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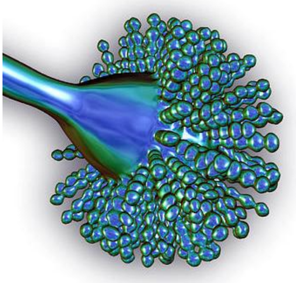
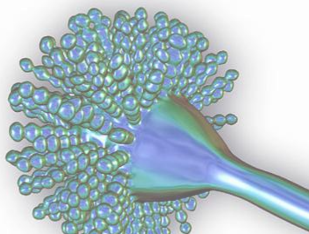
Evaluation of bio-based alternatives and natural biocides against azoles to mitigate fungal resistance in crops

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(i) Introduction

Around **120,000 tons of azoles** were marketed in the European countries **(2010–2021),** with **99%** used in agriculture.

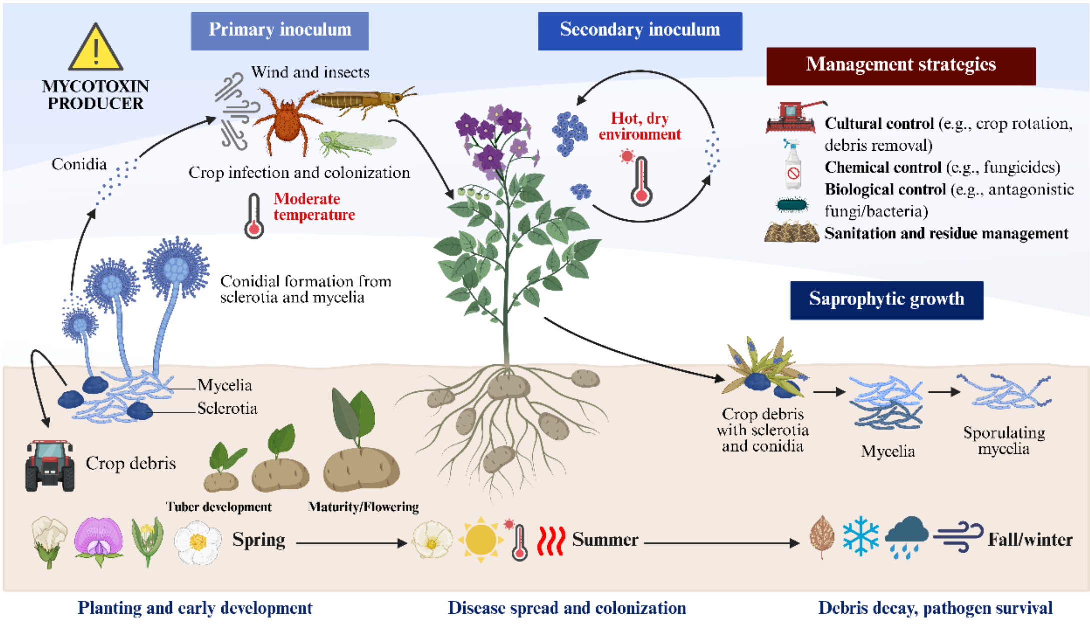
Overuse has led to **azole-resistant *Aspergillus* spp.,** especially *A. fumigatus*, with clinical resistance rates ranging from **0.7%** to **63.6%**, and mortality rates up to **100%.**

Environmental **hotspots** for resistance include **agricultural waste** and **biocide-treated wood.**

Substances like **epoxiconazole**, heavily used in crops, are now under regulatory scrutiny and may be banned.

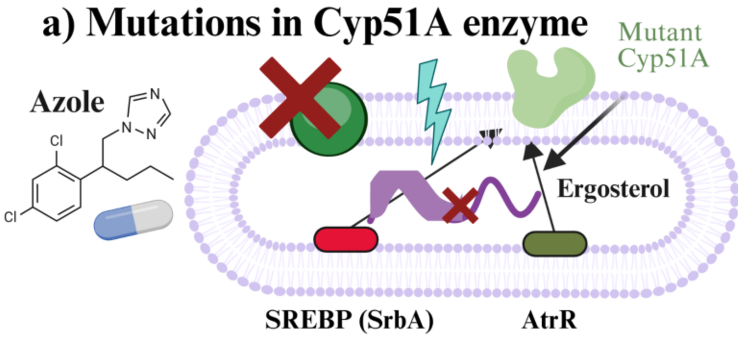
This review explores biobased alternatives to reduce azole dependence and support sustainable crop protection.

(ii) Epidemiological cycle of *Aspergillus* spp.



(iii) Mechanisms of azole resistance in phytopathogenic fungi

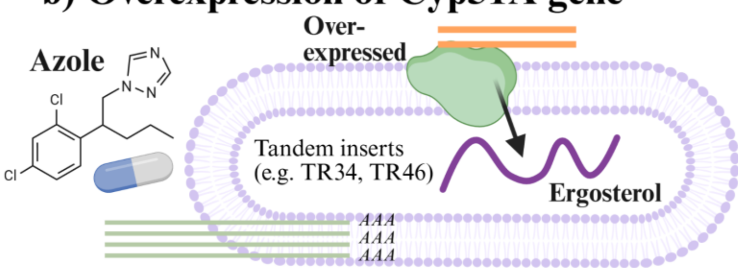
a) Mutations in Cyp51A enzyme



Pathway affected ergosterol biosynthesis

Point mutations in the *cyp51A* gene reduce azole binding affinity without affecting the enzymatic activity of Cyp51A

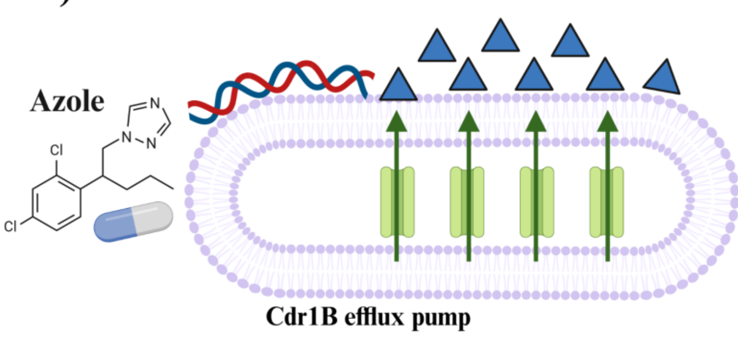
b) Overexpression of Cyp51A gene



Pathway affected: Cyp51A gene-regulated transcription

↑ mRNA → ↑ **Cyp51A** protein → higher concentration of azole is needed to inhibit the entire enzyme

c) Reduced intracellular accumulation of azoles

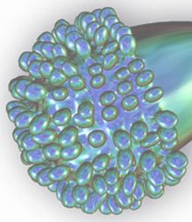


Pathway affected: Active drug transport (efflux)

Overexpression of efflux transporters (e.g., Cdr1B) actively pumps azoles out of the cell, **reducing intracellular concentrations and drug efficacy**

↓ azole intracellular concentration → **target (CYP51) is not effectively inhibited**

(iv) Biobased alternatives



- **Biopesticides:** Use natural enemies like *Bacillus thuringiensis* or fungi; safer for **non-target species** and **environment.**
- **Biostimulants:** Natural compounds that boost plant **growth** and **stress** tolerance (e.g., drought or salinity).
- **Biofertilizers:** Living **microbes** (e.g., rhizobacteria or mycorrhizae) that improve nutrient **uptake** and soil **health**.
- **Semiochemicals: Pheromones**, **allelopathy** and other communication chemicals to disrupt pest behavior (e.g., mating).
- **Plant-based products:** Essential oils and **bioactive** extracts with antimicrobiall properties (e.g., lavender or *Mentha piperita* L.)
- **Genetically modified crops:** Plants **modified** for pest resistance using **microbial** genes (e.g., cry proteins from *Bacillus thuringiensis*).
- **Cultural practices:** Crop rotation, intercropping, and mechanical controls to reduce **disease/pest pressure**.

(v) Benchmarking with azoles

CRITERIA	AZOLES	BIO-BASED ALTERNATIVES
Mode of action	Broad-spectrum; inhibits ergosterol synthesis in fungi	Often narrow-spectrum; disrupt life cycle or behavior of specific targets
Origin	Synthetic chemical compounds	Natural: microbial, plant-derived, or biological
Persistence	Long environmental half-life	Rapidly biodegradable, lower persistence
Resistance development	**High** risk of resistance buildup	**Lower** resistance pressure due to diverse mode of actions (MOAs)
Target specificity	Non-specific; may harm beneficial organisms	Often highly specific (e.g., species-specific pheromones)
Toxicity and ecotoxicity	Moderate to high; potential for human/environmental risk	**Low**; generally safe for humans, non-target organisms, and ecosystems
Regulatory pressure	**High** (many azoles under scrutiny/bans in EU, etc.)	**Lower**; often supported under organic/ecological standards
Cost and scalability	Cost-effective and widely used at large scale	Often more expensive; scaling still in development
Compatibility with Integrated Pest Management (IPM)	**Limited**; can disrupt IPM systems	**Highly** compatible with IPM
Innovation and RandD pipeline	**Mature**, but declining innovation due to resistance	**Growing field**; novel discoveries in microbes, botanicals, and fermentation

(vi) Conclusions and future perspectives

- Control of pathogens is a **long-term priority** that calls for **integrated** and **sustainable solutions.**
- Food and agricultural production are at **risk** due to the **resilience** and emerging **resistance** of ***Aspergillus* spp.**
- A combination of **strategies** is essential as **no single control method is sufficient.**
- Regulatory policy changes will **restrict** the **availability of azoles**, but for now there are **not comparably effective substitutes.**
- The way forward relies on the **continued evaluation** and pursuit of **new (bio)fungicides that meet the highest standards.**

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