

## INPLASY

Control of Alternaria Brown Spot (*Alternaria alternata* (Fr.) Keissler) in Citrus: A systematic review

INPLASY202550011

doi: 10.37766/inplasy2025.5.0011

Received: 6 May 2025

Published: 6 May 2025

Garmendia, A; Ferriol, M; Beltran R; García-Breijo F; Raigón MD; Parra MC; Merle H.

**Corresponding author:**

Hugo Merle

humerfa@upvnet.upv.es

**Author Affiliation:**

Universitat Politècnica de València.

**ADMINISTRATIVE INFORMATION**

**Support** - This research was funded by the Citrusalte SAT, Artan Frut Coop. V., Casa Blava SAT 58CV and Versol SAT 108 CV as part of a project undertaken with the Universitat Politècnica de València (Spain, UPV 20250023), of which Merle H. was the principal researcher. There was no additional external funding received for this study.

**Review Stage at time of this submission** - Completed but not published.

**Conflicts of interest** - None declared.

**INPLASY registration number:** INPLASY202550011

**Amendments** - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 6 May 2025 and was last updated on 6 May 2025.

**INTRODUCTION**

**Review question / Objective** What substances have been used to control ABS (*Alternaria brown spot*), and their effectiveness? What are the methodologies used to test the substances? Why is ABS field control failing?

**Condition being studied** *Alternaria brown spot* is one of the most critical diseases affecting susceptible mandarins worldwide, being a limiting factor for their cultivation. Although there are numerous re-ports on effective substances against the disease, field control is failing. In the literature, some of the results are contradictory, depending on the study and experimental scale. Therefore, this paper aimed to collate, analyze, and synthesize the most relevant empirical evidence to answer the following questions: (i) What substances have been used to control ABS and

what is their effectiveness? (ii) What are the methodologies used to test the sub-stances? (iii) Why is ABS field control failing and what are the main factors hindering such control?

**METHODS**

**Search strategy** First, a broad literature search to obtain all records on the topic was carried out in five databases: WoS, Scopus, Google Academics, PubMed, and Scielo. The databases were consulted in January 2025. The search string used in all databases was, "*Alternaria alternata*" AND "Citrus" and, whenever possible, it was limited to the title, abstract and key words.

**Participant or population** Citrus varieties susceptible to the pathogen. Studies that include different aspects of the pathogen in susceptible citrus varieties such as Nova or Leanri.

**Intervention** Biocontrol and natural substances for the control of the pathogen *Alternaria alternata* in citrus.

Fungicides for the control of *Alternaria* brown spot (*Alternaria alternata*) in citrus.

**Comparator** Effectiveness of substances in the laboratory and in the field.

**Study designs to be included** Studies that have conducted experiments at any scale, from laboratory to field experiments, with substances against *Alternaria alternata*.

**Eligibility criteria** Conferences, reports with no outcome of interest (remoteness of results from the main topic), reports focused on post-harvest disease and not on ABS (*Alternaria alternata*), and wrong species (seeding albinism in lemon) were excluded.

**Information sources** Five databases: WoS, Scopus, Google Academics, PubMed, and Scielo. The databases were consulted in January 2025. The search string used in all databases was, "Alternaria alterna-ta" AND "Citrus".

In all, 891 articles were retrieved from five databases. The WoS database contributed the majority of articles for this review, 39% of the total. The Scopus, Google Academics, PubMed, and Scielo databases represented, respectively, 31%, 16%, 12%, and 2% of the papers found. The databases with a broader search spectrum, such as WoS, Scopus, and Google Scholar, retrieved the largest number of records.

**Main outcome(s)** In this review, 98 reports were full-text reviewed to extract all the information about the substances and treatments used to control ABS. From the beginning, main topic reports were classified into two groups: those referring to natural substances and those referring to traditional fungicides. The details of the natural substances and fungicides used against *A. alternata*, and a summary of the methodologies used to test these substances have been provided. During this research, we have also highlighted important aspects that may be hindering the control of the disease in the field, despite the existence of substances with proven antifungal activity. In the case of the natural substances group, although there were many that showed antifungal activity in the laboratory, very few have been tested in the field. In the scarce field experiments, the results showed lower effectiveness than fungicides. Therefore, more field experiments are probably needed.

Regarding fungicides, we have found contradictory results between reports, and even little coherence between laboratory and field experiments results. For example, potassium phosphite, which did not show antifungal activity in the laboratory, showed a field effectiveness equivalent to that of fungicides. In addition, we found inconsistencies between mycelial growth assays, spore germination assays, and seedlings experiments, and variable results depending on the strains (probably due to the development of resistance).

A long period of fruit sensitivity, abundance and floatability of inoculum, rapid infections, appearance of resistance to fungicides, moderate effectiveness inhibiting the germination of conidia, uncertainty about the times of application and persistence of the products, are all handicaps that greatly hinder the control of the pathogen in real field conditions.

All this information suggests that disease control probably requires a different approach than that based only on the application of antifungal substances.

**Quality assessment / Risk of bias analysis** A reliability and significance index was provided for each primary study. These indexes were based on the number of replicates of the experiments and the methodology used in the study.

**Strategy of data synthesis** The data from the 98 records were included in two tables: one for natural substances (S1 Table) and one for fungicides (S2 Table) with the following items:

- Article identifiers: authors, year of publication, country, and title.
- Target species: plant species, variety, and disease (*A. a.* general, ABS, or post-harvest losses)
- Substance Information: group, common (commercial) name, scientific (substance) name, additional information.
- Experiment information: type (I, II; when two different types of experiments were used in the same study), concentration, and additional information (field experiments: yes/no).
- Main result: text (the results explained in the text), MIC (Minimum Inhibitory Concentration), MGI (Mycelial Growth Inhibition), EC50% (Concentration causing 50% growth inhibition), effectiveness (Type I, Type II).
- Conclusion: text.
- Interest: importance (goes from 1 to 5, and reflects the closeness to the main topic), reliability scale (goes from 1 to 3, low, medium, high; and reflects the quality and reproducibility of experiments).

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**Subgroup analysis** From the initial reports, subgroup "biocontrol and natural substances" (n = 59) and "fungicides to control ABS" (n= 54) were selected for full-text screening (n = 113). These two groups included information on the substances used to control *A. alternata* in both laboratory and field experiments, which are required to answer the research questions.

**Sensitivity analysis** All reports used similar methodologies and experimental sizes. Quality was uniform, and the robustness of the results was reflected by a reliability index.

**Country(ies) involved** Spain.

**Keywords** *Alternaria alternata*; Citrus; systematic review; fungicides; natural substances; *Alternaria Brown Spot*; Nova; Leanri; field control.

**Contributions of each author**

Author 1 - Alfonso Garmendia.

Author 2 - María Ferriol.

Author 3 - Roberto Beltrán.

Author 4 - Francisco García-Breijo.

Author 5 - María Dolores Raigón.

Author 6 - María Del Carmen Parra.

Author 7 - Hugo Merle.

Email: humerfa@upvnet.upv.es