International Journal of Natural and Health Sciences (IJNHS)
Vol.4, No.4, 2026: 491-506

Biotic Elicitor-Induced Enhancement of Bioactive Metabolite Production and Antioxidant Activity in Vitro Cultured Medicinal Plants

Yanti Puspita Sari[1*], Aloysia Sri Pujiyanti[2], Linda Oktavianingsih[3]
Universitas Mulawarman
Corresponding Author: Yanti Puspita Sari ypsman2002@yahoo.com

ARTICLEINFO

Keywords: Antioxidant Activity, Biotic Elisitors, Culture in Vitro, Bioactive Metabolites, Medicinal Plants

Received : 5 August
Revised : 20 September
Accepted : 20 October

©2026 Sari, Pujiyanti, Oktavianingsih : This is an open-access article distributed under the terms of the Creative Commons Atribusi 4.0 Internasional.

ABSTRACT

Medicinal plants are a source of bioactive metabolites, but in vitro culture productivity is often low. This study aims to analyze the influence of the type, concentration, and duration of biotic elisters on the accumulation of metabolites and antioxidant activity. Systematic literature review followed PRISMA 2020 via Scopus, Web of Science, PubMed, and ScienceDirect; 42 articles for the period 2021–2025 were analyzed descriptive-thematically. A total of 34 studies (81.0%) reported an increase in at least one metabolite, while 24 of 30 studies (80.0%) showed an increase in antioxidant activity. Fungal elicitors most often increase metabolites, while chitosan shows the highest proportion of antioxidant increases. The optimal response generally occurs at intermediate concentrations and 48 hours–7 days of exposure. Biotic elitheration has the potential to optimize phytochemical production, but it requires plant-specific, culture, dose, and time-specific standardization

DOI: https://doi.org/10.59890/ijnhs.v4i4.2
E-ISSN: 3025-9665
https://journalijnhs.my.id/index.php/ijnhs/index

INTRODUCTION

Medicinal plants are a source of bioactive metabolites, such as phenolics, flavonoids, alkaloids, terpenoids, and essential oils, which are used in the pharmaceutical, functional food, cosmetic, and nutraceetic industries. However, the production of these compounds through conventional cultivation is often unstable because it is influenced by genotype, plant age, season, soil conditions, temperature, and environmental pressure. In vitro culture offers more controlled production and is not dependent on the season. However, these cultures do not always produce optimal amounts of metabolites, so strategies are needed to increase their biosynthesis activity (Ozyigit et al., 2023).

The development of such technology is important because the increased use of medicinal plants can increase the pressure on their natural populations, especially when the harvested part is in the form of roots, rhizomes, bark, or entire plants. In the Indonesian context, Cahyaningsih et al. (2021) identified around 5,490 species of medicinal plants and designated 233 species as conservation priorities. Around 77.25% of these priority species are known to be harvested destructively. Therefore, metabolite production through in vitro culture can be an alternative to provide uniform bioactive materials while reducing dependence on wild crop harvesting (Cahyaningsih et al., 2021).

One potential approach is elitization, which is the provision of stimuli to activate the plant's defense response. Biotic elicitors, such as chitosan, yeast extracts, pectin, alginates, and components of microorganisms, can be recognized by plant cells as signals that resemble a pathogen attack. The introduction triggers the formation of reactive oxygen species, activation of protein kinases, transcription factors, and enzymes of secondary metabolite biosynthesis. This response can increase the production of phenolics, flavonoids, alkaloids, and terpenoids while strengthening the antioxidant system. Its effectiveness is influenced by the type of elitor, concentration, time of exposure, species, and type of culture used (Bhaskar et al., 2022; Jain et al., 2024).

Various studies have shown the effectiveness of biotic elifiers, but they have given mixed results. Bavi et al. (2022) found that yeast and chitosan extracts affect phenolic and terpenoid content in Zataria multiflora cell suspension cultures. Kanthaliya et al. (2023) reported that pectin 100 mg/L increased the total isoflavonoid Pueraria tuberosa by 1.68 times compared to cultures without elisters and 2.77 times compared to the parent plant. Silva-Santos et al. (2023) also showed that yeast and chitosan extracts modulate the growth, essential oil production, and antioxidant system of Lippia alba. These findings show that optimal conditions are specific to plants and culture systems (Bavi et al., 2022; Kanthaliya et al., 2023; Silva-Santos et al., 2023).

Although research on elitization is evolving, the available evidence is still fragmented by species, type of culture, elicitor, target metabolites, and methods of measuring antioxidant activity. Guru et al. (2022) discuss elicitation broadly, while Alcalde et al. (2022) focus more on adventitious root cultures and hairy roots. The Jain et al. (2024) study outlined the types and mechanisms of biotic elitors, while Karalija et al. (2025) linked them to in vitro culture and multiomics technologies. However, studies that systematically compare the relationship

between the type, concentration, and duration of elicytes with bioactive metabolite production and antioxidant activity are still limited (Alcalde et al., 2022; Karalija et al., 2025).

Based on these gaps, this study aims to systematically analyze the influence of the type, concentration, and duration of exposure to biotic elifiers on the production of bioactive metabolites and antioxidant activity of medicinal plants cultured in vitro. The study used a systematic literature review approach to identify elicitation response patterns, the most responsive metabolite groups, and factors that explain the variation in outcomes between studies. Theoretically, this study is expected to strengthen the understanding of the relationship between elicytes, defense mechanisms, and biosynthesis of secondary metabolites. In practical terms, the results can be the basis for the development of efficient, standardized, and sustainable culture protocols for the pharmaceutical, nutraceethical, and conservation needs of medicinal plants.

LITERATURE REVIEW

In Vitro Culture as a Bioactive Metabolite Production System

In vitro culture is a biotechnological approach that allows the production of plant secondary metabolites in a controlled, sustainable, and relatively independent manner from environmental conditions and the availability of plant materials in nature. Cell suspension cultures, microshoots, calluses, and hair roots can be used as a production system for phenolic compounds, flavonoids, alkaloids, terpenoids, and bioactive pigments. However, culture productivity is often lower than that of whole plants due to loss of tissue differentiation and weak activation of defense pathways. Therefore, elytication is used to mimic biological stress so that the biosynthesis pathway of defensive metabolites can be activated without causing excessive culture damage (Wawrosch & Zotchev, 2021; Jalota et al., 2024).

Physiological and Molecular Basis of Biotic Elicitation

Biotic elicitors are derived from living organisms or their biological components, such as yeast extracts, chitosan, pectin, fungal cell extracts, and microorganism culture filtrates. The introduction of elitor molecules by plant membrane receptors triggers the formation of reactive oxygen species, changes in calcium ion concentrations, activation of protein kinases, as well as regulation of transcription factors WRKY, MYB, bHLH, AP2/ERF, and NAC. The sequence further increases the expression of genes encoding the enzyme biosynthesis of secondary metabolites. Thus, the increase in metabolites is not only influenced by the type of elystor, but also the strength of the signal, the duration of exposure, the physiological conditions of the culture, and the genetic ability of the plant species to respond to biotic stress (Jan et al., 2021; Amani et al., 2024).

Yeast and Pectin Extracts as Triggers of Phenolic Compounds

Yeast extracts are widely used because they contain polysaccharides, proteins, amino acids, and cell wall components that can be recognized as danger signals by plant cells. In the culture of Ajuga integrifolia micro shoots, Ullah et al. (2021) found that yeast and pectin extracts affect biomass, phytochemical content, and antioxidant activity differently. Pectin 10 mg/L produces the highest dry biomass, while the antioxidant increase is more potent at higher concentrations of pectin. The findings show that the optimum concentration for growth is not always the same as the optimum concentration for metabolite production. In Gardenia jasminoides suspension cultures, the highest flavonoid response occurred after six days of Saccharomyces cerevisiae elication, while the highest antioxidant activity was achieved earlier. These differences show a lag between metabolite accumulation and antioxidant responses (Ullah et al., 2021; Francisca et al., 2025).

Fungal and Chitosan Elicitors in the Regulation of Metabolic Pathways

Fungal elicitors can affect metabolism extensively because they contain cell wall components and extracellular compounds that resemble infection signals. In the hair root of Salvia miltiorrhiza, elictors derived from Mucor fragilis alter primary and secondary metabolism and increase the accumulation of a number of bioactive compounds (Xu et al., 2021). A similar result was found in the hair root of Ficus carica, when cell extracts and filtrates of mushroom cultures modified the expression of phenolic biosynthesis genes, phenolic compound content, and antioxidant capacity (Amani et al., 2024). In addition, chitosan 5 mg/L was reported to increase the total betalain content by 86.9% and strengthen the antioxidant activity of Celosia argentea cell suspension cultures. However, yeast extracts do not provide a similar response, so the effectiveness of the elifier is specific to the plant and the targeted metabolite pathway (Mueangnak et al., 2025).

Antioxidant Activity and the Need for Standardization of Elicitation

Increased phenolics, flavonoids, and bioactive pigments generally correlate with increased culture ability to capture free radicals or reduce oxidants. However, the strength of the relationship is influenced by the composition of the metabolites and the antioxidant testing method used. Research on Nasturtium officinale microshoot cultures showed that elicitor types can produce different responses on DPPH, FRAP, and CUPRAC assays (Klimek-Szczykutowicz et al., 2022). In addition, the effectiveness of chitosan is influenced by the source of the material, molecular weight, degree of deacetylation, solubility, dosage, and duration of treatment, so that comparisons between publications still face standardization problems (Roy Chowdhury et al., 2025). The literature supports the need for SLRs that compare the type, concentration, and duration of elitization to metabolite production and antioxidant activity, while explaining the variation in responses by species and culture system.

METHODOLOGY

Types of Research

This study uses systematic literature review (SLR) with a descriptive-thematic synthesis design. This approach combines descriptive quantitative analysis to map the distribution and tendency of outcomes with qualitative-thematic analysis to elucidate the relationship between the type, concentration, and duration of biotic elilizer administration with bioactive metabolite production and antioxidant activity. Implementation and reporting of research follows Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 which includes the stages of identification, screening, feasibility assessment, and inclusion of articles (Page et al., 2021). Search strategies are reported based on PRISMA-S so that databases, search terms, restrictions, and search results can be replicated (Rethlefsen et al., 2021).

Population and Sample

The study population includes all international experimental articles that discuss the use of biotic elisters in in vitro cultures of medicinal plants and were published in January 2021–December 2025. The unit of analysis was selected on a criterion-based basis through the application of eligibility criteria, not through probability sampling. Inclusion criteria include peer-reviewed journal articles in English; using calluses, cell suspensions, microshoots, adventitious roots, or hair roots; apply yeast extracts, chitosan, pectin, fungal elicizers, or other biotic ingredients; have a control culture; and quantitatively measure bioactive metabolites and/or antioxidant activity. Review articles, proceedings, book chapters, field research, studies using only abiotic elifiers, articles without control groups, and articles without adequate outcome data were excluded. A total of 42 articles met all criteria and were designated as units of analysis. This study did not use human respondents or participants because the data was sourced from scientific publications.

Research Instruments

The research instrument consists of a search protocol, an article selection form, a data extraction sheet, and a methodological quality assessment sheet. The extraction sheet contains the author's name, year and country of the study, plant species, type of culture, source and type of elicizer, concentration, duration of exposure, control group, metabolites analyzed, antioxidant measurement methods, yield values, statistical significance, as well as study conclusions. The instrument was developed based on PRISMA 2020 and PRISMA-S, then tested in five articles to ensure clarity of definition and consistency of data recording. Risk of bias was assessed using six domains adapted from the JBI Critical Appraisal Tool for Quasi-Experimental Studies, namely clarity of intervention, presence of control, equivalence of treatment, adequacy of replication, validity of measurements, and accuracy of statistical analysis (Barker et al., 2024). The risk of bias is categorized as low if the entire domain is met or there is only one aspect of reporting that is unclear; some concerns if at least two aspects are unclear without critical weaknesses; and high if control, replication, measurement, or

analysis is inadequate. The validity of the content was checked based on its suitability with the research question, while reliability was maintained through independent assessment by two reviewers. Agreements between reviewers are calculated using Cohen's kappa and discrepancies are resolved by consensus.

PRISMA Data Collection and Selection Procedure

The research questions were formulated using the PECO framework, namely population in the form of in vitro cultures of medicinal plants; exposure/intervention in the form of biotic elicizers; comparator in the form of cultures without elitors; and outcomes in the form of bioactive metabolite production and antioxidant activity. Search was conducted on Scopus, Web of Science, PubMed, and ScienceDirect using a combination of terms: ("medicinal plant*" OR "herbal plant*") AND ("in vitro culture" OR callus OR "cell suspension" OR "hairy root*" OR microshoot*) AND ("biotic elicitor*" OR chitosan OR "yeast extract" OR pectin OR "fungal elicitor*") AND ("secondary metabolite*" OR phenolic* OR flavonoid* OR alkaloids* OR terpenoids* OR antioxidants*). The search syntax is tailored to the characteristics of each database.

The identification stage resulted in 443 records, consisting of 132 articles from Scopus, 104 articles from the Web of Science, 89 articles from PubMed, and 118 articles from ScienceDirect. All records are exported to Zotero for reference management. A total of 121 duplicate articles and 34 recordings that did not correspond to the period or type of publication were issued, leaving 288 articles for title and abstract filtering.

A total of 190 articles were issued at the screening stage because they did not discuss medicinal plants, in vitro cultures, or biotic elifiers. Of the 98 articles searched in full-text form, six articles could not be obtained, so 92 articles were assessed as eligible. At the full-text stage, 50 articles were excluded because the study was not conducted in vitro (n = 14), used only abiotic elifiers (n = 11), did not research medicinal plants (n = 8), did not use control groups (n = 6), did not measure relevant metabolites or antioxidant activity (n = 7), and did not provide adequate quantitative data (n = 4). Thus, a total of 42 articles were included in the final synthesis.

Screening was carried out using Rayyan independently by two reviewers. Rayyan is used to manage inclusion, exclusion, and article decisions that require further discussion, while the final decision remains determined by human reviewers (Valizadeh et al., 2022). The flow of article selection is presented in Figure 1.

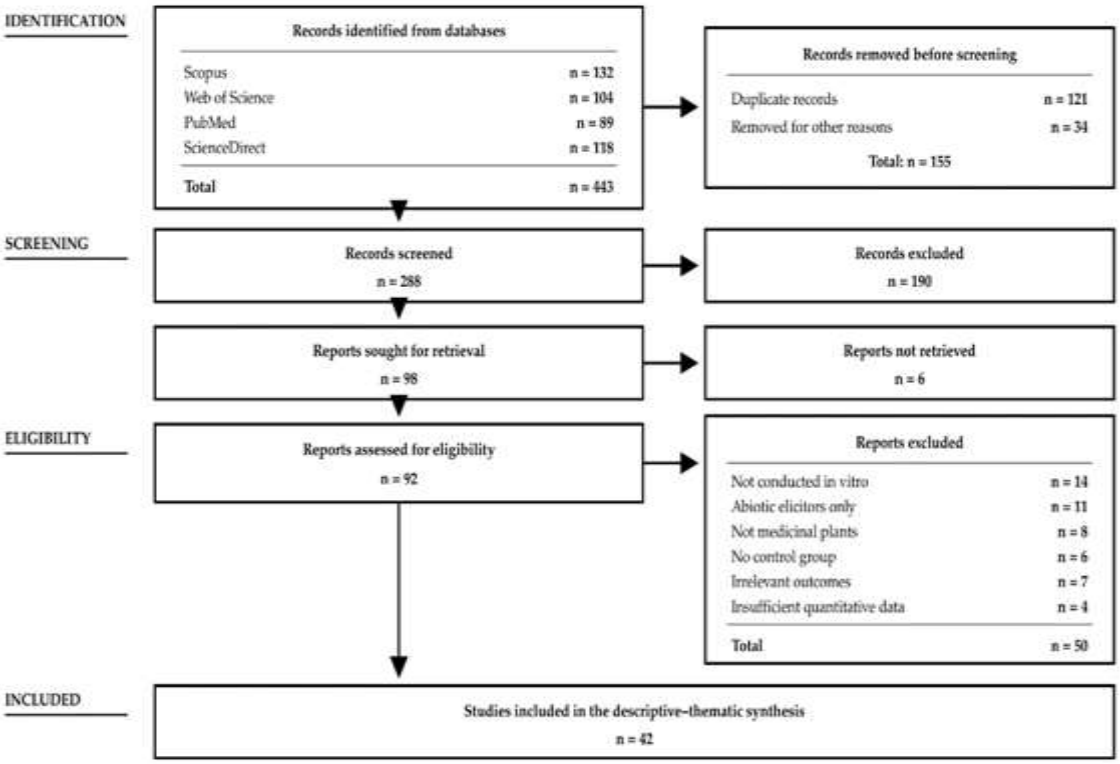


Figure 1. Article Selection Flowchart Based on PRISMA 2020

Data Analysis Techniques

The data was analyzed using Microsoft Excel 365 and R version 4.4.3. Descriptive analysis included frequency and percentage of elitor types, culture systems, metabolite groups, antioxidant testing methods, and treatment response directions. When control and treatment values are available, relative change is calculated using the formula:

Relative change (%) = ((treatment value − control value)/ control value) × 100%

Results were grouped by elicitor type, concentration, duration of exposure, plant species, culture system, target metabolites, and antioxidant testing methods. Thematic analysis was directed at five themes, namely elifier effectiveness, dose–response patterns, time-response patterns, species- and culture-specific variations, and the relationship between bioactive metabolite accumulation and antioxidant activity. Meta-analysis was not performed because there was high heterogeneity in species, culture form, elifier concentration, duration of treatment, unit of result, and analytical method. Therefore, an explicitly and structured reported non-meta-analytic synthesis is used (Cumpston et al., 2023). The results of the methodological quality assessment are used to reinforce interpretation, not as a single basis for issuing articles. Because it used publicly available secondary data and did not directly involve humans or animals, the study did not require ethical approval.

RESULTS AND DISCUSSION

Characteristics of Inclusive Studies

A total of 42 experimental articles met all PRISMA inclusion criteria. Because culture design, concentration units, duration of elicitation, and output reporting differed between studies, results were synthesized based on effect direction and response patterns, rather than through combined effect estimates. Publications are spread relatively evenly in 2021–2025, with the highest proportion in 2023 (23.8%). Cell suspension culture was the most widely used system (38.1%), followed by hair roots/adventitious (26.2%), calluses (19.0%), and shoots or microshoots (16.7%). This composition suggests that the evidence comes mainly from culture systems that have direct and relatively homogeneous contact with elicizers.

Table 1. Characteristics of 42 Included Studies

Dimensions	**Category**	**n**	**%**
Year of publication	2021	8	19,0
	2022	9	21,4
	2023	10	23,8
	2024	8	19,0
	2025	7	16,7
Sistem culture	Cell suspension	16	38,1
	Hairroot/adventitious	11	26,2
	Callus	8	19,0
	Pucuk/microshoot	7	16,7
Primary electors	Yeast extract	13	31,0
	Kitosan	11	26,2
	Mushroom Elisitor	9	21,4
	Pectin	5	11,9
	Other Biotics	4	9,5
Metabolit target	Fenolik/flavonoid	21	50,0
	Terpenoids/essential oils	8	19,0
	Alkaloid	6	14,3
	Betalain/other pigments	4	9,5
	Other groups	3	7,1
Key antioxidant tests	DPPH	15	35,7
	FRAP	6	14,3
	ABTS	5	11,9
	CUPRAC/other tests	4	9,5
	Not rated	12	28,6

Note: the classification of metabolites and antioxidant tests use the main output of each article to make the categories mutually exclusive.

Phenolics and flavonoids are the most frequently analyzed group of metabolites (50.0%). This dominance is relevant to the research objectives because these two groups of compounds commonly contribute to radical capture capacity and reduction. A total of 12 studies (28.6%) did not measure antioxidant activity, so the increase in metabolite levels in this group could not be directly interpreted

as an increase in antioxidant function. Of the 30 studies that tested antioxidants, DPPH was the most commonly used method (15 studies; 35.7% of all studies), while the use of FRAP, ABTS, and CUPRAC or other tests was more limited.

Methodological Quality and Reliability of Selection

Cross-reviewer agreement reached $\kappa = 0.84$ on title and abstract screening and $\kappa = 0.81$ on full-text assessments, indicating strong agreement. Based on the six adapted JBI assessment domains, 30 studies (71.4%) were categorised as low risk of bias and 12 studies (28.6%) had some concerns; None of the studies were categorised as being at high risk of bias. The most frequently encountered weaknesses relate to biological replication reporting, unit randomization of cultures, and justification of statistical methods. All 42 studies were included, but the findings of the study with some concerns were interpreted more carefully.

Effectiveness of Elicifier Type on Metabolites and Antioxidant Activity

Overall, 34 of 42 studies (81.0%) reported a significant improvement in at least one bioactive metabolite compared to controls. Antioxidant activity was assessed in 30 studies and increased in 24 studies (80.0% of the studies that measured it). Mushroom elicitors showed the highest proportion for increased metabolites (88.9%), while chitosan showed the highest proportion for increased antioxidant activity (87.5% of studies assessing antioxidants). The difference in proportion between groups is relatively small and stems from an unbalanced number of studies; Therefore, these results are not used to establish one type of elicitor as the best universal treatment.

Table 2. Direction of Effect by Type of Biotic Elicitor

Types of elicitors	**Studies**	**Increased metabolites**	**Antioxidants are assessed**	**Increased antioxidants**	**Dominant response**
Yeast extract	13	10 (76,9%)	9	7 (77,8%)	Phenolic, flavonoids, terpenoids
Kitosan	11	9 (81,8%)	8	7 (87,5%)	Fenolik, flavonoid, pigmen
Mushroom Elisitor	9	8 (88,9%)	6	5 (83,3%)	Phenolic acids, terpenoids
Pectin	5	4 (80,0%)	4	3 (75,0%)	Isoflavonoid, fenolik
Other Biotics	4	3 (75,0%)	3	2 (66,7%)	Response specific species
Total	42	34 (81,0%)	30	24 (80,0%)	—

Note: the percentage increase in antioxidants was calculated against the number of studies that conducted antioxidant tests on each group of elicitors.

The pattern supports the mechanism by which microbial or fungal cell wall derived molecules recognize hazard signals, then activate defense pathways and divert carbon streams to secondary metabolism. However, eight studies showed no consistent improvement. Negative or mixed responses mainly arise when high concentrations decrease growth, increase tissue tanning, or shift metabolism to compounds that are not the target of analysis. Thus, the increase in metabolites must be interpreted alongside the biomass data so that the increase in concentration per dry weight is not mistakenly considered an increase in volumetric productivity.

Concentration Pattern and Duration of Exposure

Of the 31 studies that compared at least two concentrations, 19 studies (61.3%) found intermediate concentrations to be the optimal condition, seven studies (22.6%) found the highest response at the maximum concentration tested, three studies (9.7%) at low concentrations, and two studies (6.5%) showed no clear patterns. This optimally shaped pattern suggests that elicitation works within the dose window: too low doses have not sufficiently activated defense signals, while too high doses can cause excessive stress and inhibit cell growth. In the 28 studies that compared exposure times, the optimum conditions most often occurred between 48 hours and 7 days (16 studies; 57.1%). Six studies (21.4%) peaked after more than 7 days, four studies (14.3%) in 24 hours or less, and two studies (7.1%) provided a mixed pattern. These findings answer the focus of the duration–response relationship by showing that the accumulation of metabolites does not always coincide with peak antioxidant activity. Redox signals can change quickly, whereas biosynthesis and accumulation of certain metabolites take longer.

The Relationship of Bioactive Metabolites and Antioxidant Activity

Of the 30 studies that assessed antioxidant activity, 24 studies reported an improvement, four showed mixed results depending on the test method or time of harvest, and two studies found no improvement. This tendency shows a positive relationship between elicitation, metabolite accumulation, and antioxidant activity, but does not prove a uniform causal relationship. DPPH and ABTS primarily measure radical capture ability, while FRAP and CUPRAC assess reduction capacity; Differences in reaction mechanisms, solvents, equivalent standards, and extract composition can result in different treatment ratings.

Discrepancies between total phenolic levels and antioxidant test results can also occur because total colorimetric measurements do not differentiate the potency of each compound. A small change in high-activity metabolites can increase antioxidant capacity without a large increase in total phenolics, whereas the accumulation of low-reactivity compounds can raise total levels without producing comparable functional changes. Therefore, a combination of total level analysis, chromatography profiles, biomass, and more than one antioxidant test is a more robust approach.

Summary of Representative Studies

Table 3 presents nine representative studies from 42 articles that have the most complete quantitative information or provide important findings to explain the heterogeneity of results. The summary retains the original unit so that the values are not directly compared if the culture matrix and analysis methods are different.

Table 3. Summary of Nine Representative Studies in the Synthesis of Results

Study	**Plants and culture**	**Elysizing protocol**	**Key findings**
Farjaminezhad & Garoosi (2021)	Azadirachta indica; suspensi sel	Yeast extract 25–100 mg/L; 2–6 days	The highest biomass was 580.25 g/L fresh weight and 21.01 g/L dry weight at 100 mg/L for 6 days. Azadiractin was highest at 16.08 mg/g BK at 25 mg/L for 2 days; productivity of 219.78 mg/L at 25 mg/L for 4 days.
Ullah et al. (2021)	Ajuga integrifolia; Microshoot	Yeast/pectin extract 1–200 mg/L	Pectin 50 mg/L produces the highest antioxidant activity, while yeast extract 100 mg/L is most potent for anti-inflammatory activity. Catalpol decreases in most treatments; The response depends on the external.
Xu et al. (2021)	Salvia miltiorrhiza; akar rambut	Endophytic fungal elitistor Mucor fragilis	Increases salvianolic acid B, rosmarinic acid, stearic acid, and oleic acid; changes accompanied by SmAACT, SmGGPPS, and SmPAL regulations.
Bavi et al. (2022)	Zataria multiflora; suspensi sel	Yeast and chitosan extracts; Dosage/time variation	Phenolics, flavonoids, and terpenoids change dose- and time-dependent. Increased metabolites are not identical in every combination of treatments.
Klimek-Szczykutowicz et al. (2022)	Nasturtium officinale; mikroshoot	Yeast extracts and other elicitors	The activity measured with CUPRAC, DPPH, and FRAP provided a large number of different responses, confirming that the choice of test method influenced interpretation.
Kanthaliya et al. (2023)	Pueraria tuberosa; pucuk in vitro	Pectin 100 mg/L; Comparative Elicitor	The total isoflavonoids reached 9,359.56 µg/g, which is 1.68 times that of shoots without elitors (5,573.13 µg/g) and 2.77 times that of the parent plant (3,380.17 µg/g), accompanied by increased antioxidant activity.

Amani et al. (2024)	Ficus carica cv. Sally; Hair roots	Ekstrak sel/filtrat Piriformospora indica 2–6%; 48–96 jam	DPPH was highest at 39.24% on 2% cell extracts for 72 hours (control 13.13%); FRAP 94.10 M/g BK on 4% cell extract (control 46.24). However, a 4% cell extract for 96 hours lowered a total phenolic of 39% and a total flavonoid of 36%.
Mueangnak et al. (2025)	Celosia argentea; suspensi sel	Chitosan 5 mg/L; yeast extract 100–400 mg/L	Chitosan produces betalain 4.65 mg/g BK, DPPH 96.47%, and ABTS 97.82%. In contrast, yeast extracts lower betalain, different from the tendency in some other medicinal plants.
Francisca et al. (2025)	Gardenia jasminoides; suspensi sel	Saccharomyces cerevisiae 0–7,5%; 0–6 hari	The 7.5% concentration gives flavonoids and the highest antioxidant activity; The flavonoid peak occurs on day 6, while the antioxidant peak occurs on day 2.

Integration with Supporting Research and Difference Analysis

Supporting studies show that the direction of response is primarily determined by the interaction of species × the culture system × elicitizer × dose × time. Yeast extract enhances certain externalities in Ajuga integrifolia and Gardenia jasminoides (Ullah et al., 2021; Francisca et al., 2025), but lowered betalain in Celosia argentea (Mueangnak et al., 2025). These differences are likely related to receptor sensitivity, yeast extract composition, growth phase, and target biosynthesis pathways. Thus, yeast extracts cannot be treated as an equivalent formula between studies without composition characterization and dose normalization.

Pectin 100 mg/L provides the highest isoflavonoid accumulation in Pueraria tuberosa (Kanthaliya et al., 2023), while the highest antioxidant activity in Ajuga integrifolia is obtained at 50 mg/L (Ullah et al., 2021). These differences are not direct contradictions because species, target metabolites, and key outputs are different. Rather, it reinforces the existence of an endpoint-specific optimum. Something similar is seen in Ficus carica: some fungal elitor conditions increase DPPH and FRAP, but longer exposure to certain concentrations decreases total phenolics and flavonoids (Amani et al., 2024).

Molecular evidence from the hair root of Salvia miltiorrhiza suggests that fungal elistors alter metabolites as well as gene expression of phenylpropanoid and terpenoid pathways (Xu et al., 2021). The findings provide a mechanistic explanation for the quantitative patterns in synthesis, but do not negate the influence of overstress. Meanwhile, differences in flavonoid and antioxidant peak times in Gardenia jasminoides (Francisca et al., 2025), as well as differences in CUPRAC, DPPH, and FRAP results in Nasturtium officinale (Klimek-Szczykutowicz et al., 2022), suggest that sampling time and test selection can be a source of substantive heterogeneity.

In practical terms, this synthesis supports gradual optimization, not the selection of elitors based on the name of the ingredient alone. Advanced experiments need to test the concentration and time matrix, report biomass as well as volumetric productivity, use appropriate solvent controls, and combine metabolite profiles with at least two antioxidant assays. Theoretically, the results reinforce a nonlinear response model: elicitators activate secondary defenses and metabolism at the optimal range, but can inhibit growth or divert metabolic pathways when stress intensity exceeds the culture adaptive capacity. The heterogeneity of design and reporting units limits the meta-analysis, so the strongest finding at this stage is consistency of effect direction and moderation factors, rather than a single universal measure of effect.

CONCLUSIONS AND RECOMMENDATIONS

A systematic review of 42 articles showed that biotic elisters have the potential to increase bioactive metabolite production and antioxidant activity in in vitro cultures of medicinal plants. A total of 34 studies (81.0%) reported an increase in at least one bioactive metabolite, while 24 of the 30 studies that tested for antioxidants (80.0%) showed increased activity. Mushroom elicitors have the highest proportion of increased metabolites, while chitosan provides the highest proportion of increased antioxidant activity. A total of 30 studies (71.4%) were at low risk of bias and 12 studies (28.6%) had some concern. Such effectiveness is not universal because it is influenced by species, type of culture, elifier characteristics, concentration, time of exposure, target metabolites, and testing method.

The results confirm that biotic elicitation works through a nonlinear response pattern with optimal dose range and time. Intermediate concentrations and exposure durations of 48 hours to 7 days most often result in the best response, while excessive exposure can decrease growth or inhibit the accumulation of metabolites. Therefore, further research and application are recommended using specific optimization for each plant–culture–elitor combination, reporting biomass and volumetric productivity, using appropriate controls, and combining metabolite profiles with at least two antioxidant test methods. For industrial applications, optimal conditions need to be validated in bioreactors by paying attention to culture stability, quality consistency, process safety, and economic feasibility.

FURTHER STUDY

This study was limited by the heterogeneity of the experimental design, type of culture, unit of concentration, duration of treatment, extraction method, and reporting of antioxidant activity. These differences do not allow for the incorporation of effect measures through meta-analysis and limit direct quantitative comparisons between species. The 2021–2025 publication year restriction and the use of four databases also allow for the exclusion of relevant studies published in the previous period, are in another database, or are not available in full text.

Follow-up research needs to use a factorial design that compares the type of elitor, concentration, and duration of exposure directly on a standardized culture system. Metabolomics, transcriptomics, and gene expression analysis are needed to elucidate the relationship between elitor recognition, regulation of biosynthesis pathways, metabolite accumulation, and antioxidant activity. Validation studies on bioreactors, evaluation of the stability of metabolites during subcultures, production cost analysis, and sustainability assessments are also required so that laboratory results can be translated into a consistent and scalable production platform for medicinal plant metabolites.

ACKNOWLEDGMENT

The author expresses his appreciation to the researchers whose scientific work is the source of data in this systematic review. Awards were also presented for academic input that supported the preparation, evaluation, and improvement of manuscripts.

REFERENCES

Alcalde, M. A., Perez-Matas, E., Escrich, A., Cusido, R. M., Palazon, J., & Bonfill, M. (2022). Biotic elicitors in adventitious and hairy root cultures: A review from 2010 to 2022. Molecules, 27(16), Article 5253. https://doi.org/10.3390/molecules27165253

Amani, S., Mohebodini, M., Khademvatan, S., Jafari, M., & Kumar, V. (2024). Modifications in gene expression and phenolic compounds content by methyl jasmonate and fungal elicitors in Ficus carica cv. Siah hairy root cultures. BMC Plant Biology, 24, Article 520. https://doi.org/10.1186/s12870-024-05178-2

Barker, T. H., Habibi, N., Aromataris, E., Stone, J. C., Leonardi-Bee, J., Sears, K., Hasanoff, S., Klugar, M., Tufanaru, C., Moola, S., & Munn, Z. (2024). The revised JBI critical appraisal tool for the assessment of risk of bias for quasi-experimental studies. JBI Evidence Synthesis, 22(3), 378–388. https://doi.org/10.11124/JBIES-23-00268

Bavi, K., Khavari-Nejad, R. A., Najafi, F., & Ghanati, F. (2022). Phenolics and terpenoids change in response to yeast extract and chitosan elicitation in Zataria multiflora cell suspension culture. 3 Biotech, 12(8), Article 163. https://doi.org/10.1007/s13205-022-03235-x

Bhaskar, R., Xavier, L. S. E., Udayakumaran, G., Kumar, D. S., Venkatesh, R., & Nagella, P. (2022). Biotic elicitors: A boon for the in-vitro production of plant secondary metabolites. Plant Cell, Tissue and Organ Culture, 149(1–2), 7–24. https://doi.org/10.1007/s11240-021-02131-1

Cahyaningsih, R., Magos Brehm, J., & Maxted, N. (2021). Setting the priority medicinal plants for conservation in Indonesia. Genetic Resources and Crop Evolution, 68(5), 2019–2050. https://doi.org/10.1007/s10722-021-01115-6

Cumpston, M. S., Brennan, S. E., Ryan, R., & McKenzie, J. E. (2023). Synthesis methods other than meta-analysis were commonly used but seldom specified: Survey of systematic reviews. Journal of Clinical Epidemiology, 156, 42–52. https://doi.org/10.1016/j.jclinepi.2023.02.003

Farjaminezhad, R., & Garoosi, G. (2021). Improvement and prediction of secondary metabolites production under yeast extract elicitation of Azadirachta indica cell suspension culture using response surface methodology. AMB Express, 11, Article 43. https://doi.org/10.1186/s13568-021-01203-x

Francisca, G. O., Arsiningtyas, I. S., & Mursyanti, E. (2025). Effect of addition Saccharomyces cerevisiae elicitor on total flavonoid content and antioxidant activity of Gardenia jasminoides cell suspension culture. Yuzuncu Yıl University Journal of Agricultural Sciences, 35(1), 23–35. https://doi.org/10.29133/yyutbd.1474298

Guru, A., Dwivedi, P., Kaur, P., & Pandey, D. K. (2022). Exploring the role of elicitors in enhancing medicinal values of plants under in vitro condition. South African Journal of Botany, 149, 1029–1043. https://doi.org/10.1016/j.sajb.2021.10.014

Jain, D., Bisht, S., Parvez, A., Singh, K., Bhaskar, P., & Koubouris, G. (2024). Effective biotic elicitors for augmentation of secondary metabolite production in medicinal plants. Agriculture, 14(6), Article 796. https://doi.org/10.3390/agriculture14060796

Jalota, K., Sharma, V., Agarwal, C., & Jindal, S. (2024). Eco-friendly approaches to phytochemical production: Elicitation and beyond. Natural Products and Bioprospecting, 14, Article 5. https://doi.org/10.1007/s13659-023-00419-7

Jan, R., Asaf, S., Numan, M., Lubna, & Kim, K.-M. (2021). Plant secondary metabolite biosynthesis and transcriptional regulation in response to biotic and abiotic stress conditions. Agronomy, 11(5), Article 968. https://doi.org/10.3390/agronomy11050968

Kanthaliya, B., Joshi, A., Arora, J., Alqahtani, M. D., & Abd Allah, E. F. (2023). Effect of biotic elicitors on the growth, antioxidant activity and metabolites accumulation in in vitro propagated shoots of Pueraria tuberosa. Plants, 12(6), Article 1300. https://doi.org/10.3390/plants12061300

Karalija, E., Macanović, A., & Ibragić, S. (2025). Revisiting traditional medicinal plants: Integrating multiomics, in vitro culture, and elicitation to unlock bioactive potential. Plants, 14(13), Article 2029. https://doi.org/10.3390/plants14132029

Klimek-Szczykutowicz, M., Dziurka, M., Blažević, I., Đulović, A., Apola, A., Ekiert, H., & Szopa, A. (2022). Impacts of elicitors on metabolite production and on antioxidant potential and tyrosinase inhibition in watercress microshoot cultures. Applied Microbiology and Biotechnology, 106, 619–633. https://doi.org/10.1007/s00253-021-11743-8

Mueangnak, K., Kitwetcharoen, H., Thanonkeo, S., Klanrit, P., Apiraksakorn, J., Klanrit, P., Klanrit, P., & Thanonkeo, P. (2025). Enhancing betalains production and antioxidant activity in Celosia argentea cell suspension cultures using biotic and abiotic elicitors. Scientific Reports, 15, Article 376. https://doi.org/10.1038/s41598-024-83096-x

Ozyigit, I. I., Dogan, I., Hocaoglu-Ozyigit, A., Yalcin, B., Erdogan, A., Yalcin, I. E., Cabi, E., & Kaya, Y. (2023). Production of secondary metabolites using tissue culture-based biotechnological applications. Frontiers in Plant Science, 14, Article 1132555. https://doi.org/10.3389/fpls.2023.1132555

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., … Moher, D. (2021). The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ, 372, Article n71. https://doi.org/10.1136/bmj.n71

Rethlefsen, M. L., Kirtley, S., Waffenschmidt, S., Ayala, A. P., Moher, D., Page, M. J., Koffel, J. B., & PRISMA-S Group. (2021). PRISMA-S: An extension to the PRISMA statement for reporting literature searches in systematic reviews. Systematic Reviews, 10(1), Article 39. https://doi.org/10.1186/s13643-020-01542-z

Roy Chowdhury, M., Mehmet, M., Mukherjee, J., Debnath, A. J., & Ražná, K. (2025). Chitosan as an elicitor in plant tissue cultures: Methodological challenges. Molecules, 30(17), Article 3476. https://doi.org/10.3390/molecules30173476

Silva-Santos, L., Palhares Neto, L., Corte-Real, N., Sperandio, M. V. L., Camara, C. A. G., Moraes, M. M., & Ulisses, C. (2023). Yeast extract and chitosan elicitation improves essential oil, regulates plant growth and antioxidative system in Lippia alba (Mill.) N.E. Brown (Verbenaceae). South African Journal of Botany, 163, 756–769. https://doi.org/10.1016/j.sajb.2023.10.024

Ullah, M. A., Gul, F. Z., Khan, T., Bajwa, M. N., Drouet, S., Tungmunnithum, D., Giglioli-Guivarc'h, N., Liu, C., Hano, C., & Abbasi, B. H. (2021). Differential induction of antioxidant and anti-inflammatory phytochemicals in agitated micro-shoot cultures of Ajuga integrifolia Buch. Ham. ex D.Don with biotic elicitors. AMB Express, 11, Article 137. https://doi.org/10.1186/s13568-021-01297-3

Valizadeh, A., Moassefi, M., Nakhostin-Ansari, A., Hosseini Asl, S. H., Saghab Torbati, M., Aghajani, R., Maleki Ghorbani, Z., & Faghani, S. (2022). Abstract screening using the automated tool Rayyan: Results of effectiveness in three diagnostic test accuracy systematic reviews. BMC Medical Research Methodology, 22, Article 160. https://doi.org/10.1186/s12874-022-01631-8

Wawrosch, C., & Zotchev, S. B. (2021). Production of bioactive plant secondary metabolites through in vitro technologies—status and outlook. Applied Microbiology and Biotechnology, 105(18), 6649–6668. https://doi.org/10.1007/s00253-021-11539-w

Xu, W., Jin, X., Yang, M., Xue, S., Luo, L., Cao, X., Zhang, C., Qiao, S., Zhang, C., Li, J., Wu, J., Lv, L., Zhao, F., Wang, N., Tan, S., Lyu-Bu, A. G. A., Wang, C., & Wang, X. (2021). Primary and secondary metabolites produced in Salvia miltiorrhiza hairy roots by an endophytic fungal elicitor from Mucor fragilis. Plant Physiology and Biochemistry, 160, 404–412. https://doi.org/10.1016/j.plaphy.2021.01.023