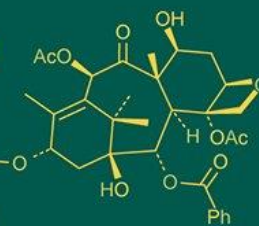
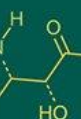
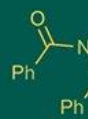


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Biological and synthetic plant growth modulators affect the seed nutritional quality of lentil (*Lens culinaris* Medik.) varieties

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Abstract

Pot experiments were conducted in winter season of 2024-25 and 2025-26 at Department of Plant Physiology, College of Agriculture, Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur, Madhya Pradesh, India, to evaluate the influence of biological and synthetic modulators on the proximate composition of lentil (*Lens culinaris* Medik.) seed. The experiments were designed in a factorial randomized/replicated thrice with three replications. Treatments compiled of two contrasting varieties, L 4717 (small seeded) and L 4727 (bold seeded), and eleven modulators Root-Knot Nematode (RKN)-affected plant extract at 10, 20 and 30 ppm, Wilt-Affected Plant Extract (WAPE) at 10, 20 and 30 ppm, salicylic acid at 100 and 200 ppm, *Trichoderma* @ 6 g kg⁻¹, fungicide @ 2 g kg⁻¹ and water-spray as control. Seed moisture, fat, protein, ash and carbohydrate contents were estimated to see the effects various treatments on nutritional composition. Significant varietal and modulator effects were observed for most proximate constituents, accompanied by pronounced genotype × modulator interactions. L 4727 recorded higher pooled protein (28.40%) and ash (3.48%), whereas L 4717 showed greater carbohydrate concentration (66.49%). WAPE @ 20 ppm produced the highest pooled protein (28.82%) and fat (3.06%) among modulators. The interaction L 4727 × WAPE @ 20 ppm recorded maximum protein (32.28%), fat (3.09%) and ash (3.70%), while L 4717 × RKN @ 20 ppm resulted in the highest carbohydrate concentration (67.81%). Salicylic acid @ 200 ppm produced the highest pooled moisture content (5.23%). The findings demonstrate that biological and synthetic modulators differentially influenced seed reserve composition in a genotype-and concentration-dependent manner. Spraying WAPE @ 20 ppm, in L 4727, was found very effective in improving nutritional quality.

Keywords: Lentil, Wilt-Affected Plant Extract (WAPE), Root-Knot Nematode (RKN)-affected plant extract, seed proximate composition, genotype × plant growth modulator interaction

Introduction

Lentil (*Lens culinaris* Medik.) is an important cool-season food legume valued for its nutritional, economic and agronomic significance. Its seed provides substantial protein and carbohydrates, together with fat, minerals and other nutritionally important constituents, making lentil an important component of nutritional security. Seed nutritional quality is a complex trait governed by genotype and modified by environmental conditions and crop management. Genotypic differences in physiological responses to drought and other environmental stresses can influence photosynthetic activity, water relations, reproductive development and ultimately seed productivity and quality (Mishra *et al.*, 2016; Sehgal *et al.*, 2017; Dash *et al.*, 2017; Dash *et al.*, 2020) [17, 20, 8, 7].

Seed composition is the integrated effect of variety and modulators during reproductive stage particularly at seed development. Photosynthetically fixed carbon is partitioned among carbohydrates, proteins and lipids, while nitrogen and mineral nutrients are assimilated, translocated and remobilized towards developing reproductive sinks. Consequently, the proximate composition of mature seed reflects the integrated effects of photosynthetic carbon assimilation, biomass accumulation, nutrient acquisition, nitrogen metabolism and source-sink regulation. Genotypic variation in growth and dry-matter partitioning can therefore contribute to differences in the accumulation of seed storage constituents.

Environmental conditions may further modify water status, chlorophyll concentration and physiological activity, thereby influencing assimilate availability during seed filling (Mondal *et al.*, 2014; Whitehead *et al.*, 2000) ^[18, 22]. Variation in lentil seed nutritional quality under contrasting moisture regimes further emphasizes the sensitivity of seed composition to the physiological environment during crop development (Bansal *et al.*, 2023) ^[3].

Physiological regulation through exogenous biological and synthetic growth modulators provides a potential strategy for modifying plant growth, stress responses and assimilate partitioning. Plant growth regulators can influence photosynthetic activity, vegetative development, reproductive processes and source-sink relationships, with potential consequences for the accumulation of seed storage compounds (Giannakoula *et al.*, 2012; Prasad *et al.*, 2023) ^[11, 19]. Such physiological regulation is particularly relevant during reproductive development, when assimilates and nutrients are progressively allocated towards developing seeds.

Salicylic acid (SA) is an important plant signalling molecule involved in stress responses, antioxidant defence and regulation of physiological processes. Its application can influence plant growth, photosynthetic performance, reproductive development and yield under adverse environmental conditions, thereby providing a potential means of modifying assimilate availability during seed formation (Azarnia *et al.*, 2020; Bouallegue *et al.*, 2025) ^[2, 4]. These physiological effects provide a rationale for examining SA as a modulator of seed nutritional composition in lentil.

Biological modulators, including beneficial microorganisms and plant-derived extracts, can influence plant growth, nutrient acquisition and stress tolerance. Plant growth-promoting microorganisms and *Trichoderma* have potential to improve plant growth, nodulation, yield and nutrient acquisition, while plant-derived extracts may influence plant defence against soil-borne pathogens and nematodes (Javeria *et al.*, 2020; Marra *et al.*, 2021) ^[12, 16]. Disease-affected plant extracts may also possess physiological signalling activity; sterilized *Fusarium* wilt-affected plant extract has been associated with enhanced physiological and stress-mitigating responses in chickpea, suggesting its potential as a biological modulator (Thakur, 2025) ^[21].

Despite increasing interest in plant growth regulators, microbial preparations and plant-derived inputs, comparative information on biological, plant-based and synthetic modulators and their effects on lentil seed proximate composition across contrasting genotypes remains limited. In particular, the physiological basis of genotype-dependent responses to Root-Knot Nematode (RKN)-affected plant extract and Wilt-Affected Plant Extract (WAPE), and their effects on the accumulation of protein, fat, carbohydrate, ash and moisture in lentil seed, remains insufficiently characterized. Therefore, the present study was undertaken to evaluate the effects of selected biological, plant-based and synthetic modulators on the proximate composition of contrasting lentil genotypes and to identify genotype-modulator combinations associated with improved seed nutritional quality.

Materials and Methods

Pot experiments were conducted in 2x11 factorial randomized design with three replications during the rabi

seasons of 2024-25 and 2025-26 at the Department of Plant Physiology, College of Agriculture JNKVV, Jabalpur, Madhya Pradesh, India. The experiment comprised of two contrasting lentil (*Lens culinaris* Medik.) varieties, L 4717 (V₁-small seeded) and L 4727 (V₂-bold seeded) and 11 modulators (i.e., T₁, control (water spray); T₂, RKN-affected plant extract @ 10 ppm; T₃, RKN-affected plant extract @ 20 ppm; T₄, RKN-affected plant extract @ 30 ppm; T₅, wilt-affected plant extract (WAPE) @ 10 ppm; T₆, WAPE @ 20 ppm; T₇, WAPE @ 30 ppm; T₈, salicylic acid @ 100 ppm; T₉, salicylic acid @ 200 ppm; T₁₀, *Trichoderma* @ 6 g kg⁻¹; and T₁₁, fungicide @ 2 g kg⁻¹. Each pot had an area of approximately 0.138 m², a diameter of 0.42 m, and contained approximately 30 kg soil. The modulators were applied at two critical developmental stages, namely the four-leaf stage and immediately before floral initiation, as specified in the experimental programme.

At physical maturity, representative seed samples from each treatments unit were collected, cleaned and used for proximate analysis. Moisture, fat, crude fibre, ash and total carbohydrate contents were estimated following the procedures described by AOAC (1980) ^[1], while total protein was determined by the Lowry *et al.* (1951) ^[15] method.

Fat content was estimated by Soxhlet extraction using petroleum ether (60-80 °C), and crude fibre was determined by sequential acid and alkali digestion followed by drying and ashing. Ash content was determined by incinerating a weighed seed sample in a muffle furnace at approximately 600 °C to constant weight. Moisture content was estimated by oven-drying the sample at 105 °C to constant weight. Total carbohydrate content was determined colorimetrically by the phenol-sulfuric acid method, using glucose as the standard and recording absorbance at 490 nm. The respective proximate constituents were expressed as percentage of seed weight, while protein content was expressed as mg g⁻¹ where applicable.

The experimental data from two years experiments was statistically analyzed as per method prescribed by Fisher, R. A. (1935) ^[10] and Cochran, W. G. and Cox, G. M. (1957) ^[6] adopted for FCRD. The analysis of variance (ANOVA) was carried out to determine the significance of the effects of individual factors and their interaction. Where the treatment effects were found to be significant, the appropriate critical difference (CD) was used for comparison of treatment means.

Results and Discussion

The proximate composition of lentil seed was significantly influenced by genotype and modulator application, with pronounced genotype × plant growth modulator interactions indicating that nutritional responses were dependent on the genetic background of the plant. L 4727 exhibited significantly higher pooled protein (28.40%) and ash (3.48%) than L 4717 (24.60 and 3.15%, respectively), whereas L 4717 accumulated greater carbohydrate concentration (66.49%) than L 4727 (62.35%). This contrasting pattern suggests differences between the two genotypes in the partitioning of assimilated carbon and nitrogen towards seed storage compounds. Such genotypic variation in nutritional composition is physiologically important because seed quality is determined not only by total assimilate production but also by the relative allocation of carbon, nitrogen and mineral nutrients to developing

seeds. This interpretation is consistent with Choukri *et al.* (2020) ^[5], who demonstrated substantial genotypic variation in lentil protein and micronutrient concentrations under contrasting environmental conditions, and Kumar *et al.* (2023) ^[14], who reported that physiological stress could modify both seed productivity and nutritional quality.

Among the plant growth modulators, substantial treatment-dependent variation was evident in seed protein concentration. WAPE @ 20 ppm produced the highest pooled protein concentration (28.82%), followed by WAPE @ 30 ppm (26.80%) and RKN @ 30 ppm (26.66%), whereas the control recorded only 23.80%. The response was particularly pronounced in the genotype $\times$ modulator combination, where L 4727 $\times$ WAPE @ 20 ppm recorded 32.28% protein, compared with 24.32% in L 4727 $\times$ control. Thus, WAPE @ 20 ppm increased protein concentration in L 4727 by approximately 32.7% relative to its control. From a plant physiological perspective, this response may indicate improved nitrogen acquisition, assimilation and/or remobilization towards developing seeds, although these processes were not directly measured in the present experiment. The finding is nevertheless consistent with the broader physiological concept that biological elicitors can modify plant metabolic activity and stress-response pathways. In the review, Thakur (2025) ^[21] reported that foliar application of sterilized *Fusarium* wilt-affected plant extract at 20 ppm in chickpea improved photosynthetic rate, chlorophyll content, water-use efficiency, proline accumulation and ROS-scavenging activity. Although the study was conducted in chickpea and under *Fusarium* stress, it provides physiologically relevant evidence that affected-plant extracts can function as biological signals capable of modifying plant metabolic and stress-response processes.

The superior protein accumulation under WAPE @ 20 ppm is also noteworthy in view of the established importance of maintaining physiological activity during seed filling. Previous studies in lentil have shown that environmental stress can substantially alter seed protein and other nutritional attributes. Choukri *et al.* (2020) ^[5] reported reductions in crude protein, iron and zinc under heat and combined heat-drought stress, while Kumar *et al.* (2023) ^[14] observed reductions in protein and mineral quality under limited soil moisture. Therefore, the higher protein concentration observed following WAPE application may reflect improved maintenance of source activity and/or assimilate allocation during reproductive development. However, because photosynthesis, nitrogen uptake and post-flowering nitrogen remobilization were not measured in the present study, this mechanism should be regarded as a physiological interpretation rather than a demonstrated causal pathway.

The response of carbohydrate concentration was almost opposite to that of protein. L 4717 recorded higher pooled carbohydrate concentration (66.49%) than L 4727 (62.35%). Among modulators, RKN @ 10 ppm maintained relatively high carbohydrate concentration (66.42%), whereas WAPE @ 20 ppm recorded the lowest treatment mean (61.58%). At the interaction level, L 4717 $\times$ RKN @ 20 ppm recorded the maximum carbohydrate concentration of 67.81%, while L 4727 $\times$ WAPE @ 20 ppm recorded only 57.74%. The reciprocal relationship between protein and carbohydrate accumulation, particularly in L 4727, suggests differential partitioning of assimilated carbon and nitrogen into storage products. Such a response is consistent with the

physiological concept that enhancement of one storage component can alter the relative contribution of other components to seed dry matter. Whitehead *et al.* and Zakeri and Bueckert, as summarized in the review, emphasized that post-flowering biomass and nitrogen allocation are important determinants of reproductive sink development and seed formation.

Fat concentration also responded strongly to the biological treatments. WAPE @ 20 ppm produced the highest pooled fat concentration (3.06%), while WAPE @ 10 ppm and salicylic acid @ 100 ppm recorded only 1.04%. The highest interaction value was observed in L 4727 $\times$ WAPE @ 20 ppm (3.09%). The simultaneous increase in protein and fat under this treatment suggests that WAPE @ 20 ppm may have promoted a broader shift in seed reserve accumulation rather than merely increasing a single biochemical constituent. Nevertheless, the magnitude of this response should be interpreted cautiously because the present experiment did not quantify the enzymatic pathways involved in lipid biosynthesis or carbon partitioning.

The response to salicylic acid was comparatively different. Salicylic acid @ 200 ppm produced the highest pooled seed moisture (5.23%) but did not maximize protein, fat or carbohydrate concentration. Previous studies in the review indicate that SA acts as a signalling molecule capable of modifying photosynthesis, antioxidant defence, membrane stability and reproductive development in lentil. Kayednezami *et al.* (2012) ^[13] reported that SA modified growth, yield components and physiological responses under salinity, while Dhar *et al.* (2025) ^[9] observed improved plant growth, branching, pod-bearing capacity and chlorophyll status following SA application. Bouallegue *et al.* (2025) ^[4] further demonstrated that SA could alleviate salinity-induced reductions in photosynthetic activity and regulate oxidative stress. These findings support the interpretation that the response of SA-treated plants is mediated through broader physiological regulation rather than direct enhancement of a particular seed constituent.

Ash concentration was also significantly affected by both genotype and modulator. L 4727 recorded higher pooled ash (3.48%) than L 4717 (3.15%), while the treatment means ranged from 2.52% with WAPE @ 30 ppm to 3.51% with WAPE @ 10 ppm and salicylic acid @ 100 ppm. The highest interaction value was recorded in L 4727 $\times$ WAPE @ 20 ppm (3.70%). Since ash represents the total mineral residue, these differences may reflect variation in mineral acquisition, translocation and deposition in the seed. However, individual mineral elements were not estimated; therefore, the response should not be interpreted as enrichment of any specific mineral nutrient.

The observed treatment-dependent changes in seed composition are consistent with earlier evidence that physiological regulation during crop growth can extend beyond biomass and yield to influence harvested seed quality. Giannakoula *et al.* (2012) ^[11] demonstrated that application of different plant growth regulators altered the biochemical composition of lentil seed, including its phenolic profile and antioxidant-related compounds. Similarly, the review indicates that *Trichoderma* can influence lentil productivity and nutritional quality beyond its conventional role in disease suppression, although responses vary considerably among strains. Marra *et al.* (2021) ^[16] reported strain-dependent effects of *Trichoderma*

on lentil growth, seed production and micronutrient accumulation.

Overall, the present findings demonstrate that biological and synthetic modulators altered seed proximate composition in a highly genotype-and concentration-dependent manner rather than producing a uniform nutritional response. The

most notable response was obtained with WAPE @ 20 ppm in L 4727, which simultaneously recorded maximum protein (32.28%), fat (3.09%) and ash (3.70%) among the variety × modulator combinations, while L 4717 × RKN @ 20 ppm favoured carbohydrate accumulation (67.81%).

Table 1: Influence of genotype and biological and synthetic modulators on proximate composition of lentil seed (*Lens culinaris* Medik.)

Treatments	Moisture (%)			Fat (%)			Protein			Ash (%)			Carbohydrate		
	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled
V1	4.030	4.000	4.010	1.890	1.830	1.860	24.620	24.610	24.600	3.140	3.130	3.150	66.560	66.430	66.490
V2	3.990	3.970	3.980	1.900	1.860	1.880	28.400	28.450	28.400	3.470	3.380	3.480	62.350	62.340	62.350
SEm±	0.008	0.002	0.004	0.005	0.003	0.003	0.011	0.038	0.015	0.019	0.019	0.015	0.049	0.041	0.034
CD (0.05)	0.023	0.006	0.013	NS	0.008	0.008	0.031	0.109	0.042	0.054	0.053	0.042	0.140	0.118	0.097
T1	3.170	3.150	3.160	3.060	3.030	3.040	23.820	23.890	23.800	3.500	3.500	3.480	65.880	66.430	66.160
T2	3.430	3.450	3.440	2.070	2.010	2.040	24.790	24.870	24.760	3.380	3.350	3.350	66.520	66.320	66.420
T3	3.570	3.550	3.560	2.140	2.020	2.080	26.500	26.470	26.480	3.320	3.320	3.430	64.660	64.650	64.650
T4	3.680	3.650	3.670	2.060	2.030	2.050	26.880	26.450	26.660	3.070	2.580	3.060	65.040	65.290	65.170
T5	3.520	3.500	3.510	1.070	1.010	1.040	26.680	26.670	26.710	3.520	3.470	3.510	65.610	65.350	65.480
T6	3.280	3.250	3.260	3.070	3.050	3.060	28.770	28.900	28.820	3.430	3.430	3.420	61.800	61.370	61.580
T7	4.390	4.360	4.370	1.070	1.020	1.050	26.720	26.840	26.800	2.500	2.500	2.520	65.350	65.290	65.320
T8	5.170	5.110	5.140	1.050	1.020	1.040	27.010	27.010	27.010	3.470	3.470	3.510	63.700	63.400	63.550
T9	5.240	5.210	5.230	2.100	2.020	2.060	24.940	24.980	24.970	3.480	3.480	3.430	64.330	64.310	64.320
T10	4.290	4.300	4.300	2.070	2.030	2.050	27.700	27.760	27.700	3.330	3.330	3.380	62.680	62.570	62.630
T11	4.340	4.300	4.320	1.110	1.060	1.090	27.850	27.970	27.770	3.380	3.380	3.360	63.410	63.290	63.350
SEm±	0.019	0.005	0.010	0.011	0.007	0.010	0.025	0.090	0.034	0.044	0.044	0.035	0.115	0.097	0.080
CD (0.05)	0.053	0.014	0.030	0.031	0.019	0.030	0.072	0.257	0.097	0.126	0.125	0.099	0.328	0.276	0.229
Interaction (V×T)															
Treatments	Moisture (%)			Fat (%)			Protein (%)			Ash (%)			Carbohydrate (%)		
	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled
V1T1	3.270	3.290	3.280	3.050	3.010	3.030	23.260	23.290	23.280	3.530	3.530	3.530	66.490	66.870	66.680
V1T2	3.360	3.400	3.380	2.060	2.010	2.040	24.230	24.050	24.140	3.270	3.200	3.230	67.550	67.340	67.440
V1T3	3.220	3.200	3.210	2.200	2.020	2.110	23.630	23.630	23.630	3.330	3.330	3.330	67.810	67.820	67.810
V1T4	3.830	3.800	3.820	2.060	2.010	2.040	25.980	24.980	25.480	2.470	2.500	2.480	66.520	66.720	66.620
V1T5	3.700	3.700	3.700	1.050	1.010	1.030	24.720	24.690	24.700	3.400	3.300	3.350	67.780	67.300	67.540
V1T6	3.170	3.100	3.130	3.050	3.010	3.030	25.210	25.500	25.350	3.170	3.170	3.170	65.640	65.220	65.430
V1T7	4.540	4.500	4.520	1.060	1.010	1.030	26.160	26.100	26.230	2.300	2.300	2.300	66.400	66.100	66.250
V1T8	5.250	5.210	5.230	1.050	1.010	1.030	24.580	24.750	24.660	3.430	3.430	3.430	65.830	65.600	65.720
V1T9	5.070	5.010	5.040	2.100	2.020	2.060	23.550	23.930	23.650	3.300	3.300	3.300	65.740	65.750	65.740
V1T10	4.340	4.300	4.320	2.060	2.010	2.040	24.260	24.050	24.230	3.170	3.170	3.170	66.760	66.470	66.620
V1T11	4.530	4.500	4.520	1.080	1.020	1.050	25.270	25.710	25.200	3.200	3.200	3.200	65.600	65.570	65.580
V2T1	3.070	3.010	3.040	3.070	3.040	3.060	24.370	24.490	24.320	3.470	3.470	3.470	65.270	65.990	65.630
V2T2	3.500	3.500	3.500	2.070	2.020	2.050	25.340	25.690	25.390	3.500	3.500	3.500	65.490	65.300	65.400
V2T3	3.920	3.890	3.900	2.080	2.020	2.050	29.370	29.310	29.330	3.300	3.300	3.300	61.510	61.480	61.500
V2T4	3.530	3.510	3.520	2.060	2.040	2.050	27.780	27.920	27.850	3.670	2.670	3.170	63.560	63.860	63.710
V2T5	3.330	3.300	3.320	1.090	1.010	1.050	28.640	28.660	28.710	3.630	3.630	3.630	63.440	63.400	63.420
V2T6	3.390	3.400	3.400	3.090	3.090	3.090	32.320	32.300	32.280	3.700	3.700	3.700	57.960	57.510	57.740
V2T7	4.230	4.210	4.220	1.090	1.030	1.060	27.280	27.580	27.360	2.700	2.700	2.700	64.290	64.490	64.390
V2T8	5.080	5.010	5.050	1.060	1.030	1.040	29.430	29.270	29.350	3.500	3.500	3.500	61.560	61.190	61.380
V2T9	5.420	5.410	5.410	2.090	2.020	2.060	26.320	26.030	26.280	3.670	3.670	3.670	62.920	62.870	62.890
V2T10	4.250	4.300	4.280	2.070	2.050	2.060	31.150	31.470	31.170	3.500	3.500	3.500	58.600	58.680	58.640
V2T11	4.150	4.100	4.130	1.140	1.100	1.120	30.440	30.220	30.330	3.570	3.570	3.570	61.220	61.010	61.120
SEm±	0.026	0.007	0.015	0.015	0.010	0.009	0.036	0.127	0.048	0.063	0.062	0.049	0.163	0.137	0.113
CD (0.05)	0.075	0.019	0.042	0.043	0.027	0.025	0.102	0.363	0.138	0.179	0.177	0.140	0.465	0.390	0.323

Conclusion

Seed proximate composition was significantly influenced by genotype and plant growth modulator treatments, with pronounced genotype × plant growth modulator interactions. L 4727 recorded higher protein (28.40%) and ash (3.48%), whereas L 4717 had greater carbohydrate content (66.49%). WAPE @ 20 ppm produced the highest protein (28.82%) and fat (3.06%). The L 4727 × WAPE @ 20 ppm combination recorded maximum protein (32.28%), fat (3.09%) and ash (3.70%), while L 4717 × RKN @ 20 ppm maximized carbohydrate (67.81%). These findings indicate differential regulation of seed reserve partitioning and

highlight WAPE @ 20 ppm, particularly in L 4727, as a promising treatment for improving lentil seed nutritional quality.

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