

**IN VITRO EVALUATION OF CHEMICAL FUNGICIDES AGAINST
Colletotrichum sp. CAUSING ANTHRACNOSE DISEASE OF HOT PEPPER**

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ABSTRACT

Anthracnose, caused by the *Colletotrichum* spp., is one of the most destructive diseases of hot pepper (*Capsicum annuum* L.), causing yield losses of 10-80% in different production areas. To identify effective chemical options, anthracnose-infected hot pepper fruits were collected from farmers' fields in Kakani, Nuwakot, and the pathogen was isolated. Five fungicides - Kingsin M (thiophanate methyl 70% WP), King's Care (trifloxystrobin 10% + tebuconazole 20% SC), Kingmil 72 (cymoxanil 8% + mancozeb 64% WP), Protector ZN (chlorothalonil 70% WP), and King's M 80 (mancozeb 80% WP) were evaluated *in vitro* at three concentrations (50, 100, and 200 ppm) using the poisoned food technique. The experiment was arranged in a completely randomized design with four replications. King's Care completely inhibited the mycelial growth of *Colletotrichum* sp. at all concentrations, while Kingmil 72 showed complete inhibition at 200 ppm. Kingsin M and King's M 80 were highly effective at higher concentrations, whereas Protector ZN (chlorothalonil) was moderately effective. The results indicate that chemical options are available against the anthracnose, with King's Care being the most effective, followed by Kingmil 72. However, these fungicides are needed to be tested *in vivo*/field conditions to confirm their efficacy.

Keywords: *Colletotrichum*, Efficacy of fungicides, laboratory evaluation, mode of action, target site

INTRODUCTION

Hot pepper (*Capsicum annuum* L.) is a major spice crop grown worldwide and is valued for its culinary, medicinal, and economic importance (Bosland & Votava, 2003). In Nepal, it is an integral component of daily diet, and is consumed in both fresh and dried forms (Chapagain, 2016). It has also gained importance as a high-value cash commodity with strong domestic and export demand (Poudyal, et al., 2023).

Hot pepper production is constrained by several biotic factors, particularly diseases caused by fungal, bacterial, and viral pathogens. Among these, anthracnose is considered one of the most destructive diseases of chilli worldwide (Saxena et al., 2016a; Manandhar et al., 1995). In Nepal, anthracnose is also among the most economically important diseases of chilli and can cause significant yield losses.

Anthracnose of chilli is caused by several *Colletotrichum* species, including *C. capsici*, *C. acutatum*, *C. coccodes* and, *C. gloeosporioides* (Timila et al., 2014). These pathogens are seed-borne and may occur both externally and internally in infected chili seeds (Meon & Nik, 1988). They can also survive

in infected plant debris, allowing the pathogens to persist between cropping seasons and cause to recurrent disease outbreaks. Under favorable environmental conditions, anthracnose can cause yield losses of up to 80% (Saxena et al., 2016b).

Cultural practices and host resistance provide limited control because resistant varieties are scarce and often lack durable resistance. Consequently, chemical fungicides remain an important component of anthracnose management. Fungicides belonging to groups such as benzimidazoles, strobilurins, triazoles, dithiocarbamates, including, mancozeb, propiconazole, chlorothalonil, and their ready-mix formulations are widely used against *Colletotrichum* spp. (Waller, 1992; Sharma & Kulshrestha, 2015). However, the continuous use of single-site fungicides can lead to the development of fungicide resistance, environmental contamination, and ecological imbalance (Brent & Hollomon, 2007). Fungicide combinations with different modes of action may provide greater efficacy and lower the risk of resistance development.

Considering the severe impact of anthracnose on chilli production and the limited availability of resistant hot pepper cultivars, this preliminary study was undertaken to identify effective fungicides against *Colletotrichum* sp. under laboratory conditions.

MATERIALS AND METHODS

The experiment was conducted in December 2024 at the laboratory of Nepal Plant Disease and Agro Associates (NPDA), Balaju, Kathmandu, Nepal.

Collection of diseased samples

Hot pepper fruits showing typical anthracnose symptoms such as circular sunken spots with pink or orange spore mass development in concentric rings were collected from farmer's fields in Kakani, Nepal, and brought to the laboratory for pathogen isolation.

Isolation, identification and culture of pathogen

Small pieces (1-2 cm) were cut from the junction of diseased and healthy tissue of infected fruits. The pieces were washed with tap water, surface sterilized in 1% sodium hypochlorite for one minute, and rinsed twice with sterile distilled water. After drying under laminar airflow, the pieces were placed onto 2% water agar (WA: 20 g agar in 1000 mL distilled water) plates and incubated at 25 ± 1 °C. Fungal growth was observed after 48 hours of incubation and observed under a stereomicroscope. A bit of hyphal tips from the growth were transferred to potato dextrose agar (PDA: 200 g peeled potato, 20 g dextrose, and 20 g agar in 1000 mL distilled water) plates and incubated at 25 ± 1 °C until full growth. The cultural and morphological characters were studied.

The fungus was identified based on cultural and morphological characters following Barnett and Hunter (1998) and confirmed as *Colletotrichum* sp. Pure cultures were maintained on PDA slants and stored at 4 °C until use. For inoculum preparation, the pathogen was grown on PDA plates to obtain fresh cultures.

Preparation of poisoned medium

PDA was used as a culture medium. Before autoclaving, 200 ml PDA was dispensed into 250 ml conical flasks as per required for the number of treatments and sterilized at 121 °C for 20 minutes. Test fungicides were weighed and prepared stock suspensions or solutions in sterile water. The fungicide suspensions or solutions were mixed with the sterilized PDA (about 50 °C) to obtain final concentrations of 50, 100, and 200 ppm (active ingredient). The medium was mixed gently to avoid air bubbles and poured into labeled Petri plates (9 cm diameter) at about 20 ml per plate.

Inoculation of pathogen onto poisoned PDA

A 5-mm mycelial disc from the actively growing culture was placed at the center of each poisoned PDA plate under aseptic conditions, while control plates contained PDA without fungicide. Plates were incubated at 25 ± 1 °C. The experiment was arranged in a completely randomized design with 16 treatments and four replications (one plate per replication).

Data recording

Data were recorded when the fungal colony in the control plate reached full growth. Radial mycelial growth was measured along two perpendicular directions and averaged. Percent inhibition of radial growth (I) was calculated using the formula by Vincent (1947):

$$I = \frac{(C-T)}{C} \times 100\%$$

Where: I = Percent inhibition of mycelial growth, C = Colony diameter (mm) in control plate, and T = Colony diameter (mm) in treated plate.

Table 1. Details of the fungicides used in this study

Trade name	Active ingredient	Chemical group	Target site/Mode of action	FRAC Code
Kingsin M	Thiophanate methyl 70% WP	Benzimidazole	β- tubulin assembly inhibition/disrupts mitosis and cell division	01
Kingmil 72%	Cymoxanil 8% + mancozeb 64%WP	Cyanoacetamide oxime + Dithiocarbamate	Cymoxanil: RNA polymerase inhibition (nucleic acid synthesis)/locally systemic; Mancozeb: Multi-site activity (enzyme inactivation by reacting with SH-groups)	27 + M 03
King's Care	Trifloxystrobin 10 % + tebuconazole 20 % SC	QoI (Strobilurin) + DMI (Triazole)	Trifloxystrobin: Inhibits mitochondrial respiration (cytochrome bc1 at Qo site); Tebuconazole: Inhibits C14-demethylase in sterol biosynthesis / disrupts fungal membrane	11 + 03
Protector ZN	Chlorothalonil 70% WP	Chloronitrile	Multi-site activity (reacts with thiol groups in enzymes/proteins)	M 05
King's M 80	Mancozeb 80%WP	Dithiocarbamate	Multi-site activity (enzyme inactivation by reacting with SH-groups)	M 03

Source: (FRAC, 2024)

All the chemical fungicides used in this study was manufactured by Shenzhen King Quenson Ind. Co., Ltd. Shenzhen, China. The level of growth inhibition was categorized as: no inhibition (0%), slight (0.1-<25%), moderate (25-<50%), high (50-<75%), very high (75-<100%), and complete inhibition (100%) (Adhikari, 2023).

Statistical analysis

Data were analyzed using Analysis of Variance (ANOVA) in R software to test the effects of fungicide, concentration, and their interaction. A heatmap was prepared using MS Excel.

RESULTS

Identification of fungal pathogen

The pathogen causing anthracnose in hot pepper was identified as *Colletotrichum* sp. Acervuli with abundant dark brown to black setae were observed on infected fruits (Fig. 1). Presence of setae is the characteristics feature of *Colletotrichum* sp. The fungus produced grey to light pinkish mass of hyaline, single-celled conidia that were cylindrical to oblong with rounded ends, mostly straight but occasionally slightly curved (Fig. 2). Based on these morphological characteristics, the pathogen was identified as *Colletotrichum* sp.



Fig. 1. Acervuli of *Colletotrichum* sp. under a stereo-microscope



Fig. 2. Conidia of *Colletotrichum* sp. showing hyaline, aseptate, cylindrical to oblong spores with rounded ends under a compound microscope.

***In-vitro* efficacy of fungicides**

The efficacy of different fungicides against *Colletotrichum* sp. was evaluated using the poisoned food technique. Significant differences were observed among treatments in mycelial growth and percentage inhibition (Table 3 and Fig. 3). The untreated control recorded maximum mycelial growth (45.00 mm). The results are shown in Fig. 4.

King's Care completely inhibited mycelial growth at all tested concentrations (50, 100, and 200 ppm). Kingmil 72 also showed complete inhibition at 200 ppm. Moderate inhibition was recorded with Kingmil 72 at 100 ppm (42.78%) and at 50 ppm (24.44%) respectively and, King's M 80 at 50 ppm showed up to 40.00% inhibition, while slight inhibition was observed with Kingmil 72 at 50 ppm (24.44%).

High inhibition was obtained with King's M 80 at 100 ppm (61.67%) and 200 ppm (71.11%). Kingsin M also showed high inhibition at all concentrations, with 61.11 - 62.22% growth reduction. Protector ZN gave moderate to high inhibition depending on concentration.

Table 2. Effect of different concentrations of chemical fungicides on the mycelial growth of *Colletotrichum* sp. on PDA at the 10th day of inoculation

Fungicides (trade name)	Fungicides (common name)	Concentration (ppm, active ingredient)	Mycelial growth (mm)	Inhibition (%)	Inhibition category
Kingsin M	Thiophanate methyl 70% WP	50	17.50 ^c	61.11	High inhibition
Kingsin M		100	17.25 ^c	61.67	High inhibition
Kingsin M		200	17.00 ^c	62.22	High inhibition
Kingmil 72%	Cymoxanil 8% + mancozeb 64%WP	50	34.00 ^b	24.44	Slight inhibition
Kingmil 72%		100	25.75 ^d	42.78	Moderate inhibition
Kingmil 72%		200	0.00 ^g	100.00	Complete inhibition
King's Care	Trifloxystrobin 10 % + tebuconazole 20 % SC	50	0.00 ^g	100.00	Complete inhibition
King's Care		100	0.00 ^g	100.00	Complete inhibition
King's Care		200	0.00 ^g	100.00	Complete inhibition
Protector ZN	Chlorothalonil 70% WP	50	26.00 ^{cd}	42.22	Moderate inhibition
Protector ZN		100	27.25 ^c	39.44	Moderate inhibition
Protector ZN		200	17.25 ^c	61.67	High inhibition
King's M 80	Mancozeb 80%WP	50	27.00 ^{cd}	40.00	Moderate inhibition
King's M 80		100	17.25 ^e	61.67	High inhibition
King's M 80		200	13.00 ^f	71.11	High inhibition
Untreated control -		-	45.00 ^a	0.00	No inhibition
Grand mean			17.77		
CV (%)			5.1		
LSD			1.27		
P - value			< 0.001		
F - test			858.40**		

contd...

Fungicides (trade name)	Concentration		
	50 ppm	100 ppm	200 ppm
Kingsin M	61	62	62
Kingmil 72%	24	43	100
King's Care	100	100	100
Protector ZN	42	39	62
King's M 80	40	62	71
Control	0.0	0.0	0.0

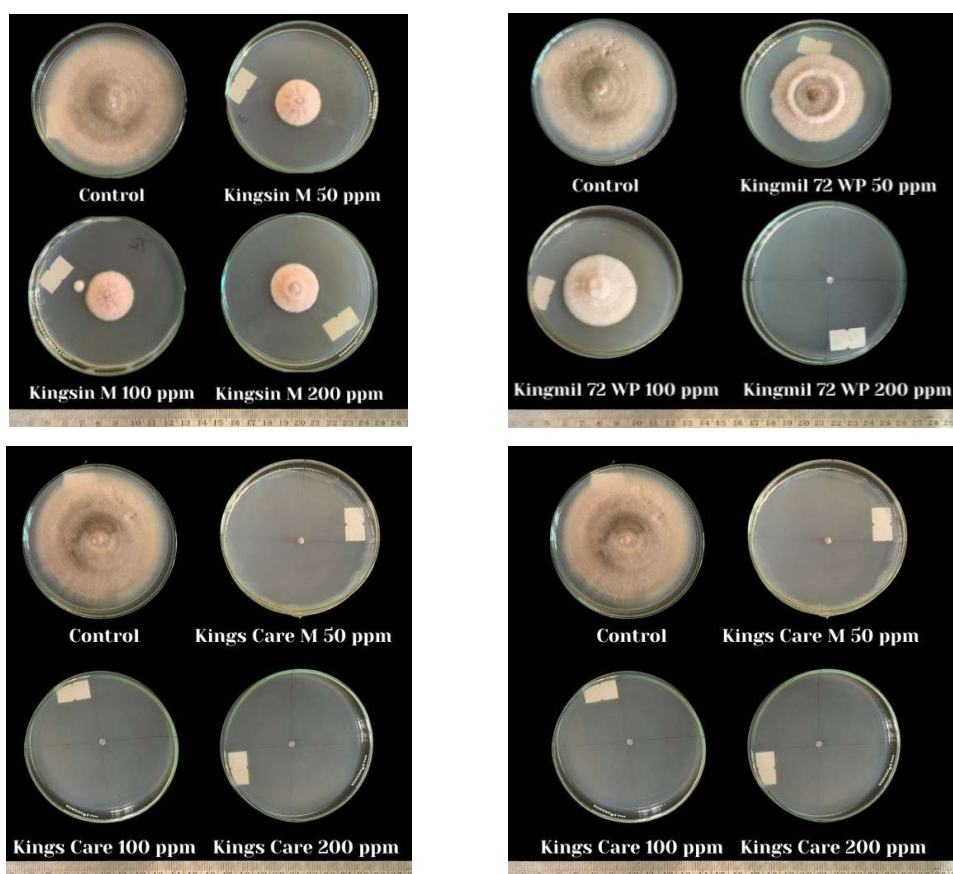


Fig 3. Heatmap illustrating summary of percent inhibition of radial mycelial growth of *Colletotrichum* sp. by chemical fungicides at different concentration.

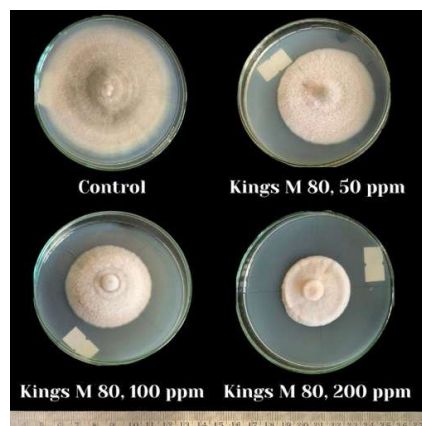


Fig. 4. Effect of different concentrations of Kingsin M (thiophanate methyl 70% WP), Kingmil 72 (trifloxystrobin 10 % + tebuconazole 20 % SC), King's Care (cymoxanil 8% + mancozeb 64%WP), Protector ZN (chlorothalonil 70% WP), and Kings M 80 (mancozeb 80%WP) on the radial growth of *Colletotrichum* sp. at the 10th day of inoculation.

DISCUSSION

The present study revealed that the efficacy of fungicides differed significantly in their ability to inhibit the growth of *Colletotrichum* sp. under *in vitro* conditions. Among the tested chemical fungicides, King's Care (50, 100, and 200 ppm) and Kingmil 72% (200 ppm) were the most effective, thereby completely inhibiting the mycelial growth of the pathogen (100% inhibition). Similar findings were reported by Nambiar et al. (2025) who found that tebuconazole 50% + trifloxystrobin 25% WG; achieved complete inhibition (100%) of mycelial growth across all concentrations (0.10%, 0.15%, and 0.20%). Poudel et al. (2025) also reported that treatment with trifloxystrobin 25% + tebuconazole 50% EC resulted in the complete inhibition of *Bipolaris sorokiniana* at concentrations of 25, 50, and 100 ppm.

Furthermore, Thakur et al. (2023) observed complete suppression of *Colletotrichum* sp. using systemic fungicides at higher concentrations. The complete inhibition observed with King's Care (trifloxystrobin 10% + tebuconazole 20% SC) may be attributed to its combination of strobilurin and triazole group fungicides. Strobilurin (QoI) inhibits mitochondrial respiration by blocking the cytochrome bc1 complex (Qo site), while tebuconazole (DMI) interferes with ergosterol biosynthesis in fungal cell membranes, disrupting membrane integrity and fungal growth (FRAC, 2024). These combined strobilurin-triazole mode of action provides dual-site fungicidal activity by disrupting electron transport in mitochondrial respiration and inhibiting ergosterol biosynthesis, resulting in effective preventive and curative control while reducing the risk of resistance development in fungal pathogens (Hollomon, 2015).

Kingmil 72%, (cymoxanil 8% + mancozeb 64%), also showed excellent efficacy at higher concentrations. It could be due to the mode of action and target site of cymoxanil and mancozeb. Cymoxanil (CYM), inhibits cell growth, biomass production, and respiration. Mechanistically, CYM

specifically targets mitochondria, inhibiting oxygen consumption in both whole cells and isolated mitochondria by suppressing cytochrome c oxidase (CcO) activity, a crucial enzyme in oxidative phosphorylation (Mendes et al., 2024). Beyond mitochondrial effects, CYM also interferes with nucleic acid metabolism, particularly by inhibiting RNA polymerase I activity, which affects RNA synthesis and subsequently disrupts fungal growth.

When combined with mancozeb, as in Kingmil 72%, the fungicide exhibits enhanced efficacy, likely due to a dual mechanism of action targeting both cellular respiration and nucleic acid synthesis. Mancozeb, a dithiocarbamate, has a multi-site contact action by reacting with sulfhydryl (-SH) groups of fungal enzymes, leading to enzyme inactivation and cell death (Bartlett et al., 2002). The synergistic action of systemic (cymoxanil) and contact fungicide (mancozeb) provides broad-spectrum protection.

CONCLUSIONS

From the study, some fungicides showed significant control against *Colletotrichum* sp. though their effectiveness varied among fungicides. It can be concluded that King's Care (trifloxystrobin 10% + tebuconazole 20% SC) is the most effective fungicide, followed by Kingmil 72 (cymoxanil 8%+ mancozeb 64%), at higher concentration for inhibiting the growth of *Colletotrichum* sp. under *in vitro* conditions. However, their effectiveness should be evaluated under *in vivo* and field conditions.

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