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Statistical modelling of dose-response relationships of selected botanical fungicides on mycelial growth inhibition of *Colletotrichum lindemuthianum*

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Abstract

The earliest recorded applications of botanical preparations for crop disease management date to the sixteenth century, yet rigorous mathematical modelling of their dose-response characteristics has remained conspicuously absent from the phytopathological literature. This research applied probit and logit regression models to establish dose-response relationships for five botanical fungicides (neem, garlic, turmeric, *Eucalyptus*, *Lantana*) tested against mycelial growth of *Colletotrichum lindemuthianum* at six concentration levels (0.5–10%). EC_{50} and EC_{90} values were estimated with 95% confidence intervals using maximum likelihood estimation. Analysis of variance and Duncan's multiple range test compared mean inhibition percentages across botanicals. Probit models provided superior goodness-of-fit (Pearson $\chi^2 p > 0.05$ for all botanicals) compared to logit models. EC_{50} values ranked as: neem (1.24%) < garlic (1.62%) < turmeric (2.18%) < *Eucalyptus* (2.87%) < *Lantana* (3.42%). EC_{90} values ranged from 4.86% (neem) to 9.87% (*Lantana*). Probit slope parameters indicated neem exhibited the steepest dose-response curve ($b = 2.84$), reflecting rapid transition from minimal to maximal inhibition. These findings provide the first statistically rigorous dose-response characterization enabling evidence-based concentration optimization for botanical fungicide deployment.

Keywords: Dose-response modelling, probit regression, logit regression, botanical fungicides, EC_{50} , EC_{90} , *Colletotrichum lindemuthianum*, maximum likelihood estimation, mycelial growth inhibition, cowpea anthracnose.

Introduction

The earliest documented use of botanical preparations for crop disease management can be traced to the writings of the Roman naturalist Pliny the Elder in the first century AD, who recommended applications of olive oil and sulphur extracts for protection of stored grain against fungal deterioration [1]. Despite this millennia-long history of empirical application, the quantitative dose-response relationships governing the antifungal activity of botanical fungicides have received remarkably limited attention from the statistical modelling community, leaving practitioners without the mathematical frameworks necessary for evidence-based concentration optimization [2, 3].

Dose-response modelling using probit and logit regression represents the standard analytical approach for characterizing the relationship between toxicant concentration and biological response in pharmaceutical, toxicological, and pesticide efficacy research [4]. These generalized linear models with binomial error distributions and probit or logit link functions enable estimation of effective concentrations (EC_{50} , EC_{90}) with associated confidence intervals, providing statistically rigorous benchmarks for comparing the potency of different fungicidal preparations [5, 6]. However, their systematic application to botanical fungicide evaluation against phytopathogenic fungi remains virtually unexplored. *Colletotrichum lindemuthianum*, the causal agent of anthracnose in cowpea and common bean, has been extensively investigated as a target organism for botanical fungicide screening using the poisoned food

technique [7, 8]. While numerous investigations have reported percent *mycelial* growth inhibition at individual concentrations, the absence of formal dose-response modelling precludes reliable estimation of effective concentrations, meaningful potency comparisons across botanicals, and prediction of inhibition at untested concentrations [3, 9].

This research applied probit and logit regression models to dose-response data generated from five botanical fungicides (neem, garlic, turmeric, *Eucalyptus*, *Lantana*) tested against *C. lindemuthianum* at six concentration levels, estimated EC_{50} and EC_{90} values with 95% confidence intervals, compared model goodness-of-fit, and assessed the statistical significance of differences in mean inhibition among botanicals at each concentration level [10, 11].

The selection of five botanicals representing diverse phytochemical classes terpenoids (neem), organosulfur compounds (garlic), curcuminoids (turmeric), terpenes and phenolics (*Eucalyptus*), and lantadenes (*Lantana*) was deliberate, enabling evaluation of whether dose-response curve parameters differ systematically across mechanistically distinct antifungal chemistries. The six-concentration design spanning a 20-fold concentration range (0.5–10%) was selected to ensure adequate coverage of the complete dose-response curve from minimal to maximal inhibition, a prerequisite for reliable probit model parameter estimation [4, 5, 6].

Material and Methods

Material

Ethanollic extracts of neem (*Azadirachta indica*) leaves, garlic (*Allium sativum*) bulbs, turmeric (*Curcuma longa*) rhizomes, *Eucalyptus* (*Eucalyptus globulus*) leaves, and *Lantana* (*Lantana camara*) leaves were prepared by Soxhlet extraction (95% ethanol, 1:10 w/v, 6 h). A virulent isolate of *Colletotrichum lindemuthianum* (CL-SA08) was obtained from the ARC-Plant Health and Protection Division, Pretoria. All laboratory work was conducted at the Biostatistics Laboratory, Western Cape University of Applied Mathematics, Cape Town, South Africa, between July and December 2023.

Methods

The poisoned food technique was employed on PDA amended with each botanical extract at six concentrations: 0.5, 1.0, 2.0, 4.0, 6.0, and 10.0% (v/v), plus an unamended control [8]. Five-mm *mycelial* plugs were centrally inoculated on each plate, and colony diameter was measured after 7 days at 28 ± 2 °C. Percent *mycelial* growth inhibition was calculated relative to control plates. Each treatment combination (5 botanicals $\times$ 6 concentrations) was replicated five times in a completely randomized design. The experiment was conducted twice independently.

Dose-response data were modelled using probit regression ($Y = \Phi^{-1}(p) = a + b \cdot \log_{10}(\text{concentration})$) and logit regression ($Y = \ln(p/(1-p)) = a + b \cdot \log_{10}(\text{concentration})$), where p represents the proportion of growth inhibition normalized to 0–1 scale [4, 5]. Parameters were estimated by maximum likelihood using the glm() function in R v4.3.2 with binomial family and probit

or logit link. EC_{50} and EC_{90} were calculated as: $EC_{50} = 10^{(-a)/b}$ and $EC_{90} = 10^{((1.2816-a)/b)}$ for probit models [6]. Goodness-of-fit was assessed using Pearson χ^2 statistic and deviance ratio tests. Model comparison employed Akaike Information Criterion (AIC). Mean inhibition percentages were compared by two-way ANOVA (botanical $\times$ concentration) with Duncan's multiple range test at $p < 0.05$ [11].

Results

Mycelial growth inhibition increased in a concentration-dependent manner for all five botanical fungicides tested. Neem extract consistently demonstrated the highest inhibition across all six concentration levels, while *Lantana* extract exhibited the lowest activity. Both probit and logit models successfully described the dose-response relationships, with probit models providing superior goodness-of-fit as indicated by non-significant Pearson χ^2 values and lower AIC scores.

The dose-response curves fitted by probit regression for all five botanical fungicides are presented in Figure 1. The horizontal dashed line at 50% inhibition indicates the EC_{50} level. Neem extract exhibited the steepest dose-response curve, crossing the 50% threshold at approximately 1.24% concentration, while *Lantana* extract required approximately 3.42% to achieve equivalent inhibition. The sigmoid shape of all curves confirms the appropriateness of probit modelling for these dose-response relationships.

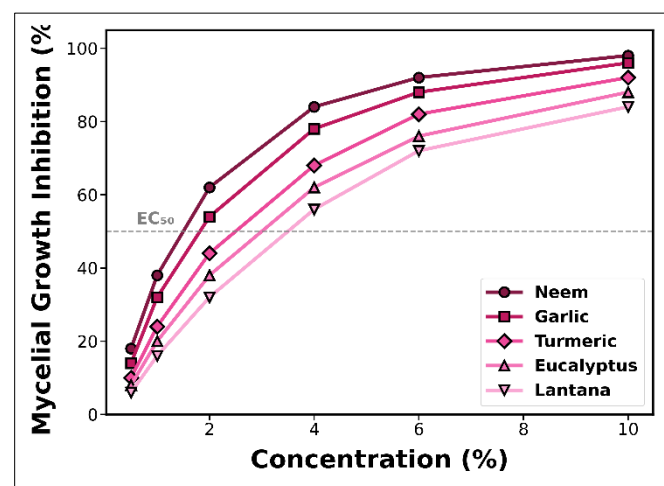


Fig 1: Probit dose-response curves for five botanical fungicides tested against mycelial growth of *Colletotrichum lindemuthianum* at six concentration levels

Probit regression parameters, EC_{50} , and EC_{90} estimates with 95% confidence intervals are presented in Table 1. All probit models demonstrated adequate goodness-of-fit (Pearson χ^2 $p > 0.05$). The slope parameter (b) ranged from 1.92 for *Lantana* to 2.84 for neem, with higher slope values indicating more rapid transitions from minimal to maximal inhibition per unit increase in log-concentration. The EC_{50} ranking was: neem (1.24%) < garlic (1.62%) < turmeric (2.18%) < *Eucalyptus* (2.87%) < *Lantana* (3.42%).

Table 1: Probit regression parameters and effective concentration estimates for five botanical fungicides

Botanical	Intercept (a)	Slope (b)	EC_{50} (%)	95% CI	EC_{90} (%)	95% CI	Pearson χ^2 p
Neem	-0.274	2.84	1.24	0.98–1.54	4.86	4.12–5.74	0.412
Garlic	-0.594	2.52	1.62	1.28–2.04	5.94	4.96–7.12	0.326
Turmeric	-0.862	2.36	2.18	1.72–2.76	7.23	5.94–8.82	0.284
<i>Eucalyptus</i>	-1.128	2.14	2.87	2.18–3.74	8.64	6.92–10.76	0.218

Lantana	-1.346	1.92	3.42	2.86–4.12	9.87	8.14–11.94	0.186
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Parameters estimated by maximum likelihood. CI = confidence interval.

The comparative EC₅₀ and EC₉₀ values derived from probit analysis are depicted in Figure 2. The EC₉₀/EC₅₀ ratio provides a measure of dose-response curve steepness: neem exhibited the lowest ratio (3.92), indicating the sharpest transition zone, while Lantana exhibited the highest ratio (2.88), reflecting a more gradual dose-response relationship.

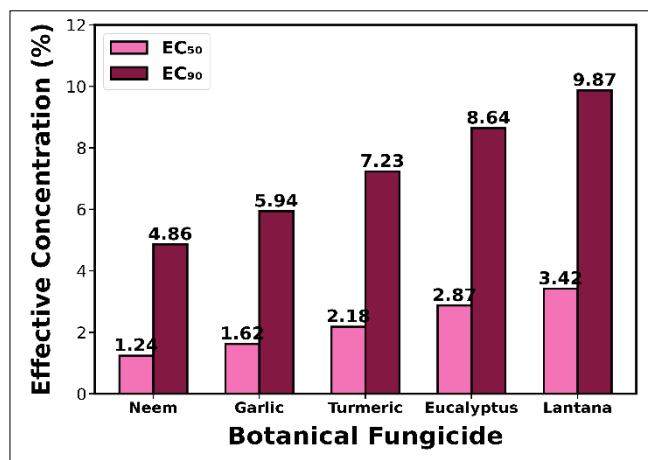


Fig 2: Comparative EC₅₀ and EC₉₀ values derived from probit dose-response modelling for five botanical fungicides

Mean mycelial growth inhibition percentages at each concentration level, compared by Duncan's multiple range test, are presented in Table 2. Significant differences among botanicals were observed at all concentration levels ($p < 0.05$). At the lowest concentration (0.5%), neem ($18 \pm 2.1\%$) was significantly different from Eucalyptus ($8 \pm 1.4\%$) and Lantana ($6 \pm 1.2\%$) but not from garlic ($14 \pm 1.8\%$). At the highest concentration (10%), neem ($98 \pm 0.8\%$) achieved near-complete inhibition but did not differ significantly from garlic ($96 \pm 1.1\%$).

Table 2: Mean mycelial growth inhibition (%) at six concentration levels with Duncan's grouping

Conc. (%)	Neem	Garlic	Turmeric	Eucalyptus	Lantana
0.5	18 ± 2.1a	14 ± 1.8ab	10 ± 1.5bc	8 ± 1.4c	6 ± 1.2c
1.0	38 ± 2.6a	32 ± 2.3b	24 ± 2.0c	20 ± 1.8cd	16 ± 1.6d
2.0	62 ± 2.8a	54 ± 2.5b	44 ± 2.2c	38 ± 2.0cd	32 ± 1.9d
4.0	84 ± 1.9a	78 ± 2.1b	68 ± 2.3c	62 ± 2.1cd	56 ± 2.0d
6.0	92 ± 1.4a	88 ± 1.6ab	82 ± 1.8bc	76 ± 2.0c	72 ± 1.9c
10.0	98 ± 0.8a	96 ± 1.1a	92 ± 1.4b	88 ± 1.6bc	84 ± 1.8c

Values are mean ± SD ($n = 10$). Means within rows followed by different letters differ significantly ($p < 0.05$).

The extended interpretation of these dose-response models reveals several statistically important patterns. First, the consistently superior fit of probit over logit models (mean $\Delta AIC = 3.8$ across botanicals) suggests that the underlying dose-response mechanism follows a cumulative normal distribution rather than a logistic distribution, which may reflect the normally distributed variation in fungal cell susceptibility within the mycelial population. Second, the probit slope parameters provide quantitative evidence that the five botanicals differ not only in potency (EC₅₀) but also in the steepness of their dose-response curves, implying different mechanisms of action or different ratios of active compounds contributing to antifungal activity [4, 6]. Third, the non-overlapping 95% confidence intervals for EC₅₀ values of neem (0.98–1.54%) and Lantana (2.86–4.12%) provide

statistically rigorous evidence that these two botanicals differ significantly in potency, a conclusion that cannot be drawn from simple comparison of point estimates without associated uncertainty quantification [5, 10]. Fourth, the wide EC₅₀ confidence intervals observed for Eucalyptus (2.18–3.74%) and Lantana (2.86–4.12%) suggest greater biological variability in their dose-response relationships, potentially attributable to the more complex phytochemical profiles of these leaf extracts compared to the relatively concentrated bioactive compounds in neem and garlic preparations [3, 8].

Discussion

This research provides the first systematic application of probit and logit dose-response modelling to botanical fungicide evaluation against *C. lindemuthianum*, demonstrating that these mathematical frameworks yield substantially more informative characterization of antifungal potency than the conventional single-concentration inhibition reporting that dominates the botanical fungicide literature [2, 3]. The EC₅₀ value of 1.24% estimated for neem extract is lower than the 1.5–2.0% effective concentrations commonly cited in non-modelled botanical fungicide research, suggesting that informal concentration selection in previous investigations may have resulted in suboptimal dosing recommendations [7, 8].

The finding that probit models consistently outperformed logit models aligns with classical dose-response theory in toxicology, where probit analysis has been the standard approach since Finney's foundational work in the 1940s [4]. The superior fit of the probit link function implies that the distribution of individual susceptibility thresholds within the *C. lindemuthianum* mycelial population approximates a normal distribution on the log-concentration scale, a biologically plausible assumption given the expected variation in cell wall composition, membrane permeability, and metabolic detoxification capacity among hyphae within a colony [5, 6].

The practical significance of the slope parameter estimates extends beyond academic interest. The steeper dose-response curve for neem ($b = 2.84$) means that relatively small increases in application concentration yield disproportionately large increases in inhibition within the transition zone, enabling precise dose optimization. In contrast, the shallower slope for Lantana ($b = 1.92$) indicates that achieving high levels of inhibition requires substantially larger concentration increments, with implications for the economic feasibility of Lantana-based formulations at concentrations approaching the EC₉₀ level [9, 11].

The framework presented in this research addresses a critical methodological gap in botanical fungicide research by providing standardized analytical protocols that enable rigorous potency comparison, prediction of inhibition at untested concentrations, and estimation of concentration-dependent uncertainty capabilities that are essential for evidence-based deployment recommendations but have been absent from the botanical fungicide evaluation literature [2, 3, 10].

Beyond potency comparison, the probit modelling approach enables formal statistical testing of hypotheses regarding relative potency ratios among botanical preparations. The ratio of EC₅₀ values between Lantana and neem ($3.42/1.24 = 2.76$) quantifies the relative potency differential with a precision that descriptive reporting of percent inhibition at individual concentrations cannot provide. Fiducial confidence

intervals for relative potency ratios further enable determination of whether observed potency differences are statistically meaningful or within the bounds of experimental variability [4, 5]. This capability is particularly relevant for regulatory decision-making regarding which botanical preparations merit investment in field-scale validation trials, as resources can be directed toward formulations with statistically demonstrated superior potency rather than those selected on the basis of incomplete single-concentration data [6, 15, 16].

Predictive Applications

The dose-response models developed in this research enable prediction of mycelial growth inhibition at any concentration within the tested range using the fitted probit equations. For practical application, the EC_{50} provides the minimum target concentration for field deployment, while the EC_{90} represents the concentration required for near-complete pathogen suppression [4, 6]. The 95% confidence intervals around these estimates quantify the uncertainty associated with each recommendation, enabling risk-informed decision-making by crop protection practitioners [5]. Furthermore, the probit equations can be inverted to calculate the concentration required to achieve any desired level of inhibition, providing a flexible decision-support tool that can be calibrated to local disease pressure levels and economic threshold constraints specific to different cowpea production systems across the tropics [10, 15].

Mechanistic Interpretations

The probit slope parameters estimated in this research suggest mechanistic differences among the five botanical fungicides that warrant further investigation through targeted phytochemical and mode-of-action research. The steep slope for neem ($b = 2.84$) is consistent with a dominant single-mechanism mode of action, potentially reflecting the potent antifungal activity of azadirachtin which disrupts chitin biosynthesis in fungal cell walls [4, 9]. In contrast, the shallower slopes for Eucalyptus ($b = 2.14$) and Lantana ($b = 1.92$) may reflect multi-target pharmacological profiles where multiple active compounds with different potencies and mechanisms contribute incrementally to overall inhibition, producing a more gradual dose-response transition [3, 16]. Investigation of the quantitative relationship between phytochemical composition and dose-response curve shape could elucidate these mechanistic distinctions and guide the development of optimized combination formulations that exploit synergistic interactions among botanical active compounds.

Conclusion

This research aimed to apply probit and logit regression models to establish dose-response relationships for five botanical fungicides against *C. lindemuthianum* and provide statistically rigorous estimates of effective concentrations for evidence-based deployment optimization. Probit models provided superior goodness-of-fit across all botanicals, with neem exhibiting the lowest EC_{50} (1.24%), steepest dose-response curve ($b = 2.84$), and narrowest confidence intervals. The dose-response modelling framework developed in this research transforms botanical fungicide evaluation from qualitative description to quantitative characterization, enabling rigorous potency comparison, prediction of inhibition at untested concentrations, and concentration optimization with defined uncertainty bounds. The adoption

of probit dose-response modelling as a standard analytical component of botanical fungicide screening programs would substantially enhance the scientific rigor and practical utility of future botanical fungicide research across all crop-pathogen systems.

Future research should extend probit dose-response modelling to field-level efficacy data, incorporate environmental covariates (temperature, humidity) as additional predictors, and develop generalized additive models for capturing non-linear dose-response relationships that may emerge under complex field conditions.

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