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Research Article

# MANAGEMENT OF GARLIC RUST (Puccinia allii) USING PROPICONAZOLE FUNGICIDE AND ITS ECONOMIC IMPORTANCE ON GARLIC (Allium sativum L.) PRODUCTION IN ENEBSIE SARMIDER DISTRICT, NORTH-WESTERN ETHIOPIA

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**ABSTRACT.** Garlic is one of the most important medicinal and spice crops in Ethiopia. It is cultivated both under irrigation and during the man cropping season. However, the production and productivity of garlic affected both biotic (living factors) and abiotic (non-living factors) factors. Among biotic factors fungus disease like garlic rust caused by *Puccinia allii* is the major problem in garlic production in most areas of northwestern Ethiopia. The objective of the study was to evaluate the appropriate rate and frequency of Propiconazole fungicide to manage garlic rust. The field experiment was conducted at Enebsie Sarmider district, Bete Emider kebele during 2022, the main cropping season. The treatments were arranged in factorial randomized complete block design with three replications. The combination of three rates (0.65, 0.75, and 1L ha<sup>-1</sup>) and four spray frequency (one, two, three and four times) of fungicide and one control treatment were evaluated. Both disease and yield-related data were collected and analyzed using SAS 9.4 software. The analysis of variance revealed that a combination of rates and spraying frequencies of fungicide significantly reduced the garlic rust disease with correspondingly increased bulb yield and yield related of garlic. The minimum terminal severity (34%), and area under disease progress curve (119.17 % days<sup>-1</sup>) were recorded from plot treated with 1L ha<sup>-1</sup> and four times application of fungicide, however the maximum garlic bulb yield increment (264.53%) was obtained from plot received 0.75L ha<sup>-1</sup> and four times application of fungicide. The economic analysis confirmed that the highest net benefit (739,840-birr ha<sup>-1</sup>) and accepted marginal rate of return (56,200%) was obtained from plots treated with 0.75L ha<sup>-1</sup> within four times sprayed plot. Generally, in the study area 0.75L ha<sup>-1</sup> with four time's sprayed frequency of fungicide was effective in controlling garlic disease and gave the highest cost-benefit advantage as compared with other treatments. This study indicated that spraying frequency is the more determining factor to control garlic rust disease than different rate of Propiconazole fungicide.

Keyword: Bulb yield; Clove weight; Disease severity; Fungicide application; Yield loss

#### INTRODUCTION

Garlic (*Allium sativum* L.) is one of most important horticulture crops in the family of Alliaceae. It is believed to have originated in Central Asia and travelled throughout the world via commerce and colonization [1]. Garlic has been used in China and India for more than 5000 years, and Egypt since 2000 B.C., however China is the largest producer and consumer of garlic in the world [2]. It is a fundamental component in many or most dishes of various countries in the world including Ethiopia [3, 4]. All parts of the garlic plant have a vital use; the cloves are

used as seed, for consumption and for medicinal purposes, the leaves, stems (scape) and flowers on the head (spathe) are also edible and are most often consumed while immature and still tender [3]. Additionally, garlic is used to heal communicable diseases effectively [5]. Garlic has been cultivated in different parts of Ethiopia, mostly in Oromia, Arsi, Bale, Shewa, Jimma, Wolaita, Haddiya, Amahara, Tigray, and southern Ethiopia. The bulk of garlic for domestic market is produced in homestead gardens of subsistence farmers [6]. Current garlic production and productivity in Ethiopia are below their potential, with the country being ranked 15th in the world [6]. According to the Central Statistical Agency [7], garlic production decreased from a hectare area coverage of 16,411.19 to a production coverage of 15,381 hectares, resulting in a total bulb yield reduction from 159,093.58 to 138,664.3 tons. The average garlic productivity also decreased from 9.7 to 9.02 tons per hectare.

However, garlic production and productivity in Ethiopia are very low due to many biotic and a biotic factor such as lack of high yielding varieties, non-availability of quality seeds, imbalanced fertilizer use, lack of irrigation facilities, lack of proper disease, and insect management and other agronomic practices, low storability, and lack of proper marketing facilities [8]. Among the various challenges, biotic factors are the main contagious and infectious diseases, of which garlic rust (Puccinia allii), white rot (Sclerotium cepivorum B.), pink rot, mosaic virus and nematodes are the most widespread and destructive pathogens of cultivated garlic crops in Ethiopia [9]. From foliar disease, garlic rust caused by *Puccinia allii* is the major destructive disease problem in almost all garlic producing regions of Ethiopia [10]. The disease affects the leaves rather than the garlic bulb directly, which has an indirect negative impact on the size and quality of the harvested bulbs and lowers their marketability. Plants with severe infections may be more vulnerable to secondary infections and may have a direct reduction in bulb output [10]. Yeshewas et al. [10], examined sixteen garlic germplasm samples in East Gojjam for yield and disease tolerance; they found that none of the cultivars exhibited a high level of rust resistance. According to Habtewold et al. [11], the presence of rust resulted in a decrease in bulb weight, bulb diameter, and number of cloves per bulb by 55.9%, 16.33%, and 33.6% respectively. The greatest recorded incidence and severity of garlic rust, ranging from 87.5 to 96.6% incidence and 49.02 to 63.5% severity, were reported. Lower mean disease incidence (36.4%) and severity (12.8%) were recorded in Eastern Amhara, Ethiopia [9].

Global garlic producers have implemented efficient management strategies, such as removing contaminated plants, eliminating volunteer plants, avoiding the pathogen's epidemic season, maintaining good field hygiene, and utilizing host resistance together with the right fungicides, and integrated use of host resistance and appropriate fungicides [11]. Rex Duo and Propiconazole fungicides were identified as effective in managing garlic rust and resulting in the highest garlic bulb yield. In 2018, a 106% garlic yield advantage was achieved in plots treated with Rex Duo fungicide [11]. Lack of improved garlic varieties in the research area is a result of the widespread use of fungicides alone to combat garlic rust. Fungicides like mancozeb, propiconazole, tebuconazole and others instead of resistance varieties to minimize the infestation of garlic rust disease. So far, management of the disease under irrigation and main rainy season production systems in all small-scale farmers have not been done in Enebsie Sarmider Districts, north-western Ethiopia. Hence, finding economical management options for this disease is a prerequisite. Therefore, the main objective of this study was to determine the appropriate rate and frequency of Tilt 250EC fungicide for the management of garlic rust and to evaluate its economic importance.

## MATERIALS AND METHODS

# Description of the Study Area

The experiment was conducted at Enebsie Sarmider district, Betemider Kebele

Administration on farmer field. Enebsie Sarmider district is located 365 km North-west of Addis Ababa capital city of Ethiopia, and 180 km to the East of Bahir Dar City the capital city of Amhara region, and at a geographical coordinate of 10<sup>0</sup> 50`00`` N latitude and 38<sup>0</sup> 16`00`` E longitude. The total area of the *district* is 106764 hectares. Its agro-ecological zone is 53% lowland, 23% mid-land, and 24% highland. The Betemider Kebele altitude ranges from 2641 m.a.s.l, average annual rain fall of the *Kebele* ranges from 1700 mm and the annual temperature ranges from 9 up to 14 degree centigrade [12]. Kebele has 2236 ha of total area, from this 2049 ha is used for crop production. Among the crops produced in this *Kebele* wheat, legumes, *teff* and garlic are the major ones. The major garlic pests were aphids, cutworm, and rust. The soil type of the district in general and the research site in particular is vertisoil and alfisoil [12]. The field site represents an important garlic growing and garlic rust prone region in Ethiopia.

# Description of the Experimental Materials

Garlic bulb (Tseday variety) was used as variety, and Propiconazole fungicide (Tilt 250EC) was used as a rate and frequency treatments for garlic rust control.

**Table 1.** Descriptions of experimental materials

	<b>Table 1.</b> Descriptions of experimental materials								
Trade name	Active	Mode of action	Product	Product	Diluting				
	ingredient		formulation	rate (L/ha)	water (L/ha)				
Propiconazole	Triazole	Systemic,	Emusifiable	0.5L	1000L				
(Tilt 250EC)		protective and	concentration						
		curative	(EC)						
Garlic variety (Tseday local variety)									

## Experimental Treatments, and Design

There were thirteen (13) treatment combinations; three rate (0.65, 0.75, 1L/ha), four frequencies (1, 2, 3, 4 times) of Tilt 250EC fungicide, and one control with a total of 39 experimental units. The study was conducted using a factorial Randomized Complete Block Design (RCBD) with three replications. Each plot was consist of 6 rows with 2m length. The space between plants, rows, plots and replication was 0.1m, 0.3m, 0.5m, and 1m wide, respectively. The net plot size (the middle four rows) was 1m x 2m (2m<sup>2</sup>), and the total gross area was 160m<sup>2</sup>.

Table 2. Treatment combination used for field experiment at Enebsie Sarmider

No.	Tilt 250EC Rates (L ha <sup>-1</sup> )	Tilt 250EC frequencies	Treatment combination
1	0.65	1	0.65 + 1  time
2	0.65	2	0.65 + 2  times
3	0.65	3	0.65 + 3  times
4	0.65	4	0.65 + 4  times
5	0.75	1	0.75 + 1  time
6	0.75	2	0.75 + 2  times
7	0.75	3	0.75 + 3  times
8	0.75	4	0.75 + 4  times
9	1	1	1 + 1 time
10	1	2	1 + 2  times
11	1	3	1 + 3 times
12	1	4	1 + 4 time
13	0	0	0 + 0 time (Control)

## Experimental Management Procedures

The design and the bed of the experiment were arranged and prepared appropriately. Uniform size garlic bulbs were planted in well prepared bed and a recommended 0.04kg urea fertilizer (half at planting and half after one month of first application), and 0.04kg NPS fertilizer (applied at planting time) were applied for each plot. The application of the fungicide began from the onset of garlic rust disease on the garlic plants. During fungicide sprayed 2m height plastic sheet was used to prevent spray drift within plots. A manual knapsack sprayer was used for applying the fungicide. Other agronomic practices were applied uniformly to each plot at the recommended level on hectare based.

## Data Collected

#### Disease Parameters

Disease incidence

Garlic leaves with symptoms of rust disease was counted and expressed in percent (%) infection, and disease incidence was calculated by using the following formula (Eqn. 1).

$$\frac{\text{Number of diseased plants}}{\text{Total plants observed}}*100$$

## Eqn. 1

Disease severity

Garlic rust severity was assessed on 10 randomly selected and pre tagged plants of the middle rows, and it was collected at 7 days interval from the time of disease appearance until the crop attained its physiological maturity (Eqn. 2).

$$\frac{\text{Area of plant part covered by disease}}{\text{Total area of plant parts observed}}*100$$

## Eqn. 2

Disease severity was rated by estimating the percentage of leaf area diseased using standard disease scoring scales of 0-5 rust severity, where, 0 = non-infected, 1 = 1-10%, 2 = 11-25%, 3 = 26-50%, 4 = 51-75%, and 5 = 76-100% of the leaf surface covered with lesion [13]. The scores were changed into percentage severity index (PSI) for analysis by using the following formula (Eqn. 3) [14].

$$\frac{Sum\ of\ numerical\ ratings}{Total\ number\ of\ plants\ scored\ *\ maximum\ score\ of\ the\ scale}*100$$

## Eqn. 3

The Area under Disease Progress Curve (AUDPC)

AUDPC values were calculated from severity for each treatment data by using the following formula (Eqn. 4) and the value was expressed in % per days [15]:

AUDPC = 
$$\sum_{i=1}^{n-1} \frac{x_{i+1} + x_i}{2} (t_i + 1 - t_i)$$

## Eqn. 4

Where, X<sub>i</sub> is the cumulative disease severity expressed as a proportion at the i<sup>th</sup> assessment; t<sub>i</sub> is the time (days after planting) at the i<sup>th</sup> observation and n is total number of assessment.

Disease progress rate

Logistic model (Eqn. 5) were used for estimation of disease progression parameters from each treatment [16]. The transformed data was regressed over time (DAS). The goodness of fit of the model was tested based on the magnitude of the coefficient of determination (R<sup>2</sup>) and residuals (SE) obtained.

Disease progress rate (Unit per day) = 
$$\text{Ln}(\frac{Y}{(1-Y)})$$

## Eqn. 5

**Percent disease control** (%): It was calculated by using the following formula (Eqn 6):

$$PDC (\%) = ((PST - PSU)/PSU) *100$$

## Eqn. 6

Where, PST = Percent severity in the treated plot, PDC = Percent disease control, and PSU = Percent severity in the untreated plot.

## Growth and Yield Related Parameters

The plant heights, bulb weight, bulb diameter, number of cloves, and clove weight of 10 randomly selected and pre tagged plants were measured. Total bulb yield weight of the middle four row plants were measured by using balance and the results transformed to tone per hectare. The relative yield losses of each treatments were determined as a percentage of that of the sprayed plots of the respective variety. Losses were calculated separately for each of the treatments with different levels of disease by using the following formula (Eqn. 7) [17].

RYL (%) = 
$$\frac{(Y1 - Y2)}{Y1} * 100$$

## Eqn. 7

Where, RL% = relative yield loss, Y1 = plots with maximum protection, Y2 = unsprayed plots or sprayed plots with varying level of disease.

Relative yield advantage calculated by the following formula (Eqn. 8) [17].

RYA (%) = 
$$\frac{\text{Bulb yield of protected plot} - \text{Bulb yield of control}}{\text{Bulb yield of control}} * 100$$

Eqn. 8

## Data Analysis

The data was subjected to analysis of variance (ANOVA) by the methods described by Gomez, [18] using SAS software 9.4 version [19]. Least Significance Difference (LSD) was used to separating mean differences among treatments. Disease infection rate was subjected to analysis of variance (ANOVA) to compare the digressive or progressive phase of the disease among treatments. The association of disease, yield and yield components was evaluated by using Pearson correlation analysis to explain the degree of their relationship based on correlation coefficient (r) value. Linear regression model was used to predict the relationship between AUDPC and bulb yield.

## Cost Benefit Analysis

Cost data were collected to compare the economic advantage of treatment combination. These included variable input costs and cost for the fungicide and labor during the implementation of the experiment. The cost benefit analysis was done according to CIMMYT, [20] to calculate the incomes and expense of each treatment used in the experiment. The cost to daily Labour was 130 birr per day. The selling price bulb yield during harvesting season of the local market was taken as 60 birr per kilogram. To calculate gross income, yield obtained from each treatment were adjusted by 10%. Adjusted total bulb yield adjusted downward by a10% to reflect the difference between the experimental yield and yield of the farmer (Eqn. 9). Total variable cost, gross benefit, net benefit (NB) (Eqn. 10), and marginal rate of return (MRR) (Eqn. 11) were the attributes used in the partial budget analysis.

Adjusted total bulb yield (ADY) = Average bulb yield — (Average bulb yield \* 0.1).

Eqn. 9

Net field benefits (NBs) = Gross benefit — Total input cost

Eqn. 10

 $MRR(\%) = \frac{Marginal \text{ net benefit}}{Total \text{ input cost (Marginal cost)}} * 100$ 

**Eqn. 11** 

## **RESULTS AND DISCUSSIONS**

## Garlic Rust Onset and Incidence

Garlic rust disease first appeared 65 days after planting with different level of incidence in each plot. At the first date of assessment, the highest incidence was 46.667%, whereas the lowest disease incidence was 34.417% (Table 3). The findings were consistent with the research conducted by Admasie *et al.* [21], who observed that the rust disease symptoms emerged 67 days after planting, and a high disease incidence (96.67%) was recorded during the initial evaluation date. Furthermore, Endalew *et al.* [9], discovered that the use of fungicides and different garlic cultivars, such as local, Tseday, and Chelenko-1 cultivars, had a significant impact on the growth of garlic rust.

Table 3. Garlic rust disease onset at Enebsie Sarmider district during 2022 cropping season

Treati	ments	Mean incidence (%)
Rate (L ha <sup>-1</sup> )	0.65	39.167 <sup>ab</sup>
	0.75	39.167 <sup>ab</sup>
	1	$35.250^{b}$
	Control	$46.667^{a}$
Frequency	1	45.833 <sup>a</sup>
	2	$37.000^{b}$
	3	34.417 <sup>b</sup>
	4	$40.000^{\mathrm{ab}}$
LSD (0.05)		7.67
CV (%)		23.47

CV = Coefficient of variation, LSD = List significant difference, L = Liter, 1-4 = Tilt 250EC applications

Analysis of variance revealed that different rate and spraying frequency of Tilt fungicide showed a significant difference (P < 0.05) on disease incidence at different date of assessments. At the final date of assessment, the lowest disease incidence (50%) was recorded from plots treated with one liter and four times spraying frequency, whereas the highest disease incidence (100%) was recorded from untreated plots (Table 4). The result in agreed with the study of Admasie  $et\ al.\ [21]$ , who found that plots treated with a 1.25 L rate in two spraying sessions had the lowest disease incidence (70%), while the highest disease incidence (100%) occurred in plots treated with a 0.75 rate in three spraying sessions. Additionally, Endalew  $et\ al.\ [9]$ , who stated that the application of Tilt 250EC fungicide had a significant effect on garlic rust.

**Table 4.** Interaction effect of rate and fungicide application frequency on garlic rust incidence at Enebsie Sarmider district during 2022 cropping season

	Treatments	Disease incidence at different date of assessment				
Rates (L ha <sup>-1</sup> )	Tilt 250EC Frequencies	72 DAS	79 DAS	86 DAS	93 DAS	
0.65	1	$70.000^{b}$	$100.00^{a}$	$100.00^{a}$	100.00 <sup>a</sup>	
	2	56.667 <sup>cde</sup>	$80.00^{b}$	$100.00^{a}$	$100.00^{a}$	
	3	$40.000^{\rm f}$	$76.67^{bc}$	$100.00^{a}$	$100.00^{a}$	
	4	$46.667^{ef}$	$53.33^{fg}$	56.67 <sup>d</sup>	63.33°	
0.75	1	66.667 <sup>bc</sup>	$100.00^{a}$	$100.00^{a}$	$100.00^{a}$	
	2	$46.667^{ef}$	93.33 <sup>a</sup>	$100.00^{a}$	$100.00^{a}$	
	3	$60.000^{bcd}$	$70.00^{cd}$	86.67 <sup>b</sup>	96.67ª	
	4	53.333 <sup>de</sup>	$60.00^{\rm ef}$	66.67°	$70.00^{\circ}$	
1	1	56.667 <sup>cde</sup>	96.67 <sup>a</sup>	96.67 <sup>a</sup>	$100.00^{a}$	
	2	$46.667^{ef}$	83.33 <sup>b</sup>	$100.00^{a}$	$100.00^{a}$	
	3	56.667 <sup>cde</sup>	63.33 <sup>de</sup>	83.33 <sup>b</sup>	83.33 <sup>b</sup>	
	4	46.66 <sup>ef</sup>	$50.00^{g}$	$50.00^{d}$	$50.00^{d}$	
	Control	83.333 <sup>a</sup>	$100.00^{a}$	$100.00^{a}$	$100.00^{a}$	
LSD (0.05)		11.260	4.1579	3.9809	4.1579	
CV (%)		11.05	6.03	5.32	5.47	

CV = coefficient of variation, LSD = List significant difference, DAS = Days after sowing, L = Liter, 1-4 = Times of Tilt 250EC applications

## Garlic Rust Severity

The analysis of variance revealed that different rate and spray frequency of Tilt 250EC fungicide showed significant difference (P < 0.05) on disease severity. The maximum level of disease severity (96.667%) was recorded from control plot followed by 78% from plots treated with 0.65L ha<sup>-1</sup> rate within one time application (Table 5). During each disease assessment, the minimum disease severity level of garlic rust was recorded from plots treated with all rates

within four times spraying frequency. This finding agreed with work of Tilahun et al. [22], who reported that the maximum disease severity (89.9%) was recorded from untreated plot, whereas the lowest disease severity (0%) was recorded from a plot treated 0.75L ha<sup>-1</sup>. Furthermore, Admasie et al. [21], who found that the control plots had the highest garlic rust severity (79.33%), whereas the plots treated with 1.5 liters and sprayed three times had the lowest disease severity (17.33%).

Table 5. Interaction effects of Tilt rates and frequency on garlic rust severity at Enebsie Sarmider

district	during	2022	cropping	caacan	
aistrict	auring	ZUZZ	cropping	season	

usinet during 2022 cropping season								
	Treatments	Diseas	Disease severity at different day of assessments					
Rate (L	Tilt 250EC frequency	72 DAS	79 DAS	86 DAS	93 DAS			
ha <sup>-1</sup> )								
0.65	1	22.000 <sup>b</sup>	38.000 <sup>b</sup>	56.667 <sup>b</sup>	78.000 <sup>b</sup>			
	2	$20.667^{bc}$	35.333 <sup>b</sup>	$50.000^{cd}$	71.333°			
	3	$20.667^{bc}$	$32.000^{\circ}$	$40.667^{e}$	47.333 <sup>f</sup>			
	4	$20.00^{bcd}$	29.333 <sup>cde</sup>	$36.667^{fg}$	41.333gh			
0.75	1	$20.667^{bc}$	35.333 <sup>b</sup>	52.667°	$76.667^{\rm b}$			
	2	$5.333^{e}$	27.333 <sup>def</sup>	$42.000^{e}$	$66.000^{d}$			
	3	19.333 <sup>cd</sup>	$30.000^{cd}$	$38.667^{ef}$	$45.333^{fg}$			
	4	$18.667^{cd}$	$27.333^{def}$	33.333gh	$37.333^{hi}$			
	1	$18.000^{d}$	31.333°	$48.667^{d}$	71.333°			
1	2	14.667 <sup>e</sup>	$26.667^{ef}$	41.333e	$60.000^{e}$			
	3	$18.000^{d}$	27.333 <sup>def</sup>	$34.667^{gh}$	39.333 <sup>h</sup>			
	4	$18.000^{d}$	$26.000^{\rm f}$	31.333 <sup>h</sup>	$34.000^{i}$			
	Control	25.333a	45.333a	69.333ª	96.667 <sup>a</sup>			
	LSD (0.05)	2.147	2.8807	3.3949	4.4003			
	CV (%)	6.31	5.06	4.17	4.01			

CV = Coefficient of variation, LSD = List significant difference, L= Lite, DAS = Days after sowing, 1-4 = Times of Tilt 250EC applications

## Terminal Garlic Rust Severity

The analysis of variance revealed that the interaction effect of rate and frequency showed a significant (P < 0.05) effect on terminal disease severity. At the final disease assessment, the highest terminal disease severity (96.667%) was recorded from untreated plot, whereas the lowest terminal severity (34%) was recorded from a plot treated with one liter and four times application (Table 6). Among spraying frequencies four times application frequency of fungicide was found most effective against rust and it gave the highest disease control. Fungicide application frequencies were effective in reducing the severity of rust disease in the current study. This finding agreed with the finding of Admasie et al. [21], who reported that the highest (79.33%), and lowest (17.33%) terminal disease severity was recorded from untreated plot and a plot treated with three times spraying frequency, respectively.

## Percent disease control

In the case of percent disease control, a combination of rate and application frequency had a positive advantage on garlic rust severity reduction. The highest percent disease control (64.84%) was recorded from plots received one L ha<sup>-1</sup> rate with four-time application, while a lowest percent disease control (19.34%) was recorded from plots treated with 0.65L ha<sup>-1</sup> rate with one time application of fungicide, respectively (Table 6). A rate of one L ha-1 with fourtime application of Propiconazole (64.84%) followed by 0.75L ha<sup>-1</sup> (61.43%) gave a maximum advantage for garlic rust disease control over other treatments and untreated plots. The result in lined with the finding of Admasie et al. [21], who reported that bulb yield increased in a high range over untreated plots when garlic was treated with foliar fungicide, Tilt 250EC and up to 71.07% yields loss was prevented by applying fungicides on garlic crop.

**Table 6.** Combined effects of rate and frequency on garlic rust terminal severity, percent disease control and area under disease progress curve

		<u>rea unaer aisease</u>	1 0	1.17D.D.G. (0)
<u> </u>	reatments	Terminal	Percent disease	AUDPC (% per
Rate (L ha <sup>-1</sup> )	Tilt 250EC frequency	severity (%)	control (%)	days)
0.65	1	$78.000^{b}$	19.34	205.83 <sup>b</sup>
	2	71.333°	26.27	188.33 <sup>cd</sup>
	3	47.333 <sup>f</sup>	51.09	149.83 <sup>ef</sup>
	4	$41.333^{gh}$	57.29	$137.17^{gh}$
0.75	1	$76.667^{b}$	20.68	197.17 <sup>bc</sup>
	2	$66.000^{d}$	31.75	159.83 <sup>e</sup>
	3	45.333fg	53.15	$143.17^{fg}$
	4	$37.333^{hi}$	61.43	126.83 <sup>hi</sup>
	1	71.333°	26.27	180.83 <sup>d</sup>
1	2	$60.000^{\rm e}$	37.95	$152.50^{ef}$
	3	39.333 <sup>h</sup>	59.36	$127.83^{hi}$
	4	$34.000^{i}$	64.84	$119.17^{i}$
Control		96.667 <sup>a</sup>	0	249.17 <sup>a</sup>
LSD (0.05)		4.40		11.08
CV (%)		4.01		3.69

CV = Coefficient of variation, LSD = List significant difference at 5%, 1-4 = Times of Tilt 250EC applications

## Area under Disease Progress Curve

The analysis of variance showed that a significant (P < 0.05) difference among treatments on AUDPC. The highest AUDPC (249.17% days<sup>-1</sup>) was recorded from untreated plot, whereas the lowest AUDPC (119.17% days<sup>-1</sup>) was recorded from plots treated with 1L ha<sup>-1</sup> of fungicide with four times frequency (Table 6). These showed that Tilt 250EC spraying frequencies and rates had a different effect on garlic rust development. The present study was supported by Tilahun *et al.* [22], who found that the maximum AUDPC was recorded from untreated control plot. Additionally, Admasie *et al.* [21], who reported that the highest AUDPC (1,860%-days) was recorded from the control plots.

## Disease Progress Curve and Infection Rate

The analysis of variance showed that a significant (P < 0.05) difference among treatments on progress curve and infection rate (Fig. 1 and Table 7).

The highest disease progress rate (0.3714 units per day) was recorded from unsprayed plot and the result revealed that the disease progress rate was faster on unsprayed control plots, whereas the lowest disease progress rate (0.0173 units per day) was recorded from a plot treated with 1L ha<sup>-1</sup> rate within four times frequency (Table 7). This result in agreed with the study of Endalew *et al.* [9], who demonstrated that the disease progression rate in unsprayed plots was significantly higher (0.3321-unit day-1) compared to treated plots.

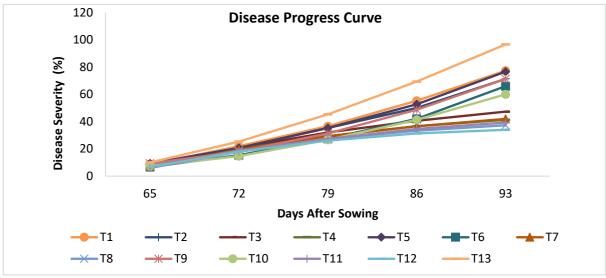


Figure 1. Garlic rust progress curve at Enebsie Sarmider district during 2022 cropping season.

<u>Hint:</u> T1= 0.65L + 1 time frequency, T2 = 0.65L + 2-times frequency, T3 = 0.65L + 3 times frequency, T4 = 0.65L + 4 times frequency, T5 = 0.75L + 1 times frequency, T6 = 0.75L + 2 times frequency, T7 = 0.75L + 3 times frequency, T8 = 0.75L + 4 times frequency, T9 = 1L + 1 times frequency, T10 = 1L + 2 times frequency, T11 = 1L + 3 times frequency, T12 = 1L + 4 times frequency, T13 = Control treatments, L = Liter.

**Table 7.** Interaction effects of Tilt 250EC rate and application frequency on disease infection rate (units per day) in each interval of fungicide application frequency

T	reatments	Infection rate in each interval of days of					
		assessment					
Rates (L ha <sup>-1</sup> )	Tilt 250EC frequency	72 DAS	79 DAS	86 DAS	93 DAS		
0.65	1	$0.1561^{bcd}$	$0.1084^{b}$	$0.1084^{b}$	0.1555 <sup>b</sup>		
	2	$0.1441^{cd}$	$0.0871^{c}$	$0.0871^{c}$	$0.1322^{b}$		
	3	0.1861 <sup>a</sup>	$0.0537^{d}$	$0.0537^{d}$	$0.0388^{c}$		
	4	$0.1656^{abc}$	$0.0475^{d}$	$0.0475^{d}$	$0.0280^{c}$		
0.75	1	$0.1333^{de}$	$0.1016^{bc}$	$0.1016^{bc}$	$0.1428^{b}$		
	2	$0.1340^{de}$	$0.0936^{bc}$	$0.0936^{bc}$	$0.1410^{b}$		
	3	$0.1594^{abcd}$	$0.0551^{d}$	$0.0551^{d}$	$0.0392^{c}$		
	4	$0.1415^{cd}$	$0.0407^{d}$	$0.0407^{d}$	$0.0250^{\circ}$		
1	1	$0.1089^{e}$	$0.1045^{bc}$	$0.1045^{bc}$	$0.1379^{b}$		
	2	$0.1118^{e}$	$0.0945^{bc}$	$0.0945^{bc}$	$0.1080^{b}$		
	3	$0.1765^{ab}$	$0.0491^{d}$	$0.0491^{d}$	$0.0286^{c}$		
	4	$0.1470c^{d}$	$0.0373^{d}$	$0.0373^{d}$	$0.0173^{c}$		
Control		$0.1594^{abcd}$	$0.1433^{a}$	0.1433 <sup>a</sup>	$0.3714^{a}$		
LSD (0.05)		0.0293	0.0185	0.0185	0.0490		
CV (%)		11.73	12.29	12.29	19.03		

CV=Coefficient of variance, LSD=List significant, DAS= Days after sowing, 1-4 = Times of Tilt 250EC applications

## Plant Height

The analysis of variance revealed that Tilt 250EC frequency had a significant (P < 0.05) effect on plant height. The highest plant height (57.942 cm) was recorded from plot treated with four times spraying frequency, whereas the lowest plant height (49.033 cm) was recorded from unsprayed plot (Table 8). This finding was in line with the finding of Tilahun *et al.* [23], who reported that significant difference was recorded on garlic plant height among fungicide spray frequencies.

**Table 8.** Main effect of fungicide frequency on garlic plant height at Enebsie Sarmider during 2022 main cropping season

Tilt 250EC frequency	Plant height (cm)	
1	51.883 <sup>b</sup>	
2	52.375 <sup>b</sup>	
3	54.617 <sup>ab</sup>	
4	57.942 <sup>a</sup>	
Control	49.033 <sup>c</sup>	
LSD (0.05)	4.2061	
CV (%)	9.33	

LSD=List significant difference, CV=Coefficient of variation, 1-4 = Times of Tilt 250EC applications

## **Bulb Weight**

The analysis of variance showed that the combined of rate and spraying frequencies had a significant (P < 0.05) effect on bulb weight. The maximum bulb weight (42.74 and 41.9g) was recorded from four times application of fungicide with one and 0.75L ha<sup>-1</sup>, respectively. On other hand the minimum bulb weight (11.52g) was obtained from untreated plot (Table 9). Comparatively better bulb weight was obtained from a treatment received four times application frequency than other treatments. Similarly, the average weight of bulbs harvested from plots treated with fungicide was significantly (P < 0.05) higher than the weight of bulbs that were harvested from unsprayed plots [24]. All the fungicide-treated plots gave higher bulb weight than the unsprayed plots [25]. Additionally, Admasie *et al.* [21], who noted that the application of fungicide resulted in an increase in bulb weight compared to unsprayed plots.

#### **Bulb Diameter**

The analysis of variance showed that the integrated of rate and spraying frequencies had a significant deference (P < 0.05) on bulb diameter. The maximum bulb diameter (4.48 cm) was recorded from 0.75L ha<sup>-1</sup> with three times spraying frequency. On other hand the minimum bulb diameter (2.58 cm) was obtained from untreated plot (Table 9). The present study was in line with the study of Habtewold *et al.* [11], who stated the highest bulb diameter (3.17 cm) was recorded from Tilt 250EC sprayed plot, while the smallest bulb diameter (2.65 cm) was obtained from unsprayed plots. Similarly, Admasie *et al.* [21], who stated that the largest bulb diameter (4.7 cm) was obtained from plots treated with 0.5 liters of fungicide, while the smallest bulb diameter (3.63 cm) was recorded from control plots.

# Clove Weight

The integrated effect of rate and spraying frequencies revealed that a significant (P < 0.05) effect on clove weight. The highest average clove weight (2.55g) was recorded from 0.75L ha<sup>-1</sup> with four times spraying frequency, whereas the lowest clove weight (0.93g) was recorded from unsprayed plot (Table 9). This finding agreed with the work of Admasie *et al.* [21], who reported that all fungicide treated plots gave the highest clove weight compared to control plots.

## Clove Numbers

The integrated effect of rate and spraying frequencies revealed that a significant (P < 0.05) difference on clove number. The highest average number of clove (17.73) was recorded from plots treated with 0.75L ha<sup>-1</sup> within three times frequency, whereas the lowest clove numbers (13.26) was recorded from unsprayed plot (Table 9). This finding agreed with the finding of Habtewold *et al.* [11], who reported that loss in cloves number per bulb was 33.6% on control plots compared with Tilt 250EC sprayed plot.

## **Bulb Yield**

Analysis of variance showed that the combined effect of rate and frequencies had a significant (P < 0.05) difference on bulb yield. The maximum bulb yield (12.79 t ha<sup>-1</sup>) was recorded from a treatment treated with four times application within a rate of 1L ha<sup>-1</sup> Tilt 250EC and in the same frequency within a rate of 0.75L ha<sup>-1</sup> (12.54 t ha<sup>-1</sup>), respectively (Table 9). The the current study revealed that in Ethiopia especially prone areas it is not possible to grow garlic crop and obtain better yield without fungicide application. Different studies from different areas demonstrated that bulb yield increase in garlic due to fungicide application. Admasie *et al.* [21], who reported that bulb yield increased in a high range over untreated plots when garlic was treated with foliar fungicide, and up to 71.07% yields loss was prevented by applying fungicides on garlic crop. Accourding to Habtewold *et al.* [11], who found that Rex Duo and Propiconazole fungicides were effective in controlling rust and produced the highest garlic yields. Furthermore, a yield advantage of up to 106% was achieved in plots treated with Rex Duo compared to unsprayed plots.

**Table 9.** Interaction effect of rates and Tilt 250EC application frequency on garlic bulb weight, diameter, clove weight, clove number, and bulb yield at Enebsie Sarmider during 2022 cropping

			season			
Trea	tments	Bulb	Bulb	Clove	Clove	<b>Bulb Yield</b>
Rates (L	Tilt 250EC	Weight (g)	Diameter	Weight (g)	Number	(t/ha)
<b>ha</b> -1)	frequency		(cm)			
0.65	1	14.247 <sup>ef</sup>	2.76 <sup>ecd</sup>	1.23 <sup>cde</sup>	11.63e	4.26 <sup>ef</sup>
	2	$16.450^{\text{def}}$	$2.93^{\rm ecd}$	1.23 <sup>cde</sup>	15.86 <sup>abcd</sup>	$4.92^{def}$
	3	23.277°	$3.53^{b}$	$1.60^{c}$	13.63 <sup>cde</sup>	$6.96^{\circ}$
	4	$32.507^{b}$	$4.14^{a}$	$2.04^{b}$	$16.86^{ab}$	$9.73^{b}$
0.75	1	$16.810^{\text{def}}$	$3.09^{bcd}$	$1.19^{\text{cde}}$	13.86 <sup>bcde</sup>	5.03 <sup>def</sup>
	2	17.773 <sup>cde</sup>	$3.03^{\text{ecd}}$	$1.20^{\text{cde}}$	14.83 <sup>abdc</sup>	$5.32^{\text{cde}}$
	3	$38.233^{ab}$	$4.48^{a}$	$2.22^{ab}$	17.73 <sup>a</sup>	$11.45^{ab}$
	4	$41.900^{a}$	$4.44^{a}$	2.55 <sup>a</sup>	16.56abc	$12.54^{a}$
1	1	$17.600^{\text{cdef}}$	$2.66^{\mathrm{ed}}$	$1.10^{de}$	$14.20^{bcde}$	$5.26^{\text{cdef}}$
	2	$20.730^{cd}$	$3.16^{bc}$	1.31 <sup>cde</sup>	13.33 <sup>de</sup>	$6.19^{cd}$
	3	38.767 <sup>a</sup>	$3.10^{bcd}$	$1.29^{\text{cde}}$	$14.00^{\text{bcde}}$	11.60 <sup>a</sup>
	4	$42.740^{a}$	$3.23^{bc}$	$1.46^{\rm cd}$	14.23 <sup>bcde</sup>	$12.79^{a}$
Co	ontrol	$11.527^{\rm f}$	$2.58^{\rm e}$	$0.93^{e}$	13.26 <sup>de</sup>	$3.44^{f}$
LSI	D <sub>(0.05)</sub>	14.71	0.49	0.41	3.12	1.84
	7 (%)	6.14	8.96	16.46	12.74	14.32

CV = Coefficient of variation, LSD = List significant difference at 5%, 1-4 = Times of Tilt 250EC applications

## Relative Yield Loss

The yield loss assessment was calculated for all treatments relative to yield of a maximum protected plot of 1L ha<sup>-1</sup> rate with four times spraying frequency. The highest relative yield loss (73.1%) was recorded on unsprayed control plot, whereas the lowest relative yield loss was (0%) recorded from plots treated with 1L ha<sup>-1</sup> with four times spraying frequency followed by 2.267% yield loss from plots treated with 0.75L ha<sup>-1</sup> with four times spraying frequency (Table 10). The integrated rate and frequency of fungicide reduced garlic bulb yield losses as compared with unsprayed plot. This indicates how much garlic rust disease was damaging garlic plants during favorable condition when effective management practice have not been intended. According to Tilahun *et al.* [22], who stated all fungicide-treated plots reduced bulb yield losses of garlic as compared with control plots. In addition to this, Admasie *et al.* [21] reported that a 41.55% bulb yield loss was recorded from unsprayed plot and 71.07% yield increase was obtained from the maximum protected plot.

## Relative Yield Advantage

The relative yield advantage was calculated from all treatments as compared with the unsprayed control plots. The highest bulb yield advantage (271.8%) was obtained from plots treated with 1L ha<sup>-1</sup> rate within four times frequency, whereas the lowest yield advantage (23.83%) was obtained from plots treated with 0.65L ha<sup>-1</sup> within one-time spraying frequency (Table 10). The present result in agreed with the work of Admasie *et al.* [21], who reported that a 70.07% yield advantage was obtained from plot received 1.5L ha<sup>-1</sup> fungicide. Moreover, Habtewold *et al.* [11], reported that the most significant increase in garlic yield (106.08%) was observed in plots treated with Rex Duo fungicide.

**Table 10.** Relative yield loss and yield advantage of garlic rust disease at Enebsie Sarmider during 2022 main cropping season

Treatments	Adjusted bulb yield	Relative bulb yield	Relative yield advantage
	(t/ha)	loss (%)	(%)
R1×F1	4.26	66.69	23.83
$R1 \times F2$	4.92	61.53	43.02
$R1 \times F3$	6.96	45.58	102.32
$R1 \times F4$	9.73	23.921	82.84
$R2 \times F1$	5.03	60.67	46.22
$R2 \times F2$	5.32	58.40	54.65
$R2 \times F3$	11.45	10.47	232.84
$R2 \times F4$	12.5	2.267	264.53
$R3 \times F1$	5.26	58.87	52.90
$R3 \times F2$	6.19	51.60	79.94
$R3 \times F3$	11.60	9.30	237.20
$R3 \times F4$	12.79	0	271.8
Control	3.44	73.10	0

 $R1 = 0.65L \text{ ha}^{-1}$ ,  $R2 = 0.75L \text{ ha}^{-1}$ ,  $R3 = 1L \text{ ha}^{-1}$ , F = Tilt 250EC frequency, 1-4 = Times Tilt 250EC applications

## Correlation Association of Garlic Rust with Yield and Yield Related Parameters

The association of disease and yield parameters was examined by using simple correlation analysis. Disease parameters: AUDPC, final severity and rate of infection were very highly significant (P < 0.01) strongly correlated to each other (Table 11). This result also supported by the work done by Admasie *et al.* [21], who reported that the disease parameters, the final percentage severity index and AUDPC, were highly significantly (P < 0.01) correlated with each other.

Table 11. Correlation analysis between garlic rust disease, and garlic yield and yield related

	parameters								
	FS	AUDPC	RI	PH	BW	BD	CW	CN	TY
FS	1	0.97***	0.94***	-0.52**	-0.88***	-0.66**	-0.63**	-0.37*	-0.88**
<b>AUDPC</b>		1	$0.93^{**}$	-0.47**	-0.82***	-0.59**	-0.55**	-0.37**	-0.82***
RI			1	-0.52**	-0.79***	-0.61**	-0.61**	-0.34**	-0.79***
PH				1	$0.50^{**}$	$0.41^{**}$	$0.43^{**}$	$0.31^{**}$	$0.50^{**}$
BW					1	$0.70^{***}$	$0.68^{**}$	$0.48^{**}$	$1.00^{***}$
BD						1	$0.91^{***}$	$0.65^{**}$	$0.70^{**}$
CW							1	$0.44^{**}$	$0.68^{**}$
CN								1	$0.48^{**}$
TY									1

AUDPC = Area under disease progress curve, FS = Final severity, RI = Rate of infection, PH = Plant height, BW = Bulb weight, BD = Bulb diameter, CW = Clove weight, CN = Clove number, TY = Total yield, \*\*\* = Correlation is strongly correlated, \*\* = Correlation is moderately correlated, ns = Weakly correlated

Yield parameters: plant height, bulb weight, bulb diameter, clove weight, clove number, total yield was very highly significant (P < 0.01) and negatively correlated with disease parameters such as final severity, AUDPC and rate of infection (Table 11). This result agreed with the research findings of Endalew *et al.* [2021], who reported that area under disease progress curve was highly and negatively associated with all recorded growth, yield and yield contributing parameters. Similarly, Admasie *et al.* [9] reported that bulb yield was negatively correlated with percentage severity index and AUDPC. And also, he reported that bulb weight and clove weight were positively correlated with bulb yield. Additionally, Tilahun *et al.* [23], who reported that correlation analysis revealed significant (P < 0.001) negative relationships between garlic rust severity and total yield, and clove weight.

# Linear Regression between AUDPC and Total Bulb Yields

A linear regression model was used to predict the relationship between AUDPC and bulb yield. AUDPC was considered as independent variable, whereas bulb yield as dependent variable and regressed to estimate bulb yield loss due to disease. AUDPC is an analytical model to indicate the relationship of yield loss with disease effects. Thus, as AUDPC (% days<sup>-1</sup>) increases, the bulb yield decreases and goes to zero asymptotes, which indicates the inverse relationship between AUDPC and bulb yield of garlic. In general, the linear regression graph indicates that as the AUDPC increased, there was a decreasing trend in garlic bulb yield (Fig. 2). The equation of the model was Y = -0.0769x + 20.3 which indicated that, for every one unit increase in AUDPC ((% days<sup>-1</sup>) there was a corresponding 76.9t ha<sup>-1</sup> garlic yield loss that occurred at Enebsie Sarmider district during 2022 main cropping season (Figure 2). The relationship as indicated by regression model, 100% loss in garlic yield was predicted due to garlic rust (*Puccinia allii*). This result is in agreement with Admasie *et al.* [21], who reported that for every one unit increase in AUDPC, there was an increment of garlic yield loss (3.1979 kg ha<sup>-1</sup>), and predicted garlic yield loss (78.48%) due to garlic rust disease.

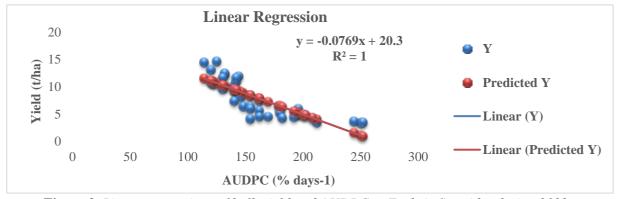


Figure 2. Linear regressions of bulb yield and AUDPC at Enebsie Sarmider during 2022.

# Cost Benefit Analysis

The result of partial budget analysis showed an increment in net benefit which resulted in higher total bulb yield. The highest net benefit (753,090-birr ha<sup>-1</sup>) was recorded from treatment of 1L ha<sup>-1</sup> within four times frequency (Table 12). This result agrees with the research result of Tilahun *et al.* [22], who reported that the highest net benefit was obtained from three times sprayed plot. The application of Rex Duo fungicide resulted in a financial gain of 46818.125 Ethiopian Birr (ETB) per hectare [11].

**Table 12.** Economic analysis of rate and spraying frequency of Tilt 250EC on the management of

agulia must at	Enghaia	Camaidan	dunina	2022	anannina saasan
gariic rusi ai	Eneosie	sarmaer	auring	2022	cropping season

TRT	TBY (t/ha)	ABY (t/ha)	PP	TIC (birr)	GB (birr/ha)	NB (birr)
R1×F1	4.74	4.26	60	7,847.5	255,600	247,752.5
$R1 \times F2$	5.47	4.92	60	9,230	295,200	285,970.0
$R1 \times F3$	7.74	6.96	60	10,522.5	417,600	407,077.5
$R1 \times F4$	10.82	9.73	60	11,860	583,800	571,940.0
$R2 \times F1$	5.59	5.03	60	8,022.5	301,800	293,777.5
$R2 \times F2$	5.91	5.32	60	953	319,200	318,247.0
$R2 \times F3$	12.73	11.45	60	11,047.5	687,000	675,952.5
$R2 \times F4$	13.95	12.54	60	12,560	752,400	739,840.0
$R3 \times F1$	5.85	5.26	60	8,460	315,600	307,140.0
$R3 \times F2$	6.89	6.19	60	10,410	371,400	360,990.0
$R3 \times F3$	12.90	11.60	60	12,360	696,000	683,640.0
$R3 \times F4$	14.22	12.79	60	14,310	767,400	753,090.0
Control	3.83	3.44	60	6,480	206,400	199,920.0

TRT = Treatments, TBY = Total bulb yield, ABY = Adjusted bulb yield, PP = Price per kilogram, TIC = Total input cost, GB = Gross benefit, MC = Marginal cost, NB = Net benefit, R1 =  $0.65L\ ha^{-1}$ , R2 =  $0.75L\ ha^{-1}$ , R3 =  $1L\ ha^{-1}$ , F = Tilt 250EC frequency, 1-4 = Times of Tilt 250EC applications

To calculate the MRR, the dominance analysis was carried out by listing the treatments in order to increase the total variable cost. According to CIMMYT, [20] any treatments that have net benefit less or equal to the previous treatment was dominated and eliminated from further analysis. The finding of the current study indicated those four times application of Tilt 250EC fungicide at seven day's interval was effective in reducing the severity of garlic rust. Even if three- and four-times spraying frequencies with all rates of Tilt 250EC were the best management options of garlic rust. But partial budget analysis revealed that 0.75L ha<sup>-1</sup> with four times frequency was the most profitable and gave maximum marginal rate of return (56,200%) compared to the other treatments (Table 13). Admasie *et al.* [21], who discovered that the optimal net benefit, marginal net benefit, and marginal rate of return were achieved by three times application of pesticides compared to untreated controls. The use of Rex Duo fungicide also provided an increased net benefit and maximum marginal rate of return (1440.6%) [11].

Table 13. Dominance analysis for garlic bulb yield affected by rate and fungicide frequencies

TRT	TIC (birr)	NB (birr)	Dominance	MRR (%)	Ranks
R2×F2	953	318,247.0		0.00	
Control	6,480	199,920.0	Dominated		
$R1 \times F1$	7,847.50	247,752.5	Dominated		
$R2 \times F1$	8,022.50	293,777.5	Dominated		
$R3 \times F1$	8,460	307,140.0	Dominated		
$R1 \times F2$	9,230	285,970.0	Dominated		
$R3 \times F2$	10,410	360,990.0		42,743.0	3
$R1 \times F3$	10,522.50	407,077.5		46,087.5	2
$R2 \times F3$	11,047.50	675,952.5		268,875.0	4
$R1 \times F4$	11,860	571,940.0	Dominated		
$R3 \times F3$	12,360	683,640.0		7,687.5	6
$R2 \times F4$	12,560	739,840.0		56,200.0	1
$R3 \times F4$	14,310	753,090.0		13,250.0	5

TVC=Total variable cost, NB=Net benefit, R1 = 0.65 liter, R2 = 0.75 liter, R3 = 1 liter, F = Tilt frequency, 1-4 = Times applications, MRR=Marginal rate of return

## **CONCLUSIONS**

Garlic is one of the most produced and edible spices in Ethiopia including Amhara region. The result of this study revealed that the integrated rate and frequency of Propiconazole fungicide showed a significant difference in disease parameters, yield and yield components. The combination of three- and four-times spraying frequency with all fungicide rates reduced disease severity with a corresponding increase in bulb yield and yield components. Plots treated four times at each fungicide rate gave the maximum bulb yield as compared to one, two- and three-times sprayed plots in all treatments. This indicates that spraying frequency is the more determining factor to control garlic rust disease than different rate of Propiconazole fungicides. However, four times application of fungicide at 7 days interval was most effective in reduction of garlic rust disease level and increased garlic bulb yield with acceptable marginal rate of returns. After economic analysis the highest MRR was obtained from a plot treated with 0.75L ha<sup>-1</sup> within four times application at low garlic rust disease pressure in the study area. The detrimental impact of fungicide application is evident, as spraying one to four times is not recommended when disease pressure is low in cultivated varieties and when it does not occur during the growing season. However, when the disease becomes severe and the variety loses its resistance over the growing period, wise application of fungicide at the prescribed rate, interval, and timing was necessary. Prior to fungicide application, farmers should monitor disease occurrence and assess infestation levels. Based on these findings, it is advised that end users in the study area and similar regions utilize 0.75L ha<sup>-1</sup> of Propiconazole fungicide with a four-time spraying frequency during severe garlic rust epidemics. To mitigate the adverse effects of fungicides, farmers should implement integrated management options to develop a sustainable disease management strategy tailored to their production system and location.

**Author contribution statement:** Yigermal Melkamu: Conceived and designed the experiments, performed the experiment, and analyzed the data; Adane Fentaye: Wrote the article, discussed, and interpreted the data. Adane Fentaye and Yigermal Melkamu: Wrote and edit the final paper.

**Data availability statement:** The data presented in this study are available upon request from the corresponding author.

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