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Communication

Comparative Efficacy of Systemic and Combination Fungicides for the Control of Alternaria Leaf Spot of Cabbage

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Abstract: Alternaria leaf spot of cabbage, caused by the *Alternaria brassicicola*, affects leaves of cabbages and often results in head rots causing severe decline in yield. In this work, the effects of systemic and combination fungicides on *A. brassicicola* mycelia growth in vitro and disease severity in field trials were investigated. The results of in vitro evaluation revealed that both fungicides significantly inhibited ($p < 0.05$) the growth of *A. brassicicola* under in vitro conditions. However, metalaxyl-M 6% was less effective with 100 μg/mL having only 30 ± 3.5% inhibition. On the other hand, 100 μg/mL of mancozeb 63% + carbendazim 12% had 94 ± 3.5% growth inhibition of *A. brassicicola*, respectively, under the same conditions. Dose-response analysis of the efficacy of the two fungicides showed that the LC_{50} of metalaxyl-M 6% and mancozeb 63% + carbendazim 12% were 125.52 ppm and 57.22 ppm, respectively, indicating the superiority of combination fungicide over systemic fungicide alone. Field studies showed that while manure type significantly impacted on biomass production ($p < 0.001$), it did not significantly affect disease severity. On the other hand, the frequency of fungicide application impacted on disease severity, with biweekly application leading to a significant reduction in disease severity after 10 weeks.

Keywords: cabbage; *Alternaria brassicicola*; metalaxyl; mancozeb; carbendazim

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1. Introduction

Cabbage (*Brassica oleracea* L.) is a significant fresh vegetable crop grown globally. Brassica plants have been the subject of much scientific interest because of their agricultural and horticultural importance, with six species (including *B. oleracea*) having evolved through the combination of three chromosomes from three earlier species as described by the triangle of U theory [1] and confirmed by genetic studies [2]. Although these crucifers are biennial crops, they are usually grown as annual plants. They are compatible with the climatic conditions found in various parts of the world. However, their production and quality are often impacted negatively by Alternaria leaf spot disease [3].

Alternaria leaf spot is potentially caused by three pathogens namely, *Alternaria brassicicola*, *Alternaria brassicae* and *Alternaria raphanin*, with *A. brassicicola* being the most common in cabbages. *Alternaria brassicicola* is considered a necrotrophic (causing death) plant-pathogenic fungus and like other *Alternaria* species has been shown to secrete numerous toxic secondary metabolites and proteins that cause cell death via toxin production in plants or by directly damaging cells [4]. Symptoms include head rot that initially appears as small brown spots on an otherwise healthy head, while leaf symptoms include round, brown spots [5]. As the disease spreads, leaves can develop enough spots that they begin to merge to form large necrotic areas. The result is a significant decrease in quality, yield and value. Once present, Alternaria can persist in residues, and in some cases develop resting spores that allow them to survive in the soil [6].

Management of Alternaria disease is a very important step to avoid extreme economic losses. To manage this disease, scientists have employed a variety of strategies including the use of pesticides, plant activators and plant extracts, but the most often utilized strategy to lessen disease severity is the introduction of resistant varieties [7,8]. The disease can most effectively be managed by genetic resistance since it is lasting, ecologically safe and locally appropriate. However, owing to the limited availability of resistant varieties in many parts of the world, the commonest management strategy employed by farmers is the use of fungicides [8]. Although metalaxyl-M is an eye irritant and was withdrawn from outdoor seed treatment in countries of the European Union in June 2021, its use in greenhouse conditions continues to be authorized [9]. In addition, due to the need for an immediate response to Alternaria disease of brassica plants without the availability of resistant varieties, mancozeb 63% + carbendazim 12% remains among the most commonly used fungicides around the world owing to its broad spectrum of application and low toxicity [10,11]. Generally, due to their quick effect with respect to lessening the severity of infection, ease of application and widespread availability in the market, fungicides are used to treat infections in most developing countries, where resistant cultivars are not readily available [7,12]. Farmers need to quickly control disease outbreaks when they are severe, which often requires the use of commercial fungicides. Several fungicides with different modes of actions, including systemic and protectant (non-systemic), have been tested with varying degrees of success in managing the disease [8,13]. In addition, organic and inorganic nutrients have also been shown to enhance the performance of Brassica crops under different pathogen treatments.

Notwithstanding the progress made in fungicide development, there is an increasing need for comparative studies of in vitro and in vivo efficacies of both systemic and combination fungicides. Therefore, this study was aimed at assessing the effectiveness of different doses of two fungicides in vitro. One of the fungicides (metalaxyl-M 6%) is a systemic fungicide, while the other (mancozeb 63% + carbendazim 12% Wettable Powder, WP) is a combination fungicide. The results of this study revealed the superiority of the combination fungicide over systemic fungicide alone for the control of Alternaria disease of cabbage.

2. Materials and Methods

2.1. Study Design and Sampling Location

An in vitro control study was performed in the pathology laboratory of the Department of Crop Science, University of Nigeria Nsukka, in 2019. Thereafter, the field experiment was conducted in the departmental teaching and research farm in 2020/2021. The experimental design was a split plot experiment in randomized complete block design. Randomization was used to neutralize the effect of systematic biases [14]. Soil and organic manure elemental compositions were determined using Thermo Solaar S4 Atomic Absorption Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). In addition, the effect of two organic manure types (poultry manure and pig manure) on biomass production of *B. oleracea* was determined through plant-height measurement. To avoid destructive harvesting, plant heights were measured bi-weekly by measuring the distance from soil surface to shoot tips [15,16].

2.2. Isolation and Purification of Fungi

Infected leaves and heads with water-soaked area were used for the isolation of the fungi. Small bits of 5 mm size were taken from the junction of diseased and healthy portions with the help of sterilized blades. These bits were surface-sterilized with sodium hypochlorite (0.1%) for 10 to 20 s and washed thrice with sterilized distilled water and subsequently transferred to potato dextrose agar (PDA) in a Petri plate under aseptic conditions and incubated for 3–4 days at 25 ± 1 °C. The isolated fungi were purified via the hyphae tip method. The root and stem of the diseased plant was cut into 5–6 cm pieces, washed with tap water and surface-sterilized with 2% sodium hypochlorite for 2 min. The piece was then plated on PDA with 10 µL of streptomycin antibiotic (30 mg/L) for the isolation of suspected fungi in Petri dishes. All plates were incubated at 25 ± 1 °C for

7 days. Different slides were prepared for the identification of the pathogens from each Petri dish, which have pathogen cultures. Examination of the slides was performed under a light microscope (AmScope, Irvine, CA, USA), where they were identified by morphological characteristics such as septations, spore shape and colony growth.

2.3. Pathogenicity Test

To determine whether the isolates can cause infection, virulence test assays of the isolates were carried out on the three crucifers. Mycelia plugs (5 mm in diameter) from a 6-day-old PDA culture of *A. brassicicola* were placed on the surface of the uninfected cabbage over artificial wound for easy penetration of the pathogen, kept moist with a piece of moistened absorbent cotton and incubated in a growth chamber at 25 °C under a 12 h photoperiod and 85% relative humidity. After 6 days, lesion diameters were measured as the mean of two diameters at perpendicular angles. This experiment was carried out in triplicates.

2.4. In Vitro Evaluation of Fungicides

Two fungicides were added to separate conical flasks containing 80 mL of PDA media at concentrations of 10, 20, 40, 80 and 100 µg/mL. The two fungicides examined were (1) metalaxyl-M 6% (traded as Red Force in Nigeria) and (2) mancozeb 63% + carbendazim 12% Wettable Powder, WP (traded as Green Force in Nigeria). A total of 20 mL of the media was poured into a Petri dish of 90 cm diameter. The freshly growing mycelium from the selected culture plate was cut 5 mm with a borer and inoculated at the center of the Petri dish under aseptic conditions in an isolation chamber. Controls were maintained without any fungicides. An in vitro experiment was conducted in a completely randomized design with factor A being the fungicides and factor B the isolates, replicated three times. Mycelial radial growth was measured 5 days after inoculation for all treatments and the inhibition percent of mycelium by different concentrations of chemicals was calculated. The percentage of growth inhibition was calculated and arcsine-transformed prior to statistical analysis using the percentage-transformation formula Inhibition rate = ASIN·(SQRT·(ab/100) × 180/3.1415926).

2.5. Analysis of the Effectiveness of Combination Fungicide in Field Trials

Based on the result of in vitro studies, mancozeb 63% + carbendazim 12% was used for field studies. The experimental design was a split-plot experiment in randomized complete block design. The foliar spray regimens were replicated three times. A portion of land 20 m long by 16 m wide with an area of 320 m^2 was cleared, ploughed, harrowed and ridged. Beds were prepared 2 weeks prior to transplanting and well-cured manure (poultry or pig, 20 ton/ha) was applied on the beds. In each bed, measuring 1 m × 1 m, 12 seedlings were transplanted with a spacing of 25 cm in between plants. Four plants were sampled in each bed. Cabbage seedlings inoculated with *A. brassicicola* were transplanted from the departmental nursery to the already-made beds. Inoculation followed the method described by Macioszek et al. [17]. In brief, the second leaves of *B. oleracea* seedlings were inoculated with two drops (10 µL per drop) of *A. brassicicola* conidial suspension at a concentration of 5×10^5 conidia per ml of distilled water. A total of 50 mL of mancozeb 63% + carbendazim 12% (100 ppm) was applied per plant as foliar spray at different frequencies. The spray regimens followed are described in Table 1, while disease severity (a measure of fungicide effectiveness against pathogen) was calculated using the method and scale of Aba et al. [18]. In brief, the extent of coverage of the pathogen (as percentage of leaf surfaces) was determined per plant. Based on this, a parametric score of 0 was assigned to plants with 0% infection; a score of 1 to plants with 1–25% infection; a score of 2 for 26–50% infection; a score of 3 for 51–75% infection; and a score of 4 for 76–100% infection. Finally, the mean values of the scores per treatment were used to assess the effectiveness of each treatment.

Table 1. Spray regimen of mancozeb 63% + carbendazim 12% (100 ppm) used during the field study.

Treatment	Spray Regimen	Weeks after Transplanting					Description
		2	4	6	8	10	
Treatment 1	No spray	-	-	-	-	-	Control
Treatment 2	2-weekly	+	+	+	+	+	5 times
Treatment 3	4-weekly	+	-	+	-	+	3 times
Treatment 4	6-weekly	+	-	-	+	-	2 times

"+" indicates application; "-" indicates no application.

2.6. Data Analysis

The data were collected at 2, 4, 6, 8 and 10 weeks after transplanting. Statistical analysis was performed using Microsoft Excel, version 16 (Microsoft, Redmond, WA, USA) and R language, version 4.3.0 [19]. One-way analysis of variance (ANOVA) was used to compare the mean values under different treatments with that of the control, followed by Tukey's all-pairwise comparisons. In all cases, the normality of variances was tested via the Shapiro–Wilk method [20], while homogeneity of variances was tested using Levene's test [21]. Differences were considered significant at $p < 0.05$.

3. Results and Discussion

3.1. Physicochemical Properties of Soil and Organic Manure

The physicochemical properties of the soil from the experimental sites before planting revealed that the soil was not fertile. The percentage nitrogen (2.44), phosphorus (30.28) and potassium (2.00) were low. Cation exchange capacity (13.60 meq/100 g) and base saturation (19.93) was relatively low. Organic matter (2.44%) and organic carbon (1.42%) depict the low fertility of the soil of the study area. The pH was 6.2 (slightly acidic). The soil was classified as sandy loam. The percentages of organic matter, organic carbon, nitrogen, phosphorus and potassium in poultry manure were 77.98, 22.70, 4.17, 3.13 and 2.41% respectively. On the other hand, the percentages of organic matter, organic carbon, nitrogen, potassium and phosphorus in pig manure were 52.69, 18.96, 2.68, 2.09 and 1.36%, respectively. These results indicate that poultry manure has higher levels of nutrients essential for plant growth than pig manure. In a recent study of different organic and inorganic manure sources, Adekiya et al. [22] found that although all organic manure increased soil nitrogen, phosphorus and potassium levels over NPK fertilizer, poultry manure had the highest values of soil nutrients (except soluble organic matter, for which rabbit manure had the highest amount).

3.2. In Vitro Efficacy of Systemic versus Combination Fungicide

Pathogenicity test revealed that *A. brassicicola* recorded a virulence rate of 16.67 ± 6.40% on cabbage after 6 days of inoculation. The results of in vitro evaluation revealed that both fungicides significantly inhibited ($p < 0.05$) the growth of *A. brassicicola* under in vitro conditions. However, metalaxyl-M 6% was less effective with 100 μg/mL having only 30 ± 3.5% inhibition. On the other hand, 80 μg/mL and 100 μg/mL of mancozeb 63% + carbendazim 12% had 70 ± 5.6% and 94 ± 3.5% growth inhibition of *A. brassicicola*, respectively, under the same conditions (Figure 1).

Dose-response analysis of the efficacy of the two fungicides showed that the LC_{50} of metalaxyl-M 6% and mancozeb 63% + carbendazim 12% to *A. brassicicola* were 125.52 ppm and 57.22 ppm respectively, indicating the superiority of combination fungicide over systemic fungicide alone (Figure 2). In this case, LC_{50} refers to the concentration of fungicide that is lethal to 50% of *A. brassicicola* mycelia. Similarly, while 28.92 ppm of mancozeb 63% + carbendazim 12% is lethal to 10% of *A. brassicicola*, it requires 78.82 ppm of metalaxyl-M 6% to achieve the same result (Figure 2). The observed difference in the efficacy of the two fungicides is related to their mode of action. Metalaxyl-M 6% is a systemic fungicide that inhibits protein and nucleic acid synthesis, with RNA production being particularly affected so that mitosis is inhibited [23,24]. On the other hand, mancozeb 63% + carbendazim 12% is a combination fungicide composed of both systemic (carbendazim) and protectant (mancozeb) fungicides [8]. Carbendazim is a benzimidazole derivative that primarily blocks nuclear division during fungal-cell division and invariably inhibits DNA and RNA biosynthesis [25–27]. Mancozeb is a non-systemic fungicide that is widely used as a contact fungicide to control fungal diseases [28]. It interferes with enzymes containing sulphydryl groups, disrupting several biochemical processes within fungal-cell cytoplasm and mitochondria [29]. As a result, carbendazim-containing combination fungicides have wide application for the control of diseases in agriculture [8,30]. Previous studies have shown that combination fungicides are more effective for the treatment of fungal diseases. For example, in a study of six fungicides for the treatment of Botrytis gray mold of chickpea, Rashid et al. [8] found that mancozeb 63% + carbendazim 12% was most effective, resulting in the lowest disease severity (3.33 score on a scale of 1–9) and the highest increase (38%) of grain yield. Similar results were found for combination treatment in sunflower [12].

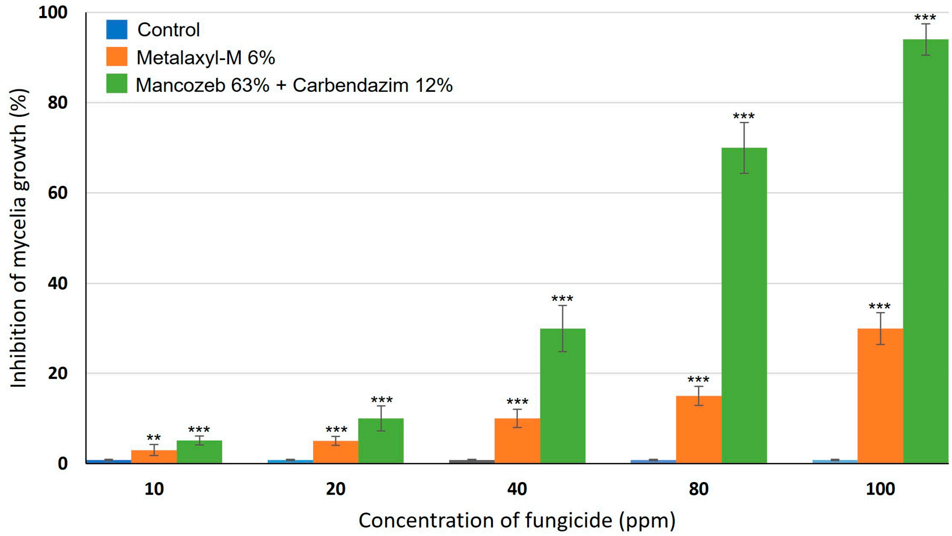

Figure 1. Effect of fungicide concentration on percentage growth inhibition of *A. brassicicola* in vitro. Significance code: '***' $p < 0.001$; '**' $p < 0.01$.

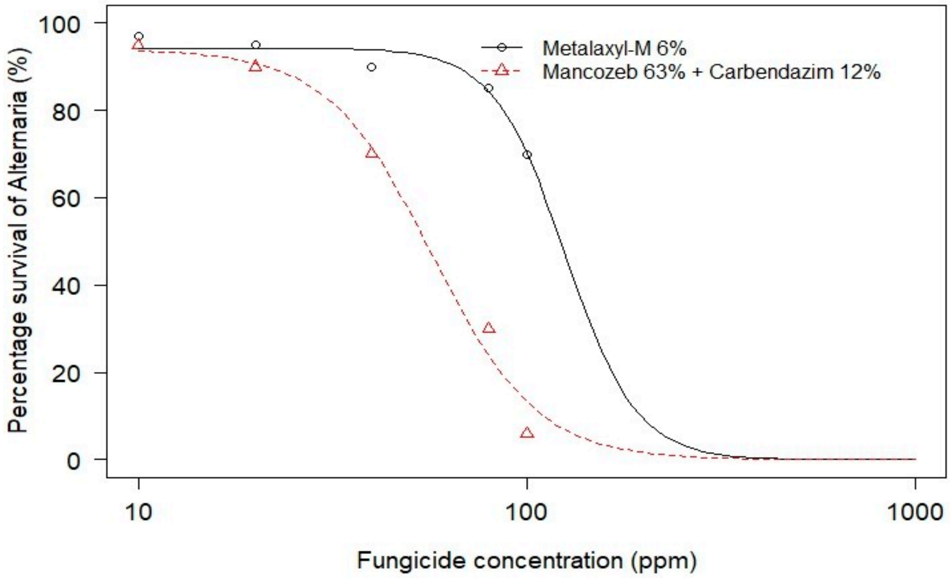

Figure 2. Dose-response curves of *A. brassicicola* exposed to different fungicides. Plot shows percentage survival of *A. brassicicola* in response to fungicide concentration modeled using a 3-parameter log-logistic model.

3.3. Effect of Manure Type on Plant Biomass

It was observed that manure type significantly affected cabbage plant height (Figure 3). Organic manure is considered more effective than chemical fertilizers owing to its benefits to crops and soil. Poultry manure led to significantly greater plant height ($p < 0.001$) than pig manure in all the treatment regimens (Figure 3). The higher growth rate observed in cabbage plants under poultry manure amendment can be explained by the low C:N ratio, which often leads to faster mineralization and early release of nutrients [22]. The carbon-to-nitrogen ratio of organic manure influences the growth of microorganisms involved in organic carbon mineralization, and consequently enhances the decomposition and mineralization of nitrogen by plants [31].

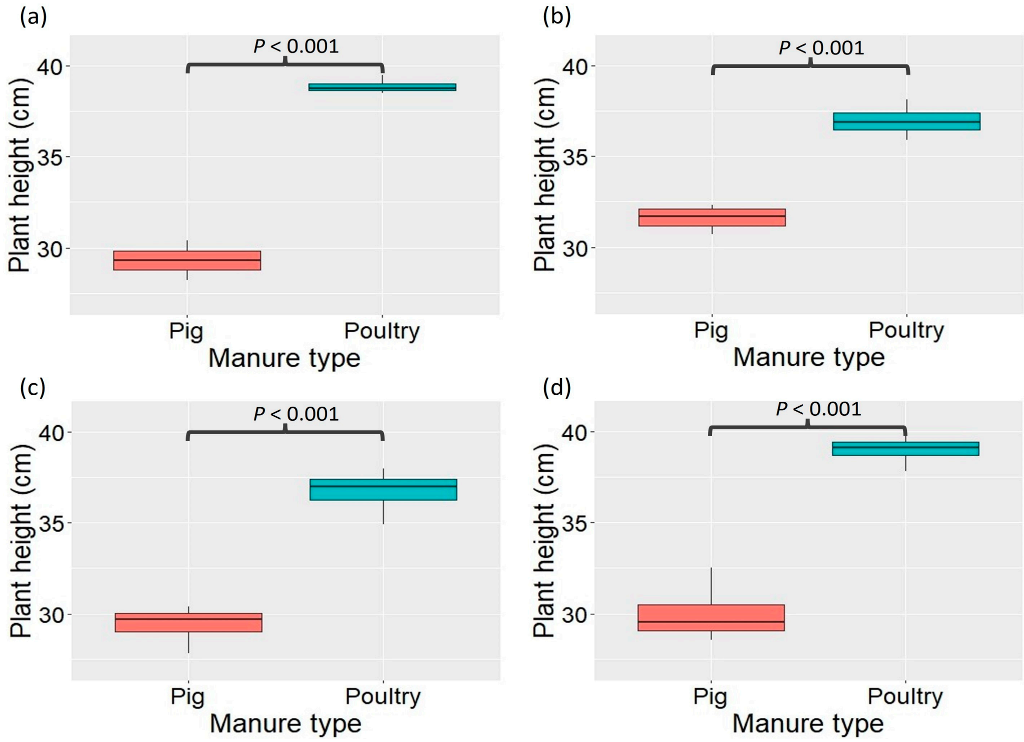

Figure 3. Boxplots showing significant differences in the mean values of *B. oleracea* plant height (cm) versus manure type under different fungicide spray regimens, namely, (**a**) no spray (control), (**b**) six-weekly spray (two times), (**c**) four-weekly spray (three times) and (**d**) two-weekly spray (five times).

The findings in this study were in harmony with some previous experiments on cabbage production [32]. In a separate study of the effect of different organic manure sources (cow, pig, poultry and rabbit) and NPK fertilizer on the growth and yield of okra, it was observed that poultry manure led to the greatest yield, while rabbit manure and NPK fertilizer each gave the least yield [22]. Similarly, Fagwalawa and Yahaya [33] found that when compared to other organic-manure sources investigated, poultry manure significantly increased the growth and yield of vegetable plants. Poultry manure positively affects nitrogen uptake and dry-matter accumulation [34]. Abd El-Monem and Hamed [35] and Zhou et al. [36] observed that chicken manure and other organic manure sources slowly releases nutrients that anchor root development, leading to higher yield and better quality, while improving soil quality. Organic soil amendment sustains crop-production systems since it forms an integral source of N and C, in addition to being an important part of soil pH moderation [37,38]. Furthermore, organic manure provides secondary nutrients and amino acids which are required by plants for photosynthetic activities, cell division, plant growth and dry-matter accumulation [39], and are necessary for enzymatic activities and soil-organic-carbon quality and quantity [40].

3.4. Effectiveness of Combination Fungicide in the Field Study

The analysis of the effect of fungicide on disease severity revealed that mancozeb 63% + carbendazim 12% significantly ($p < 0.05$) reduced disease severity 10 weeks after transplanting (Table 2). The study revealed that variability existed among the spray regimens of mancozeb + carbendazim used in the management of *A. brassicicola* in field conditions, with the highest disease severity recorded in the control (no spray) treatment and the lowest severity in the bi-weekly treatments (Table 2). The variability observed in incidence and severity of diseases could therefore be attributed to frequency of spray of mancozeb + carbendazim as well as pesticide chemistry. Previous studies have shown that for many fungal diseases, the most effective method of control and sometimes the only one available for disease control is the frequent application of chemical sprays on plants, seeds or into the soil [41]. Similarly, McGrath [42] observed that a higher dosage of pesticides led to better control of phytophthora blight disease of cucumber. While manure type significantly ($p < 0.001$) affected plant biomass under all spray treatments, it did not have the same effect on disease severity for the all treatment regimens. This observation may be explained by the fact that both manures were organic in nature. Previous studies have shown that when compared to conventional farms, soils from organic farms were more suppressive with respect to various soil-borne and foliar diseases [43–45]. Since the two manures used in this study were both organic in nature, they had similar effects on disease severity. Hence, the effect of manure type on disease severity was not always significantly different. Instead, the most significant differences observed were associated with variation in spray regimen or frequency.

Table 2. Mean values (±SE) of disease severity scores showing the effect of manure type and fungicide spray regimen on Alternaria disease on cabbage.

Manure	Regimen	Weeks after Transplanting				
		2	4	6	8	10
Poultry	No spray	1.33 ± 0.10^{a}	1.67 ± 0.09^{a}	1.33 ± 0.12^{a}	1.33 ± 0.08^{a}	1.33 ± 0.05^{a}
	2×	1.00 ± 0.12^{ab}	1.33 ± 0.10^{ab}	1.33 ± 0.13^{a}	1.33 ± 0.10^{a}	1.33 ± 0.10^{a}
	3×	1.00 ± 0.08^{ab}	1.33 ± 0.06^{ab}	1.33 ± 0.09^{a}	1.33 ± 0.09^{a}	1.33 ± 0.11^{a}
	5×	0.67 ± 0.06^{b}	1.00 ± 0.05^{b}	1.00 ± 0.04^{ab}	1.00 ± 0.07^{ab}	1.00 ± 0.07^{ab}
Pig	No spray	2.00 ± 0.11^{c}	2.33 ± 0.14^{c}	1.67 ± 0.11^{c}	1.67 ± 0.05^{c}	1.67 ± 0.08^{c}
	2×	1.00 ± 0.07^{ab}	1.33 ± 0.11^{ab}	1.33 ± 0.04^{a}	1.33 ± 0.05^{a}	1.33 ± 0.12^{a}
	3×	1.00 ± 0.04^{ab}	1.00 ± 0.07^{b}	1.00 ± 0.05^{ab}	1.00 ± 0.10^{ab}	1.00 ± 0.05^{ab}
	5×	0.00 ± 0.02^{d}	0.33 ± 0.02^{d}	0.67 ± 0.03^{b}	0.67 ± 0.05^{b}	1.00 ± 0.07^{ab}

2×, 3× and 5×: Spray frequencies of 2 times, 3 times and 5 times, respectively. Mean values in the same column with different superscript are significantly different from each other ($p < 0.05$).

In conclusion, in vitro studies revealed that combination fungicides that are composed of systemic and non-systemic (contact) compounds (mancozeb 63% + carbendazim 12%) were more effective than systemic fungicide alone (metalaxyl 6%). The spray regimens (2×, 3× and 5×) of the combination treatment (mancozeb 63% + carbendazim 12%) were positive for the control of *A. brassicicola* under field conditions. However, spraying fortnightly (5×) was the most effective regimen for the management of *A. brassicicola* and in turn effectively controlled Alternaria leaf spot disease in field trial.

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References

1. Prakash, S.; Bhat, S.R.; Quiros, C.F.; Kirti, P.B.; Chopra, V.L. Brassica and its close allies: Cytogenetics and evolution. *Plant Breed. Rev.* **2009**, *31*, 21–187.
2. Xue, J.-Y.; Wang, Y.; Chen, M.; Dong, S.; Shao, Z.-Q.; Liu, Y. Maternal inheritance of U's triangle and evolutionary process of *Brassica* mitochondrial genomes. *Front. Plant Sci.* **2020**, *11*, 805. [CrossRef] [PubMed]
3. Matić, S.; Tabone, G.; Garibaldi, A.; Gullino, M.L. Alternaria leaf spot caused by *Alternaria* species: An emerging problem on ornamental plants in Italy. *Plant Dis.* **2020**, *104*, 2275–2287. [CrossRef]
4. Ohm, R.A.; Feau, N.; Henrissat, B.; Schoch, C.L.; Horwitz, B.A.; Barry, K.W.; Condon, B.J.; Copeland, A.C.; Dhillon, B.; Glaser, F.; et al. Diverse lifestyles and strategies of plant pathogenesis encoded in the genomes of eighteen *Dothideomycetes fungi*. *PLoS Pathog.* **2012**, *8*, e1003037. [CrossRef]
5. Cabrefiga, J.; Salomon, M.V.; Vilardell, P. Improvement of Alternaria leaf blotch and fruit spot of apple control through the management of primary inoculum. *Microorganisms* **2023**, *11*, 101. [CrossRef]
6. Thomma, B.P.H.J. *Alternaria* spp.: From general saprophyte to specific parasite. *Mol. Plant Pathol.* **2003**, *4*, 225–236. [CrossRef] [PubMed]
7. Viriyasuthee, W.; Jogloy, S.; Saksirirat, W.; Saepaisan, S.; Gleason, M.L.; Chen, R.S. Biological control of Alternaria leaf spot caused by *Alternaria* spp. in Jerusalem artichoke (*Helianthus tuberosus* L.) under two fertilization regimes. *Plants* **2019**, *8*, 463. [CrossRef]
8. Rashid, M.H.; Hossain, M.A.; Kashem, M.A.; Kumar, S.; Rafii, M.Y.; Latif, M.A. Efficacy of combined formulations of fungicides with different modes of action in controlling Botrytis gray mold disease in chickpea. *Sci. World J.* **2014**, *2014*, 639246. [CrossRef]
9. European Food Safety Authority (EFSA); Bellisai, G.; Bernasconi, G.; Brancato, A.; Carrasco Cabrera, L.; Ferreira, L.; Giner, G.; Greco, L.; Jarrah, S.; Leuschner, R.; et al. Evaluation of confirmatory data following the Article 12 MRL review for metalaxyl-M. *EFSA J.* **2021**, *19*, e06996.
10. Gullino, M.L.; Tinivella, F.; Garibaldi, A.; Kemmitt, G.M.; Bacci, L.; Sheppard, B. Mancozeb: Past, present, and future. *Plant Dis.* **2010**, *94*, 1076–1087. [CrossRef]
11. Zhou, T.; Guo, T.; Wang, Y.; Wang, A.; Zhang, M. Carbendazim: Ecological risks, toxicities, degradation pathways and potential risks to human health. *Chemosphere* **2023**, *314*, 137723. [CrossRef] [PubMed]
12. Mathivanan, N.; Prabavathy, V.R. Effect of carbendazim and mancozeb combinationon Alternaria leaf blight and seed yield in sunflower (*Helianthus annus* L.). *Arch. Phytopathol. Plant Prot.* **2007**, *40*, 90–96. [CrossRef]
13. Khan, M.M.; Khan, R.U.; Mohiddin, F.A. Studies on the cost-effective management of Alternaria blight of rapeseed-mustard (*Brassica* spp.). *Phytopathol. Mediterr.* **2007**, *46*, 201–206.
14. Yu, F.; Qiu, F.; Meza, J. Chapter 12—Design and Statistical Analysis of Mass-Spectrometry-Based Quantitative Proteomics Data. In *Proteomic Profiling and Analytical Chemistry*, 2nd ed.; Ciborowski, P., Silberring, J., Eds.; Elsevier: Boston, MA, USA, 2016; pp. 211–237.
15. Eze, M.O.; George, S.C.; Hose, G.C. Dose-response analysis of diesel fuel phytotoxicity on selected plant species. *Chemosphere* **2021**, *263*, 128382. [CrossRef] [PubMed]
16. Eze, M.O.; Thiel, V.; Hose, G.C.; George, S.C.; Daniel, R. Bacteria-plant interactions synergistically enhance biodegradation of diesel fuel hydrocarbons. *Commun. Earth Environ.* **2022**, *3*, 192. [CrossRef]
17. Macioszek, V.K.; Gapińska, M.; Zmienko, A.; Sobczak, M.; Skoczowski, A.; Oliwa, J.; Kononowicz, A.K. Complexity of *Brassica oleracea–Alternaria brassicicola* susceptible interaction reveals downregulation of photosynthesis at ultrastructural, transcriptional, and physiological levels. *Cells* **2020**, *9*, 2329. [CrossRef]
18. Aba, S.; Eze, S.; Ishieze, P.; Omeje, T.E.; Ugwuoke, K. Controlling phytophthora blight of cucumber (*Cucumis sativus*) in a derived savannah, using different spray intervals of a registered fungicide. *Plant Pathol. Quar.* **2018**, *8*, 122–130. [CrossRef]
19. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2018.
20. Ghasemi, A.; Zahediasl, S. Normality tests for statistical analysis: A guide for non-statisticians. *Int. J. Endocrinol. Metab.* **2012**, *10*, 486–489. [CrossRef]
21. Levene, H. Robust tests for equality of variances. In *Contributions to Probability and Statistics; Essays in Honor of Harold Hotelling*; Ingram, O., Harold, H., Eds.; Stanford University Press: Stanford, CA, USA, 1960; pp. 278–292.

22. Adekiya, A.O.; Ejue, W.S.; Olayanju, A.; Dunsin, O.; Aboyeji, C.M.; Aremu, C.; Adegbite, K.; Akinpelu, O. Different organic manure sources and NPK fertilizer on soil chemical properties, growth, yield and quality of okra. *Sci. Rep.* **2020**, *10*, 16083. [CrossRef] [PubMed]
23. Sukul, P.; Spiteller, M. Metalaxyl: Persistence, degradation, metabolism, and analytical methods. *Rev. Environ. Contam. Toxicol.* **2000**, *164*, 1–26.
24. Fisher, D.J.; Hayes, A.L. Mode of action of the systemic fungicides furalaxyl, metalaxyl and ofurace. *Pestic. Sci.* **1982**, *13*, 330–339. [CrossRef]
25. Zhou, Y.; Xu, J.; Zhu, Y.; Duan, Y.; Zhou, M. Mechanism of action of the benzimidazole fungicide on *Fusarium graminearum*: Interfering with polymerization of monomeric tubulin but not polymerized microtubule. *Phytopathology* **2016**, *106*, 807–813. [CrossRef]
26. Clemons, G.P.; Sisler, H.D. Localization of the site of action of a fungitoxic benomyl derivative. *Pestic. Biochem. Physiol.* **1971**, *1*, 32–43. [CrossRef]
27. Hammerschlag, R.S.; Sisler, H.D. Benomyl and methyl-2-benzimidazolecarbamate (MBC): Biochemical, cytological and chemical aspects of toxicity to Ustilago maydis and Saccharomyces cerevesiae. *Pestic. Biochem. Physiol.* **1973**, *3*, 42–54. [CrossRef]
28. Carniel, L.S.C.; Niemeyer, J.C.; Filho, L.C.I.d.O.; Alexandre, D.; Gebler, L.; Klauberg-Filho, O. The fungicide mancozeb affects soil invertebrates in two subtropical Brazilian soils. *Chemosphere* **2019**, *232*, 180–185. [CrossRef] [PubMed]
29. Thiruchelvam, M. Mancozeb. In *Encyclopedia of Toxicology*, 2nd ed.; Wexler, P., Ed.; Elsevier: New York, NY, USA, 2005; pp. 5–8.
30. Davidse, L.C. Antimitotic activity of methyl benzimidazol-2-yl carbamate (MBC) in *Aspergillus nidulans*. *Pestic. Biochem. Physiol.* **1973**, *3*, 317–325. [CrossRef]
31. Wolf, B.; Snyder, G. *Sustainable Soils: The Place of Organic Matter in Sustaining Soils and Their Productivity*, 1st ed.; CRC Press: Boca Raton, FL, USA, 2003.
32. Vimala, P.; Illias, M.; Salbiah, H. *Effect of Rates of Organic Fertiliser on Growth, Yield and Nutrient Content of Cabbage (Brassica oleracea var. capitata) Grown under Shelter*; International Society for Horticultural Science (ISHS): Leuven, Belgium, 2006.
33. Fagwalawa, L.D.; Yahaya, S.M. Effect Organic Manure on the Growth and Yield of Okra. *Imp. J. Interdiscip. Res.* **2016**, *2*, 130–133.
34. Geng, Y.; Cao, G.; Wang, L.; Wang, S. Effects of equal chemical fertilizer substitutions with organic manure on yield, dry matter, and nitrogen uptake of spring maize and soil nitrogen distribution. *PLoS ONE* **2019**, *14*, e0219512. [CrossRef]
35. Abd El-Monem, A.; Hamed, M. Response of some barley cultivars productivity and available soil nutrients to chicken manure rates under sandy soil conditions. *J. Soil Sci. Agric. Eng.* **2017**, *8*, 715–721. [CrossRef]
36. Zhou, Z.; Zhang, S.; Jiang, N.; Xiu, W.; Zhao, J.; Yang, D. Effects of organic fertilizer incorporation practices on crops yield, soil quality, and soil fauna feeding activity in the wheat-maize rotation system. *Front. Environ. Sci.* **2022**, *10*, 2292. [CrossRef]
37. Liang, Q.; Chen, H.; Gong, Y.; Fan, M.; Yang, H.; Lal, R.; Kuzyakov, Y. Effects of 15 years of manure and inorganic fertilizers on soil organic carbon fractions in a wheat-maize system in the North China Plain. *Nutr. Cycl. Agroecosyst.* **2012**, *92*, 21–33. [CrossRef]
38. Rinaldi, S.; De Lucia, B.; Salvati, L.; Rea, E. Understanding complexity in the response of ornamental rosemary to different substrates: A multivariate analysis. *Sci. Hortic.* **2014**, *176*, 218–224. [CrossRef]
39. Iqbal, A.; He, L.; Ali, I.; Ullah, S.; Khan, A.; Akhtar, K.; Wei, S.; Fahad, S.; Khan, R.; Jiang, L. Co-incorporation of manure and inorganic fertilizer improves leaf physiological traits, rice production and soil functionality in a paddy field. *Sci. Rep.* **2021**, *11*, 10048. [CrossRef] [PubMed]
40. Zhang, C.; Zhao, Z.; Li, F.; Zhang, J. Effects of organic and inorganic fertilization on soil organic carbon and enzymatic activities. *Agronomy* **2022**, *12*, 3125. [CrossRef]
41. Agrios, G. *Plant Pathology*, 5th ed.; Elsevier Academic Press: Burlington, MA, USA, 2004.
42. McGrath, M.T. Fungicide resistance in cucurbit powdery mildew: Experiences and challenges. *Plant Dis.* **2001**, *85*, 236–245. [CrossRef]
43. Liu, B.; Tu, C.; Hu, S.; Gumpertz, M.; Ristaino, J.B. Effect of organic, sustainable, and conventional management strategies in grower fields on soil physical, chemical, and biological factors and the incidence of Southern blight. *Appl. Soil Ecol.* **2007**, *37*, 202–214. [CrossRef]
44. Joshi, D.; Hooda, K.; Bhatt, J.; Mina, B.; Gupta, H. Suppressive effects of composts on soil-borne and foliar diseases of French bean in the field in the western Indian Himalayas. *Crop Prot.* **2009**, *28*, 608–615. [CrossRef]
45. Aba, S.; Baiyeri, P.; Tenkouano, A. Impact of poultry manure on growth behaviour, black sigatoka disease response and yield attributes of two plantain (*Musa* spp. AAB) genotypes. *Tropicultura* **2011**, *29*, 20–27.