



Research Article

Comparative Effectiveness of Some Novel Fungicides Against Soil-Borne Pathogens of Chili

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Abstract | Soil-borne fungal pathogens are considered the most critical pathogens responsible for the enormous losses in chili crops. The diseases caused by them are often challenging to control due to different incidence level and limited knowledge of their epidemiological features. Advanced accessibility and a simple application process for chemical control systems have made it practical and widely accepted. In the present study, ten different fungicides viz., Shincar, Antracol, Alliete, Ridomil Gold, Moncut, Systhane, Kocide, Evito, Nativo, and Topsin M at 200, 400, 600, 800, and 1000 ppm concentrations were tested in in vitro by poisoned food method against *Rhizoctonia solani*, *Verticillium albo-atrum* and *Macrophomina phaseolina*. Our results showed that Nativo and Evito at 800-1000 ppm and Systhane and Kocide at 1000 ppm can significantly inhibit the mycelial growth of *R. solani* leading to 100% growth inhibition. Additionally, three fungicides, namely Systhane, Shincar, and Topsin M, were highly effective at all concentrations and caused 100% growth inhibition of *M. phaseolina*, followed by Antracol (87.22%) at 800 ppm. The Nativo, Evito and Moncut were moderately effective at 1000 ppm, causing more than 78% mycelial growth inhibition of *M. phaseolina*. Moreover, the ten fungicidal treatments were considerably effective against *V. albo-atrum*, with Moncut and Shincar being the most effective at all concentrations. This was followed by Evito (86.66%), Kocide (80%), and Systhane (79.94%) at 1000 ppm. While Topsin M and Systhane caused the slightest inhibition, i.e., 17.52% and 10% at 200 ppm. The chemicals demonstrated an escalating inhibitory trend as the concentration increased further.

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Introduction

Chili (*Capsicum annum* L.) is an economically important and widely grown crop worldwide (Olatunji and Afolayan, 2018). Both green and dry

chili and peppers cover an area of 3.69 million hectares and produce 4.03 million tonnes (dry + green) worldwide (FAOSTAT, 2022). It is commonly used in many food items worldwide as well as cosmetics, pharmaceuticals, and ornaments (Bashir *et al.*,

2018; Tripodi and Kumar, 2019). It serves as a vital reservoir of human nutrients, abundant in Vitamins A, B, C, E, and P, while also being high in potassium, magnesium, and iron. In contrast to red chili, fresh green chili possesses a higher amount of vitamin C than citrus fruits (Kongkachuichai *et al.*, 2015). Chili production faces various constraints, including fungal, bacterial, viral infection, and abiotic stress (Abdel-Fattah and Al-Amri, 2012; Zulfitri *et al.*, 2020). These pathogens cause many diseases, from sowing seeds in the nursery to the field till harvesting (Kavitha *et al.*, 2005; Goldberg, 2010; Divya and Sudini, 2013). Damping-off, root rots, and wilts are common and devastating diseases and affect the crop in either pre or post-harvest stage, which causes significant losses (Pakdevaraporn *et al.*, 2005). Soil-borne fungal pathogens, including *Phytophthora capsici*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Phytophthora nicotianae*, *Fusarium solani*, *Fusarium oxysporum*, *Verticillium dahliae*, *Verticillium albo-atrum*, and *Pythium spp.* has been reported for causing these diseases (Abdel-Monaim and Ismail, 2010; Abdel-Monaim *et al.*, 2014). Soil-borne pathogens, especially *Macrophomina phaseolina*, *Rhizoctonia solani*, and *Verticillium albo-atrum*, attack chili plants on roots, leaves, and stems and cause 70% yield losses in the field (Fradin and Thomma, 2006; Jabeen *et al.*, 2016). *M. phaseolina* (Tassi) Goid is responsible for diseases in elevated temperatures (30-35°C) and limited moisture conditions (Ghosh *et al.*, 2018). It impacts over 500 cultivated and wild plant species globally (Cohen *et al.*, 2022). *M. phaseolina* produces microsclerotia in the soil and affect various foods crops and their physiological process such as chlorophyll, proline, and sugar content under the favorable environmental conditions (Jadon and Shah, 2012). Root rot and damping-off caused by *R. solani* is a significant threat to nursery and young chili plants. *R. solani* is soil and seedborne pathogen (Sivalingam *et al.*, 2006). The pathogen is challenging to control because of its ability to survive long-term in the soil and organic debris and cause disease incidence of 33.2% in seedlings and 40.2% in the field (Varma *et al.*, 2020). *R. solani* is responsible for wilting and death of mature chili plants (Ashwini and Srividya, 2016). Several practices are available to avoid the disease, such as cultural practices, biological control, resistant varieties, crop rotation, and soil solarization (Van *et al.*, 2016), but these methods contain limitations. Like non-availability of resistant varieties and/or biocontrol-based products

for large-scale field application (Amini and Sidovich, 2010). Chemical control is the most broadly used and preferred method for controlling pathogens. Despite all the health and environment risks of fungicides, it has been proven effective in managing strategies (Dahal and Shrestha, 2018). Some agro pesticides and fungicides have been reported against many pathogens, such as carbendazim, carboxin, chlorothalonil, companion, copper oxychloride, mancozeb, metalaxyl, thiram, propiconazole azoxystrobin and iprodion (Hao *et al.*, 2020). Out of these two fungicides carbendazim and mancozeb are considered as the most effective control agents. Typically, fungicides function by employing a shared mechanism centered on microtubule polymerization. This approach effectively regulates fungal cell division, contributing to an efficient crop protection strategy (Abrar Ul Hassan *et al.*, 2021). Therefore, the objective of the present study was to evaluate the ten fungicides at different doses against soil-borne fungal pathogens associated with the chili plants.

Materials and Methods

Collection of specimen, isolation and identification of pathogens

To extract soil-borne fungi affecting chili plants, roots were gathered from diverse fields within the Mirpurkhas district of Sindh province, Pakistan. A total of three chili field selected in Mirpurkhas district and five infected plants were collected from each field. These collected plants were placed into individual paper bags, each labeled with its corresponding location, and transported to the laboratory for the purpose of isolating root-associated fungi. The plants were carefully washed with tap water to eliminate any adhering soil particles from the roots. Subsequently, the infected segments of the roots were dissected into small 1 cm pieces, using sterilized scissors. These root segments were subjected to surface sterilization using a 5% NaClO solution for 30 seconds. Following this, they underwent three washes with sterile distilled water and were then arranged on Petri dishes containing PDA medium. Streptomycin sulfate and penicillin were added to the PDA medium at a concentration of 1 ml/L. A five root samples were put in each Petri dish and seal with parafilm. The plates were incubated at 27°C ± 2°C for 3 days. The resulting fungal colonies exhibited a variety of colors, sizes, and shapes on the PDA medium. For more comprehensive analysis, these fungal colonies

were subjected to purification through hyphal tip transfer onto fresh PDA plates. The identification of isolated fungi was accomplished by assessing their morphological characteristics, utilizing identification keys provided by Booth (1971), Ellis (1971), Barnett and Hunter (1972), and Singh (1982).

In vitro screening of fungicides against roots infecting fungi:

Ten fungicides viz., Shincar, Antracol, Aliette, Ridomil Gold, Moncut, Systhane, Kocide, Evito, Nativo, and Topsin M at 200, 400, 600, 800, and 1000 ppm were evaluated against *Rhizoctonia solani*, *Verticillium albo-atrum*, and *Macrophomina phaseolina* by food poisoned technique (Maitlo et al., 2013). The details of fungicides, including brand names, chemical names, chemical groups, active ingredients, and formulations, are shown in Table 1. Before pouring, the concentrations of fungicides were mixed in a PDA with the help of sterilize glass pipette. Fungicide-free mediums were used as control. After solidification of PDA, a 5 mm disk of 8 days old culture was transferred to the center of the Petri plate. All treatments were repeated three time with four replications. Mean values were calculated of each treatment. The inoculated plates were incubated at 27°C for seven days. Two perpendicular lines were drawn on the back of the Petri plates and crossed in the middle of the plate to measure the radial colony growth of the tested fungi. The colony growth (mm) was measured using a scale every 24 hours until the control plate was filled in any treatment. The mycelial growth inhibition percentage was recorded as per given by formula (Vincent, 1947):

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

Where; PI= Percent inhibition of fungal mycelial growth; C= Fungal mycelial growth in control plates; T= Fungal mycelial growth in treated plates.

Data analysis

The experiment was conducted using a completely randomized design (CRD) with four replications. To compare the mean values, the least significant difference (LSD) test was performed at a significance level of p = 0.05. Additionally, Duncan's Multi-Range Test was employed for comparing the means at the same significance level. All statistical analyses were carried out using Statistix version 8.1.

Results and Discussion

Isolation of fungi from infecting roots

A total of 18 fungi, namely, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Alternaria alternata*, *A. solani*, *Curvularia lunata*, *Colletotrichum capsici*, *C. gloeosporioides*, *Fusarium solani*, *F. oxysporum*, *Macrophomina phaseolina*, *Pythium dilense*, *P. aphanidermatum*, *Phytophthora capsici*, *Rhizoctonia solani*, *Rhizopus oryzae*, *R. stolonifer*, and *Verticillium albo-atrum* were isolated from the roots with different frequency. Among all pathogens, *F. solani* appeared with the highest frequency (9.37%) isolated from chili roots, followed by *R. solani* (9.11%), *F. oxysporum* (8.85%), *A. flavus* (6.5%), *A. fumigatus* (6.45%), *M. phaseolina* (6.25%), *P. aphanidermatum* (5.77%), *V. albo-atrum* (5.4%), *P. dilense* (5.29%), *A. solani* (4.55%), *A. alternata* (4.5%), *A. niger* (4.3%), *R. stolonifer* (3.23%), *C. gloeosporioides* (3.12%), *P. capsici* (2.61%), *R. oryzae* (2.61%), *C. capsici* (2.5%) and *C. lunata* (2.12%) (Figure 1).

Table 1: Details of fungicides tested against *M. phaseolina*, *R. solani* and *V. albo-atrum*.

Trade name	Active ingredient	Chemical Group	Manufacturer/Distributor in Pakistan
Alliete	Fosetyl Aluminium	Ethyl Phosphonate	Bayer Crop Science
Antracol	Propine	Dithiocarbamates	Bayer Crop Science
Evito	Fluoxastrobin	Dihydro-dioxazines	Arysta Life Science Pakistan
Kocide	Copper hydroxide	Inorganic	FMC Corporation
Moncut	Flutolanil	Benzamides	Arysta Life Science Pakistan
Nativo	<i>Tebuconazole + Trifloxystrobin</i>	Oximino acetates and Triazoles	Bayer Crop Science
Ridomil Gold	Mancozeb + Mefenoxam	Dithiocarbamates and Acylalanines	Syngenta Pakistan Limited
Sythane	Myclobutanil	Triazole	FMC Corporation
Shinar	Carbendazim	benzimidazole	FMC Corporation
Topsin M	Thiophanate-methyl	Thiophanates	Arysta Life Science Pakistan

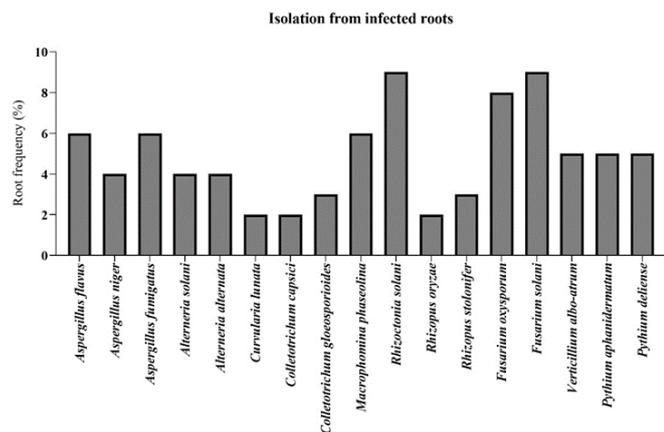


Figure 1: Frequency isolation of fungi from infected portions of roots collected from Mirpurkhas.

Effect of different fungicides on colony growth of fungi

Under *in-vitro* conditions, ten different fungicides *viz.*, Antracol, Aliette, Evito, Kocide, Moncut, Nativo, Ridomil Gold, Systhane, Shincar, and Topsin M were tested for their effects on colony growth of the *R. solani*, *V. albo-atrum*, and *M. phaseolina*. Each fungicide was tested with five concentrations *viz.*, 200, 400, 600, 800, and 1,000 ppm. All fungicides produced a significant reduction in the growth of fungi compared to control. An increase in the concentration of all the ten fungicides in the medium showed a significant gradual reduction in the growth of pathogenic fungi. The LD₅₀ values were calculated according to the inhibition percentage of the tested

fungus. For this purpose, the inhibition percentages are converted to probit units.

Effect of different fungicides on Macrophomina phaseolina

Ten different fungicides were employed at various doses to assess their impact on the growth inhibition of *Macrophomina phaseolina*. Among the investigated fungicides, Systhane, Shincar, and Topsin M appeared highly effective at all doses (200-1000 ppm), which yielded 100% inhibition, followed by Antracol 1000 ppm caused 100% inhibition of *Macrophomina phaseolina*. Antracol at 600 and 800 ppm causes 87.22% and 70.55% inhibition of mycelial growth. Among all concentrations, the highest concentration (1000 ppm) of Nativo, Evito, Moncut, and Aliette caused more than 70% inhibition of *M. phaseolina*. Kocide and Ridomil Gold were ineffective at all doses (Figure 2).

Based on LD₅₀ values, *M. phaseolina* was found to be the most sensitive pathogen against Shincar with an LD₅₀ value of 87.1065, followed by Topsin M (91.2182), Systhane (97.4625), Nativo (323.2536), Antracol (384.4707), Evito (510.8925), Moncut (557.9459) and Aliette (652.02). The Kocide and Ridomil Gold fungicide were infective with LD₅₀ value Kocide (871.5312) and Ridomil Gold (874.0793), respectively (Figure 3).

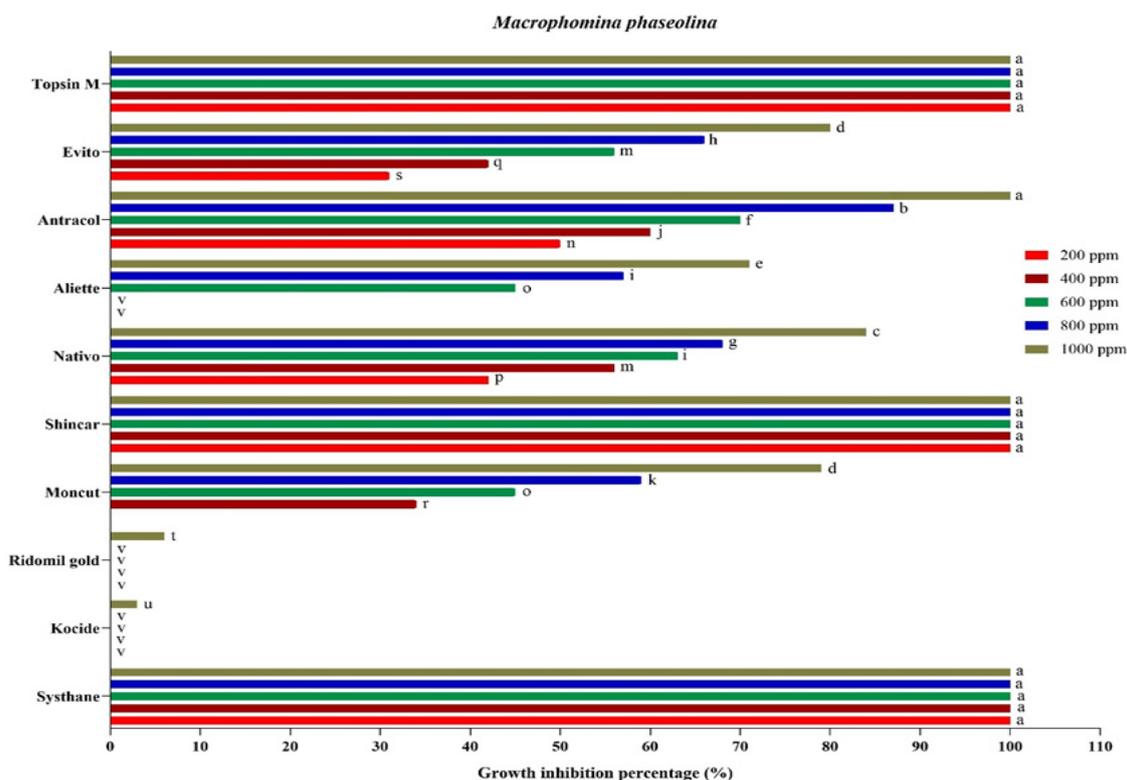


Figure 2: Response of *Macrophomina phaseolina* to different concentrations of various fungicides.

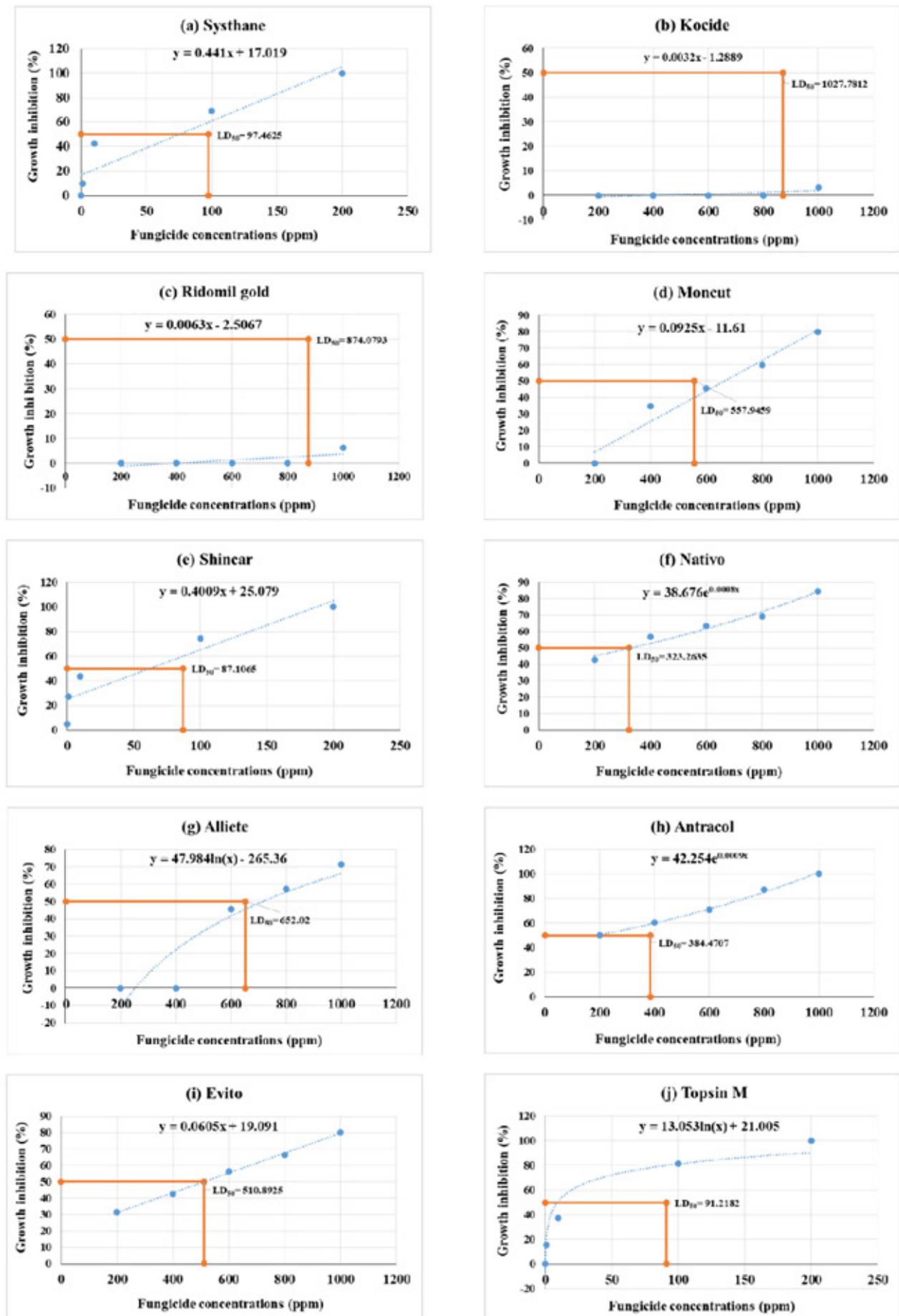


Figure 3: LD₅₀ values of different fungicides against *Macrophomina phaseolina*.

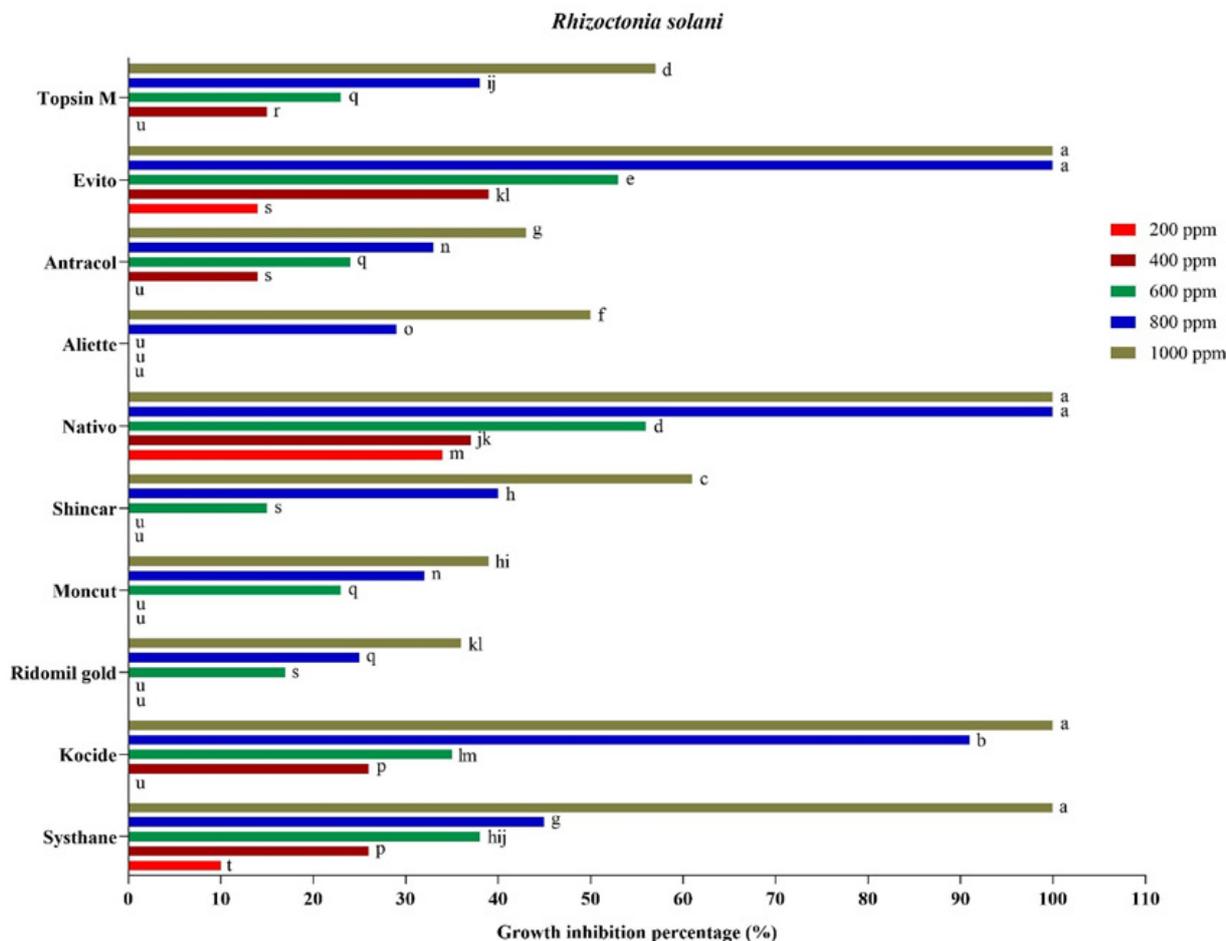


Figure 4: Response of *Rhizoctonia solani* to different concentrations of various fungicides.

Effect of different fungicides on Rhizoctonia solani

Out of ten fungicides, only four fungicides resulted in 100% inhibition of *Rhizoctonia solani* at higher concentrations; these include Systhane at 1,000 ppm, Kocide at 800-1000 ppm, Nativo at 800-1000 ppm, and Evito at 800-1000 ppm. In comparison, Shincar at 1000 ppm caused 61.66% inhibition. More than 50% inhibition of *R. solani* has been observed in Nativo at 600 ppm, Evito at 600 ppm, Topsin M at 1000 ppm, and Aliette at 1000 ppm. All the remaining fungicides produced either no or less than a 50% reduction in colony growth compared to the control (Figure 4). In the present study, it was proved that *R. solani* was most sensitive against four different fungicides at higher concentrations. However, 50% inhibition of *R. solani* was calculated with different fungicides. The lowest LD₅₀ value was recorded with Nativo (538.8762), followed by Evito (588.4488), Moncut (616.6486), Antracol (636.5374), Topsin M (639.1904), Kocide (669.9019), Shinar (682.1744), Ridomil Gold (735.9251), Systhane (759.8890) and Allie (819.613), respectively (Figure 5).

Effect of different fungicides on Verticillium albo-atrum

Against *Verticillium albo-atrum*, all tested fungicides caused a significant reduction in the colony growth. At all concentrations, two fungicides, Moncut and Shincar, resulted in 100% inhibition, followed by Evito 1000 ppm, which caused 86.6% inhibition. While Systhane at 800-1000 ppm, Kocide at 600-1000 ppm, Ridomil Gold at 1000 ppm, Antracol at 600-1000 ppm, Aliette at 600-1000 ppm, Evito at 600-800 ppm, and Topsin M at 800-1000 ppm exhibited more than 60% inhibition. Additionally, Ridomil Gold at 800 ppm, Nativo at 400 ppm, Aliette at 400 ppm, Antracol at 400 ppm, and Evito at 400 ppm reduced the growth of *V. albo-atrum* by 50-57%. Other concentrations were less effective (Figure 6). However, the LD₅₀ values of ten different fungicides for the 50% inhibition of *V. albo-atrum* were determined. The significantly minimum LD₅₀ value was recorded with Moncut (107.5457), followed by Shinar (118.298), Aliette (457.8515), Evito (487.7099), Kocide (489.4386), Antracol (518.9959), Ridomil Gold (520.5342), Systhane (575.7236), Nativo (593.2692), and Topsin M (612.6594), respectively (Figure 7).

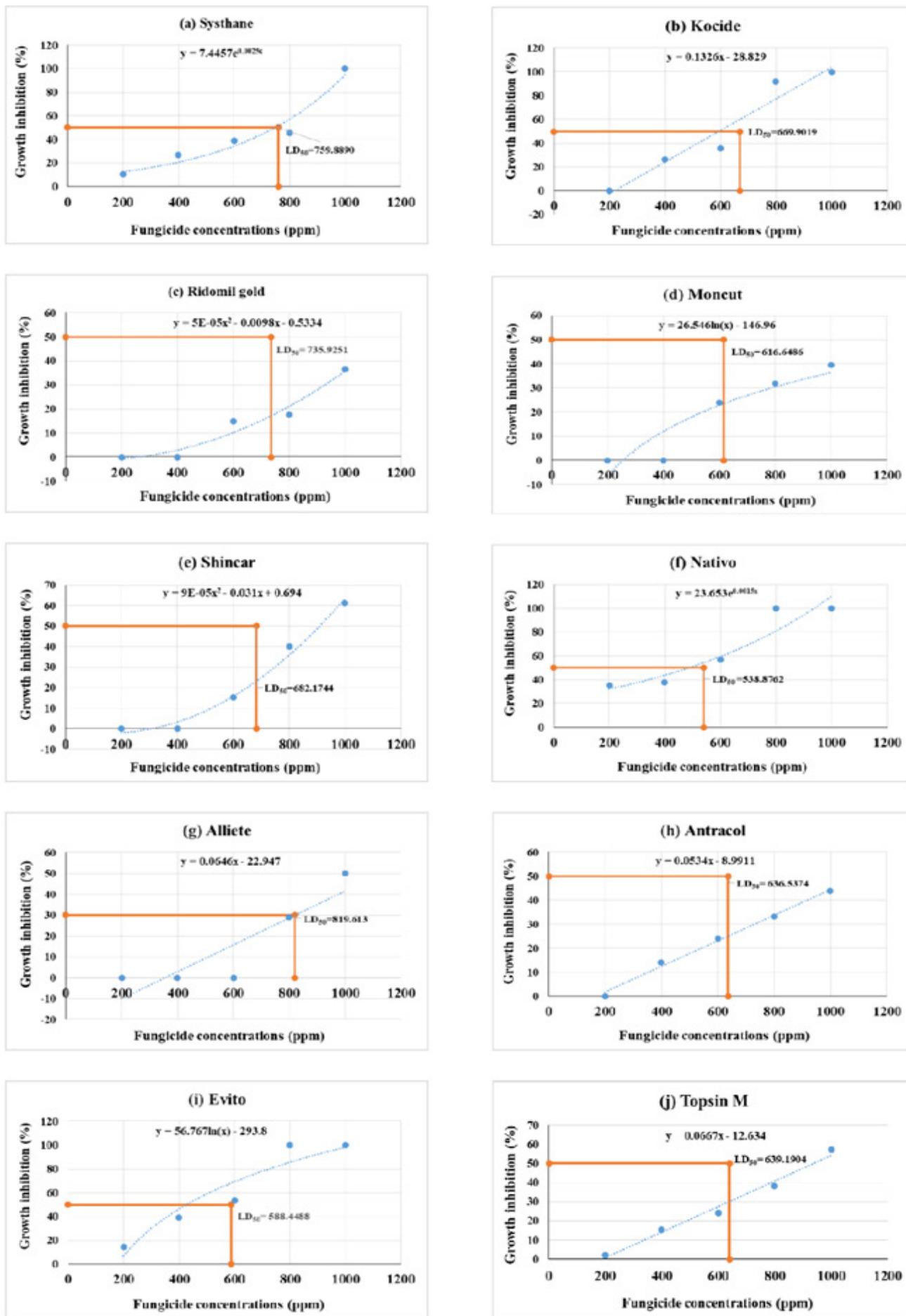


Figure 5: LD₅₀ values of different fungicides against *Rhizoctonia solani*.

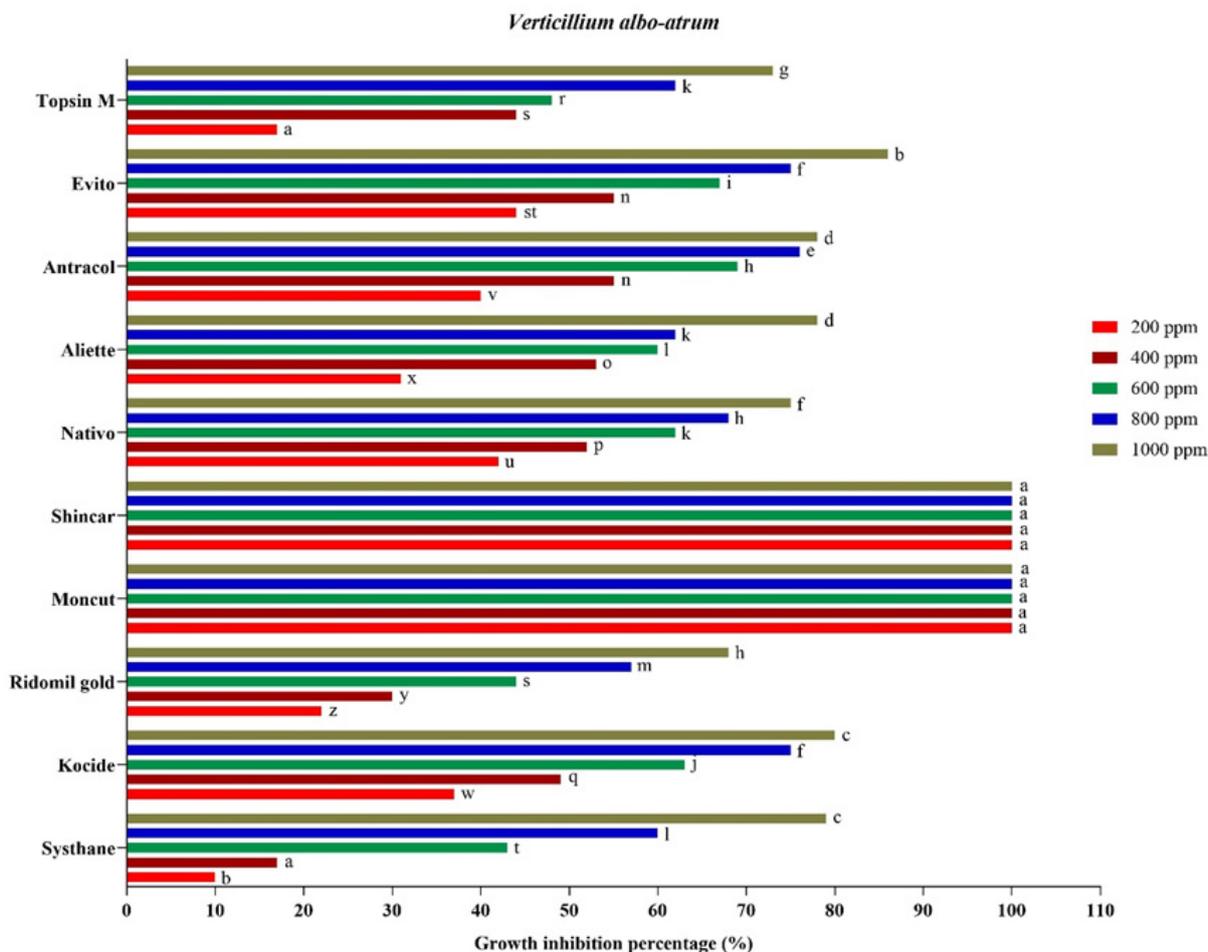


Figure 6: Response of *Verticillium albo-atrum* to different concentrations of various fungicides.

Soil-borne fungal pathogens are considered the most critical pathogens responsible for the enormous losses in chili crops. The use of fungicides against soil-borne diseases can help to manage the crop. In the present study, ten fungicides were used for their effectiveness against three soil-borne fungal pathogens. Several chemical pesticides and fungicides are available in the market and also reported in previous studies for reduce the mycelial growth of different fungal pathogens, such as carbendazim, carboxin, chlorothalonil, companion, copper oxychloride, mancozeb, metalaxyl, thiram, propiconazole azoxystrobin and iprodion (Hao *et al.*, 2020). In addition, among all fungicides, two fungicides carbendazim and mancozeb have been reported highly effective control agents. Typically, fungicides function by employing a shared mechanism centered on microtubule polymerization. This approach effectively regulates fungal cell division, contributing to an efficient crop protection strategy (Abrar UI Hassan *et al.*, 2021). Among all fungicides, Nativo, Evito at 800-1000 ppm, and Sythane and Kocide at 1000 ppm proved to be the most effective against *R. solani*. Other workers are also found Nativo highly effective for *R. solani* (Persaud *et al.*,

2019; Karkee and Mandal, 2020; Rashid *et al.*, 2020). Evito and myclobutanil also successfully inhibited the growth of *R. solani* (Eliwa *et al.*, 2021; Daniels and Latin, 2013; Davis *et al.*, 1997). Carbendazim is one of the most influential and broad-spectrum systemic fungicides that inhibit a wide range of pathogens (Amini and Sidovich, 2010). In present studies, Sythane, Shincar (carbendazim), and Topsin M were highly effective against *M. phaseolina*. In another study, carbendazim caused the remarkable inhibition of *M. phaseolina* and *F. oxysporum* under *in vitro* conditions (Karibasappa *et al.*, 2020; Dahal and Shrestha, 2018). Similarly, Topsin M (thiophanate methyl) is also well recognized broad-spectrum fungicide, which effective against large number of fungal pathogens (Nasir *et al.*, 2012; Khanzada *et al.*, 2005). Myclobutanil effectively controls soybean rust, anthracnose, and bacterial pustule (Sangawongse, 1991). Against *V. albo-atrum*, two fungicides, Moncut (Flutolanil) and Shincar (carbendazim), at all concentrations, proved excellent performance and caused complete inhibition of fungus. Carbendazim also found to check the growth *V. chlamydosporium* (De *et al.*, 2009). It inhibited the conidial germination and colony growth

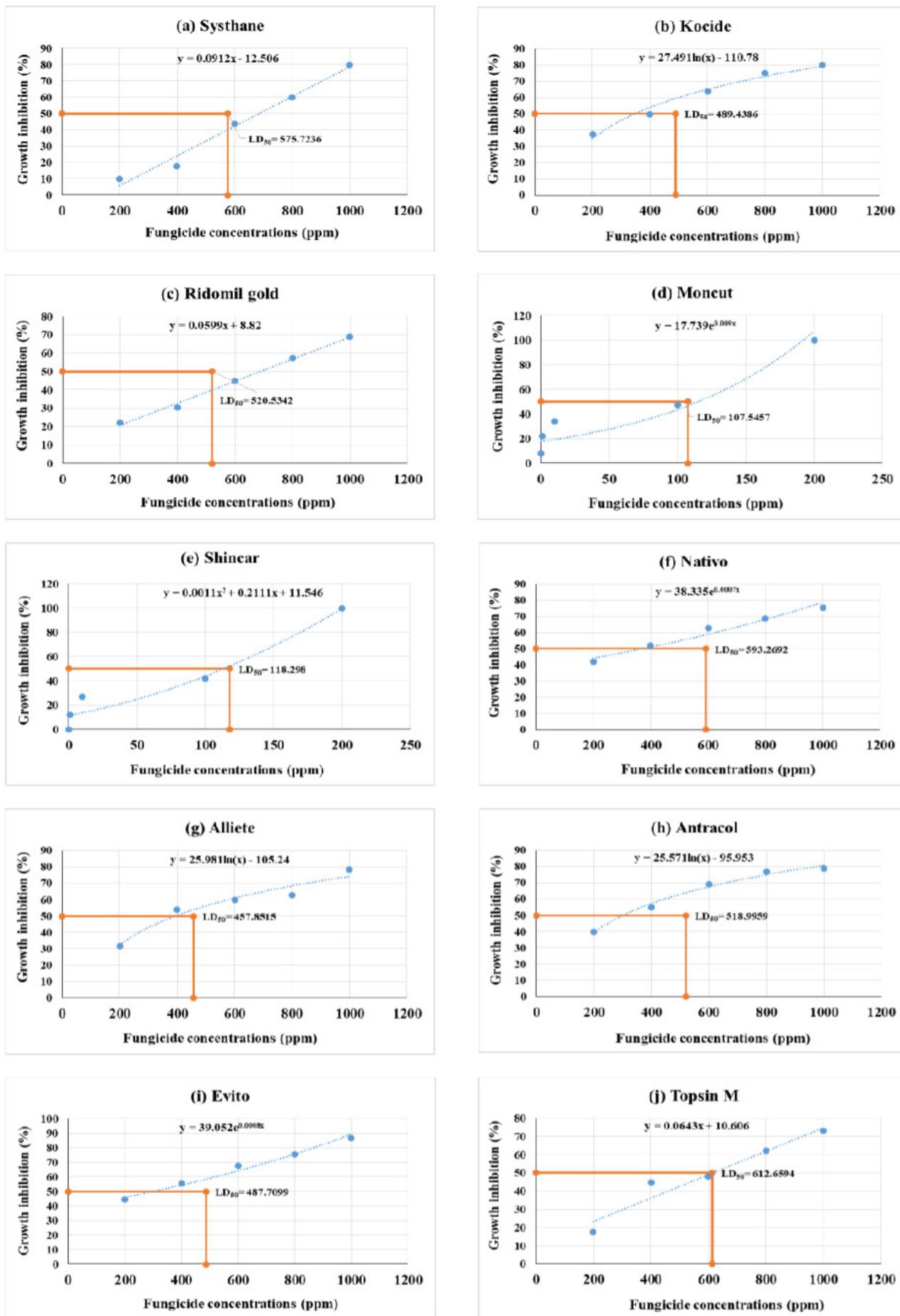


Figure 7: LD₅₀ values of different fungicides against *Verticillium albo-atrum*.

(Bhat *et al.*, 2017). Similarly, Hussain *et al.* (2020), found carbendazim is the most effective fungicides against *Alternaria solani*, leading complete inhibition. Moncut is the most commonly used fungicide to control soil fungal diseases. Moncut fungicide is widely used for controlling rice sheath blight and other plant diseases caused by fungal pathogens (Motoba *et al.*, 1988). Hirooka *et al.* (1990) reported that moncut fungicides reduced the complete mycelial growth of *R. solani*.

Conclusions and Recommendations

Significant variability in the effectiveness of fungicides has been noted. Only specific fungicides demonstrate efficacy against *M. phaseolina*, *R. solani* and *V. albo-atrum*. In areas experiencing high disease prevalence, substances such as Systhane, Shincar, Nativo, Antracol, Evito, and Topsin M are commonly employed. In the present study, 50% growth inhibition also checked through LD₅₀ formula and found that *R. solani* was highly sensitive against Nativo fungicides. Therefore, *M. phaseolina* was found to be the most sensitive pathogen against Shincar and Topsin M fungicides, while *V. albo-atrum* was noted with Moncut and Shincar fungicides.

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Novelty Statement

The present study reveals the response of three different soil-borne pathogens viz., *Macrophomina phaseolina*, *Rhizoctonia solani* and *Verticillium albo-atrum* to 10 old and novel synthetic fungicides.

Author's Contribution

Nabeel Akhtar: Conducted this research and prepared the manuscript for publication.

Owais Iqbal: Reanalyze the data, revised and finalized the manuscript writing.

Imtiaz Ahmed Nizamani: Supervised and helped in experimental setup.

Rehana Naz Syed: Evaluated and revised the final version of manuscript.

Abdul Mubeen Lodhi: Analyzed, edited and approved the manuscript.

All authors have read and agreed to the published

version of the manuscript.

Conflicts of interest

The authors have declared no conflict of interest.

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