UKR Journal of Agriculture and Veterinary Sciences (UKRJAVS)
Homepage: https://ukrpublisher.com/ukrjavs/
Email: submit.ukrpublisher@gmail.com
Volume 2, Issue 2 (March-April) 2026
ISSN: 3107-6173 (Online)

OF DOSAGE RESPONSE CURVES TO FLUDIOXONIL, NOT ALL PHYTOPATHOGENIC FUNGI FOLLOW S-CURVE PATTERNS

Jane Ifunanya Mbadianya [1]*, Dongya Shi[2] and Chang-jun Chen[3]

[1]Nnamdi Azikiwe University, Awka, Nigeria, P.M.B. 5025
[2&3]College of Plant Protection, Nanjing Agricultural University, Key Laboratory of Pesticide Science, Jiangsu Province, Nanjing 210095, China

***Corresponding Author**: Jane Ifunanya Mbadianya

DOI: *https://doi.org/10.5281/zenodo.19638812*

Article History
Original Research Article
Received: 01-03-2026
Accepted: 05-04-2026
Published: 18-04-2026

Citation: Jane Ifunanya Mbadianya, Dongya Shi, Chang-jun Chen. (2026). OF DOSAGE RESPONSE CURVES TO FLUDIOXONIL, NOT ALL PHYTOPATHOGENIC FUNGI FOLLOW S-CURVE PATTERNS. UKR Journal of Agriculture and Veterinary Sciences (UKRJAVS), 2(2), 18-26.

Abstract

BACKGROUND*: Fludioxonil is a broad-spectrum fungicide widely used for the management of seed-borne and postharvest fungal diseases. This study aimed to characterize the dose–response relationships of phytopathogenic fungi exposed to fludioxonil. Six fungal pathogens including; Fusarium fujikuroi, Alternaria alternata, Botrytis cinerea, Colletotrichum gloeosporioides, Colletotrichum acutatum, and Sclerotinia sclerotiorum were selected to evaluate their response-to-dosage curves (RTDCs) to fludioxonil.*

RESULTS*: The response-to-dosage curves (RTDCs) of the six plant pathogens to fludioxonil varied among species. In fludioxonil-sensitive isolates, Fusarium fujikuroi exhibited a fluctuating response, while Alternaria alternata, Botrytis cinerea, Colletotrichum gloeosporioides, C. acutatum, and Sclerotinia sclerotiorum displayed typical sigmoidal (S-shaped) dose–response patterns. Among resistant isolates, B. cinerea and F. fujikuroi maintained S-curve responses at higher concentrations, whereas resistant A. alternata isolates did not follow this pattern.*

CONCLUSION*: Among the six phytopathogenic fungi examined, Fusarium fujikuroi exhibited a distinctive response-to-dosage curve, highlighting the diversity of RTDCs among plant pathogens in response to MAPK (mitogen-activated protein kinase) inhibitors.*

Keywords: *six phytopathogenic fungi, fludioxonil, response-to-dosage curves (RTDC), Fusarium fujikuroi, fluctuating sensitivity.*

1. INTRODUCTION

Fludioxonil is a non-systemic fungicide belonging to the phenylpyrrole class and was first commercialized by Syngenta in 1993 (Raaijmakers et al., 2002). It has broad-spectrum antifungal activity and is widely applied for the management of seed-borne and postharvest diseases (Förster et al., 2007; Paranjape et al., 2014). Owing to its high level of crop safety and strong inhibitory effects on target pathogens, fludioxonil is considered one of the most effective fungicides for seed treatment, forming a durable protective barrier around emerging seedlings. Its mode of action involves interference with a fungal hybrid histidine kinase, OS-1, which belongs to group III histidine kinases (Jeschke, 2017).

Despite nearly three decades of extensive use, resistance to fludioxonil has been reported only sporadically in field populations. Resistance development is primarily associated with mutations in the OS-1 protein, which plays a key role in fungal osmotic signal transduction (Cui et al., 2002; Dry et al., 2004; Ochiai et al., 2001; Oshima et al., 2002; Yoshimi et al., 2004). Furthermore, alterations within the HAMP domains located in the N-terminal region of OS-1 have been shown to confer high levels of resistance to fludioxonil (Duan et al., 2014; Fillinger et al., 2012; Ren et al., 2016; Vignutelli et al., 2002). In fludioxonil-resistant field isolates of *Botrytis cinerea* and *Fusarium fujikuroi*, mutations are predominantly found in the histidine kinase, adenylyl cyclase, methyl-accepting chemotaxis protein, and phosphatase (HAMP) domains of the N-terminal region. In contrast, laboratory-generated mutants often harbor mutations either within the HAMP domains or in the HATPase c domain located in the C-terminal region (Ren et al., 2016).

During in vitro sensitivity assays, fludioxonil has occasionally demonstrated unexpectedly high efficacy at low concentrations, which deviates from the typical dose–response relationship. To investigate this phenomenon, six phytopathogenic fungi namely: *Fusarium fujikuroi, Alternaria alternata, Botrytis cinerea, Colletotrichum gloeosporioides, Colletotrichum acutatum,* and *Sclerotinia sclerotiorum* were selected for sensitivity evaluation. The aim of this study was to characterize the dose-response relationships of fludioxonil in both sensitive and resistant fungal isolates. The findings are expected to provide guidance for optimizing the practical application of fludioxonil under field conditions.

2. MATERIALS AND METHODS

2.1 Fungal strain

Isolates representing six phytopathogenic fungi including; *Fusarium fujikuroi, Alternaria alternata, Botrytis cinerea, Colletotrichum gloeosporioides, Colletotrichum acutatum,* and *Sclerotinia sclerotiorum* were obtained from infected tissues of strawberry, tomato, rice, grape, celery, and cucumber plants. Diseased samples were collected from agricultural fields in Jiangsu and Shandong provinces, China, between 2023 and 2025 and were subsequently used to evaluate the dose–response relationships to fludioxonil. Prior to experimentation, all fungal isolates were maintained on potato dextrose agar (PDA) slants at 4 °C.

2.2 Fungicide and media

Fludioxonil (98% active ingredient) of pure technical grade (provided by Yangzhou Younuo Chemical Co, Ltd, CN) was dissolved in methanol to obtain 10^4 µg/ml stock solution. The stock solution was preserved in the dark at 4 ^{0}C. PDA was prepared with 200 g of potato, 20 g of glucose and 16 g of agar L^{-1} of distilled water.

2.3 Determination of sensitivity of the six fungal pathogens to fludioxonil

Sixteen isolates of *Fusarium fujikuroi*, four isolates each of *Alternaria alternata*, *Colletotrichum gloeosporioides*, and *Colletotrichum acutatum*, twelve isolates of *Botrytis cinerea*, and six isolates of *Sclerotinia sclerotiorum* were cultured on potato dextrose agar (PDA) at 25 °C for five days. Actively growing mycelial discs (5 mm in diameter) were excised from the peripheral region of each colony, approximately one-third of the distance from the colony edge, and transferred to fresh PDA plates for subsequent assays.

The sensitivity of all fungal isolates to fludioxonil was evaluated using the mycelial growth inhibition method. PDA media were amended with fludioxonil at a range of concentrations (0, 0.0414, 0.1234, 0.3703, 1.111, 3.333, 10, 30, 90, and 270 µg mL^{-1}) to establish dose–response relationships. Mycelial plugs were placed at the center of the amended PDA plates and incubated at 25 °C. Because *C. gloeosporioides, C. acutatum,* and *S. sclerotiorum* failed to grow at concentrations above 0.0414 µg mL^{-1}, additional assays were conducted using lower fludioxonil concentrations of 0.0052, 0.0104, and 0.0207 µg mL^{-1}. For fludioxonil-resistant isolates of *F. fujikuroi*, higher concentrations (100, 200, 400, and 800 µg mL^{-1}) were applied.

Colony diameters were measured when the control colonies (without fludioxonil) reached approximately 8 cm in diameter. Growth measurements were obtained by averaging two perpendicular colony diameters, with the initial plug diameter (5 mm) subtracted. Mycelial growth inhibition rates were calculated and subjected to arcsine transformation prior to statistical analysis. Each treatment included three replicates per isolate, and the entire experiment was conducted twice.

2.4 STATISTICAL ANALYSIS

The data were analyzed using analysis of variance (ANOVA) with the aid of GENSTAT 12.0 Release 4.23 (GENSTAT 2009) statistical tool. Means were later compared with Fisher's protected least significant difference at 5% probability level as outlined by (Obi, 2002).

3. RESULTS

3.1 Of six phytopathogenic fungi, five follow S-models of response-to-dosage curves

The results indicated that fludioxonil concentrations, fungal species, and their interaction significantly influenced mycelial growth inhibition ($P \leq 0.05$) compared with the untreated controls. *Colletotrichum gloeosporioides*, *C. acutatum*, and *Sclerotinia sclerotiorum* exhibited typical sigmoidal (S-shaped) dose–response curves, with increasing sensitivity observed as fludioxonil concentrations increased (Figures 1, 2, and 4, respectively). Fludioxonil showed strong antifungal activity against these pathogens, with a minimum inhibitory concentration (MIC) of 0.0404 µg mL^{-1}.

Similarly, fludioxonil-sensitive isolates of *Botrytis cinerea* and *Alternaria alternata* displayed comparable sigmoidal response patterns (Figures 3 and 7, respectively), indicating a concentration-dependent inhibition of mycelial growth.

3.2 *F. fujikuroi* possesses unique response-to-dosage curve

As shown in Figure 5, *Fusarium fujikuroi* displayed a unimodal dose–response pattern. The first peak in growth inhibition (58.8%) occurred at 0.0414 µg mL⁻¹, which corresponded to the minimum inhibitory concentration, followed by a decline as fludioxonil concentrations increased. A second unimodal response was observed at 10 µg mL⁻¹, where inhibition reached 57.57%. Overall, sensitivity to fludioxonil fluctuated across concentrations and gradually stabilized at 90 µg mL⁻¹, aligning with a sigmoidal response pattern. In contrast, fludioxonil-resistant isolates of F. fujikuroi exhibited restored sensitivity at concentrations of 100 µg mL⁻¹ and above, characterized by a typical S-shaped dose–response curve.

3.3 Response-to-dosage curves of fludioxonil-resistant and -sensitive strains differs in *A. alternata*, similar in *B. cinerea* and *F. fujikuroi*

Among the fludioxonil-resistant isolates, differential responses to increasing fungicide concentrations were observed. Higher concentrations of fludioxonil resulted in enhanced inhibitory effects against resistant isolates of *Botrytis cinerea* (Figure 3) and *Fusarium fujikuroi* (Figure 6). In contrast, increasing fludioxonil concentrations did not lead to a corresponding increase in mycelial growth inhibition in resistant isolates of *Alternaria alternata* (Figure 7), indicating a reduced or absent concentration-dependent response.

4. DISCUSSIONS

This study evaluated the dose–response relationships of six phytopathogenic fungi to fludioxonil and demonstrated that pathogen responses do not universally conform to a classical sigmoidal model. Five of the six tested species exhibited typical S-shaped dose–response curves, indicating increasing sensitivity with rising fludioxonil concentrations. *C. gloeosporioides, C. acutatum,* and *S. sclerotiorum* were highly sensitive to fludioxonil and required relatively low concentrations for effective inhibition. In addition, nine out of twelve isolates of *Botrytis cinerea* and two isolates of *A. alternata* were fludioxonil-sensitive, displaying S-curve patterns with a minimum inhibitory concentration (MIC) of 0.04014 µg mL⁻¹.

These findings align with previous reports highlighting the broad-spectrum efficacy of fludioxonil in protecting crops such as maize, soybean, potato, cotton, sunflower, and various vegetables from fungal pathogens including *Alternaria spp., Colletotrichum spp., B. cinerea,* and *Sclerotinia spp*. (Lawry et al., 2017; Paranjape et al., 2014). Earlier studies have consistently shown that fludioxonil is highly effective at inhibiting mycelial growth and spore germination of *B. cinerea* when compared with other fungicides (Leroux et al., 2002; Zhao et al., 2010; Kim et al., 2016). Similarly, *in vitro* evaluations by Iacomi-Vasilescu et al. (2004) identified fludioxonil as the most effective fungicide against several *Alternaria* species, strongly suppressing spore germination and germ tube elongation, particularly in *A. brassicicola*. Fludioxonil has also been reported as an effective option for managing anthracnose caused by *Colletotrichum spp*. in chili and bell pepper production systems (Gao et al., 2017; Harp et al., 2014). Moreover, the high baseline sensitivity of *S. sclerotiorum* to fludioxonil and its successful application in controlling oilseed rape stem rot have been well documented (Duan et al., 2013).

In contrast to the other pathogens examined, *F. fujikuroi*, a seed-borne pathogen, exhibited a distinct and unstable dose–response pattern. Its sensitivity fluctuated across concentrations, producing a unimodal response with inhibition peaks at 0.3703 µg mL⁻¹ and 10 µg mL⁻¹, before stabilizing at higher concentrations (90 µg mL⁻¹). Despite this atypical response behavior, the strong antifungal activity and favorable crop safety profile of fludioxonil support its continued use as a seed treatment fungicide against *F. fujikuroi* (Paranjape et al., 2014).

Resistant isolates of *B. cinerea, F. fujikuroi,* and *A. alternata* displayed altered response patterns, often characterized by unimodal curves. Growth of resistant *B. cinerea* isolates was suppressed at elevated fludioxonil concentrations (90 µg mL⁻¹), while resistant *F. fujikuroi* isolates regained sensitivity at concentrations ≥100 µg mL⁻¹, following an S-shaped response with an MIC of 800 µg mL⁻¹. These observations are consistent with earlier findings that fludioxonil resistance is associated with mutations in histidine kinase genes involved in osmotic signal transduction pathways (Cui et al., 2002; Dry et al., 2004; Ochiai et al., 2001; Oshima et al., 2002; Yoshimi et al., 2004). Although resistance in *B. cinerea* has been reported infrequently (Baroffio et al., 2003; Myresiotis et al., 2007; Vignutelli et al., 2002; Ziogas and Kalamarakis, 2001), the present study confirms that higher fungicide doses may partially restore efficacy, particularly under high disease pressure, as previously suggested by Van et al. (2011).

Conversely, increasing fludioxonil concentrations did not enhance inhibition of resistant *A. alternata* isolates, indicating that dose escalation alone is ineffective for managing these populations. To delay resistance development, integrated resistance-management strategies should be implemented, including fungicide rotation or mixtures with compounds possessing different modes of action (Wu et al., 2015). Given the observed cross-sensitivity within phenylpyrrole fungicides, combination strategies may improve control efficacy while reducing resistance risk (Duan et al., 2013).

In summary, *F. fujikuroi* exhibited a unique and unstable dose–response behavior to fludioxonil, whereas the remaining pathogens largely followed sigmoidal response patterns. Effective management of *F. fujikuroi* should therefore include fludioxonil seed treatment followed by fungicides with alternative modes of action during later growth stages. For fludioxonil-resistant populations, increased concentrations improved efficacy against *B. cinerea* and *F. fujikuroi*, but not against resistant *A. alternata* isolates. Overall, this study provides a scientific basis for optimizing fludioxonil application rates in the field and enhances understanding of pathogen dose–response dynamics to MAPK (mitogen-activated protein kinase) pathway inhibitors.

ACKNOWLEDGEMENTS

This work was supported by Chinese key R & D Plan (2016YED0201007, 2018YFD0201201 and 2018YFD0201000), Agricultural Science and Technology Projects of Jiangsu Province, China [PZCZ201715 and CX (18)2005], Postgraduate Research &Practice Innovation Program of Jiangsu Province（No. KYCX18_0670).

ETHICAL STATEMENT

This review was written in accordance with ethical guidelines. Informed consent was obtained from all authors, confidentially was maintained and no harm was caused

FUNDING

This work was not funded

CONFLICT OF INTEREST

This manuscript has no conflict of interest

REFERENCES

1. Baroffio, C. A., Siegfried, W., and Hilber, U. W. (2003). Long-term monitoring for resistance of Botryotinia fuckeliana to anilinopyrimidine, phenylpyrrole, and hydroxyanilide fungicides in Switzerland. *Plant disease*, 87(6), 662-666.
2. Cui, W., Beever, R. E., Parkes, S. L., Weeds, P. L., and Templeton, M. D. (2002). An osmosensing histidine kinase mediates dicarboximide fungicide resistance in Botryotinia fuckeliana (Botrytis cinerea). *Fungal Genetics and Biology*, 36(3), 187-198.
3. Dry, I. B., Yuan, K. H., and Hutton, D. G. (2004). Dicarboximide resistance in field isolates of Alternaria alternata is mediated by a mutation in a two-component histidine kinase gene. *Fungal Genetics and Biology*, 41(1), 102-108.
4. Duan, Y.-B., Ge, C.-Y., and Zhou, M.-G. (2014). Molecular and biochemical characterization of Sclerotinia sclerotiorum laboratory mutants resistant to dicarboximide and phenylpyrrole fungicides. *Journal of Pest Science*, 87(1), 221-230.
5. Duan, Y., Ge, C., Liu, S., Chen, C., and Zhou, M. (2013). Effect of phenylpyrrole fungicide fludioxonil on morphological and physiological characteristics of Sclerotinia sclerotiorum. *Pesticide Biochemistry and Physiology*, 106(1-2), 61-67.
6. Fillinger, S., Ajouz, S., Nicot, P. C., Leroux, P., and Bardin, M. (2012). Functional and structural comparison of pyrrolnitrin-and iprodione-induced modifications in the class III histidine-kinase Bos1 of Botrytis cinerea. *PLoS One,* 7(8), e42520.
7. Förster, H., Driever, G., Thompson, D., and Adaskaveg, J. (2007). Postharvest decay management for stone fruit crops in California using the “reduced-risk” fungicides fludioxonil and fenhexamid. *Plant Disease*, 91(2), 209-215.
8. Gao, Y.-Y., He, L.-F., Li, B.-X., Mu, W., Lin, J., and Liu, F. (2017). The potential of fludioxonil for anthracnose control on China chili fruit. *Phytoparasitica*, 45(3), 281-292.
9. Harp, T., Kuhn, P., Roberts, P. D., and Pernezny, K. L. (2014). Management and cross-infectivity potential of Colletotrichum acutatum causing anthracnose on bell pepper in Florida. *Phytoparasitica*, 42(1), 31-39.
10. Iacomi-Vasilescu, B., Avenot, H., Bataille-Simoneau, N., Laurent, E., Guénard, M., and Simoneau, P. (2004). In vitro fungicide sensitivity of Alternaria species pathogenic to crucifers and identification of Alternaria brassicicola field isolates highly resistant to both dicarboximides and phenylpyrroles. *Crop Protection*, 23(6), 481-488.
11. Jeschke, P. (2017). Latest generation of halogen-containing pesticides. *Pest Management Science*, 73(6), 1053-1066.
12. Kim, J.-O., Shin, J.-H., Gumilang, A., Chung, K., Choi, K. Y., and Kim, K. S. (2016). Effectiveness of different classes of fungicides on Botrytis cinerea causing gray mold on fruit and vegetables. *The Plant Pathology Journal*, 32(6), 570.
13. Lawry, S. M., Tebbets, B., Kean, I., Stewart, D., Hetelle, J., and Klein, B. S. (2017). Fludioxonil

induces Drk1, a fungal group III hybrid histidine kinase, to dephosphorylate its downstream target, Ypd1. *Antimicrobial Agents and Chemotherapy*, 61(2), e01414-01416.

14. Leroux, P., Fritz, R., Debieu, D., Albertini, C., Lanen, C., Bach, J., and Chapeland, F. (2002). Mechanisms of resistance to fungicides in field strains of Botrytis cinerea. *Pest Management Science*, 58(9), 876-888.

15. Myresiotis, C., Karaoglanidis, G., and Tzavella-Klonari, K. (2007). Resistance of Botrytis cinerea isolates from vegetable crops to anilinopyrimidine, phenylpyrrole, hydroxyanilide, benzimidazole, and dicarboximide fungicides. *Plant Disease*, 91(4), 407-413.

16. Obi, I. (2002). Statistical methods of detecting differences between treatment means and research methodology issues in laboratory and field experiments. Nsukka, Nigeria.

17. Ochiai, N., Fujimura, M., Motoyama, T., Ichiishi, A., Usami, R., Horikoshi, K., and Yamaguchi, I. (2001). Characterization of mutations in the two-component histidine kinase gene that confer fludioxonil resistance and osmotic sensitivity in the os-1 mutants of Neurospora crassa. *Pest Management Science*: formerly *Pesticide Science*, 57(5), 437-442.

18. Oshima, M., Fujimura, M., Banno, S., Hashimoto, C., Motoyama, T., Ichiishi, A., and Yamaguchi, I. (2002). A point mutation in the two-component histidine kinase BcOS-1 gene confers dicarboximide resistance in field isolates of *Botrytis cinerea*. *Phytopathology*, 92(1), 75-80.

19. Paranjape, K., Gowariker, V., Krishnamurthy, V., and Gowariker, S. (2014). The pesticide encyclopedia: Cabi.

20. Raaijmakers, J. M., Vlami, M., and De Souza, J. T. (2002). Antibiotic production by bacterial biocontrol agents. Antonie van leeuwenhoek, 81(1), 537-547.

21. Ren, W., Shao, W., Han, X., Zhou, M., and Chen, C. (2016). Molecular and biochemical characterization of laboratory and field mutants of Botrytis cinerea resistant to fludioxonil. *Plant Disease*, 100(7), 1414-1423.

22. Van Den Bosch, F., Paveley, N., Shaw, M., Hobbelen, P., and Oliver, R. (2011). The dose rate debate: does the risk of fungicide resistance increase or decrease with dose? *Plant Pathology*, 60(4), 597-606.

23. Vignutelli, A., Hilber-Bodmer, M., and Hilber, U. W. (2002). Genetic analysis of resistance to the phenylpyrrole fludioxonil and the dicarboximide vinclozolin in Botryotinia fuckeliana (Botrytis cinerea). *Mycological Research*, 106(3), 329-335.

24. Wu, D. X., Zhang, R. S., Han, X., Wang, J. X., Zhou, M. G., and Chen, C. J. (2015). Resistance risk assessment for fludioxonil in *Stemphylium solani*. *Annals of Applied Biology*, 167(2), 277-284.

25. Yoshimi, A., Tsuda, M., and Tanaka, C. (2004). Cloning and characterization of the histidine kinase gene Dic1 from Cochliobolus heterostrophus that confers dicarboximide resistance and osmotic adaptation. *Molecular Genetics and Genomics*, 271(2), 228-236.

26. Zhao, H., Kim, Y., Huang, L., and Xiao, C. (2010). Resistance to thiabendazole and baseline sensitivity to fludioxonil and pyrimethanil in Botrytis cinerea populations from apple and pear in Washington State. *Postharvest Biology and Technology*, 56(1), 12-18.

27. Ziogas, B., and Kalamarakis, A. (2001). Phenylpyrrole fungicides: Mitotic instability in *Aspergillus nidulans* and resistance in *Botrytis cinerea*. *Journal of Phytopathology*, 149(6), 301-308.

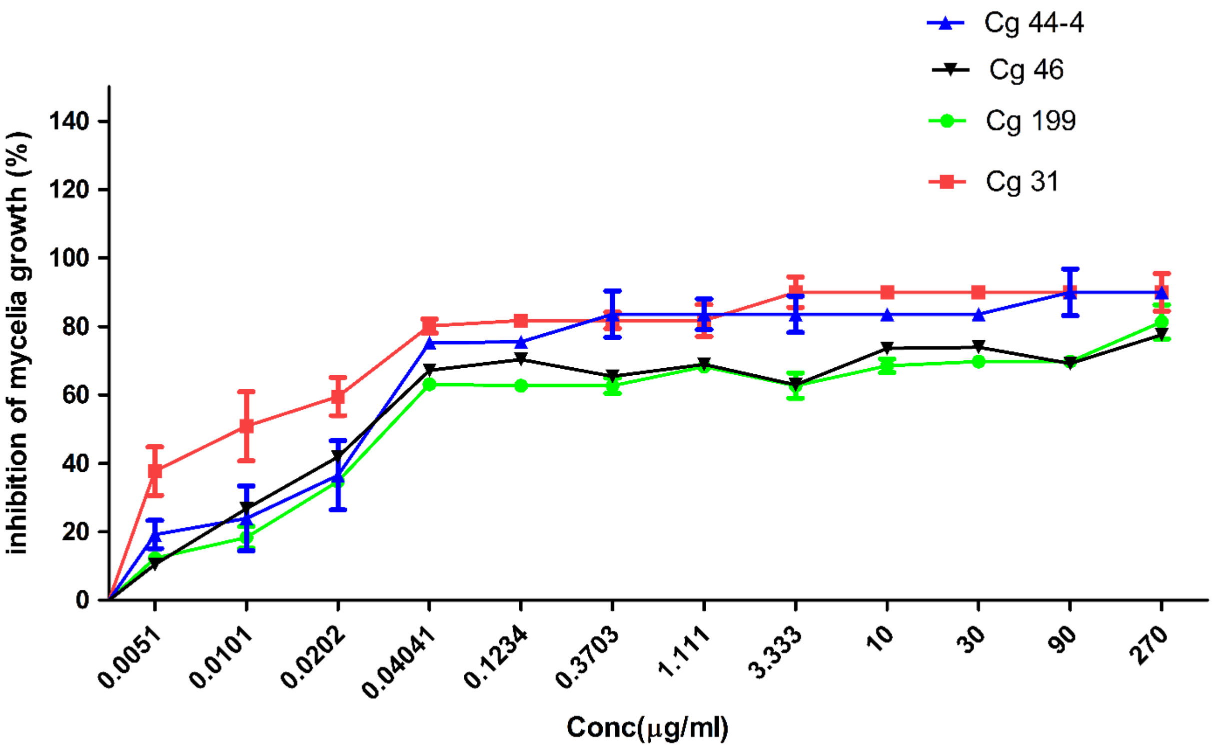

Fig 1: Response-to-dosage curves of *C. gloeosporioides* isolates to fludioxonil. All the four strains were sensitive to fludioxonil. Gg means *C. gloeosporioides* isolates.

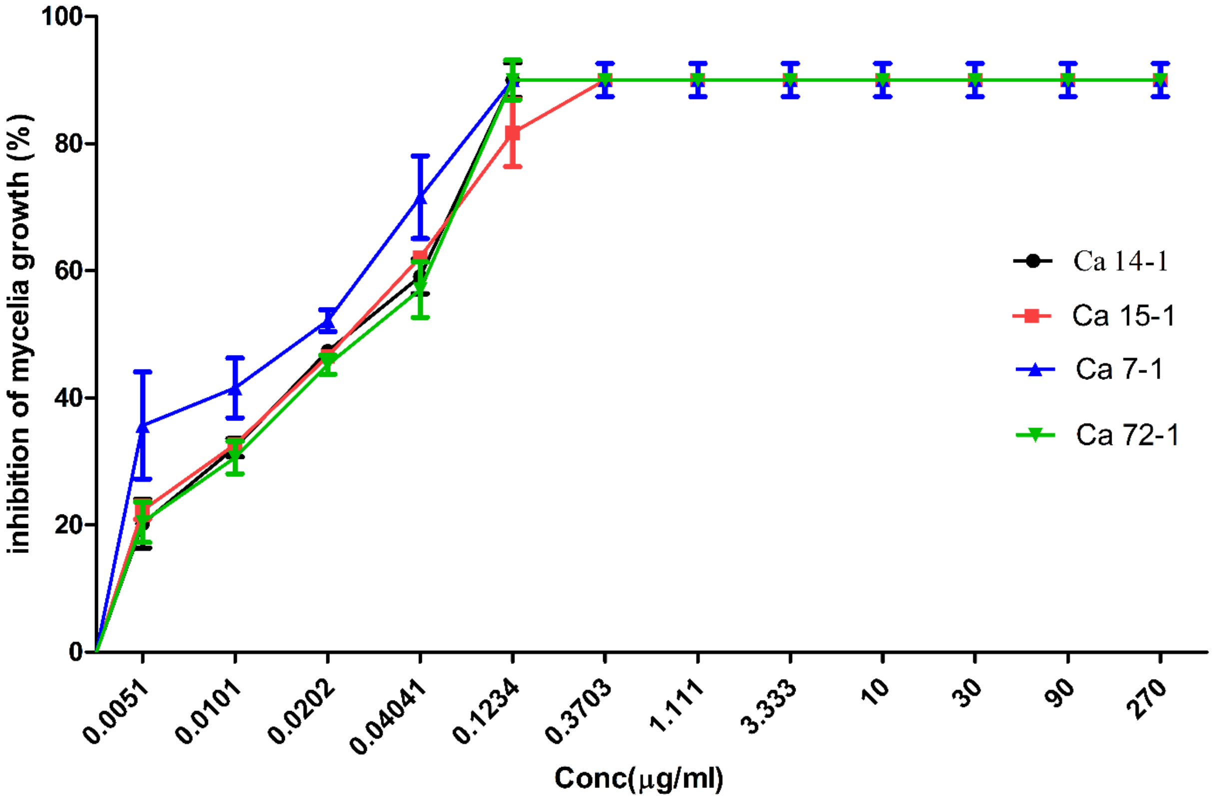

Fig 2: Response-to-dosage curves of *C. acutatum* to fludioxonil. The four strains were sensitive to fludioxonil. Ca means *C. acutatum* isolates.

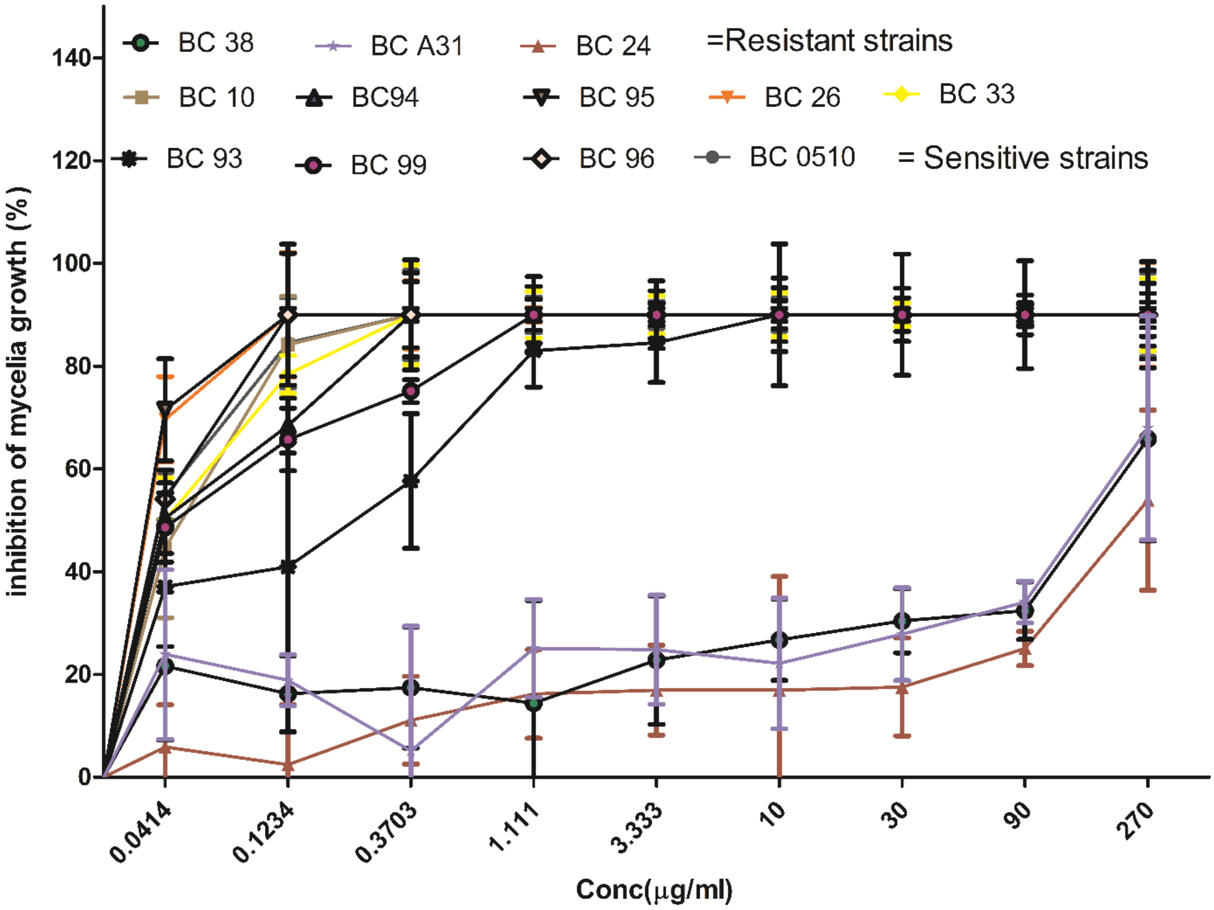

Fig 3: Response to-dosage curves of *B. cinerea* to fludioxonil**.** Bc 0510, Bc 10, Bc 26, Bc 33, Bc 93, Bc 94, Bc 95, Bc 96, and Bc 99 were sensitive to fludioxonil while Bc 24, Bc 38 and Bc A31 were fludioxonil resistant strains. Bc means *B. cinerea*

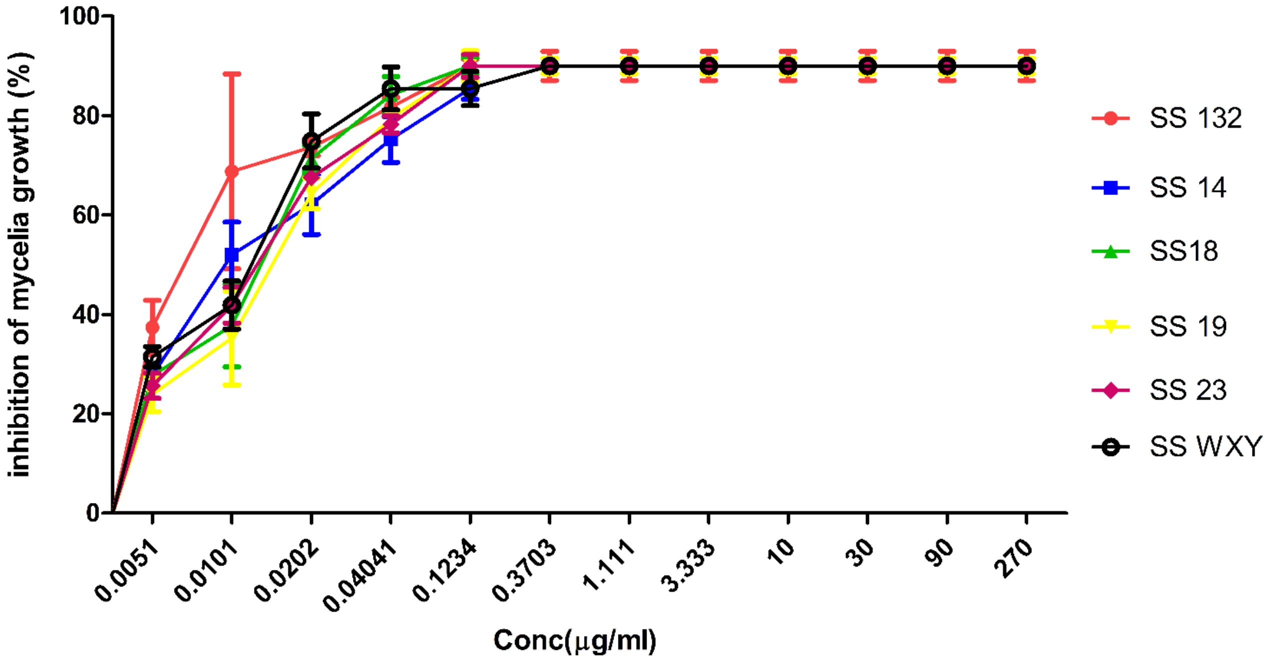

Fig 4: Response-to-dosage curves of *S. sclerotiorum* to fludioxonil. All the six strains were sensitive to fludioxonil. SS means *S. sclerotiorum.*

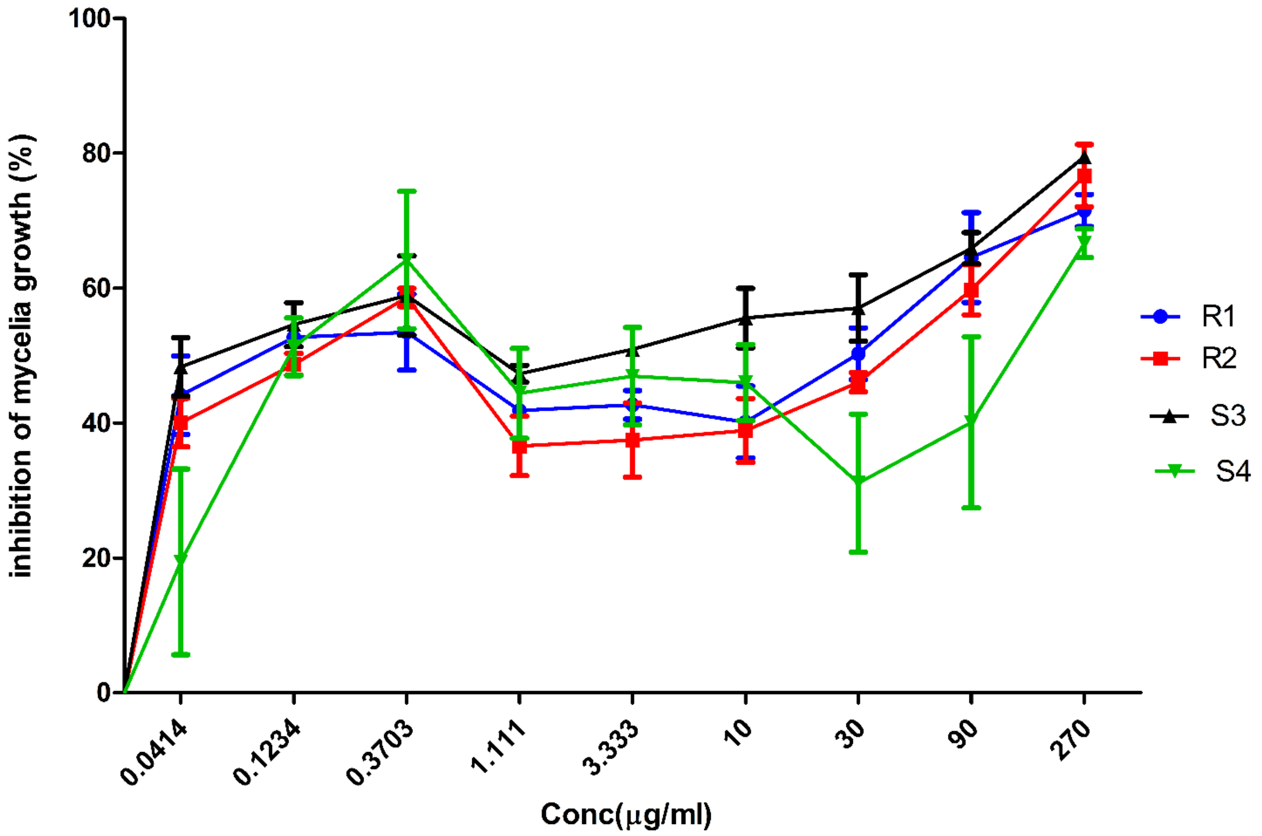

Fig 5: Response-to-dosage curves of *F. fujikuroi* to fludioxonil. The four strains were sensitive to fludioxonil. R1, R2, S3 and S4 are *F. fujikuroi* isolates.

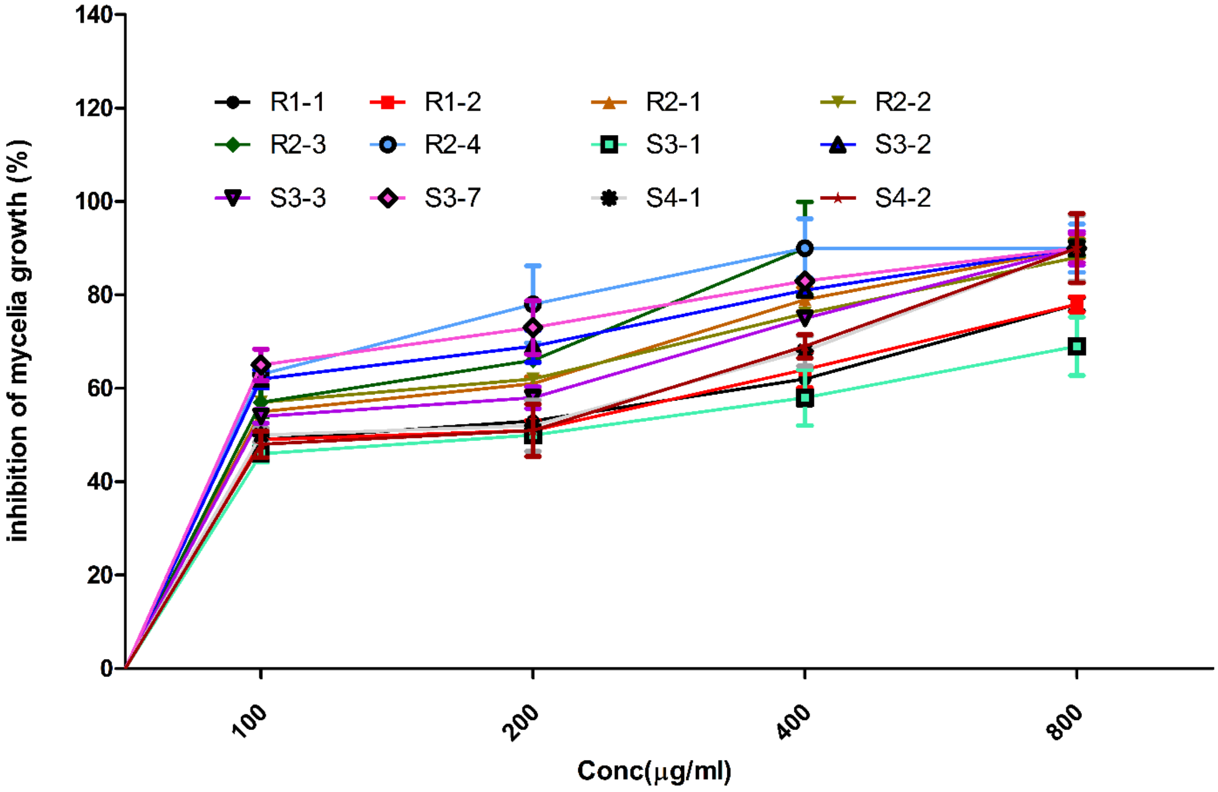

Fig 6: Response-to-dosage curves of *F. fujikuroi* to fludioxonil. RI-1, R1-2, R2-1, R2-2, S3-1, S3-2, S3-3, S3-7, S4-1 and S4-2 were the fludioxonil resistant mutants from parents' isolates (R1, R2, S3 and S4).

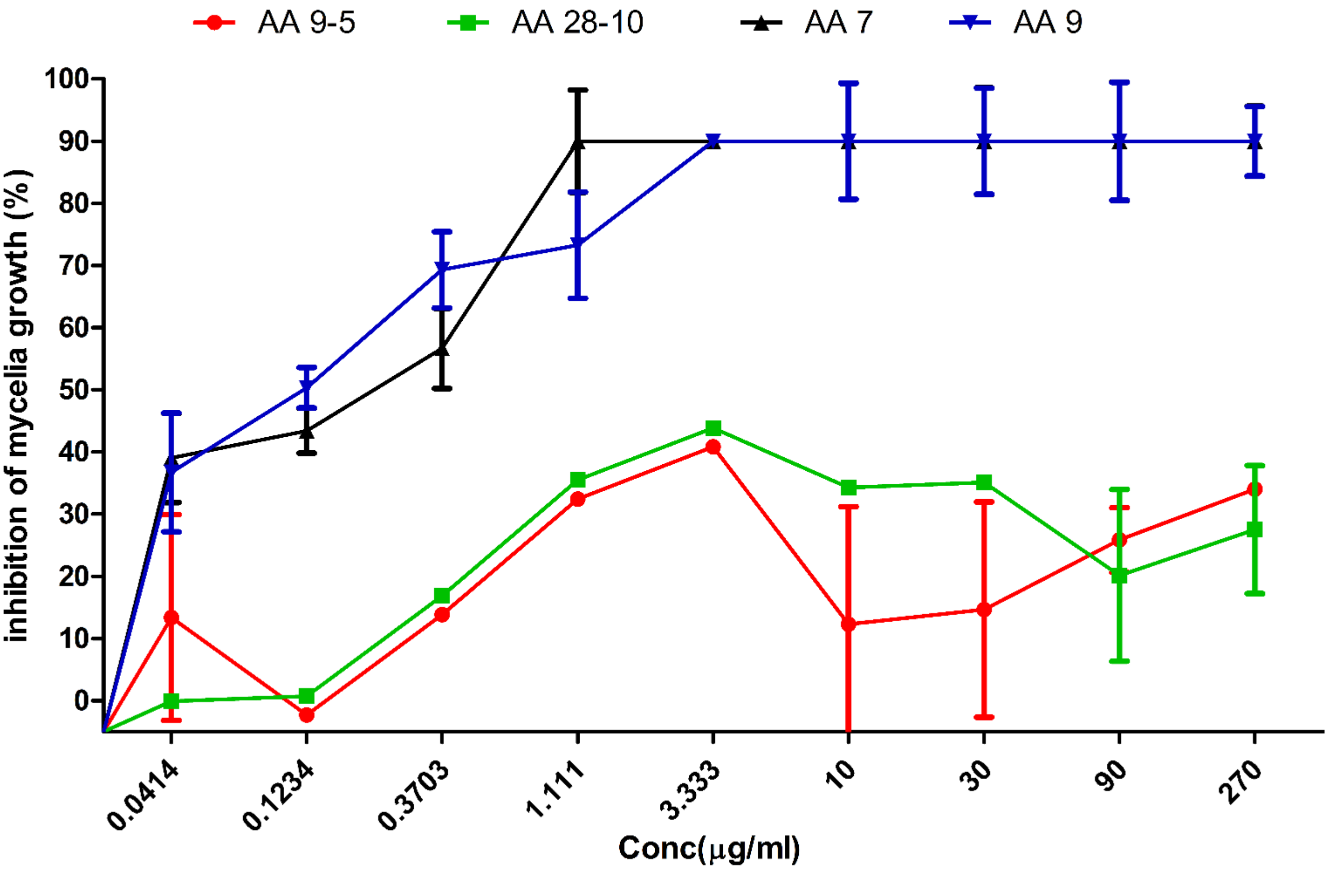

Fig 7: Response-to-dosage curves of *A. alternata* to fludioxonil. AA 7 and AA 9 were fludioxonil sensitive strains while AA 9-5 and AA 28-10 were the fludioxonil resistant strains. AA means *A. alternata* isolates.