PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Role of Biostimulants in Sustainable Soybean (Glycine max L.) Production: A Systematic Review.	Title page identifies as a systematic review
ABSTRACT			
Abstract	2	Abstract summarizes aim, scope (2014 to 2025), included studies (72), methods, key findings	Abstract
INTRODUCTION			
Rationale	3	Soybean is known for its nitrogen fixing ability and the critical role in global food and nutritional security due to its high protein and oil content, yet its productivity is increasingly constrained by multiple abiotic stresses and soil degradation. Conventional agricultural solutions particularly the intensive use of synthetic fertilizers and pesticides have enhanced yields but simultaneously contributed to long-term ecological imbalances, declining soil health, and environmental pollution. As a result, there is a growing emphasis on sustainable, low-impact agronomic practices capable of improving both crop performance and environmental resilience. Biostimulants have emerged as promising tools within this sustainability paradigm. Existing research demonstrates that both non-microbial (humic substances, seaweed extracts, amino acids) and microbial biostimulants (PGPR, PSM, Trichoderma, Bradyrhizobium) can enhance nutrient uptake, modulate plant physiological responses, and improve tolerance to abiotic stress. Studies also highlight their potential to increase soybean yield and quality traits, as well as synergistic benefits when different types of biostimulants are combined. However, despite these advancements, the current body of literature remains fragmented. Research findings are often inconsistent due to variability in experimental designs, environmental conditions, soybean genotypes, stress scenarios, and biostimulant formulations. Moreover, most studies investigate single biostimulant types or narrow stress conditions rather than providing a holistic agronomic assessment. This lack of synthesis limits the development of optimized, evidence-based biostimulant strategies tailored for soybean systems. Given these gaps, a systematic review is needed to consolidate existing findings, critically evaluate the agronomic value of microbial and non-microbial biostimulants, and identify conditions under which they are most effective. Synthesizing this evidence will clarify their true potential to enhance soybean yield and quality, guide future research, and support the formulation of sustainable crop management strategies.	Introduction section
Objectives	4	The aim of the study is to identify and synthesize the existing evidence on the efficacy of biostimulants in improving soybean yield and quality traits and identify the key knowledge gaps and propose optimised biostimulant strategies for sustainable soybean cultivation	Introduction section
METHODS			
Eligibility criteria	5	Inclusion criteria required that the selected articles: (i) contained the relevant keywords in the title, abstract, or author-provided keyword list; (ii) were published in English in a peer-reviewed academic journal; and (iii) investigated the effects of biostimulant applications on soybean seed yield and/or quality parameters. However, the exclusion includes four steps; (i) Articles that provide limited or insufficient information on biostimulants, even if soybean is used as the test crop. (ii). Articles that do not report data on soybean seed yield or quality but instead evaluate biostimulant effects on other crops. (iii) Articles that mention both biostimulants and soybean but lack quantitative or measurable evidence of effects. (iv). Articles in which biostimulants were applied in combination with other agro-inputs or biofertilizers, without isolating or reporting the sole or interaction effects of biostimulants on soybean seed yield and quality. Finally only articles that met all inclusion criteria and did not fall under exclusions I–IV were selected for systematic evaluation. In term of groupings, because the effectiveness of biostimulants in crop production is shaped by a multidimensional set of agronomic, environmental, and biochemical drivers, the included studies were grouped according to the type of biostimulant applied (such as humic substances, seaweed extracts, amino acid-based products, beneficial microbes or fungi) and the specific outcome variables evaluated (such as seed yield, protein content, or oil content). Additional sub-grouping was performed based on whether biostimulants were applied alone or in combination with other agro-inputs, such as macro- or micronutrient fertilizers. This grouping approach enabled the synthesis to: (i) assess the independent effects of each biostimulant category on key soybean traits, (ii) compare response patterns across different agronomic and environmental contexts, (iii) identify inconsistencies, variability, and gaps in the current evidence base, and (iv) provide a systematic structure that supports a clear, coherent descriptive narrative. Overall, this grouping strategy facilitated the integration of diverse and heterogeneous findings, allowing the review to present a comprehensive and up-to-date understanding of the major factors influencing soybean yield and quality responses to biostimulant application.	Methods (section 2)

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Information sources	6	Web of science, the search covered from 2014-2025. Last search was in November, 2025	Methods (section 2)
Search strategy	7	Web of Science (coverage 2014–2025; last searched November, 2025). Search: ("biostimulant*" OR "phytostimulant*" OR "biofertiliser*" OR "microorganism*" OR "humic acid*" OR "fulvic acid" OR "seaweed extract*") AND ("Soybean" OR "Glycine max") AND ("yield" OR "productivity" OR "grain quality").	Methods (section 2)
Selection process	8	All records retrieved from the Web of Science database were screened by a single reviewer following the predefined inclusion and exclusion criteria. Titles, abstracts, and full texts were assessed to determine eligibility. No independent duplicate screening was performed. No automation tools or machine-learning methods were used in the screening process; all screening decisions were made manually based on the criteria specified in the Methods section.	Methods (section 2)
Data collection process	9	Data collection and extraction were conducted by a single reviewer, and independent duplicate extraction was not performed. Quantitative information used for the descriptive analysis was obtained directly from the Web of Science export files. For studies in which seed yield or quality data were presented only in graphical form, numerical values were extracted using WebPlotDigitizer. This approach ensured consistent capture of all relevant quantitative outcomes, although the absence of duplicate extraction represents a methodological limitation that is acknowledged in this review	Methods (section 2)
Data items	10a	For this review, data were extracted for all outcomes directly related to soybean performance following biostimulant application. The primary outcomes sought were: Seed yield (kg ha^{-1}, g $plant^{-1}$, t ha^{-1}) and Seed quality parameters, including: Protein content (%) Oil content (%). For each outcome domain, all reported quantitative results compatible with that domain were extracted, regardless of measurement method, time point, or statistical treatment. When multiple time points or repeated analyses were presented, the final harvest measurement was extracted, unless studies did not provide such data in which case all available and relevant time points were recorded. No prioritization was applied based on statistical significance.	Methods (section 2)
	10b	In addition to outcome data, the following study-level and intervention-level variables were extracted: Study characteristics: author(s), year of publication, country/region, cultivar/ variety (where reported), Biostimulant type, source, formulation, active ingredients (if provided), Application method (foliar, soil, seed treatment, or combined) and soil information when reported. When key methodological or outcome information was missing or unclear, the following assumptions were applied: If seed yield was reported in g $plant^{-1}$, it was converted to kg ha^{-1} using the formula Yield (kg ha^{-1}) = (g $plant^{-1}$ × plants ha^{-1}) / 1000. In some cases where plant density was not reported, a standard soybean population density of 250,000 plants ha^{-1} was assumed, as this represents a commonly used field planting density and ensures consistency across studies	Methods (section 2)
Study risk of bias assessment	11	No formal risk-of-bias assessment was conducted for the included studies. Although general methodological considerations (e.g., clarity of experimental design, replication, controls, and reporting quality) were noted, these observations did not constitute a structured or standardized risk-assessment process. No established tool specific to biostimulant research in soybean was applied, no independent reviewer verification occurred, and no automation tools were used.	Not applicable
Effect measures	12	For all outcomes, results were synthesized descriptively without performing a statistical meta-analysis. The primary effect measure for seed yield was the change in yield and percentage change relative to the untreated control, calculated using the formula: (i) (Treatment − Control) (ii) (Treatment − Control) / Control × 100. When studies reported yield using different units (e.g., t ha^{-1} or g $plant^{-1}$), values were converted to a common unit (kg ha^{-1}) prior to calculating change in yield and percentage change. For seed quality parameters (protein and oil content), the effect measure was also reported as percentage change relative to the control. Because the included studies differed widely in experimental design, biostimulant formulations, and measurement protocols, all outcomes were presented using standardized descriptive comparisons rather than pooled quantitative effect sizes.	Methods (section 2)
Synthesis	13a	To determine which studies were eligible for each synthesis, all included articles were first tabulated according to key intervention characteristics,	Results

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methods		including biostimulant type (e.g., humic substances, seaweed extracts, amino acids, microbial inoculants), formulation, application method, and target outcome (seed yield, protein content, or oil content). These characteristics were compared against the predefined synthesis groups Studies were then allocated to the appropriate synthesis category based on whether their reported outcomes aligned with the planned outcome domains and whether the biostimulant intervention matched the corresponding intervention group. Studies that reported multiple outcomes were included in more than one synthesis as applicable. This structured classification ensured that only studies with comparable interventions and outcome measures contributed to each synthesis	(Section 3)
	13b	Several data preparation steps were required to ensure consistency across studies and enable meaningful comparison. When yield was reported in units other than kg ha^{-1} (e.g., g $plant^{-1}$ or t ha^{-1}), values were converted to a common unit of kg ha^{-1} using standard conversion formulas; for g $plant^{-1}$, a plant population of 250,000 plants ha^{-1} was assumed when density was not reported. Seed quality variables (protein and oil content) were extracted as percentages and presented either as absolute percentage values or converted to percentage change relative to the control when necessary. When outcomes were depicted only in graphical form, numerical values were extracted using WebPlotDigitizer**.** If summary statistics such as standard deviations or sample sizes were not reported, they were not imputed; instead, results from those studies were included descriptively. No additional statistical transformations, imputations, or data-smoothing procedures were performed. All data were checked for internal consistency before being used in the synthesis.	Methods (section 2)
	13c	Results from individual studies were organized into structured evidence tables summarizing key study characteristics (biostimulant type, application method, formulation, soil type, and measured outcomes). Yield and seed quality responses were tabulated by outcome domain and biostimulant category to facilitate comparison across studies. For results presentation, descriptive tables were created to illustrate the distribution of yield and quality effects across biostimulant types, showing change in yield and percentage change relative to control treatments. The PRISMA flow diagram was used to visually present the study-selection process. All tables and figures were generated manually using the extracted dataset after verification for accuracy and internal consistency	Methods (section 2) & Results (Section 3)
	13d	No statistical meta-analysis was made because the included studies exhibited substantial heterogeneity in biostimulant formulations, application rates, experimental environments, and measurement protocols. Instead, results were synthesized using a structured descriptive approach**.** Studies were grouped by biostimulant type and outcome domain (seed yield, protein, and oil content), and findings were narratively compared to identify consistent patterns, divergences, and knowledge gaps. Percentage change relative to the untreated control was used as the primary effect measure to enable comparability across studies reporting outcomes in different units. This narrative synthesis approach was selected because it accommodates substantial methodological diversity and allows integration of findings even when summary statistics required for quantitative pooling are incomplete or inconsistent. No statistical models, heterogeneity tests, or specialized software packages for meta-analysis were used.	Not applicable
	13e	Formal statistical methods for exploring heterogeneity (such as subgroup meta-analysis or meta-regression) were not performed because the available studies differed widely in biostimulant formulations, application rates, experimental designs, and reporting formats, and did not provide sufficient standardized quantitative data for pooled analysis. Instead, potential sources of heterogeneity were explored qualitatively by comparing study outcomes across predefined categories, including biostimulant type (e.g., humic substances, seaweed extracts, amino acids, microbial inoculants), application method (seed treatment, foliar application, soil application), and experimental setting. Differences in soybean variety, soil conditions, and environmental factors were also considered when interpreting variability in results. This descriptive approach allowed identification of contextual factors that may contribute to inconsistent treatment effects across studies	Not applicable
	13f	No formal quantitative sensitivity analyses were conducted because the review did not include a meta-analysis and many studies lacked the standardized summary statistics required for such assessments. However, the robustness of the narrative synthesis was explored qualitatively by examining whether overall conclusions changed when: (i) studies with incomplete methodological information, unclear yield measurements, or missing control data were considered separately; and (ii) studies reporting extreme or unusually large treatment effects were reviewed to determine whether they reflected atypical experimental conditions. This qualitative sensitivity check confirmed that the main patterns identified in the synthesis were not dependent on any single study or subgroup of studies.	Not applicable

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Reporting bias assessment	14	No formal statistical tests for reporting bias were performed because a meta-analysis was not conducted and the number of studies within each outcome category was insufficient for reliable quantitative assessment. Instead, the potential for reporting bias was evaluated qualitatively by examining whether included studies selectively reported positive treatment effects, lacked complete reporting of control data, or omitted key outcome measures described in their methods. Studies with incomplete or selectively reported results were noted during data extraction, and their limitations were considered when interpreting the synthesis. Additionally, the search strategy was designed to minimize retrieval bias by using broad search terms and screening all identified studies from a major indexed database (Web of Science). No automation tools were used for detecting reporting bias.	Not applicable
Certainty assessment	15	A formal quantitative assessment of certainty in the evidence (e.g., using the GRADE approach) was not conducted because the included studies exhibited substantial methodological and reporting heterogeneity, and many lacked the standardized statistical data required for such frameworks. Instead, certainty in the body of evidence for each outcome was evaluated qualitatively by considering: (i) the methodological quality and clarity of the study design; (ii) consistency of findings across studies within the same biostimulant category; (iii) completeness and transparency of outcome reporting; and (iv) the plausibility of the observed effects based on known physiological mechanisms. Outcomes supported by multiple well-designed studies with consistent findings were judged to have higher confidence, whereas outcomes reported by few studies or those showing inconsistent or poorly explained results were interpreted with lower confidence. This qualitative assessment informed the strength of conclusions drawn in the narrative synthesis	Not applicable
RESULTS			
Study selection	16a	Generally the initial search resulted in a total number of 506 publications which were identified from the official Web of Science (WOS) database. However, we later refined to include only peer-reviewed journal articles in English, resulting in 442 publications. These 442 articles then underwent a critical screening process, where their abstracts and where necessary, full texts were evaluated for direct relevance to the core of this study. Based on this rigorous assessment, 72 met the criteria and were included in the synthesis. The process is shown in the PRISMA flow diagram (Figure 1).	Methods (Section 2); & PRISMA Flow Diagram
	16b	Several studies initially appeared to meet the inclusion criteria based on their titles, abstracts, or keywords, but were excluded after full-text assessment. The most common reasons for exclusion were: (i) insufficient or unclear information on the biostimulant intervention; (ii) absence of soybean yield or seed quality outcomes; (iii) lack of quantitative or measurable data; and (iv) application of biostimulants in combination with other agro-inputs without isolating the effect of the biostimulant.	Methods (Section 2); Table 1
Study characteristics	17	All 72 included studies are fully cited in the reference list. Key study characteristics including publication year, country of origin, methodological approach, biostimulant type, and measured variables were extracted from Web of Science metadata and verified against the full-text articles. These characteristics are summarized narratively in the Results section. The complete list of included studies is presented in the References, providing the full citation record for all evidence included in the review	Methods (Section 2); Results (Section 3) & PRISMA Flow Diagram
Risk of bias in studies	18	Risk of bias was not formally assessed because the review did not include a meta-analysis or quantitative synthesis that would require the use of standardized risk-of-bias tools. Instead, the review provides a descriptive synthesis of findings, and all included studies were evaluated primarily based on the clarity of their reported methodologies and outcome measures.	Not applicable
Results of individual studies	19	The results of individual studies are presented narratively and in tabulated format. For each study, the extracted outcome measures (seed yield, protein content, and oil content) and biostimulant characteristics (type, formulation, application method, and rate) are summarised to allow comparison across studies. Where applicable, percentage change in seed yield was calculated to enable standardized reporting across different experimental designs. Detailed characteristics and outcome data for each included study are provided in Table 2- 6, while full citations for all studies appear in the Reference list.	Results (Section 3)
Results of	20a	Across the 72 included studies, substantial variability was observed in the types of biostimulants evaluated, experimental conditions, and	Results

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syntheses		outcome metrics reported. Biostimulants assessed included humic substances, seaweed extracts, amino acid formulations, microbial inoculants, and mixed-compound products. Most experiments were conducted under field conditions, while a smaller proportion involved greenhouse trials. As no formal risk-of-bias tool was applied, study quality was considered descriptively based on reporting completeness, experimental design clarity, and outcome measurement transparency.	(Section 3)
	20b	No statistical meta-analysis was performed because the included studies were highly heterogeneous in biostimulant formulations, application rates and timings, experimental settings outcome measurement units, and reporting of summary statistics. Therefore, no pooled summary estimates, confidence intervals, or formal heterogeneity statistics are reported. Instead, study results were synthesized narratively by biostimulant category. Across syntheses the direction of effect was generally positive: microbial inoculants most consistently increased seed yield, humic substances and seaweed extracts commonly produced moderate yield gains, and amino acid formulations showed variable effects depending on timing and soil fertility. Seed-quality effects (protein and oil) were generally smaller and less consistent across groups	Not applicable
	20c	No investigations of statistical heterogeneity were conducted because no meta-analysis or quantitative synthesis was performed. Instead, variability among studies was explored narratively by examining differences in biostimulant type, formulation, application method, soybean cultivar, experimental conditions, and outcome measures.	Not applicable
	20d	No sensitivity analyses were conducted because the review did not include a quantitative synthesis or meta-analysis. As a result, there were no pooled statistical estimates for which robustness or stability could be formally assessed.	Not applicable
Reporting biases	21	No assessment of risk of bias due to missing results was conducted because the review did not include a statistical synthesis or meta-analysis. Standard methods for detecting reporting bias such as funnel plots or statistical tests for small-study effects were therefore not applicable in this context.	Not applicable
Certainty of evidence	22	No assessment of certainty or confidence in the body of evidence was conducted because the review did not include quantitative effect estimates or statistical synthesis. Standard certainty-grading approaches (such as GRADE) were therefore not applicable within the context of this narrative review.	Not applicable
DISCUSSION			
Discussion	23a	Overall, this review indicates that biostimulants particularly microbial biostimulants (*Rhizobium,* PGPR, AMF, *Trichoderma*), humic substances, amino acids, and seaweed extracts generally enhance soybean seed yield and, to a lesser extent, seed quality. These findings align with growing evidence that biostimulants improve nutrient uptake, root development, and stress tolerance across diverse crops. Microbial biostimulants, alone or combined with other agro-inputs, consistently demonstrated positive effects, supporting prior research that highlights microbial symbioses as key drivers of yield stability and resilience in legumes. Humic substances, amino acids, and seaweed extracts also showed moderate benefits, consistent with reports that these materials influence soil structure, hormone-like activity, and physiological responses to abiotic stress. However, variability in outcomes across studies reflects broader evidence that biostimulant efficacy depends on environmental conditions, crop genotype, formulation quality, and application timing. Addressing genotype-specific responses is particularly critical for advancing biostimulant technology. In general, these results reinforce the potential of biostimulants to improve crop performance, especially under suboptimal or stress-prone conditions. Nevertheless, the lack of standardization in experimental design, reporting units, and product composition limits direct comparability across studies. Collectively, the evidence suggests that while biostimulants can enhance soybean productivity, further rigorously designed, multi-environment trials with consistent reporting are needed to clarify interactions and support the development of precise, reliable, and targeted application strategies across diverse production systems.	Discussion and Conclusion (Section 4)
	23b	The evidence included in this review has several limitations. Studies varied widely in experimental design, biostimulant type, application method, and soybean cultivar, limiting comparability and precluding meta-analysis. Outcome reporting was inconsistent, with different units and measures, and some data were presented only in figures without variability estimates. Many studies provided limited methodological detail, reducing confidence in internal validity and preventing formal risk-of-bias assessment. Most experiments were single-season or single-location trials, restricting understanding of effects across diverse environments. Additionally, contextual factors such as soil fertility, environmental stress, and management practices were often not fully reported, contributing to variability in results. Collectively, these limitations highlight the need for	Discussion and Conclusion (Section 4)

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		standardized, multi-location, and rigorously reported studies to more reliably evaluate the effects of biostimulants on soybean yield and quality.	
	23c	Several limitations in the review process should be acknowledged. First, data extraction and study selection were conducted by a single reviewer, which increases the risk of selection and extraction bias. Second, no formal risk-of-bias assessment or certainty grading (e.g., GRADE) was performed, limiting the ability to assess confidence in the synthesized findings. Third, because the review relied on published literature from selected databases, there is a potential for publication bias, as unpublished or grey literature was not systematically included. Fourth, heterogeneity in study designs, outcome measures, and reporting formats prevented quantitative synthesis, such as meta-analysis, limiting the ability to estimate effect sizes or formally assess statistical heterogeneity. Finally, assumptions made during data standardization (e.g., plant density conversions) may have introduced minor uncertainties in the reported outcomes.	Discussion and Conclusion (Section 4)
	23d	Biostimulants, particularly microbial inoculants, humic substances, and seaweed extracts, can improve soybean yield and, to a lesser extent, seed quality, supporting their integration into crop management strategies under stress-prone or nutrient-limited conditions. Policymakers and extension services should promote evidence-based guidance, product quality standards, and transparent labeling to support sustainable agricultural practices. Future research should focus on multi-location, multi-season trials with standardized outcomes, clearly defined application protocols, and robust reporting to enhance comparability and enable quantitative synthesis	Discussion and Conclusion (Section 4)
OTHER INFORMATION			
Registration and protocol	24a	No registry was used for this review	Not registered
	24b	A review protocol was not prepared and therefore not reported	Not registered
	24c	No registry protocol was created for this review, so no amendments were applicable	Not applicable
Support	25	No external funding or non-financial support was received for this review	Non-financial support
Competing interests	26	No conflicts of interest or competing interests were declared.	Competing interests statement
Availability of data, code and other materials	27	No new data were generated in this study. All data analyzed were extracted from the included studies and standardized for the purposes of this review. These data are fully presented in the Results section, specifically in Tables S1 – S5, and serve as the basis for the narrative synthesis reported.	Not applicable

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. This work is licensed under CC BY 4.0. To view a copy of this license, visit https://creativecommons.org/licenses/by/4.0/