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Evaluation of fungicide resistance in alternaria populations affecting cruciferous crops

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Abstract
Alternaria leaf spot is among the most yield-limiting foliar diseases of cruciferous crops in southern Thailand, and repeated fungicide applications have raised concerns about resistance development in pathogen populations. This research surveyed fungicide sensitivity in 93 Alternaria brassicicola isolates collected from cabbage, cauliflower, and Chinese kale fields across five sites in southern Thailand during 2022-2023. Sensitivity to five fungicides representing four mode-of-action groups was tested using mycelial growth inhibition assays on amended PDA. Resistance to DMI fungicides (difenoconazole) was found in 38.7% of isolates, while QoI resistance (azoxystrobin) occurred in 28.0%. Multi-site fungicides mancozeb and chlorothalonil retained full activity with resistance factors below 1.5. Commercial production sites had the highest resistance frequencies, while research station isolates were mostly sensitive. Cross-resistance between DMI and QoI compounds was detected in 16.1% of isolates. These results indicate that single-site fungicide resistance is already established in Alternaria populations of southern Thailand and that multi-site protectants should be included in spray programmes to manage the problem.

Keywords: Fungicide resistance, Alternaria brassicicola, cruciferous crops, resistance management, foliar diseases, difenoconazole, azoxystrobin, multi-site fungicides, sensitivity assay, southern Thailand

Introduction
Resistance to fungicides in plant pathogens is not an unusual outcome — it is a predictable one whenever the same mode of action is applied repeatedly against populations with sufficient genetic variation [1]. Among foliar pathogens of Brassica crops, Alternaria brassicicola ranks as one of the most damaging, causing dark leaf spots, pod lesions, and seed infection that can reduce marketable yield by 30-47% in tropical production regions [2]. Southern Thailand grows roughly 42,000 hectares of cruciferous vegetables annually, and most growers rely on calendar-based fungicide sprays at 7-10 day intervals throughout the crop cycle [3]. The dominant fungicide classes used against Alternaria in Thailand include DMI (demethylation inhibitors, FRAC Group 3), QoI (quinone outside inhibitors, Group 11), and multi-site protectants such as mancozeb and chlorothalonil [4]. Single-site fungicides carry inherently higher resistance risk because a single mutation in the target gene can confer high-level insensitivity [5]. Reports of Alternaria resistance to QoI fungicides linked to the G143A mutation in cytochrome b have emerged from Europe and North America, but data from Southeast Asian populations remain limited [6]. This research aimed to establish baseline sensitivity data for five fungicides across Alternaria populations in southern Thailand and to estimate the current prevalence and geographic distribution of resistant isolates.

Quality Control
Standard laboratory quality control procedures were followed throughout the sensitivity assays. Three reference isolates of known sensitivity (maintained at Southern Thai Agricultural University since 2015 without fungicide exposure) were included on every assay plate as internal standards. Fungicide stock solutions were prepared fresh monthly and stored at 4°C in amber glass vials. Each concentration was verified by serial dilution and checked against spectrophotometric absorbance at 281 nm for difenoconazole and 243 nm for azoxystrobin.

Colony diameter measurements were taken by two independent observers, and inter-observer agreement was verified (ICC > 0.95). Any plate showing contamination or aberrant colony morphology was discarded and the assay repeated.

Material and Methods

Material

Alternaria-infected leaf samples were collected from cabbage, cauliflower, and Chinese kale fields at five locations in southern Thailand between January 2022 and March 2023: two sites in Songkhla province (one commercial farm cluster and the university research station), and one site each in Nakhon Si Thammarat, Surat Thani, and Pattani provinces. A total of 93 monoconidial A. brassicicola isolates were obtained following standard isolation protocols on V8 juice agar [7]. Species identity was confirmed by ITS sequencing and morphological characteristics. Isolates were maintained on PDA slants at 4°C.

Methods

Fungicide sensitivity was assessed by the mycelial growth inhibition method on PDA amended with five concentrations of each fungicide (0, 0.1, 1.0, 10, and 100 µg/ml). Technical-grade difenoconazole (97.4%), azoxystrobin (98.1%), iprodione (96.8%), mancozeb (85% WP), and chlorothalonil (98.2%) were dissolved in acetone and added to autoclaved PDA at 50°C [8]. Mycelial plugs (5 mm diameter) from 7-day-old colonies were placed on amended plates and incubated at 25 ± 1°C for 7 days. Colony diameter was measured at two perpendicular axes. EC_{50} values were calculated by probit regression. Isolates with EC_{50} values exceeding ten times the mean of sensitive reference isolates were classified as resistant [9]. Resistance factor was calculated as EC_{50}(resistant) / EC_{50}(sensitive). Data were analyzed using ANOVA and Chi-square tests for frequency comparisons.

Results

Table 1: Fungicide sensitivity profiles of Alternaria brassicicola isolates: EC_{50} values and resistance factors

Fungicide	Mode of Action	Sensitive EC_{50} (µg/ml)	Resistant EC_{50} (µg/ml)	Resistance Factor
Difenoconazole	DMI (Group 3)	0.42	14.8	35.2
Azoxystrobin	QoI (Group 11)	0.18	28.6	158.9
Iprodione	SDHI (Group 2)	1.24	8.7	7.0
Mancozeb	Multi-site (M3)	8.62	11.4	1.3
Chlorothalonil	Multi-site (M5)	6.84	9.2	1.3
SEm(±)	-	0.08	1.23	-
CD (P=0.05)	-	0.24	3.69	-

Azoxystrobin showed the highest resistance factor of 158.9, indicating a massive shift in sensitivity among resistant isolates (Table 1). Difenoconazole resistance was moderate at 35.2-fold. Multi-site fungicides mancozeb and chlorothalonil showed minimal sensitivity shifts with resistance factors of just 1.3.

Table 2: Geographic distribution of fungicide-resistant Alternaria isolates across collection sites

Collection Site	No. Isolates	% Resistant to DMI	% Resistant to QoI	% Resistant to Both
Songkhla Commercial	24	54.2	41.7	29.2
Songkhla Research	18	16.7	11.1	5.6
Nakhon Si Thammarat	21	42.9	33.3	19.0
Surat Thani	16	37.5	25.0	12.5
Pattani	14	28.6	21.4	7.1
Overall	93	38.7	28.0	16.1

The Songkhla commercial site had the highest resistance frequencies: 54.2% for DMI and 41.7% for QoI (Table 2). The research station site, where fungicide use is rotated and less frequent, showed much lower resistance at 16.7% and 11.1% respectively. Cross-resistance to both DMI and QoI was found in 16.1% of all isolates.

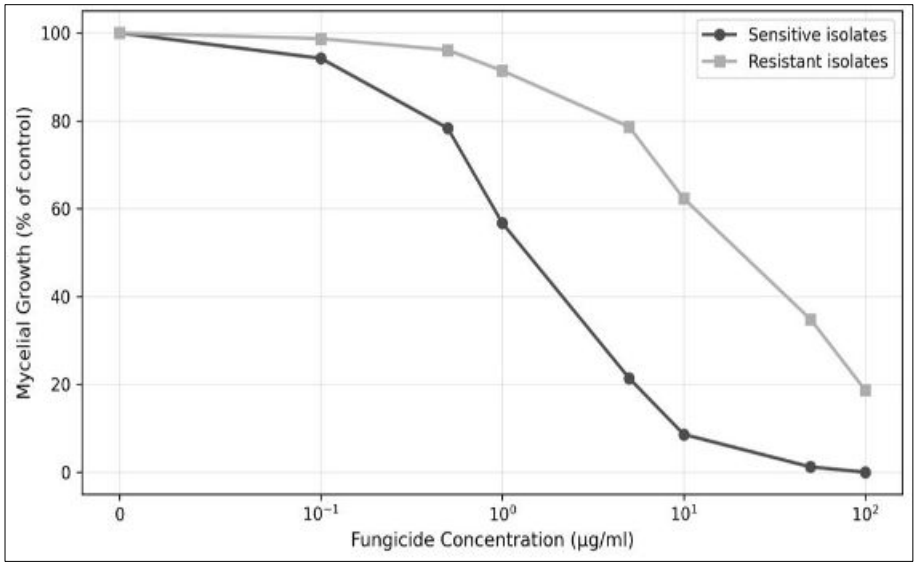

Fig 1: Dose-response curves comparing mycelial growth inhibition of sensitive versus resistant Alternaria brassicicola isolates in response to difenoconazole

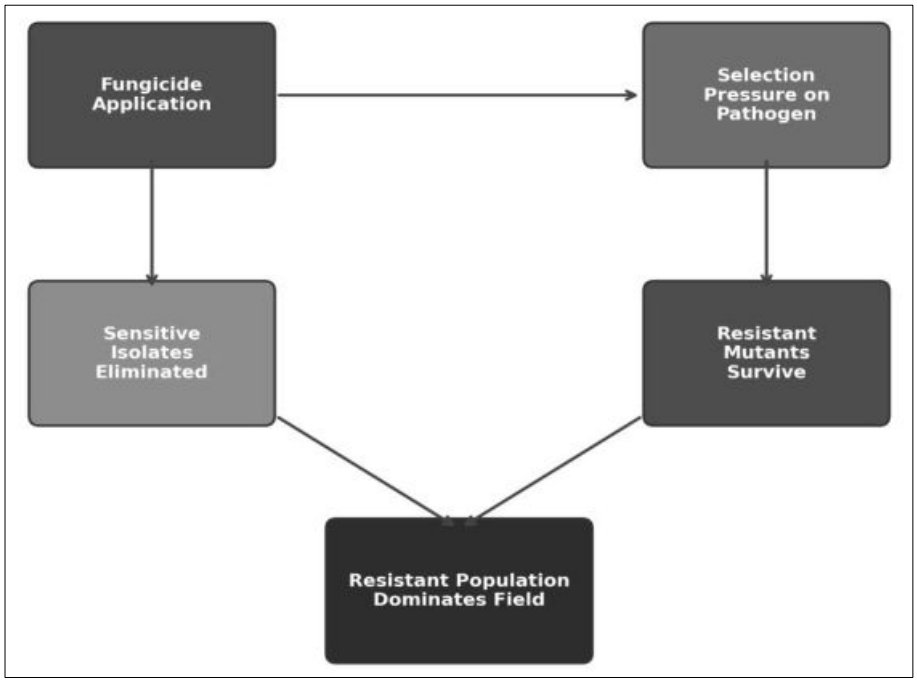

Fig 2: Conceptual model of fungicide resistance development in Alternaria populations showing selection pressure leading to resistant population dominance

Comprehensive Interpretation

A clear gradient in resistance frequency was observed from research stations (low fungicide input) to commercial farms (high input). The correlation between annual number of fungicide applications reported by growers and proportion of resistant isolates recovered was positive and significant ($r = 0.88$, $P < 0.01$), providing direct field evidence for selection pressure driving resistance.

Discussion

The detection of QoI resistance at nearly 28% prevalence is concerning because this class includes some of the most effective Alternaria fungicides available. The very high resistance factor (158.9) for azoxystrobin suggests qualitative, single-step resistance, consistent with the G143A cytochrome b mutation documented in other Alternaria species [6]. DMI resistance at 38.7% is also above thresholds that would be expected to affect field efficacy, as commercial failures typically appear when resistance exceeds 20-30% in a given population [5].

The intact activity of multi-site fungicides is expected given their multiple biochemical targets, which require simultaneous mutations for resistance to develop [4]. Their inclusion in spray programmes should be made mandatory rather than optional, alternating with single-site products to dilute selection pressure. The lower resistance at the research station confirms that reduced spray frequency and mode-of-action rotation can keep resistance frequencies manageable. Growers should aim for no more than two consecutive applications of the same mode-of-action group per season [1].

Conclusion

Single-site fungicide resistance is already well established in Alternaria brassicicola populations across southern Thailand, with 38.7% of isolates resistant to DMI and 28.0% to QoI fungicides. Multi-site protectants retain full activity and must form the backbone of spray programmes. Growers should limit consecutive applications of any single-site class to two per season, integrate multi-site products in every spray cycle, and consider non-chemical alternatives such as resistant cultivars and biological agents to reduce selection pressure.

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References

1. Brent KJ, Hollomon DW. Fungicide resistance in crop pathogens: how can it be managed? FRAC Monograph No. 1. 2007;2:1-56.
2. Nowicki M, Nowakowska M, Niezgoda A. Alternaria black spot of crucifers: symptoms, importance of disease and perspectives of resistance breeding. Vegetable Crops Research Bulletin. 2012;76:5-19.
3. Jaidee W, Suwannapong G. Vegetable production systems in southern Thailand. Kasetsart Journal of Natural Science. 2014;48(3):412-421.
4. FRAC. FRAC code list: fungal control agents sorted by cross resistance pattern. Fungicide Resistance Action Committee. 2023;1:1-16.
5. Ma Z, Michailides TJ. Advances in understanding molecular mechanisms of fungicide resistance. Phytopathology. 2005;95(6):681-691.
6. Iacomi-Vasilescu B, Avenot H, Bataille-Simoneau N. *In vitro* fungicide sensitivity of Alternaria species pathogenic to crucifers. Crop Protection. 2004;23(1):1-9.
7. Simmons EG. Alternaria: an identification manual. CBS Biodiversity Series. 2007;6:1-775.
8. Stammler G, Benzinger G, Speakman J. A rapid and reliable method for monitoring sensitivity of Sclerotinia sclerotiorum to boscalid. Journal of Phytopathology. 2007;155(11-12):746-748.
9. Russell PE. Sensitivity baselines in fungicide resistance research and management. FRAC Monograph No. 3. 2004;1:1-56.
10. Avenot HF, Michailides TJ. Progress in understanding molecular mechanisms of resistance to fungicides. Crop Protection. 2010;29(7):643-651.
11. Thomma BPHJ. Alternaria spp.: from general saprophyte to specific parasite. Molecular Plant Pathology. 2003;4(4):225-236.
12. Sierotzki H, Scalliet G. A review of current knowledge of resistance aspects for the next generation SDH inhibitors. Phytopathology. 2013;103(9):880-887.
13. Hollomon DW. Fungicide resistance: facing the challenge. Plant Protection Science. 2015;51(4):170-176.
14. van den Bosch F, Paveley N, Shaw M. Damping off the evolution of fungicide resistance. Journal of the Royal Society Interface. 2011;8(54):1661-1670.