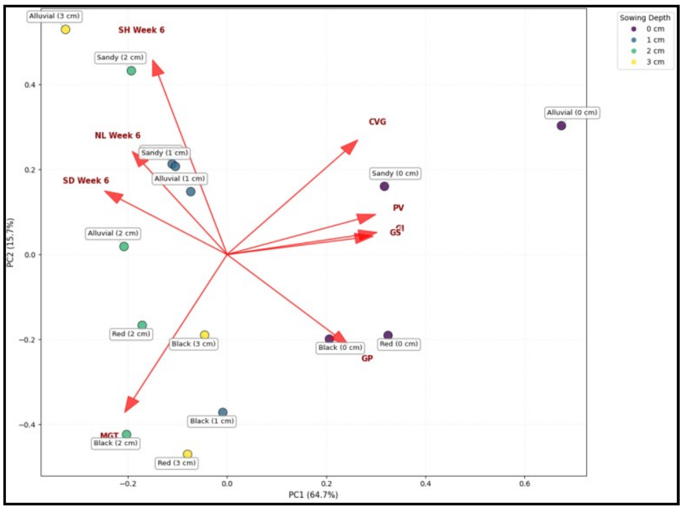

Fig. 2. PCA Scatter Plot

The correlation analysis revealed significant relationships among the measured growth parameters, providing insights into the coordinated development of *Trachyspermum ammi* seedlings (Fig. 3). Germination percentage showed strong positive correlations with key germination indices, particularly with Germination Index (r = 0.78) and Germination Speed (r = 0.80), indicating that seeds with higher germination rates tended to exhibit faster and more uniform emergence. Mean Germination Time demonstrated consistent negative correlations with all vigor parameters, including Germination Index (r = -0.56), Germination Speed (r = -0.66), and Peak Value (r = -0.82), confirming that prolonged germination periods were associated with reduced seedling vigor.

These patterns suggest germination traits are functionally linked in this species, with high-performing seeds excelling across multiple metrics simultaneously. Among the morphological growth parameters, distinct correlation patterns emerged. The number of leaves showed moderate positive correlation with germination percentage (r = 0.43) but negative association with Mean Germination Time (r = -0.48), suggesting that early germination promotes better foliar development. Seedling height correlated strongly with stem diameter (r = 0.69), reflecting coordinated growth in vertical and radial dimensions. However, both parameters showed inverse relationships with germination speed, with height (r = -0.71) and stem diameter (r = -0.67) both decreasing as germination speed increased. This apparent trade-off between rapid germination and subsequent seedling growth may reflect differential resource allocation patterns during early development. The biological interpretation of these correlations suggests several important patterns in seedling establishment. The strong intercorrelations among germination parameters support the concept of an integrated "germination vigor" axis, where seeds tend to perform consistently well or poorly across multiple germination metrics. This finding aligns with the PCA results and reinforces the hypothesis that germination traits are evolutionarily co-adapted in *Trachyspermum ammi*. The negative correlation between seedling height and germination speed may indicate a physiological trade-off, where rapidly germinating seedlings allocate more resources to root establishment at the expense of shoot elongation. This interpretation is supported by the positive association between stem diameter and germination percentage, suggesting that high-germination cohorts prioritize structural robustness over height growth. The weak correlation between number of leaves and seedling height suggests these traits may be regulated through independent developmental pathways, possibly involving different hormonal controls. In contrast, the strong relationship between stem diameter and seedling height likely reflects fundamental biomechanical constraints, where taller seedlings require thicker stems for structural support. These correlations have important practical implications for cultivation and breeding. The apparent antagonism between germination speed and later growth traits suggests breeding programs may need to prioritize either early establishment characteristics or mature plant vigor. The consistent relationship between germination percentage and stem diameter indicates that germination success could serve as a useful proxy for predicting seedling robustness in early-stage selection.

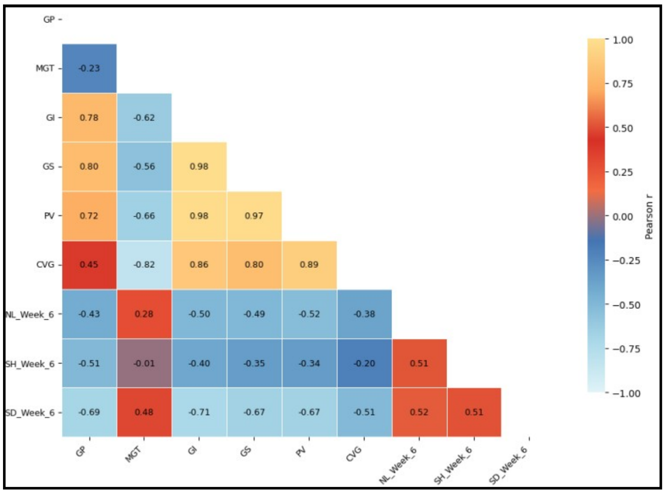

Fig. 3. Correlation Matrix Heatmap

CONCLUSION

The study demonstrated that sowing depth and soil type significantly influence the germination and seedling growth of *Trachyspermum ammi* (Ajwain). Shallow sowing (0 cm) promoted the highest germination percentage (GP), germination speed (GS), and germination index (GI), particularly in alluvial soil, while deeper sowing (2–3 cm) drastically reduced these parameters, with complete failure observed in sandy soil at 3 cm. Intermediate depths (1–2 cm) balanced germination vigor and subsequent seedling growth, with 1 cm emerging as optimal for stem diameter and leaf development. Soil type modulated these effects, with alluvial soil showing resilience across depths and sandy soil exhibiting sensitivity to deep sowing. These findings highlight the critical role of sowing depth in optimizing early establishment, a key factor for crop productivity. For farmers, these results provide actionable insights: shallow sowing (0–1 cm) in alluvial or sandy soils maximizes germination and early growth, while deeper sowing should be avoided, especially in sandy soils. The study underscores the importance of tailoring sowing practices to soil type to enhance seedling vigor and uniformity, ultimately improving yield potential for this economically valuable medicinal plant.

A limitation of this study is its focus on early growth stages under controlled conditions, which may not fully reflect field performance under variable environmental stresses such as drought or pest pressure. Additionally, the study did not explore the long-term effects of sowing depth on mature plant yield or secondary metabolite production, which are crucial for Ajwain's commercial value. Future research should investigate the interaction of sowing depth with other agronomic factors (e.g., irrigation, nutrient availability) in field trials. Studies on the physiological mechanisms behind depth-related stress responses, as well as the impact of these practices on yield and bioactive compound accumulation, would further refine cultivation guidelines. Expanding this work to other small-seeded medicinal plants could also contribute to broader agricultural advancements. In summary, this study advances the understanding of *T. ammi* ecophysiology and offers practical recommendations for farmers, while identifying avenues for future research to optimize cultivation practices and enhance crop resilience.

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Author Contributions: RK, KNO, and SS designed and conducted the experiments. BAJ supervised the research and provided critical revisions. Data collection and analysis were performed by RK and KNO. The manuscript was written and reviewed by all authors, who approved the final version.

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