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Research Article

EVALUATION OF DIVERSE MEDIUMS AS AN ECO-FRIENDLY PEAT ALTERNATIVE

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ABSTRACT. The excessive reliance on chemical fertilizers in regions of high agricultural productivity has well-documented detrimental impacts on both environmental quality and human health. Fertigation has emerged as a promising strategy to reduce chemical fertilizer usage while sustaining crop performance. This study aimed to identify alternative *Rhizobium* carrier materials that are free from hazardous metals and organic contaminants, while offering greater convenience, stability, and suitability for the long-term survival and practical application of *Rhizobium* in agriculture. The survivability of *Rhizobium* was assessed in a range of carrier substrates, including peat, perlite, pumice, mushroom compost, sawdust, barley powder, sewage sludge, coal, K-humate, perlite + K-humate, pumice + K-humate, and sawdust + K-humate. The results indicated that K-humate, coal, barley powder, and sawdust can effectively support *Rhizobium* survival, presenting viable alternatives to the conventional peat medium. Moreover, *Rhizobium* isolated from different carrier materials exhibited significantly different effects on selected yield components of bean (*Phaseolus vulgaris* L.) plants, with the differences being statistically significant ($p < 0.01$). These findings provide valuable insights into the potential of alternative carrier materials for maintaining *Rhizobium* viability and functionality, thereby enhancing the feasibility of environmentally sustainable legume production systems and reducing dependence on conventional chemical fertilization.

Abbreviations: CFU, colony-forming unit; CRD, completely randomized design; SPAD, Soil Plant Analysis Development; YMA, yeast mannitol agar

Keywords: *Peat, Medium, Bacteria, Bean, Eco-friendly*

INTRODUCTION

In contemporary agricultural research, a key objective is to enhance crop productivity while maintaining the ecological balance of the soil. Commercial legume inoculant formulations are generally prepared using carriers in powder or granular form, as well as liquid cultures. Among these, peat has long been regarded as the preferred medium for agricultural use [1,2].

An ideal carrier material for transporting beneficial microorganisms should possess several important characteristics: a high water-holding capacity, absence of harmful chemicals that could impair microbial viability, ecological safety, and the capacity to be easily adjusted to a neutral or near-neutral pH. In addition, it should be readily available in large quantities and at a reasonable cost. While liquid inoculants offer simplicity in production and ease of application to seeds or soil, they often suffer from reduced bacterial viability over time [3,4].

Composts and other organic or mineral substances have been explored as possible substitutes for peat in microbial inoculant formulations. The survival of bacteria on

inoculated seeds is primarily affected by dehydration, toxic exudates from the seed coat, and elevated temperatures. A variety of materials have been trialed as carriers for seed or soil inoculation. Rhizobium in peat-based inoculants shows notable resistance to acidic fertilizers and maintains high viability for extended periods when used as a seed coating. Even under delayed seed germination or suboptimal soil pH, peat-based products tend to yield more favorable results [5,6].

Historically, peat has been the predominant carrier medium in both research and field applications. However, concerns regarding the limited availability of high-quality peat suitable for inoculant production have prompted researchers to examine alternative materials. Studies have shown that substances such as sugarcane flour, coal dust, and lignite powder can support Rhizobium survival and, in some cases, result in substantial yield gains. For instance, the use of coal dust alone or in combination with lignite powder has been reported to enhance soybean yields by 53–123% [7].

Against this background, the present study aims to identify alternative carrier materials capable of replacing peat for use with Rhizobium bacteria, which are vital for biological nitrogen fixation. The investigation evaluates the suitability of selected materials for sustaining bacterial viability following a six-month incubation period after inoculation.

MATERIALS AND METHODS

Sterilization of carrier materials used in the experiment

The research used various materials, including peat, perlite, pumice, mushroom compost, sawdust, barley powder, sewage sludge, coal, K-Humate, perlite combined with K-Humate, pumice combined with K-Humate, and sawdust combined with K-Humate. The reference carrier employed was composed of peat material. The carrier materials were dried in ambient air and subsequently cleaned of any debris or waste. They were then crushed and filtered using a 250 µm screen mesh. The heterogeneous components were combined in a 1:1 proportion based on dry weight. The pH of all carrier materials was adjusted to a range of 6.5–7.5 using H_2SO_4. After moistening (without forming mud), the carriers were enclosed in 100 g polyethylene bags and sealed at high temperature. They were then pierced with a needle and sterilized in an autoclave at 121 °C for 1 hour. Table 1 presents the results of various physical and chemical analyses of the carrier materials used in the experiment.

Table 1. *Physical and chemical properties of some carrier materials used in the research*

	Properties	S	BP	P	MC	K-Humate	CO	PE	SS
	pH	6.52	6.70	7.62	7.60	9.82	7.64	7.11	7.83
	EC (mS/cm)	0.34	4.31	0.16	12.82	24.93	0.40	0.17	5.78
	Field Capacity	69.69	81.53	42.16	59.84	47.23	23.07	88.76	65.89
%	**Wilting point**	47.69	72.45	21.98	36.61	44.77	7.36	79.37	57.21
	Org. matter	99.5	84.9	4.62	62.2	66.7	77.8	2.47	54.98
	Organic C	56.37	44.49	---	32.21	48.65	62.62	0.06	31.89
	Total N	0.22	1.27	---	1.9	1.14	1.78	0.19	4.31

	Properties	S	BP	P	MC	K-Humate	CO	PE	SS
	Ca	1253	1803	742.5	40954	13740	1116	686.9	41397
	K	403	17986	282	19367	84761	220	175	12390
	Mg	135	333.2	78.77	4547	625.4	232.5	60.3	9563
	Na	171.66	582.77	210.42	1980	12237	458.73	288.17	1015
H_2O soluble	**P**	50.75	1658	23.75	699.07	62.20	22.06	17.20	12811
	B	3.71	9.60	5.01	17.08	74.18	21.99	9.84	42.86
$mgkg^{-1}$	**Cu**	4.21	9.37	3.44	28.56	34.37	3.15	3.25	399
	Fe	12.93	49.30	116.59	45.46	7271	27.68	20.78	5320
	Mn	7.54	9.18	2.83	76.14	55.46	2.27	1.72	357.04
	Zn	3.51	14.75	1.87	13.72	20.81	1.63	1.50	2155
	Cd	0.08	0.12	0.09	0.13	0.16	0.12	0.07	9.9
Total	**Cr**	1.19	2.62	3.80	13.61	9.96	23.86	1.66	490
	Ni	0.64	1.82	7.35	33.08	7.25	33.88	3.01	182
	Pb	8.24	73.05	8.31	17.77	38.63	145.63	16.71	118

S: Sawdust; BP: Barley powder; PE: Perlite; P: Pumice; MC: Mushroom compost; SS: Sewage sludge, CO: Coal

Multiplication and purification of bacterial strains used in the experiment

The study employed a specific strain of *Rhizobium phaseoli* that is compatible with beans. This strain was cultivated on a medium called YMA (Yeast Mannitol Agar), as described by Somasegaran and Hoben [8].

Incubation of the carrier materials

Following sterilization, the bags were transferred to a germ-free setting and inoculated with 10 mL of a contaminant-free rhizobia suspension containing 1×10^8–10^9 CFU/mL. Following inoculation, the bags were agitated to ensure uniform dispersion and then placed in an incubator at a temperature of 28 °C for approximately 6 months.

Setting up and conducting the greenhouse trial

After the incubation of the carrier materials (peat, perlite, pumice, mushroom compost, sawdust, barley powder, sewage sludge, coal, K-Humate, perlite + K-Humate, pumice + K-Humate, and sawdust powder + K-Humate), a trial was conducted to assess the efficacy of cultivating and propagating bacteria using the Akman 98 bean variety. The cultivar was acquired from the Eskişehir Agricultural Research Institute. The rhizobium bacteria, after incubation and confirmation through biochemical tests, were evaluated against the reference bacteria. This was done by cultivating Akman 98 bean plants in a sterile greenhouse environment devoid of nitrogen, using the modified Leonard bottle-jar system. The bean seeds were immersed in a 0.5% sodium hypochlorite solution for 2–3 minutes, followed by thorough rinsing with sterile distilled water; this process was repeated six times. The seeds were placed in Petri dishes containing 0.8% water agar. The dishes were then kept at a temperature of 28 °C for a period of 3 days to facilitate germination. The sprouted seeds were placed in the modified Leonard bottle-jar system. A total of 750 mL of nitrogen-free Jensen nutrient solution was diluted at a ratio of 1:5 and poured into the jars. KNO3 was added to the solutions of the nitrogenous control plants at a rate of 70 ppm N. Three seedlings that had germinated on the agar medium were transplanted into the bottle-jar system. The bacterial solution was introduced into each jar at a rate of 10 mL per jar. Following the inoculation procedure, the jars were relocated to the greenhouse. Several measurements were taken on plants harvested after the flowering stage, including root and aboveground length, aboveground fresh and dry weight, root fresh and dry weight, number and weight of nodules, nitrogen content of the aboveground parts and roots, and leaf SPAD value. The plant materials were dried in an

oven at a temperature of 70 °C until a constant weight was reached. Subsequently, the weight and nitrogen content of both the aboveground parts of the plant and the roots were measured.

Statistical analysis

The data obtained from the greenhouse trial, which was established using a completely randomized design (CRD), were subjected to analysis of variance using the Minitab 19 statistical program. Treatments with significant differences were grouped using Duncan's multiple comparison test.

RESULTS

Greenhouse Trial Results

Carrier materials, including K-humate, peat, and their mixtures, demonstrated notably high chlorophyll levels, which influence various aspects of plant growth and development. This analytical approach, widely adopted in agricultural research, enables the evaluation of plant leaves for chlorophyll content, mineral composition, and water status, as well as the visual quality of leaves and flowers. It also allows for the identification and quantification of diseased leaves, assessment of disease severity, and evaluation of the effects of biotic and abiotic stress factors on crop growth and yield, alongside the assessment of fruit quality and classification of vegetables and fruits based on color.

Variation in chlorophyll levels among treatments was evident, with the highest concentration recorded in the K-humate carrier material and the lowest in the control, a pattern consistent with previous findings [9].

Plant Growth Parameters

Plant height in the greenhouse experiment, using bacteria isolated from various carrier materials, ranged from 18.83 cm to 29.17 cm (Table 2a and b). Statistically significant differences were detected among carriers ($p < 0.01$). The tallest plants (29.17 cm) were observed with bacteria from the reference material (peat), while the shortest (18.83 cm) occurred with the coal application (Figure 1).

Table 2a. *Some yield elements obtained from the greenhouse trial of Akman 98 bean variety plants with bacteria inoculated into different carrier media*

Medium	(SPAD value)	Length (cm)		Shoot (g)		Root (g)	
		Plant	Root	Wet weight	Dry weight	Wet weight	Dry weight
S	35.72bc	26.67ab	17.67ab	15.37abc	3.42a	14.50cde	1.00c
BP	36.77bc	25.50bcd	15.50abc	14.04a-d	2.96ab	18.67a	1.87ab
PE	30.46fg	25.67abc	17.50abc	12.90cd	3.12ab	15.10b-e	1.23abc
P	31.30ef	25.33bcd	17.33abc	13.27cd	3.16ab	13.30ef	1.26abc
MC	32.59def	22.00def	18.33a	14.00a-d	3.43ab	13.00ef	1.23abc
P+KHU	34.57cd	26.33ab	16.17abc	15.67abc	3.70ab	17.00a-d	1.53abc
PE+KHU	39.90a	23.67b-e	13.67bc	13.42bcd	3.66ab	17.87abc	1.48abc
S+KHU	39.82a	24.67b-e	17.00abc	14.67a-d	3.56ab	17.67abc	1.84ab
CO	36.95b	18.83f	16.00abc	14.77abc	3.20ab	13.33ef	1.13bc
KHUMAT	41.29a	22.00def	15.17abc	16.47ab	3.89ab	19.56a	1.89a
SS	30.79ef	24.83b-e	16.17abc	13.67bcd	3.56ab	14.17de	1.36abc
PEAT	40.64a	29.17a	18.33a	17.17a	4.09a	18.13ab	1.61abc
NC	33.08de	22.27c-f	13.60c	15.80abc	3.53ab	16.93a-d	1.37abc
C	28.12g	21.33ef	16.50abc	11.53d	2.60b	10.53f	1.06c

The difference between the means indicated by different letters is statistically significant ($p<0.01$)

S: Sawdust; BP: Barley powder; PE: Perlite; P: Pumice; MC: Mushroom compost; SS: Sewage sludge, CO: Coal; PO+KHU: Pumice+K-Humate; PE+KHU: Perlite+K-Humate; TT+KHU: Sawdust+K-Humate, PEAT: Reference culture; NC: Nitrogenous control; C: Control

Table 2b. *Some yield elements obtained from the greenhouse trial of Akman 98 bean variety plants with bacteria inoculated into different carrier media*

Medium	Nodulation values			% N	
	Number	Weight (g)	Diameter (mm)	Plant	Root
S	89.00c	1.27a	4.00bcd	1.84cd	1.13b
BP	112.33a	0.80ab	4.33bc	2.09bc	0.99bc
PE	47.67ef	0.71bc	2.67cd	1.15fg	0.91bc
P	82.33cd	1.00ab	3.00cd	1.65cde	0.88bc
MC	76.00cd	0.95ab	3.33cd	1.23efg	0.65c
P+KHU	93.67abc	0.72bc	3.67bcd	1.60def	1.01bc
PE+KHU	91.67bc	1.22ab	4.67bc	1.66cde	0.98bc
S+KHU	62.33de	1.14ab	4.00bcd	1.84cd	0.93bc
CO	111.67ab	0.74b	4.67bc	1.87cd	1.17b
KHUMAT	112.33a	1.20ab	7.33a	2.42b	1.28ab
SS	28.67fg	0.17d	3.33cd	1.25efg	0.82bc
PEAT	113.67a	1.21ab	6.00b	3.25a	1.69a
NC	13.00gh	0.20cd	1.67de	2.07bcd	1.20b
C	0.00h	0.00d	0.00e	1.00g	0.65c

The difference between the means indicated by different letters is statistically significant ($p<0.01$)

S: Sawdust; BP: Barley powder; PE: Perlite; P: Pumice; MC: Mushroom compost; SS: Sewage sludge, CO: Coal; PO+KHU: Pumice+K-Humate; PE+KHU: Perlite+K-Humate; TT+KHU: Sawdust+K-Humate, PEAT: Reference culture; NC: Nitrogenous control; C: Control.

Root length ranged from 13.60 cm to 18.33 cm, with the nitrogenous control medium producing the shortest roots and the peat reference culture producing the longest. Plant wet weight varied from 11.53 g to 17.17 g, with peat yielding the highest values and the absolute control the lowest. Wet root weight ranged from 10.53 g to 19.56 g, with the highest value obtained from K-humate and the lowest from the control (Figure 1).

Dry weight differences among treatments were also statistically significant ($p < 0.01$). The highest dry weight (4.09 g) occurred in plants inoculated with bacteria from the peat carrier, while plants from the control carrier recorded the lowest dry weights.

Nodulation and Nitrogen Content

The highest number of nodules (114) was obtained from plants inoculated with bacteria from the peat material. The heaviest total nodule weight (1.27 g) was recorded from plants inoculated with bacteria from the sawdust carrier. Nodule size differed significantly across carrier materials ($p < 0.01$), with the largest diameters observed in plants treated with K-humate.

Nitrogen content in bean plants also varied significantly, with peat showing the highest levels. Sawdust, barley powder, and the nitrogenous control were among the most effective carriers in terms of plant performance, while perlite, pumice, and mushroom compost were moderately effective. Sewage sludge and the absolute control exhibited the lowest efficiencies.

Overall Efficiency Ranking

Analysis of efficiency levels in *Akman 98* bean plants indicated statistically significant differences among carrier materials ($p < 0.01$). The results suggest a gradient of effectiveness among the carrier materials (Fig. 1).

- High effectiveness: peat, K-humate, sawdust, barley powder, nitrogenous control
- Moderate effectiveness: perlite, pumice, mushroom compost
- Low effectiveness: sewage sludge, absolute control

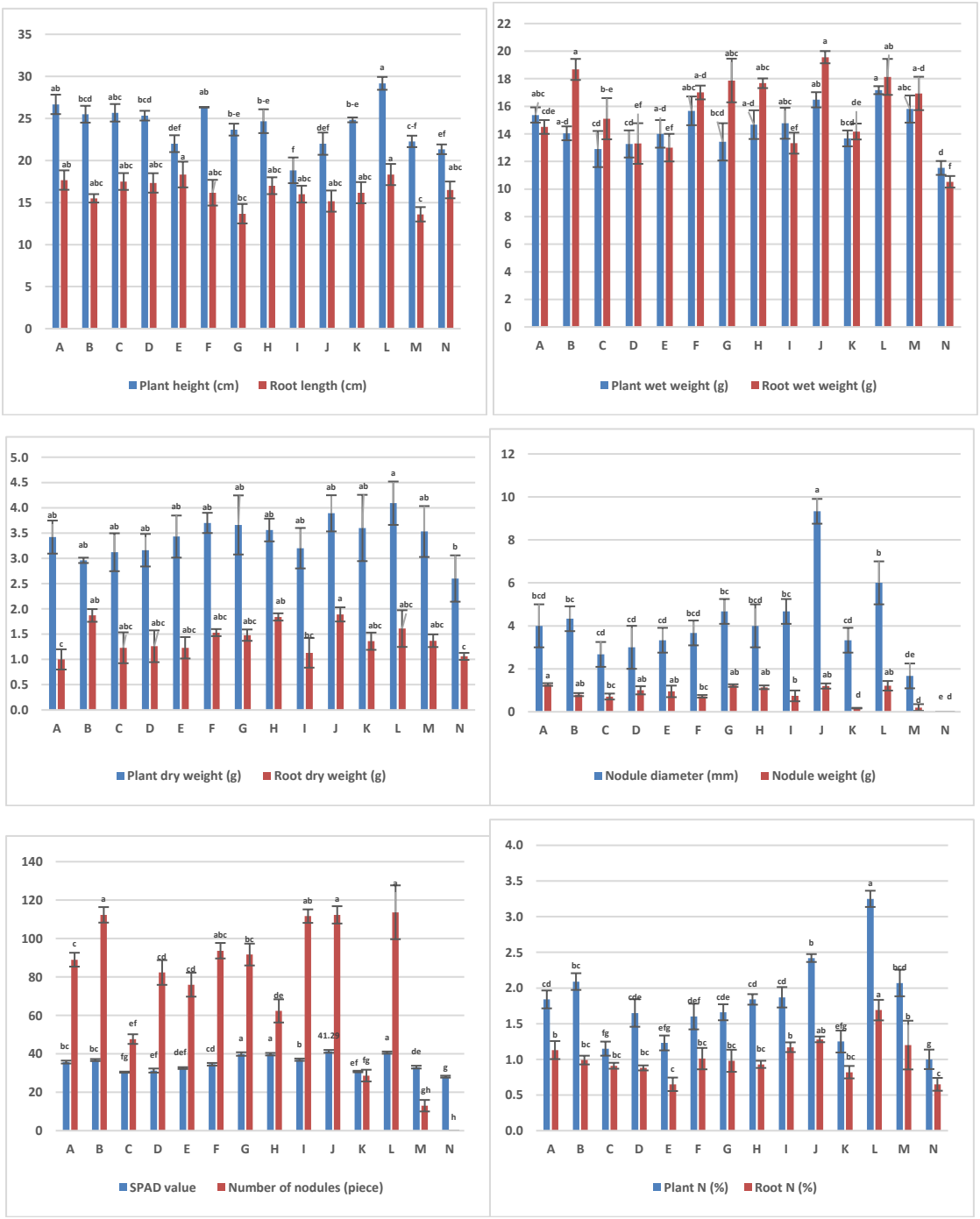


Fig. 1. *Some yield elements obtained from the greenhouse trial of Akman 98 bean variety plants with bacteria inoculated into different carrier media (A: sawdust; B: barley powder; C: perlite; D: pumice; E: mushroom compost; F: pumice+K-humate; G: perlite+K-humate; H: sawdust+K-humate; I: coal; J: K-humate; K: sewage sludge; L: peat; M: nitrogenous control; N: control). Panels, left to right and top to bottom: plant height and root length (cm); plant and root wet weight (g); plant and root dry weight (g); nodule diameter (mm) and nodule weight (g); SPAD value and number of nodules; plant and root N (%). Different letters above the bars indicate significant differences ($p < 0.01$)*

DISCUSSION

This study evaluated the survivability of *Rhizobium* in a range of carrier materials after a six-month incubation period, aiming to identify practical, safe, and effective alternatives to peat for long-term inoculant production. Peat has historically been the preferred carrier due to its favorable physicochemical properties and long record of success in legume inoculation [2, 10]. However, limitations in availability, cost, and environmental sustainability have prompted extensive research into alternative carriers [11, 5, 12].

The results align with earlier findings that carrier type significantly influences rhizobial survival and effectiveness [13, 14]. Among the tested materials, peat consistently supported high bacterial populations, confirming its role as a benchmark standard. However, several other materials including K-Humate, coal, barley powder, and sawdust also sustained viable populations and promoted favorable plant performance. Similar to the studies of Ashok et al. [15] and Ferreira & Castro [16], these carriers maintained high cell counts during storage and supported nodulation and yield comparably to peat.

Our findings on perlite, pumice, and mushroom compost as moderately effective carriers are consistent with Daza et al. [17] and Albareda et al. [12], who demonstrated that such materials can match peat in sustaining viable rhizobial populations, nodulation potential, and nitrogen fixation rates. The high performance of K-Humate observed in this study both in wet root biomass and nodule size can be attributed to its organic matter content, humic substances, and favorable pH buffering capacity, which improve microbial longevity and plant-microbe interactions [18; 19].

The influence of pH on rhizobial performance was also evident, reinforcing the work of Singleton et al. [20] and Elkoca & Kantar [21], which identified an optimal pH range of 6.5–7.0 for effective nodulation and persistence. Acidic or alkaline extremes can impair infection efficiency, reduce nodule functionality, and accelerate population decline. This underlines the need for carriers that can buffer pH changes during storage and after inoculation [22].

Notably, differences in chlorophyll concentration among treatments mirrored differences in nitrogen content and plant biomass, supporting the positive correlation between leaf chlorophyll readings and nitrogen status documented by Reeves et al. [23] and Matsunaka et al. [24]. As Sara et al. [25] observed, chlorophyll is strongly linked with vegetative growth, and in legumes, this is often directly enhanced by effective symbiotic nitrogen fixation. In our trial, materials yielding higher chlorophyll and nitrogen levels, such as peat, K-Humate, and sawdust, also produced taller plants, heavier root systems, and greater dry matter, consistent with Kacar et al. [26], who described the critical nutrient support the host plant provides during infection and nodule development.

The high nodule counts in peat treatments confirm its enduring reliability, yet the maximum nodule weight recorded for sawdust derived inoculants suggests that certain carriers may promote fewer but more metabolically active nodules. This observation aligns with previous findings that nodule size can sometimes be a better indicator of nitrogen fixation efficiency than nodule number alone [27].

While perlite, pumice, and mushroom compost were moderately effective, sewage sludge and the absolute control produced the lowest results across measured variables. This reinforces the importance of selecting carriers with balanced physical properties, low contamination risk, and minimal inhibitory compounds [1, 28].

In conclusion, this study confirms that multiple alternative carriers particularly K-Humate, coal, barley powder, and sawdust are viable replacements for peat

in *Rhizobium* inoculant production, offering competitive survival rates and positive agronomic impacts after extended storage. These findings complement earlier work by Albareda et al. [12] and Ashok et al. [15] and highlight the potential of carrier diversification to support sustainable agriculture. Given the influence of carrier pH, nutrient content, and moisture retention on rhizobial viability and plant performance, careful selection of material ideally sourced from renewable or waste resources can simultaneously improve productivity, reduce environmental impact, and extend inoculant shelf life.

Declaration of competing interest. The authors declare that they have no conflict of interest.

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