Effect of Nitrogen and Fungicidal Spray Rates on Incidence and Severity of Garlic Rust (Puccinia allii) at Haramaya, Ethiopia

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Abstract
Garlic is one of the most important crops in eastern Ethiopia. The production of the crop is threatened by a number of biotic and abiotic factors. Garlic rust, caused by Puccinia allii, is one of the most important biotic constraints in the area. A field experiment was conducted to determine the effect of nitrogen fertilizer and propiconazole fungicide spray rates on incidence and severity of garlic rust on the variety 'Tsedey' under natural condition at Haramaya University experimental site in 2013 main cropping season. The experiment was conducted using four levels of N fertilizer (0, 46, 69, and 92 kg ha^{-1}) and four different rates of propiconazole (0, 187.5, 250, and 312.5 g a.i ha^{-1}). Propiconazole was sprayed at an interval of 10 days. The experiment was laid out as RCBD in a factorial arrangement with three replications. The fungicide treatments resulted in different levels of disease severity on the variety used. The upper two rates of propiconazole were significantly ($p \leq 0.01$) effective in reducing levels of incidence and severity of garlic rust. There was generally no significant difference between applications of both rates of propiconazole. Each of the three rates of N fertilizer affected disease levels. Disease severity of up to 80% was recorded in unsprayed plots. Since the data were generated from one season experiment, it is desirable if the experiment is repeated for one more year to generate reliable and conclusive data to use the results in sustainable, environment friendly and effective integrated garlic rust management strategies and thereby stabilizing garlic production in Ethiopia.
Keywords: AUDPC, disease incidence, disease severity, Puccina allii, propiconazole.

1. INTRODUCTION

Garlic (*Allium sativum* L.) is a member of the family Alliaceae and is produced as an annual crop for seed, fresh market and processed products (Cantwell, 2000). It is grown worldwide, with about one million hectares yielding over 10 million tons each year (Meredith, 2008).

World garlic cultivation increased from 771,000 in 1989/90 to 1,204,711 ha of land in 2007 with total production from 6.5 to 15.68 million tons, and productivity from 8.43 to 13.02 t/ha, respectively (FAO, 2007). Africa is the major producer of garlic and out of the total world production; 3.5 million is produced in the continent (FAO, 2000). In Ethiopia, it is grown over an area of 21,258.43 hecares, with an annual production of 222,547 and productivity of 10.47 tonnes per ha respectively (CSA, 2013). The production is carried out throughout the country both under irrigation and rain fed conditions in different agro-climatic conditions (CACC, 2003).

Despite its importance, garlic productivity in many parts of the world is low due to genetic and environmental factors affecting its yield and yield related traits (Nonnecke, 1989**).** Insects and fungi are the major pests of the crop in different parts of the world (Janet and Tammy, 2008). In addition, diverse crop management problems and the nature of propagation limit the supply of production too.

Among the fungal diseases, garlic rust caused by P*uccinia allii* is the major destructive disease of garlic in many countries of the World (Koike *et al*., 2001). It is also the major disease problem in almost all garlic producing regions of Ethiopia (Tesfaye and Habtu, 2003). The disease does not attack the garlic bulb directly, but its damage on the leaves has indirect effect of reducing the size and quality of the bulbs at harvest (Tahir *et al*., 2006), thereby reducing their marketability.

To control this disease, quite a few options are available. No resistant garlic varieties have been identified so far. Because of wind dispersal and easy spread of the disease, cultural practices can also hardly work. This leaves us with only one available option, the chemical control (Tahir *et al*., 2006). Findings for best control of garlic rust and increase in yield with mancozeb, triadimefon, triadimenol, propiconazole and tebuconazole have been reported (Garcia *et al*., 1994). However, empirical data indicating the effect of different rates of propiconazole fungicide application on incidence and severity of garlic rust are not available. In this connection, determining the optimum fungicide rate of the effective fungicide against garlic rust is desirable for the development of a disease management strategy.

Besides, it is well established that nitrogen availability can influence the resistance of plants to plant pathogens (Marschner, 1995). Therefore, there is a research need to evaluate different levels of N fertilizer and propiconazole application rates which would help to provide a practical solution for garlic rust problem in the area. Several field experiments conducted on wheat crop have shown that the decrement of disease severity due to fungicide application depended on N fertilizer rate in some year-location combinations (Olesen *et al*. 2000).

These interactions indicate that the optimal N fertilizer rate will depend on the success of the disease control. The general relationships reported in the literature also suggest that a reduction in N fertilizer may reduce the need for disease control. But in spite of the occurrence of the disease (garlic rust) in most parts of the country every year, no research attempts have been directed to quantify the effect of fungicide control on optimal rate of N fertilizer and vice versa on garlic rust disease development in Ethiopia in general and in the highlands of Hararghe in particular. Therefore, the objective of this study was to determine the effects of nitrogen fertilizer and fungicidal spray rates on incidence and severity of garlic rust.

2. MATERIALS AND METHODS

2.1. Description of Experimental Site

The field experiment was conducted at the Haramaya University experimental station located at 42^{o}3'E longitude, 9^{o}26'N latitude and at an altitude of 1980 meters above sea level (Anon, 1990), East Hararghe Zone, Oromia, Ethiopia.

2.2. Experimental Materials

Four different rates of propiconazole fungicide (0, 187.5, 250, and 312.5 g a.i. ha^{-1}) and four levels of N fertilizer application (0, 46, 69, and 92 kg N ha^{-1}) were used as experimental materials (treatments).

2.3. Experimental Design and Management

The experiment was laid out in 4 X 4 factorial treatment combinations of four fungicide rates and four nitrogen rate treatments using a randomized complete block design (RCBD) with three replications. The plot size used was 1.5 m x 2 m consisting of five rows. Triple super phosphate was used as standard fertilizer and applied in rows during planting at a rate of 200 kg ha^{-1}.

2.4. Disease Assessment

Garlic rust incidence and severity were assessed on weekly basis by counting the number of plants visibly diseased (for incidence), and by estimating the percentage of leaf area affected (for severity) from the three middle rows of each plot. Twenty-four plants (eight plants per row) were randomly pre-tagged and the first rust reading was taken on 16 October, 68 days after planting.

2.4.1. Disease incidence

Disease incidence was assessed when symptoms appear for the first time and it was calculated with the following formula;

Disease incidence = No. of infected plants from the samples taken/total No. of plants assessed (100)

2.4.2. Disease severity

Disease severity was rated using 24 randomly pre-tagged garlic plants in the three central rows, using standard disease scales of 1-5 rust severity, where, 1 = 1 - 10%, 2 = 11 - 25%, 3 = 26 - 50%, 4 = 51 - 75%, and 5 = 76 - 100% of the leaf surface covered with lesion (Koike *et al*., 2001) and average severity of the 24 plants per plot was used for statistical analysis. The scores were changed into percentage severity index (PSI) for analysis using the formula of Wheeler (1969).

$$PSI = Snr/Npr*Msc(100)$$

Where, Snr = the sum of numerical ratings, Npr = the number of plant rated, Msc = the maximum score of the scale. Mean disease severity from each plot was used in data analysis. The area under disease progress curve (AUDPC) was calculated for each treatment from the assessment of disease severity using the following formula (Campbell and Madden, 1990).

$$AUDPC = \sum 0.5\ [\ (x_i + x_{i+1})\ (t_{i+1} - t_i)]$$

Where, n = total number assessment times, t_i = time of the ith assessment in days from the first assessment date, x_i = percentage of disease severity at ith assessment. AUDPC was expressed in percent-days, because severity (x) was expressed in percent and time (t) in days (Shaner and Finney, 1977). AUDPC values were used in the analysis of variance to compare amount of disease among plots with different treatments.

2.5. Statistical Analysis

Data on disease incidence, severity and area under disease progress curve (AUDPC) were subjected to analysis of variance (ANOVA) to determine the treatment effects. Comparisons of means to determine the significantly different variables were conducted using least significant difference (LSD) test. All data were analyzed using General Linear Model (GLM) procedure of SAS statistical version 9.2 Software (SAS, 2009).

The data on disease severity were transformed using logistic transformation, ln[y/(1-y)] (Vander Plank, 1963), before statistical analysis. The transformed data were then regressed over time and the apparent infection rates were analyzed by analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1. Effect of Nitrogen and Fungicidal Spray Rates on Incidence and Severity of Garlic Rust

The effects of treatments involving different rates of the systemic fungicide propiconazole and different rates of the N fertilizer were evaluated in the disease parameters measured.

3.1.1. Disease onset and level of incidence

Garlic rust first appeared on 12 October 2013; 66 days after planting and assessment started on 14 October 2013. At the time of the disease appearance, all plots were not free of the disease. During the second disease assessment, however, the disease progress increased in the unfertilized plots compared with fertilized ones. The different rates of N fertilizer were significantly different in their respective reaction to the disease incidence unlike the second and third assessment dates. This might be due to the effect of the chemical sprayed 68 days after planting onwards. However, propiconazole fungicide and N fertilizer rates didn't show interaction effects in all the three assessment dates (Table 1).

Table 1. ANOVA table for garlic rust incidence at different days after planting at Haramaya during 2013 cropping season

Source	DF	Mean Squares		
		68 DAP	75 DAP	82 DAP
Replication	2	3.26	68.36	87.89
N fertilizer (N)	3	143.23***	472.01**	350.48*
Propiconazole	3	56.42^{ns}	9994.58***	11965.06***
N fertilizer*Propiconazole	9	59.32^{ns}	84.27^{ns}	200.01^{ns}
CV (%)		22.9	14.49	13.40
R^2 (%)		0.74	0.94	0.93

%CV = Coefficient of variation, R^2 = coefficient of determination, ***Very highly significant (P < 0.001), **highly significant (P < 0.01), *Significant (P < 0.05), ns = non significant and DAP = Days after planting

The different propiconazole treatments had varying effects on disease incidence. The lowest rate of propiconazole, 187.5 g ha^{-1}, did not significantly affect disease incidence regardless of the spray. Levels of disease incidence in plots treated with the higher rates (250 to 312.5 g ha^{-1}) of propiconazole were significantly lower than that of the control (Figure 1).

Propiconazole fungicide at the rate of 250 g ha^{-1}significantly reduced the rust incidence at 82 DAP as compared to all other rates and the unsprayed check plots (Figure 1). In general, treatments with propiconazole at the rate of 250 g ha^{-1} resulted in comparatively lower disease incidence than any of the dosages tested but not significantly different with the higher one i.e. 312 g ha^{-1}. This indicated that the effects of both rates of propiconazole on rust incidence were comparable in the present study.

3.1.2. Disease development

Disease progress was compared among the different foliar spray schedules using disease severity, area under the disease progress curve (AUDPC) and apparent infection rate (r). All the three methods are useful tools in quantifying disease epidemics. The apparent infection rate helps to determine whether disease develops in one treatment faster than the other, while AUDPC enables to predict yield based on the level of disease induced stress (Jones, 1998).

3.1.2.1. Disease severity

The propiconazole fungicide rate treatments were significantly different (p < 0.001) in their effects on disease severity at 110 days after planting (Table 2).

Table 2. Garlic rust severity at different days after planting at Haramaya during 2013 cropping season

Source	DF	Mean Squares						
		68 DAP	75 DAP	82 DAP	89 DAP	96 DAP	103 DAP	110 DAP
Replication	2	5.72	3.78	7.87	7.65	0.12	0.23	7.8
N fertilizer	3	1.54*	1.94^{ns}	76.97*	28.69*	19.88^{ns}	20.28**	11.5*
Propiconazole	3	0.2^{ns}	1208**	4630.7***	7260.5***	12887.7**	14265.5***	20889.2***
N*Propiconazole	9	1.54^{ns}	1.72^{ns}	26.7^{ns}	20.93^{ns}	5.96^{ns}	6.68^{ns}	4.80
CV (%)		14.5	13.5	14.9	12.0	5.9	5.9	5.7
R^2 (%)		0.69	0.98	0.98	0.99	0.99	0.99	0.99

%CV = Coefficient of variation, ***Very highly significant (P < 0.001), **Highly significant (P < 0.01), *Significant (P < 0.05), ns = non significant, R^2 = coefficient of determination and DAP = Days after planting

According to Seid and Mengistu (1993) three fungicides including propiconazole were promising as compared to the untreated check for the management of rust in garlic. Trials conducted in Europe and Brazil also showed that mancozeb and propiconazole have been used for garlic rust control (Garcia, *et al.*, 1994). The result of the present study for propiconazole fungicide is in agreement with the finding of Yonas (2010), where spraying of propiconazole at a rate of 250 g ha^{-1} on weekly basis significantly reduced garlic rust severity.

On the other hand, there was significant difference ($p \leq 0.05$) between the different rates of N fertilizer with regard to disease severity (table 2) and the disease progress was also slightly higher in plots which were not fertilized than the rest three rates (110 DAP). The maximum amount of disease severity (38.27%) was recorded on unfertilized plots, while the lowest amount of disease severity (36.11%) was observed on plots which received 46 kg ha^{-1} (Figure 2). Both the higher rate and unfertilized ones, however, had effect on the severity of the disease. The study was in agreement with the discussion by (Agrios, 2005) that adequate nitrogen fertilizer reduces the rate of infection by diseases. In contrast, the survey report of Yonas (2010) pointed out that there was no variation in both garlic rust incidence and severity among fertilized and non-fertilized fields.

One possible explanation for the discrepancy might be the soil type. According to Alemayehu (2013), the experimental soil has contents of medium total Nitrogen. Such findings signify that the soil requires application of nitrogen relative to the needs of the plant for optimum yield in particular according to the specific recommendations for the crops grown.

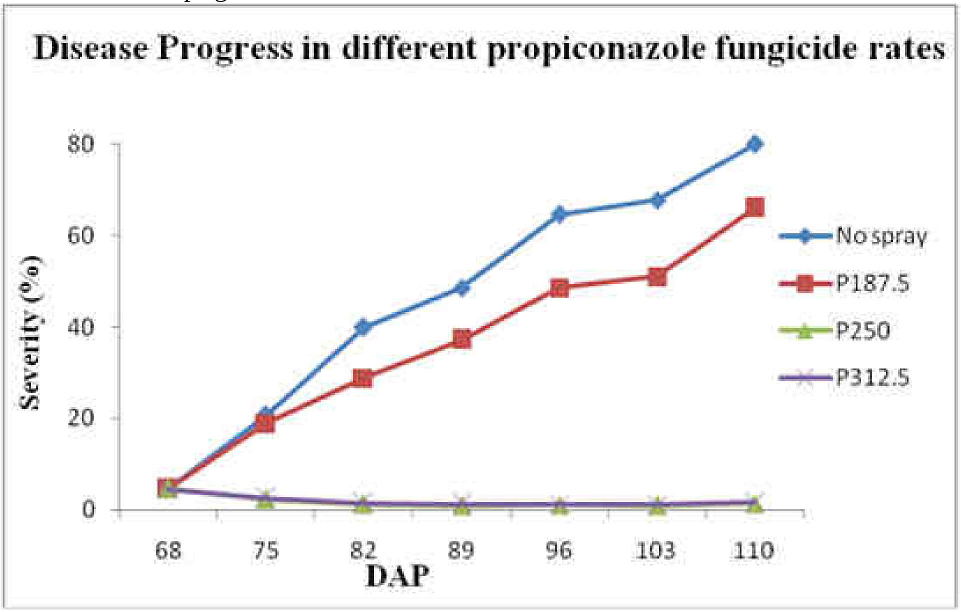

Figure 1. Rust incidence and severity on garlic treated with different rate of propiconazole fungicide during the 2013 main season

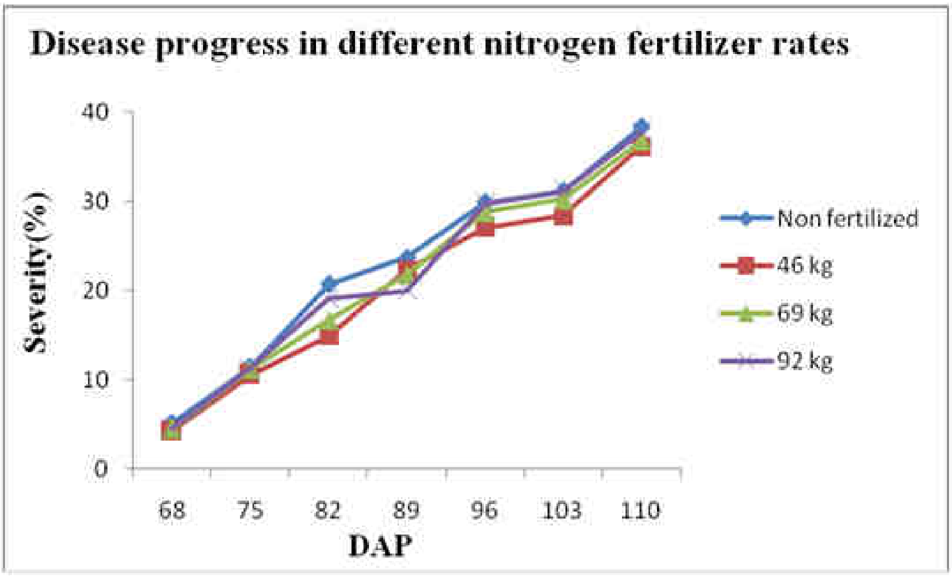

Figure 2. Garlic rust incidence and severity with different rate of N fertilizer in 2013 main cropping season

3.1.2.2. Area under disease progress curve (AUDPC)

The area under disease progress curve (AUDPC) is a very convenient summary of plant disease epidemics that incorporates initial intensity, the rate parameter, and the duration of the epidemic which determines final disease intensity (Madden *et al*., 2008). Information on disease progress is important for interpretation of epidemics and development of effective disease control measures.

Interactions between effects of N fertilizer level x fungicide spray rate treatments exhibited highly significant ($P \leq 0.01$) differences in terms of AUDPC (Table 3).

3.1.2.3. Apparent infection rate

According to Jones (1998), disease progress rate helps to determine whether disease develops in one treatment faster than the other. Based on this fact the present experiment evaluated the overall mean of all the treatment effects on disease progress rate. The rate of disease progress significantly differed among the treatments. The interactions between effects of N fertilizer level x fungicide spray rate treatments were significant ($p \leq 0.01$) in terms of reducing the rate of disease progress. Moreover, among the fungicide spray rates, lower infection rate was recorded from the plots treated with propiconazole fungicide at 250 g ha^{-1} on all levels of N fertilizer than the rest of foliar spray rates except propiconazole at 312.5 g ha^{-1}. This might be due to its being systemic fungicide with protectant and eradicant activity at this rate.

Table 3. Effect of N fertilizer and propiconazole fungicide spray rates on AUDPC and DPR of garlic rust at Haramaya during 2013 main cropping season

Treatments			
Fungicide spray rates (g a.i ha^{-1})	N fertilizer levels (kg ha^{-1})	AUDPC (%-days)	DPR (Units/day)
Control	92	2071.4^{ab}	0.109^{a}
	69	1988.8^{b}	0.104^{ab}
	46	1803.9^{c}	0.097^{bc}
	0	2089.0^{a}	0.112^{a}
	92	1553.6d^{e}	0.091^{cd}
187.5	69	1502.1^{e}	0.087^{d}
	46	1486.2^{e}	0.085^{d}
	0	1613.9^{d}	0.092^{cd}
	92	65.1^{f}	0.029^{efg}
250	69	58.1^{f}	0.025^{gh}
	46	48.5^{f}	0.019^{i}
	0	90.1^{f}	0.038^{ef}
	92	66.6^{f}	0.034^{efg}
312.5	69	64.6^{f}	0.025^{fgh}
	46	52.9^{f}	0.025^{hi}
	0	98.9^{f}	0.043^{e}
CV (%)		5.5	15.3
LSD (0.05)		84.4	0.009

AUDPC = Area under disease progress curve, DPR= Disease progress rate, CV = Coefficient of variation and LSD = Least significant difference

4. CONCLUSION

In Ethiopia, garlic (*Allium sativum* L.) is one of the most widely grown bulb crops next to onion and it is grown under a wide range of climatic and soil adaptation. Garlic yield is, however, reduced due to many factors of which diseases are economically important problems. Garlic rust, which is caused by *Puccinia allii*, is the most common and economically important foliar disease of the crop. Garlic rust was reported in Ethiopia in the late 1990's. However, no research efforts have been directed to develop suitable methods for its management in eastern Ethiopia. In the present study, systemic fungicide, propiconazole was evaluated at 187.5, 250, 312.5 g ha^{-1} rates including unsprayed control whereas N fertilizer was applied at 46, 69, and 92 kg ha^{-1} rates including unfertilized plots. The treatments were tested for their effects on incidence and severity of the garlic rust disease. The 2013 main crop season seemed to be very conducive for epidemics development of garlic rust. The fungicide treatments created different severity levels. Up to 79.99% disease severity was exhibited when the plots were left unsprayed with propiconazole while the lowest severity **(**1.30%**)** and (1.53%) was observed when propiconazloe was sprayed at 250 and 312.5 g a.i ha^{-1}, respectively, at ten days interval. These rates significantly reduced the degree of disease (AUDPC).

On the other hand, fertilizer rate treatments had differing response to the disease under natural infection. Nitrogen fertilizer rate at 46 kg ha^{-1} showed lower levels of disease incidence as well as lower levels of final severity. The highest levels of disease incidence and disease severity were obtained from unfertilized plots and plots fertilized with the higher fungicide rate (92 kg ha^{-1} propiconazole), respectively. The result showed that optimum rate of N fertilizers could produce an apparent benefit by reducing leaf senescence though they do not directly reduce the disease. Garlic rust is still capable of causing significant losses, even when garlic is grown with adequate, or more than adequate, nitrogen fertilizer. Thus, from this experiment, the mechanism and exact effect of fertilizer in reducing the development of garlic rust is not clearly understood and need further research to ascertain its role under controlled experiment in the greenhouse.

In conclusion, rust is an important production-limiting disease of garlic that calls for due attention to achieve economic management in eastern Hararghe. The results of the present study showed that substantial level of garlic rust suppression could be achieved through propiconazole fungicide treatments and to some extent through adequate rate of N fertilizer application. Thus to maintain a tolerable disease severity, fields must be fertilized with adequate level and the use of N fertilizer above 69 kg ha^{-1} should be avoided. The result of this study indicated that propiconazole fungicide at the rate of 250 g ha^{-1} provided best management of the disease. Hence, this result can be used in the management of rust by both commercial and small-scale poor farmers, provided that this finding should be confirmed through repeated experiments. Based on the current findings, it can be concluded that the use of optimum rates of nitrogen fertilizer and propiconazole fungicide would reduce the garlic rust incidence and severity.

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