



Research article

Determination of the rate and frequency of Natura 250 EW fungicide for the management of cedar rust from Gesho (*Rhamnus prinoides*) in the north west Amhara Region, Ethiopia

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ABSTRACT

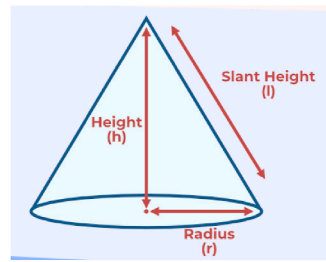
Rhamnus prinoides (Gesho) is grown in the homesteads of farmers in western Amhara. The leaves, twigs, and stems of Gesho are indispensable ingredients in traditional beverages. Its production has recently suffered from cedar rust caused by the fungus *Gymnosporangium*. To manage this disease, different fungicides have been recommended. Therefore, this research was designed to determine the rate and frequency of the use of the Natura 250 EW system for managing this disease in Gesho during 2020. Five application rates of Natura 250 EW and three spray frequencies were used as treatments to manage the disease in infected Gesho plants via the Randomized Complete Block Design (RCBD). Statistically significant differences ($P \leq 0.05$) were found between treatments for most parameters. Among these 0.75 liter ha⁻¹ treatments, two spray applications resulted in the lowest severity and greatest relative efficacy, followed by three spray applications of 0.50 liter ha⁻¹ at Bahir Dar Zuria district. However, at Yilmana Densa district, the lowest severity and greatest relative efficacy were found for the 0.75 liter ha⁻¹ spray, followed by the 0.50 liter ha⁻¹ spray, compared with the other treatments. Even though 0.75 liter ha⁻¹ had greater relative efficacy and lower disease severity than the other treatments, there was no statistically significant difference between the three sprays of 0.50 liter ha⁻¹ at both locations. Therefore, three sprays of Natura 250 EW at 0.50 liter ha⁻¹ in 15-day intervals should be recommended and demonstrated for the management of cedar rust disease on Gesho.

1. Introduction

The *Rhamnus prinoides*, L'Herit also known as Gesho in Amharic, is an East African evergreen shrub, belongs to the family Rhamnaceae, and order Rhamnales [1]; [2]. It is found in eastern, central, southern, and western Africa including Ethiopia and Eritria [1]. It is native to Ethiopia, Botsana, South Africa, Eritrea, Angola, Sudan, Cameroon, Lesetho, Uganda, Nambia, and Swaziland [3]. It has long been used to cure a variety of ailments, such as atopic dermatitis, ear, nose, and throat infections, pneumonia, arthritis, brucellosis, fever, dyspepsia, and exhaustion [2]. Gesho is cultivated on the homestead of each farmer in the western Amhara region of Ethiopia. The leaves, twigs, and stems of Gesho has indispensable social value ingredients in traditional beverages named 'Tella and Tej' [4] as well as for cash generation for farmers in rural and urban holdings. According to Negash et al. [5], in place of commercial hops, Gesho leaves are a good source of antioxidants, essential oils, and bittering agents. It is also used as a cash crop and traditional

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$$A = \pi r (r + \sqrt{h^2 + r^2})$$

Fig. 1. Cone and its area formula.

medicine ([3,6]). However, in recent times, producer farmers have been raised the question about the solution of the newly emerged diseases on Gesho plants. Based on that a survey was conducted during 2017/2018 to identify the disease and quantify the extent of damage severity and its distribution in the main Gesho-producing areas of western Amhara [7]. According to survey results, 92.11 % of Gesho fields in six districts were infected by the disease, 19.74 % of which were severely infected (>20 %) [7]. Hence, the disease identified as Cedar apple rust disease was caused by a fungus in the genus *Gymnosporangium* [7]. The disease severity ranged from 0 to 45 %, and the overall mean severity recorded was 8.57 %, where the highest mean severity was recorded at Mecha. This disease affects apple, cedar, and juniper trees and is widespread in North America and Europe [8]; [9]. Since the *Rhamnaceae* and *Rasaceae* family belong to the same Rosales order, it is anticipated that the disease will impact *R. prinoides*. Because *R. prinoides* is comprised in the family of *Rhamnaceae* [7]. This disease is new for our country Ethiopia on Gesho not more than a decade and the plant was also healthy before the occurrence of this rust disease on it. The occurrence of the disease on the new environment and host might be the result of climate change and global warming. According to Chakraborty et al. [10] that the epidemiology of diseases will be impacted by variations in temperature, precipitation, and the frequency of extreme occurrences. Since many research on climate change have shown that Climate change and global warming are causing new diseases and pests to emerge, which negatively impacts crop output in terms of yield and quality [11–13]; harsh weather might induce fungal diseases to have variations in their pathogenicity and aggressiveness [14,15]. Hence, intervention is needed to prevent the spread and increase in damage severity of this newly introduced disease in Gesho [7]. Farmers require agricultural fungicides to quickly manage disease epidemics when they are severe [16]. Finally, an evaluation of fungicides for the management of cedar rust disease was performed on a farmer's field in the Yilmana Densa district. As a result, three fungicides were selected to manage pathogen development (Nativo SC 300, Natura 250 EW, and Noble 250 WP) [17]. Hence, fungicides are regarded as the second line of defense after host resistance and are crucial to our efforts to control plant diseases [18]. However, the rate and frequency of application were not determined to give up the final recommendation; hence, this study was conducted to determine the effective and economically acceptable rate and frequency of Natura 250 EW fungicide application for the management of cedar rust disease in Gesho.

2. Materials and methods

2.1. Description of the study area

The experiment was conducted in the Yilmana Densa district Goshey Kebele and the Bahir Dar Zuria district Debretsion Laguna Kebele in 2020/2021 from October to February. According to Wallelign et al. [17] findings, the disease is more severe during coldest months, which run from October to January, and it also develops more quickly when the temperature drops and vice versa. Yilmana Densa and Bahir Dar Zuria districts are part of the West Gojjam Zone in the Amhara Region of Ethiopia. Yilmana Densa is bordered on the south by Kuarit, on the southwest by Sekela, on the west by Mecha, on the north by Bahir Dar Zuria, and east by Gonji Kolela districts. The district comprises three agroecological zones: Dega, Woina Dega, and Kola. In comparison to Woina-Dega, the Kola and Dega portions of the district have a relatively low population density. The temperature ranged from 15 to 24 °C, and the rainfall amount ranged from 1200 to 1600 mm. The region receives 1437 mm of rainfall on average per year; 54 % falls during July and August, and only 3 % during the dry months. It is situated between 15°37' N and 37°25' E [19]. Lake Tana, the southern and northern Gonder Zones, the Yilmana Densa district, the Dera district, the Abay River, and the Mecha and Achefer districts are the boundaries of the Bahir Dar Zuria district [20]. The midland agro-climatic zone is the only zone in this area. The average yearly temperature is between 10 and 32 °C, and the average annual rainfall is between 800 and 1250 mm. Its altitude varies from 1750 to 2300 m.a.s.l [20]. The 39 infected trees were selected based on the level of infection at each location, and a total of 78 Gesho trees were selected. These selected trees were maintained by labeling or tagging individually and protected them from animal disturbance by fencing around them. Additionally, the farmers were signed agreements to maintain or protect the plant until the experiment was completed. Natura 250 EW (Tebuconazole) and Nativo SC 300 (trifloxystrobin 100 g/lit + tebuconazole 200 g/lit) were chosen for the trial. Among the selected fungicides, Nativo was unavailable on the market then; we decided to use only Natura 250 EW (tebuconazole).

2.2. Treatments and experimental design

There were two treatment factors: the first factor was five rates of fungicide treatment (0, 0.125, 0.25, 0.5, and 0.75 liter ha⁻¹),

Table 1

Mean effect of treatments on disease severity and RE (%) in the Bahir Dar Zuria district during 2020/2021.

No.	Treatments	Initial severity (%)	DS 15th DA 1st spray	DS 30th DA 1st spray	DS 45th DA 1st spray	DS 60th DA 1st spray	RE (%)
1	0.75 L/ha * 1	48.61	40.28	32.78 ^{bc}	32.78 ^{bcd}	32.78 ^{bcd}	59.30
2	0.75 L/ha * 2	35.00	30.83	26.11 ^c	23.06 ^d	20.28 ^f	74.82
3	0.75 L/ha * 3	50.83	43.61	35.83 ^{bc}	29.45 ^{cde}	26.11 ^{ef}	67.59
4	0.5 L/ha (FR) *1	46.95	45.28	44.17 ^{bc}	45.28 ^{bc}	46.39 ^{bc}	42.41
5	0.5 L/ha (FR) *2	33.89	30.00	28.89 ^{bc}	28.61 ^{cde}	28.89 ^{def}	64.13
6	0.5 L/ha (FR) *3	42.50	38.33	34.05 ^{bc}	27.17 ^{de}	21.67 ^{ef}	73.10
7	0.25 L/ha * 1	49.17	44.16	42.78 ^{bc}	44.17 ^{bcd}	48.06 ^{bc}	40.34
8	0.25 L/ha * 2	40.00	37.22	35.56 ^{bc}	34.17 ^{bcd}	33.33 ^{cdef}	58.62
9	0.25 L/ha * 3	56.67	48.61	44.17 ^{bc}	36.66 ^{bcd}	33.89 ^{b-f}	57.93
10	0.125 L/ha * 1	29.44	30.00	31.39 ^{bc}	35.55 ^{bcd}	37.78 ^{bcd}	53.10
11	0.125 L/ha * 2	49.17	48.05	47.22 ^b	48.61 ^b	50.56 ^b	37.23
12	0.125 L/ha * 3	37.22	37.50	39.72 ^{bc}	41.39 ^{bcd}	44.72 ^{bcd}	44.48
13	Control	45.56	60.56	66.39 ^a	76.39 ^a	80.55 ^a	0.00
Mean		43.46	41.11	39.17	38.71	38.85	
CV (%)		32.75	30.14	28.44	26.82	25.80	
Sign level		ns	ns	*	***	***	

Footnote: FR = Factory recommendation, 1, 2 & 3 = number of sprays, RE = relative efficacy, DS = disease severity, DA = days after.**Table 2**

Mean effect of treatments on disease severity and RE (%) in Y/Denisa district during 2020/2021.

No.	Treatments	Initial severity (%)	DS 15th DA 1st spray	DS 30th DA 1st spray	DS 45th DA 1st spray	RE (%)
1	0.75 L/ha * 1	43.61	37.78	31.39 ^{cd}	30.83 ^{def}	61.06
2	0.75 L/ha * 2	50.28	42.50	31.67 ^{cd}	25.28 ^f	68.07
3	0.75 L/ha * 3	52.50	35.83	30.28 ^{cd}	20.00 ^f	74.74
4	0.5 L/ha (FR) *1	50.56	43.61	41.67 ^{bcd}	41.39 ^{cdef}	47.72
5	0.5 L/ha (FR) *2	63.05	57.78	49.44 ^{abc}	48.89 ^{bcd}	38.25
6	0.5 L/ha (FR) *3	38.61	33.61	25.56 ^d	21.11 ^f	73.34
7	0.25 L/ha * 1	55.00	51.94	51.67 ^{abc}	54.45 ^{bc}	31.22
8	0.25 L/ha * 2	44.72	36.39	30.56 ^{cd}	28.33 ^{ef}	64.22
9	0.25 L/ha * 3	48.61	41.94	36.11 ^{cd}	31.67 ^{def}	60.00
10	0.125 L/ha * 1	50.55	48.61	49.44 ^{abc}	50.56 ^{bcd}	36.14
11	0.125 L/ha * 2	63.33	62.22	60.28 ^{ab}	65.28 ^{ab}	17.54
12	0.125 L/ha * 3	63.05	54.45	51.39 ^{abc}	52.22 ^{bcd}	34.04
13	Control	48.33	61.67	71.39 ^a	79.17 ^a	0.00
Mean		51.35	46.79	43.14	42.24	
CV (%)		35.76	33.32	30.63	30.38	
Significant level		ns	ns	**	***	

Footnote: FR = Factory recommendation, 1, 2 & 3 = number of sprays, RE = relative efficacy, DS = disease severity, DA = days after.

including no treatment and the second factor was three spray frequencies (one, two, and three times spray). The spraying was performed at 15th-day intervals after the first spray. There were 13 treatments in total. These treatments were arranged in the randomized complete block design (RCBD) with three replications in factorial combination. Each infected tree was considered as a plot. The fungicide concentration was calibrated with each rate of application per hectare estimated or calculated on the tree canopy basis using the cone surface area formula (<https://byjus.com/cone-formula>) (Fig. 1). Whole plant parts were sprayed during treatment until the leaves and twigs were fully wet. During treatment, we used plastic sheets to control spray drift.

2.3. Data collection and statistical analysis

Disease severity (%), and fresh and dry leaf biomass yields (g) data were collected. The disease data were recorded for each selected *Gesho* tree branch, leaf, and stem for each treatment before and after treatment application. The number of infected leaves per branch, number of infected branches, and/or number of twigs per tree were used to rate the percentage of disease severity, and expressed as a percentage of total plants as described by Araújo et al. [21] and Bock & Chiang [22]. The leaves of each *Gesho* plant were harvested separately as a plot. Then immediately, after harvesting each plant leaves were weighted and take it as a fresh leaf biomass yield. Finally dry leaf biomass yields were taken after the sun drying of the harvested leaves of each plant/plot.

$$DS(\%) = \frac{ILA}{TLAI} * 100$$

where: DS = disease severity, ILA = infected leaf area, and TLAI = total leaf area inspected [22,23].

The relative efficacy (RE) of each treatment over the control or untreated plot was calculated by using the formula of Zadoks [24]:

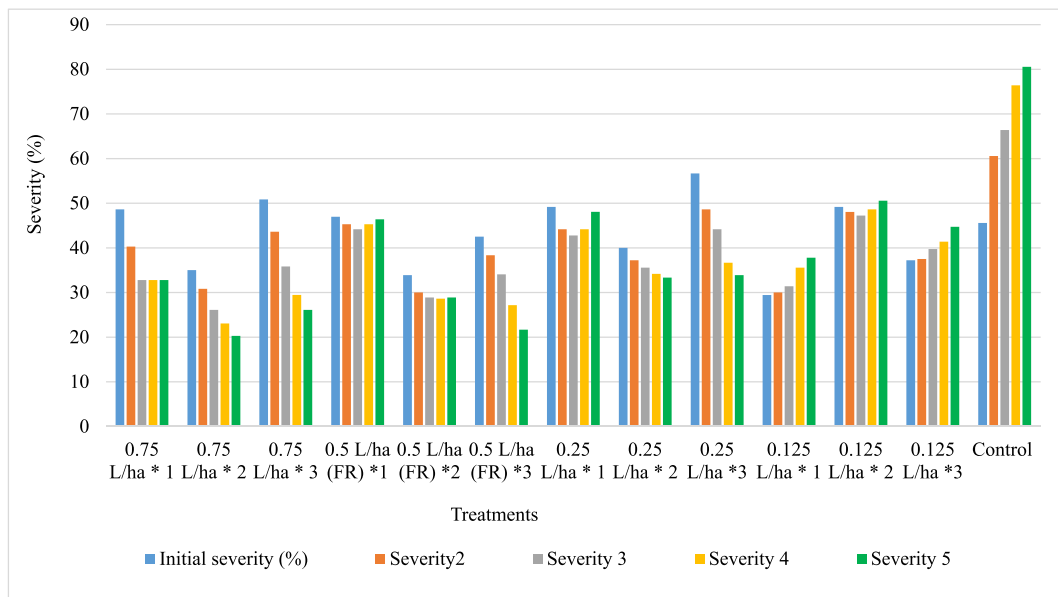


Fig. 2. The progression of disease severity in the Bahir Dar Zuria district during 2020/2021.

$$RE (\%) = \frac{(UNP - TP)}{UNP} * 100$$

where: RE = relative efficacy of treatments as a percentage, UNP = percent of disease severity in the untreated plot, and TP + percent of disease severity in the treated plot.

The collected data were analyzed using SAS version 9.0 software [25]. Fisher's least significant difference (LSD) was used for mean separation. The graphs were sketched using Excel.

3. Results and discussion

3.1. The effect of fungicide rates on disease severity

There was a highly significant difference ($P < 0.001$) between the treatments for disease severity starting from the 3rd data scoring. However, the initial (before spray) and the 2nd disease severity scores were not revealed as a statistically significant difference ($P \leq 0.05$) between treatments at both locations (Tables 1 and 2). This indicated that the selected experimental trees had comparable disease infection levels. The lowest terminal severity (20.28 %) was recorded on the treatment of two times spray of 0.75 liter ha^{-1} followed by three times spray of 0.50 liter ha^{-1} /factory recommendation (21.67 %) at Bahir Dar Zuria (Table 1). However, in the Yilmana Denisa district, the lowest terminal severity (20.00 %) was recorded on the treatment of three-time sprays of 0.75 liter ha^{-1} , followed by the three spray treatments of 0.50 liter ha^{-1} (21.11 %) (Table 2). Even though 0.75 liter ha^{-1} of the two and three times spray had stronger relative efficacy and lower terminal disease severity than the other treatments, no statistically significant difference was found between the three sprayings of the factory recommendation rate (0.50 liter ha^{-1}) at either location. Therefore, the rate of 0.75 liter ha^{-1} is not economically efficient compared with the result obtained for the other treatment (0.50 liter ha^{-1} ; factory recommendation); moreover, we have seen that the 0.75 liter ha^{-1} treatment had a negative toxic effect (burning effect) on young Gesho leaves and shoots, which were subsequently dried and defoliated. This result is agreed with study of Sharma et al. [26] that plants exposed to pesticides experience toxicity, which manifests as burns, necrosis, chlorosis, stunting, and leaf twisting as a result of numerous factors, including the rate of application of pesticides. On the other hand, the highest disease severity (80.55 and 79.17 %) and the lowest relative efficacy (0.00 %) were found on the untreated plots at Bahir Dar Zuria and Yilmana Denisa district, respectively (Tables 1 and 2). This result is in line with the study of Sanyang et al. [27] that regarding disease severity, there was a highly significant difference ($P < 0.05$) between the sprayed and un-sprayed plots. Throughout the recording period, unsprayed plots had three times the disease infection rate of sprayed plots. Similarly, the highest relative efficacy (74.82 % and 74.74 %) was calculated from the treatment of two and three times sprays of 0.75 liter ha^{-1} , followed by three times sprays of 0.50 liter ha^{-1} (73.10 % and 73.34 %) at Bahir Dar Zuria and Yilmana Denisa district, respectively (Tables 1 and 2). As we have shown, disease progression decreases from the initial severity after fungicide spray because the fungicide tebuconazole (Natura) is systemic and has protective, curative, and eradicating effects, with acro-peritoneal translocation in the xylem [28]. Tebuconazole is also known as a DMI (demethylation inhibiting fungicide), i.e., it works by affecting the cell walls of fungi by suppressing spore germination and fungal growth and interfering with the production of ergosterol (a molecule essential for fungal formation). As a result, the formation of fungus is slowed, and the process is

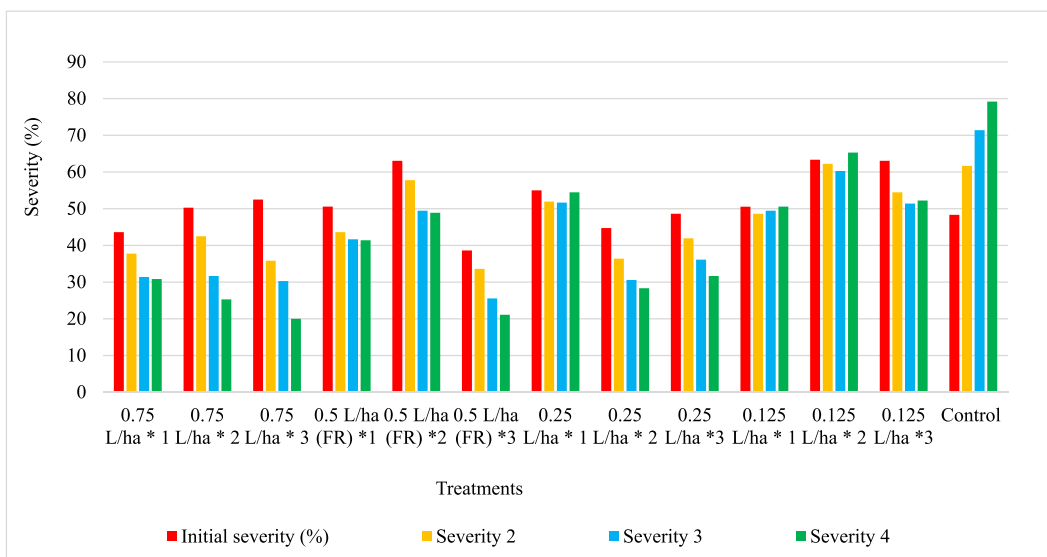


Fig. 3. The progression of disease severity in the Yilmana Densa district during 2020/2021.



Fig. 4. The status and symptoms of Cedar rust disease on Gesho twigs and leaves.

eventually stopped (Fig. 5). Because of this unique mode of action, tebuconazole is considered to be fungal-static or growth-inhibiting rather than fungicidal or fungal-killing. One spray of most of the treatments and even three sprayings of the minimum rate (0.125 liter ha^{-1}) had a slight increase in disease development at both locations compared with the other treatments, even though the fungicide application reduced or retarded disease development (Figs. 2 and 3). This is in line with the results of Kelman et al. [29] that various fungicides are available that are intended to control plant diseases by either killing or preventing the growth of the microorganisms that cause the disease. Whereas, the disease progress in the control or untreated plots was highly increased (Figs. 2–4).

3.2. The effect of fungicide rate on the yield of Gesho

Statistically highly significant differences ($P \leq 0.001$) were found between the treatments for the dry biomass yield of *Gesho* at Yilmana Denisa. Considerable difference ($P \leq 0.05$) was also found in the combined dry biomass yield of the two locations (Table 3). However, fresh and dry biomass did not show significant differences ($P \leq 0.05$) between treatments in Bahir Dar Zuria and fresh biomass at Yilmana Denisa district. This is agreed with the result of Jaskulska et al. [30] that the protection provided by fungicides had no discernible impact on leaf yield. Even though, there is no significant difference between them the highest dry and fresh biomass



Fig. 5. Regenerated twigs and leaves of Gesho after fungicide spray.

Table 3

Mean effect of treatments on fresh and dry biomass yield (g) in the Bahir Dar Zuria and Yilmana Denisa districts during 2020/2021.

No.	Treatments	Bahir Dar Zuria		Yilmana Denisa		Combined	
		Fresh Biomass (g)	Dry Biomass (g)	Fresh Biomass (g)	Dry Biomass (g)	Fresh Biomass (g)	Dry Biomass (g)
1	0.75 L/ha * 1	665.30	394.00	529.91	326.85 ^{abcd}	597.63	360.43 ^{bc}
2	0.75 L/ha * 2	758.80	452.70	644.37	237.94 ^d	701.60	345.31 ^{bc}
3	0.75 L/ha * 3	534.60	317.70	631.04	359.24 ^{ab}	582.81	338.48 ^c
4	0.5 L/ha (FR) *1	1175.20	627.90	549.64	419.14 ^a	862.40	523.54 ^a
5	0.5 L/ha (FR) *2	501.90	289.60	443.40	255.98 ^{cd}	472.63	272.78 ^c
6	0.5 L/ha (FR) *3	952.90	535.20	710.49	419.14 ^a	831.68	477.19 ^{ab}
7	0.25 L/ha * 1	569.70	365.30	582.48	359.95 ^{ab}	576.11	362.65 ^{bc}
8	0.25 L/ha * 2	508.70	279.40	399.72	237.94 ^d	454.20	258.65 ^c
9	0.25 L/ha *3	399.10	276.30	586.08	361.87 ^{ab}	492.57	319.09 ^c
10	0.125 L/ha * 1	602.50	340.50	620.78	384.97 ^a	611.65	362.75 ^{bc}
11	0.125 L/ha * 2	752.40	438.20	454.49	267.94 ^{abcd}	603.44	353.06 ^{bc}
12	0.125 L/ha *3	788.50	443.20	543.73	320.85 ^{abcd}	666.12	382.02 ^{bc}
13	Control	744.30	408.60	445.94	353.73 ^{abc}	595.11	381.17 ^{bc}
Mean		688.76	397.59	549.39	331.20	619.07	364.39
CV (%)		47.29	38.07	20.77	17.73	39.53	30.76
Sign level		ns	ns	ns	***	ns	*

Footnote: ½ AFR 1–3 = half above factory recommendation 1–3 times spray, FR1–3 = Factory recommendation 1–3 times spray, 1/2 UFR1–3 = half under factory recommendation 1–3 times spray, ¼ UFR1–3 = 1/4th under factory recommendations 1–3 times spray.

yields were found on the treatment of one time and three times spray of 0.50 litter ha⁻¹ (Table 3). The results of Schierenbeck et al. [31] agreed with our result that the fungicide application enhanced biomass product. According to Vyska et al. [32] findings plant diseases cause large crop losses in horticulture, forestry, and agriculture.

4. Conclusion and recommendations

Cedar rust disease has a high impact on ‘Gesho’ leaf production, as we have observed in the study areas and based on farmers’ sayings. Therefore, providing disease management options is becoming necessary. Among the management options, fungicide application is one option for urgent disease epidemics. Therefore, based on these results, two to three spray applications at a rate of 0.75 litter ha⁻¹ and three spray applications at the factory recommendation (0.50 litter ha⁻¹) had greater efficacy and lower disease severity, but there was no significant difference between the two treatments. As a result, using three times the factory recommendation rate can reduce the incidence of cedar rust disease in Gesho plants efficiently and economically without damaging the plants. Therefore, three sprayings of Natura 250 EW with a factory recommendation rate of 0.50 litter ha⁻¹ within 15-day intervals should be recommended and demonstrated on hot spot areas for the management of cedar rust disease in Gesho. The effects of disease on the quality of the product, disease epidemiology, and other management options, rather than fungicides, need further research.

Data availability statement

The data that support the findings of this study are available from the corresponding author, [Gebremariam Asaye], upon reasonable request.

CRediT authorship contribution statement

Gebremariam Asaye Emrie: Writing – original draft. **Kindu Demissie Fikadu:** Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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