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*Research Article*

# Evaluating Fungicides for the Management of Rust (*Uromycladium acaciae*) on Black Wattle Nursery Seedlings in Awi Zone, Amhara Regional State, Ethiopia

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Black wattle (*Acacia mearnsii* De Wild) provides numerous benefits for Ethiopian smallholder farmers due to its fast growth and wide adaptability. It is used for soil improvement and provides economic benefits through charcoal production and the use of wood as biomass fuel. However, in recent years, nursery stocks and young plantations have become susceptible to the wattle rust disease. No fungicides are currently registered in Ethiopia for control of wattle rust. A field experiment was conducted to evaluate the effectiveness of fungicides at varying rates for control of black wattle rust at Endewuha and Surta nursery sites, in Awi Zone, northern Ethiopia. Two systemic fungicides, namely, Amistar Top and Amistar Xtra, were evaluated at rates of $1\,L{\cdot}ha^{-1}$ and $0.5\,L{\cdot}ha^{-1}$ along with an unsprayed control in a randomized complete block design with three replications. Both fungicides at all rates significantly reduced rust infection at both sites. The lowest disease severity, along with increased plant height and collar diameter, was observed in the fungicide-treated plots compared to the untreated control. There was no significant difference in diseases intensity reduction and seedling height and root collar diameter among fungicide rates. The shortest height and the smallest root collar diameter were recorded on the untreated plots at both sites. The percentage reductions in disease damage compared to the control ranged from 94.6% to 96.72% at the Surta site and 94.6%–96.25% at the Endewuha site. Amistar Top or Amistar Xtra fungicide can effectively control acacia rust damage during the seedling stage, ensuring healthy and vigorous planting stock. The choice of the fungicide and application rate will depend on availability and cost, rather than on fungicide group. Further investigation is needed to assess the performance of both treated and untreated seedlings, as well as the progression of the disease in plantation sites.

**Keywords:** black wattle; disease; fungicides; incidence; invasive pathogens; nursery stock; severity

## 1. Introduction

*Acacia mearnsii* De Wild, commonly known as black wattle, is a tree with a range of uses including afforestation and reforestation, acidified soil reclamation, and degraded land rehabilitation [1–3]. It was introduced to the highlands of central and northwest Ethiopia in the early 1990s from Australia to tackle fire wood shortages worsened by deforestation [1] and become the most promising tree species in Ethiopia due to its ecological and socioeconomic importance [4].

Today, black wattle provides a wide range of economic benefits for Ethiopian smallholder. In the Awi Zone, particularly in the districts of Fagita-Lakoma, Ankasha Gagusa, and Banja, more than half of the land is covered by black wattle plantations [5]. Smallholder farmers benefit from charcoal and fuel wood production, which generate income, create jobs, and reduce rural-to-urban migration [6]. The value chain of black wattle involves women and men in activities such as nursery management, plantation establishment, harvesting, charcoal production, transportation to central markets, and wholesale or retail trade. In addition, the sector generates considerable tax revenue for local governments, with trucks taxed based on the quantity of charcoal transported [6, 7].

Black wattle was considered as disease free in Ethiopia for long time. However, in recent years, nursery stocks and young wattle plantations have become vulnerable to wattle rust and other insect pests [4] Rust is the most significant production constrain to black wattle seedling production causing seedling loss in South Africa and Australia [8]. In Ethiopia, wattle rust was first reported in Awi Zone in 2021 and the fungal pathogen, *Uromycladium acaciae* has been confirmed as the cause agent of rust disease [9]. The disease has caused seedling death, stunting, and dieback in black wattle nursery and young plantations in the Awi Zone, Gamo Zone, Gurage highlands, and Oromia region [9]. Due to the monoculture of black wattle trees, the rust disease has caused severe damage and complete loss in all districts of Awi Zone, particularly in Fagita-Lekoma, Akanksha, and Banja districts [8, 10]. Infected seedlings become weak and die in the nursery and plantation sites. Consequently, seedling production and plantation establishment have become impossible in Awi Zone.

Wattle rust can be controlled by application of fungicides, silviculture management, and planting resistant materials [8]. As it is difficult to obtain resistant planting materials quickly, immediate intervention is needed to reduce the disease's impact on wattle seedling production in the short-term using fungicides. Different chemical fungicides have been successfully evaluated and applied for wattle rust management in South Africa [8]. However, previous to this study, no fungicides evaluation or other chemical control research has been conducted in Ethiopia for the management of wattle rust.

Thus, this study aims to evaluate the effectiveness of fungicides in controlling wattle rust at the nursery stage and to assess their influence on seedling growth in Awi Zone of Northern Ethiopia.

## 2. Materials and Methods

*2.1. Site Description.* The experiments were conducted at two nursery sites in Awi Zone, Amhara Regional State in 2022. These sites include the Endewuha nursery site in the Fageta-Lekoma district and the Surta nursery site in the Banja District (Figure 1). The area has a high presence of wattle rust disease due to suitable weather conditions, monoculture cropping practices, and plantation coverage of over 50% of the land area. Fageta-Lekoma District is located at 10°57′23″–11°11′21″N and 36°40′01″–37°05′21″ E, with an altitude of 2560 m above sea level at the Endewuha site. The annual temperature ranges from 10°C to 25°C [6]. Banja District is located at 10°52′00″ to 11°02′44″ N and 36°38′26″ to 37°07′08″ E, with an altitude of 2512 m above sea level at the Surta site. The annual temperature ranges from 16°C to 26°C [11].

*2.2. Fungicides.* This study consisted of two fungicides, Amistar Xtra and Amistar Top commonly used in Ethiopia for controlling rust diseases on various agricultural crops (Table 1).

Fungicide treatments were applied twice during the nursery period. The first application was conducted when the rust disease symptoms first appeared, and the second application was made 30 days after the first application. The untreated plots were used as a control for disease monitoring. Fungicides were applied over the foliage using a manual knapsack sprayer with a 15-L capacity. To minimize fungicide drift during application, adjacent plots were covered with plastic sheeting.

*2.3. Experimental Design and Treatments.* Nursery pot filling was carried out using a standard nursery substrate consisting of forest soil, compost, and sand in a ratio of 3:2:1. Seeds were collected from healthy trees and planted in pots size of $1 \times 1.5$ m in the nursery. The experiment consisted of five treatments (one control and four fungicide treatments, as shown in (Table 2)), where each treatment was replicated three times in a randomized complete block design (RCBD). The spacing between the blocks and rows was 1 m, and the spacing between adjacent plots was 0.5 m. The same experimental design was used at both nursery sites. The seeds were sown on February 8, 2022. Each treatment plot contained four hundred seedlings, and 15 plots were randomly arranged in three rows for the application of the four fungicide treatments (using two different rates of application).

*2.4. Data Collection.* The data on disease occurrence (incidence and severity) were recorded at three-time points, viz., 7 days after the first spray, 1 month after the first spray, and 1 month after the second spray. The rust incidence data were collected from a 1 m$^2$ area of each plot in the nursery bed and calculated based on the total number of affected seedlings and the total number of plants evaluated as indicated below:

$$\text{Rust incidence} = \frac{\text{Number of infected wattle plants}}{\text{Total number of wattle plants assessed}} * 100. \tag{1}$$

For evaluating seedling height and root collar diameter, 20 randomly selected plants were sampled from each plot and measured using a measuring tape and caliper for each treatment, respectively. The height of the nursery plants (in meters) and the root collar diameter (in centimeters) were recorded for the seedlings during the final assessment date.

To study the effects of fungicide treatments on disease progression, a damage rating scale from 0 to 4 was utilized to assess disease severity [7] (Table 3). Disease severity on the

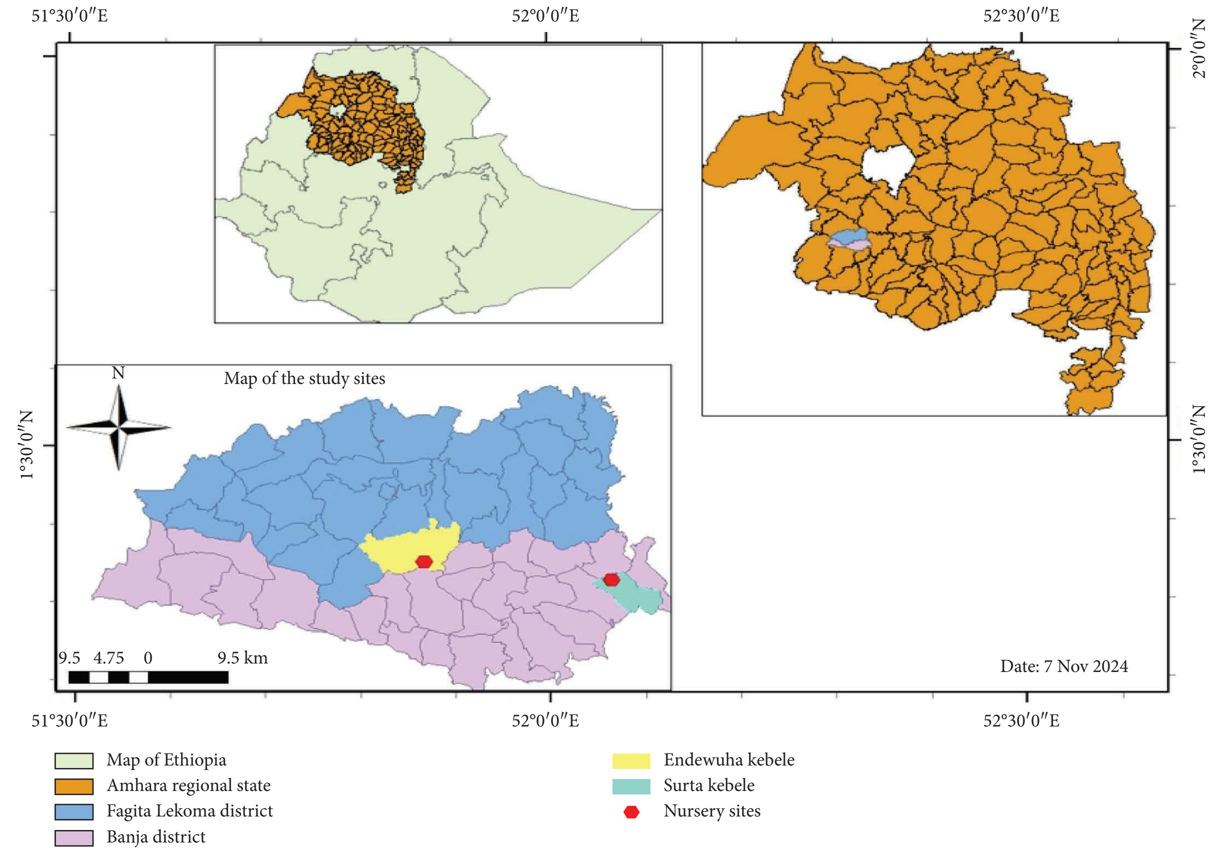


Figure 1: Map showing the location of the fungicide trials.

Table 1: Details of the tested fungicide, including the active ingredients and mode of action.

| S/n | Trade name | Active ingredients | Mode of action |
|---|---|---|---|
| 1 | Amistar Top | Azoxystrobin(strobilurin) ($200\,gL^{-1}$) + Difenoconazole (triazole) ($125\,gL^{-1}$) | Systemic |
| 2 | Amistar Xtra | Azoxystrobin(strobilurin) ($200\,gL^{-1}$) + Cyproconazole (triazole) ($80\,gL^{-1}$) | Systemic |

Table 2: Treatments.

| Treatments | Fungicides and their application rate |
|---|---|
| $T_0$ | Control |
| $T_1$ | Amistar Top1 L per hectare |
| $T_2$ | Amistar Top0.5 L per hectare |
| $T_3$ | AmistarXtra1 L per hectare |
| $T_4$ | AmistarXtra 0.5 L per hectare |

experimental seedlings was quantified by visually estimating the combination of uredosori masses, defoliation, deformed pinnules, stem, and branch lesions observed on the seedlings. The percentage of severity index (PSI) was calculated as follows:

$$\text{PSI} = \frac{\text{sum of individual numerical rating}}{\text{Total no.of seedling assessed x Maximumscore in scale}} * 100. \tag{2}$$

Adopted from [7].

Table 3: Procedures used for recording seedling disease severity of the seedlings.

| Scale | Percentage area affected |
|---|---|
| 0 | 0% (no sign of telia) |
| 1 | Telia present on leaf pinnae, branches and stem, defoliation and deformed pinnae; branches and stem with a lesion (covering 1%–25% sample seedlings) |
| 2 | Telia present on leaf pinnae, branches and stem, defoliation and deformed pinnae; branches and stem with a lesion (covering 26%–50% of sample seedlings) |
| 3 | Telia present on leaf pinnae, branches and stem, defoliation and deformed pinnae; branches and stem with a lesion (covering 51%–74% of sample seedlings) |
| 4 | Telia present on leaf pinnae, branches and stem, defoliation and deformed pinnae; branches and stem with a lesion (covering > 75% of sample seedlings) |

The percentage of rust severity reduction was calculated as follows:

$$\text{Rust severity reduction}\,(\%) = \frac{\text{wattle plant on untreated plot} - \text{treated plot}}{\text{Wattle plant on untreated plot}} * 100. \quad (3)$$

*2.5. Data Analysis.* Data regarding the incidence and severity of diseases, and diseases growth measurements (plant height and root collar diameter), were analyzed using analysis of variance (ANOVA) performed with statistical analysis system (SAS) version 9.2 software. The least significant difference (LSD) at 5% was used to separate treatment means.

## 3. Results

*3.1. Effect of Fungicides Treatment on Severity and Incidence of Wattle Rust Diseases.* During the experiment, there was high level of wattle rust disease incidence and severity at both sites. The untreated plots showed an incidence of 100% and 98.16% at Endewuha and Surta sites on the third date of assessment, respectively (Table 4). At the same time, the severity index in the control plots remained high at both sites, showing values of 29.40% at Endewuha and 28.93% at Surta (Table 4). At the first assessment date (108 DAS) for Endewuha site and (115 DAS) for Surta site, there were no significant differences in disease severity and incidence between the treated and control plots at both sites (Table 4). The results showed that diseases development was significantly lower in the treated plots ($p \leq 0.05$) compared to the control plots at both sites. After the second time fungicide application, the disease incidence and severity significantly decreased in treated plots compared to untreated plots during the second and third assessment dates (Table 4). The average disease severity of fungicide treatments recorded during the final assessment at Surta site ranged from 1.06% to 1.56%. The lowest severity (1.06%) was observed from plots treated with Amistar Top (1Lha$^{-1}$), while the highest (1.56%) was observed from plots treated with Amistar Xtra (0.5Lha$^{-1}$). The average disease severity from treated plots at Endewuha site ranged from 1.1 to 1.60 on the last assessment date.

Amistar Top Fungicide at 0.5Lha$^{-1}$ significantly reduced disease severity, resulting in healthier wattle plants compared to control at both Surta and Endewuha sites (Figures 2(a) and 3(a)). Similarly, Amistar Xtra fungicide at the same rate also demonstrated effective reduction in disease severity(Figures 2(b) and 3(b)). Untreated plots exhibited deformed leaves, stunted growth, and defoliation (Figures 2(c) and 3(c)). At Endewuha site, disease severity was reduced by an average of 94.6%–96.25% across fungicide treatments (Table 5). At Surta site, the mean percentage reduction of disease damage ranged from 94.6% to 96.72%. The highest disease control (96.72%) was achieved when Amistar Top applied at 1Lha$^{-1}$, while the lowest disease reduction was found when Amistar Xtra was applied at 0.5Lha$^{-1}$

*3.2. Effect of Fungicide Application on Seedling Height and Root Collar Diameter.* Fungicide treatments significantly ($p \leq 0.05$) increased seedling height and root collar diameter compared to untreated controls. At the final evaluation, plant height ranged from 0.40 to 0.49 m at Endewuha site and 0.40–0.45 m at Surta site (Table 6). However, within fungicide-treated plots, seedling height and root collar diameter did not significantly differ. The control treatments resulted in the lowest height and smallest root collar diameter at both sites (Table 6). The tallest seedling height (0.49 m) was observed from the treatment that received Amistar Top fungicide (1Lha$^{-1}$), while the shortest (0.40 m) was found in the unsprayed treatment at both sites. The largest root collar diameter (0.40 cm) was observed in the Amistar Top sprayed treatment (1 Lha$^{-1}$), whereas the smallest root collar diameter (0.32 cm) was recorded in unsprayed control, which was significantly lower than fungicide treatments (Table 6).

## 4. Discussion

Several research reports have indicated that wattle rust is one of the main diseases damaging wattle at nursery and plantation sites [8, 9]. Recent outbreaks of *U. acaciae*, the causal agent of wattle rust, threaten black wattle seedlings and young plantations, particularly in the Awi Zone of Amhara region (9). This rust fungus is causing significant damage to wattle

Table 4: Effect of fungicide application on wattle rust incidence and severity at Endewuha and Surta sites.

| Treatments | Endewuha site | | | | | | Surta site | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Percent incidence | | | Percent severity | | | Percent incidence | | | Percent severity | | |
| | 108 DAS | 138 DAS | 168 DAS | 108 DAS | 138 DAS | 168 DAS | 115 DAS | 145 DAS | 175 DAS | 115 DAS | 145 DAS | 175 DAS |
| $T_0$ | 4.72 | $21.01^a$ | $100^a$ | 1.53 | $6.26^a$ | $29.4^a$ | 4.30 | $15.66^a$ | $98.16^a$ | 1.43 | $4.60^a$ | $28.93^a$ |
| $T_1$ | 4.72 | $5.45^b$ | $3.93^b$ | 1.40 | $1.60^b$ | $1.1^b$ | 4.20 | $5.39^b$ | $3.83^b$ | 1.16 | $1.60^b$ | $1.43^b$ |
| $T_2$ | 4.40 | $4.46^b$ | $3.85^b$ | 1.50 | $1.13^b$ | $1.60^b$ | 3.60 | $3.90^b$ | $3.66^b$ | 1.13 | $1.46^b$ | $1.56^b$ |
| $T_3$ | 4.00 | $3.50^b$ | $3.37^b$ | 1.50 | $1.40^b$ | $1.23^b$ | 4.03 | $3.50^b$ | $2.46^b$ | 1.13 | $1.06^b$ | $1.06^b$ |
| $T_4$ | 4.03 | $3.80^b$ | $3.67^b$ | 1.53 | $1.43^b$ | $1.36^b$ | 4.30 | $4.06^b$ | $3.86^b$ | 1.43 | $1.46^b$ | $1.36^b$ |
| MEAN | 4.37 | 7.64 | 22.96 | 1.52 | 2.36 | 6.94 | 4.14 | 6.50 | 22.40 | 1.25 | 2.04 | 6.87 |
| CV | 9.89 | 13.66 | 1.5 | 15.10 | 10.72 | 12.60 | 13.02 | 14.20 | 3.14 | 14.42 | 22.25 | 8.64 |
| Signif. ($p < 0.05$) | Ns | ** | ** | Ns | ** | *** | Ns | ** | ** | Ns | ** | *** |

*Note:* Means followed by the same letter in the same column are not statistically different at 0.05%; DAS = days after sowing; significance = $0.001^{***}$, $0.01^{**}$, $0.05^{*}$; CV = coefficient of variation.

(a) (b) (c)

Figure 2: Comparison of the treated plot with untreated plot at the last date of assessment (175 days) at Surta site: plot treated with Amistar Top fungicide at a rate of 0.5 L·ha$^{-1}$ (a), plot treated with Amistar Xtra fungicide at a rate of 0.5 L ha$^{-1}$ (b), and untreated plots (c).

(a) (b) (c)

Figure 3: Comparison of the treated plot with an untreated plot at the last date of assessment (168 days) at the Endewuha site: plot treated with Amistar Top fungicide at a rate of 0.5 L·ha$^{-1}$ (a), plot treated with Amistar Xtra fungicide at a rate of 0.5 L·ha$^{-1}$ (b), and untreated plots (c).

Table 5: Effect of fungicide treatments on reduction of rust disease severity.

| Experimental nursery site | Treatments | Severity at the last date of assessment | Severity reduction (%) |
|---|---|---|---|
| Endewuha | $T_0$ (Control) | 29.40 | — |
| | $T_1$ (Amistar Top (1 L·ha$^{-1}$)) | 1.10 | 96.25 |
| | $T_2$ (Amistar Top (0.5 L·ha$^{-1}$)) | 1.60 | 94.60 |
| | $T_3$ (AmistarXtra (1 L·ha$^{-1}$)) | 1.23 | 95.81 |
| | $T_4$ (AmistarXtra (0.5 L·ha$^{-1}$)) | 1.36 | 95.37 |
| Surta | $T_0$ (Control) | 28.93 | — |
| | $T_1$ (Amistar Top (1 L·ha$^{-1}$)) | 1.43 | 96.72 |
| | $T_2$ (Amistar Top (0.5 L·ha$^{-1}$)) | 1.56 | 94.60 |
| | $T_3$ (AmistarXtra (1 L·ha$^{-1}$)) | 1.06 | 96.33 |
| | $T_4$ (AmistarXtra (0.5 L·ha$^{-1}$)) | 1.36 | 95.29 |

seedlings, resulting in a reduction in stocking, mortality of seedlings, reduction in wood products, and ultimately resulting in economic losses for growers if not controlled [8, 13–15]. In this study, the application of Amistar Top (Azoxystrobin) strobilurin (200 gL$^{-1}$) + Difenoconazole (triazole) (125 gL$^{-1}$) and AmistarXtra (Azoxystrobin) strobilurin (200 gL$^{-1}$) + Cyproconazole (triazole) (80 gL$^{-1}$) fungicides effectively controlled wattle rust, as evidenced by the improved tree seedling growth and reduction in disease severity symptoms. This finding also aligns with previous studies on forest trees, which found strobilurin/Difenoconazole or Azoxystrobin/cyproconazole as effective treatments for tree rust disease control [13, 16, 17]. These active ingredients are also used to combat fungal diseases in other crops like fruits, vegetables, and wheat in Ethiopia [18].

Untreated plots exhibited deformed leaves, stunted growth, and defoliation, indicating the experiment was conducted under high disease pressure, and the wattle

Table 6: Effect of fungicide on plant height and root collar diameter at Endewuha and Surta sites on the last date of assessment.

| Treatments | Endewuha | | Surta | |
|---|---|---|---|---|
| | Seedling height (m) | Collar diameter (cm) | Seedling height (m) | Collar diameter (cm) |
| $T_0$ (control) | $0.40^b$ | $0.32^b$ | $0.40^b$ | $0.32^b$ |
| $T_1$ (Amistar Top ($1\,L\cdot ha^{-1}$)) | $0.49^a$ | $0.40^a$ | $0.45^a$ | $0.38^a$ |
| $T_2$ (Amistar Top ($0.5\,L\cdot ha^{-1}$)) | $0.48^a$ | $0.38^a$ | $0.45^a$ | $0.36^a$ |
| $T_3$ (AmistarXtra ($1\,L\cdot ha^{-1}$)) | $0.48^a$ | $0.38^a$ | $0.43^{ab}$ | $0.36^a$ |
| $T_4$ (AmistarXtra ($0.5\,L\cdot ha^{-1}$)) | $0.47^a$ | $0.37^a$ | $0.43^{ab}$ | $0.34^{ab}$ |
| MEAN | 0.46 | 0.37 | 0.43 | 0.35 |
| CV | 4.38 | 4.58 | 5.89 | 5.34 |
| Signif. ($p < 0.05$) | * | * | * | * |

*Note:* Means followed by the same letter in the same column are not statistically different at 0.05%. DAS = days after planting; significance:$^*$ = 0.001$^{***}$, 0.01 $^{**}$, 0.05$^*$; CV = coefficient of variation.

seedlings were highly susceptible to rust disease. This trial suggested that black wattle production in Awi Zone is not economically feasible without the application of fungicides, which aligns with finding of [8], and uncontrolled wattle rust causes seedling death, deformed petioles and rachises, gummosis and stunted under favorable conditions. Other work [12, 13] has reported that wattle rust can cause defoliation and growth losses of up to 20%–40% in one year if not controlled, and in severe cases, it can even kill young trees. Fungicide treatment reduced disease severity by 94.6%–96.7% and increased plant size compared to controls, a result consistent with fungicide control of wattle rust in South Africa 70%–90% reduction [17]. The result showed that the application of Amistar Top and AmistarXtra fungicides was effective in reducing the impact of rust apart from increasing tree performance. Fungicide efficacy in reducing wattle rust severity and incidence was statistically similar across all assessment dates, indicating both fungicides are effective at application rates of $0.5\,L\ ha^{-1}$ and $L\ ha^{-1}$. The lower spray dose provided adequate protection against the rust fungus and may save the volume required as well as the cost. Nursery growers can use Amistra Top or AmistraXtra at a low rate to control rust during the nursery stage. Therefore, the choice of fungicide and application rate will depend on availability and cost, rather than on fungicide group. Active ingredients used in this study, Azoxystrobin and Difenoconazole, can enhance plant defense by indirectly influencing certain populations of endophytes [19] without harming, beneficial microbes essential for the normal growth of plants when used at the recommended dosage [19, 20].

Foliar spraying of the systematic fungicide in the nurseries improved wattle seedling growth by controlling rust disease. However, on plots that not received fungicides retarded seedlings were observed. The increased in seedling height after the application of fungicides could be due to the reduction of rust stress, which ultimately results in the proper growth function of the seedlings. Previous research reports showed that application of Amistar Top and AmistarXtra fungicides promotes the growth of plants by preventing disease caused by *Uromycladium acaciae* resulting in effective disease management [17]. Therefore, the result shown in this study provides a sound management option to reduce seedling loss by controlling wattle rust infection and increasing tree performance. Moreover, according to [13], after applications of Azoxystrobin-based fungicides, the reduction of wattle rust severity is greater, leading to beneficial effects on height and diameter growth. The reduced growth observed on the control treatment is could be due to rust infestation cause reduction of photosynthesis to plant. Pathogen infection often affects host growth and developmental by disrupting nutrients transport and reducing photosynthesis [21]. Fungicides controlled wattle rust and promoted healthy nursery growth during the experiment. This highlights the importance of developing sustainable and effective management options for wattle rust in Ethiopia.

## 5. Conclusion

This study tested two fungicides at two application rates and found significant differences in the health and growth of seedlings between sprayed and unsprayed treatments. Wattle rust caused a considerable and negative effect on the growth of tree seedlings, leading to stunted development and reduced overall health of the seedlings on untreated plots. Amistar Top or Amistar Xtra fungicides and their application rates (0.5 $Lha^{-1}$and $Lha^{-1}$) were superior to the control in terms of reducing disease incidence and severity, and increasing seedling height and root collar diameter. Therefore, fungicide management of rust disease in nurseries improves seedling quality, providing wattle growers with healthier planting stock. Although the active ingredients differed, no significant difference was noted between the Amistra Top or AmistraXtra fungicides and their application rates in reducing disease infection. This shows that both fungicides are effective at application rates of 0.5 $Lha^{-1}$and $Lha^{-1}$. Nursery growers can use either Amistra Top or AmistraXtra to control rust damage during the nursery stage at low rate. The choice of the fungicide and application rate will depend on availability and cost, rather than on the fungicide group. Further investigation is needed to evaluate the field performance of both sprayed and unsprayed seedlings after out-planting. This should also include assessing the possibility of reinfection of rust and fungicide treated performance at the plantation site.

## Data Availability Statement

The data used to support the study are available from the corresponding author upon request.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Author Contributions

Kumela Regasa: data collection, analysis, methodology, writing–original draft, and writing–review & editing; Agena Anjulo: methodology and writing–review & editing; Weldesenbet Beze and Mesfin Wondafrash: data collection, methodology, and writing–review & editing; Brett Hurley: funding acquisition, project administration, and writing–review & editing; Ilaria Germishuizen: writing–review & editing; Simon Lawson and Madaline Healey: project administration and writing–review &editing.

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