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Evaluation of lentil varieties and seedbed types for the management of lentil Fusarium wilt disease (*Fusarium oxysporum* f. sp. *lentis*) in central highlands of Ethiopia

Tolesa Bedasa* and Asrat Zewdie

Ethiopian Institute of Agricultural Research, Debre Zeit Agricultural Research Center, P. O. Box 32, Debre Zeit, Ethiopia.

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An experiment was conducted at Chefe Donsa naturally infested field with *Fusarium oxysporum* f. sp. *lentis* in order to evaluate the effect of lentil genotypes and seedbed types as components of integrated management option. A factorial experiment including lentil variety and seedbed type, each at four levels, was carried out in a split-plot design with three replications. The four lentil genotypes were ILL-590 (susceptible check), Alemaya, Derash and Denbi and four seedbed types were flat bed, open raised bed, tie-raised bed and farmer's practice. Raised seedbed exhibited relatively lower disease incidence than among the seedbed types. Interaction of the used varieties and seedbed types was significant in wilt reduction. The highest wilt incidence (ca. 67.5%) was recorded on ILL-590, susceptible lentil line, planted on flat bed, whereas, the lowest (ca. 8.8%) Fusarium wilt incidence was noted on cultivar Derash planted in raised bed. A combination of cultivar Derash and raised bed resulted significantly ($P<0.05$) higher grain yield (3827.0 kg ha^{-1}) than all other treatment combinations. Significantly ($P<0.05$) lower grain yields (in the order of 68.0 kg ha^{-1}) were obtained from integration of the susceptible genotype (ILL-590) with flatbed than all other treatment integration. The highest (899.4% unit/days) in AUDPC values were observed by flat seedbed type and ILL-590, while the lowest (114.0% unit/days) in AUDPC values were obtained by raised seedbed type and Derash variety. Wilt incidence and AUDPC values were significant and negatively correlated with yield parameters. It was concluded that using moderately resistant variety (Derash) with raised seedbed significantly reduced Fusarium wilt incidence and exhibited reasonably high yields.

Keywords*:* Lentil, management, wilt, *F. oxysporum* f. sp. lentis*.*

INTRODUCTION

Lentil (*Lens culinaris medikus*) is a high value cool season pulse crop and contains about 25% protein in its seeds (Zia et al., 2011). Its production is concentrated in the northwest provinces of Australia, Bangladesh, China,

*Corresponding author: E-mail: naafsijira@gmail.com.

Ethiopia, India, Middle East, Nepal, North America, Syria and Western Asia (Abraham, 2015). Ethiopia is the leading producer of lentil in Africa, followed by Morocco and Tunisia and is seventh in the world (Abraham, 2015). Its total area and production in Ethiopia is about 113,684 ha and 0.17 million tons, respectively, with an average yield of 1.2 tons ha^{-1} (CSA, 2017). The major lentil-producing regions in Ethiopia are Oromia, Amhara, Tigray and the Southern Nations, Nationalities and Peoples' (SNNP) (Senait et al., 2006).

Lentil plays a significant role in human and animal nutrition and in maintenance and improvement of soil fertility (Sarker and Kumar, 2011). Lentil has a good potentiality for increasing farm income (Das et al., 2013). Ethiopian farmers' produce the lentil crop mainly, for food, cash income, animal feed and more importantly to restore soil fertility (Altaf et al., 2014). Farmers and their families use it to make the local *Nifro* (boiled lentil), *Sambusa* (boiled whole lentil that is roasted in oil after wrapping with paste of wheat flour), and *Shorba* (soup) and *wot* (local soup for moistening and eating along with *Injera* (flat pancake) or bread).

Lentil Fusarium wilt (FW) (*F. oxysporum* f. sp. *lentis: Fol*) plays a major role in reducing lentil yield (Pouralibaba et al., 2015) and causes severe damage to leaves, stems, roots and pods (Singh et al., 2015; 1999). This pathogen can cause infection at all stages of plant growth with more incidences at flowering and podding stages than early vegetative stage (Chavdarov, 2006). Under field conditions, the typical wilting can appear within three to four weeks after sowing in susceptible variety (Taylor et al., 2007).

The yield of lentil remains low (1.5 tons ha^{-1}) in Ethiopia (CSA, 2017) and still relatively low compared to its yield potential (3.6 tons ha^{-1}) with well managed production due to biotic and abiotic stresses (Kumar et al., 2017). This low lentil production is attributed to various diseases, insect pests, poor agronomic practices, and lack of improved cultivars and crop protection technologies (Ghazanfar et al., 2010). In Ethiopia, lentil wilt/root rot complex caused by *F. oxysporum* F. sp. *lentis, Rhizoctonia solani*, *Macrophomina phaseolina*, *Sclerotium rolfsii*, Ascochyta blight (*Didymella lentis*) and rust (*Uromyces viciae fabae*) are the most important biotic factors causing lentil yield reductions (Ahmed and Ayalew, 2006; Negussie et al., 2006).

Vertisols are characterized by severe waterlogged soil during the rainy season due to its expansion, flaking and crust formation characteristics that reduces its percolation rate (Deckers et al., 2001). Excess soil moisture and waterlogged soil creates favourable condition for the development of wilt/root rot pathogen (Midmore, 2015). This causes breakdown of host resistance to Fusarium wilt of lentil, probably through retarding phenylalanine ammonia lyase (PAL) enzyme(s) activity (Midmore, 2015). Poor germination of seed in soils at or near saturation provided limited oxygen diffusion through thick water films surrounding the seed (Richard and Guerif, 1988). The availability of water must be balanced with aeration to meet requirements for germination and crop establishment (Dasberg and Mendel, 1971). The permeability of the roots to water reduced the oxygen levels and eventually the roots lose their ability to control water movement (Braunack and Dexter, 1989).

Several attempts have been directed to minimize the effects of pathogen on plants by seedbed preparation practices that are quite rare for legumes, particularly for lentil. Inadequate seedbed preparation contributes to favorable condition for Fusarium wilt pathogen. To avoid this problem, a set of appropriate seedbed type is very important to improve the soil physical conditions and well drainage system to release excess soil moisture. Solution for this and similar other problems can be realized by cultivating improved lentil crops on appropriate seedbed to make adequate drainage in soils with excess soil moisture to prevent root diseases (Feiza et al., 2010). A number of lentil FW resistant varieties had been identified at national and international levels to manage this risk through the use of wilt-sick plot technique. Resistant varieties can be highly economical and practicable method of disease management, but varieties should be resistant to all the races prevalent in the area (Kelly et al., 1994). However, none of the control measures are found to be effective and adequate individually at field level (James and Pandey, 2017). Thus, developing an integrated disease management approach was suggested to be essential to combat Fusarium wilt of lentil for increased and sustainable yields. Therefore, the objective of this study was to evaluate the effects of lentil genotypes and seedbed preparation methods on Fusarium wilt development.

MATERIALS AND METHODS

Field experiment was conducted with naturally infested with wilt/root rot causing pathogens at Chefe Donsa research sites in 2017/2018 cropping season. Chefe Donsa is located at latitude 08°57′N, longitude 39°06″E, altitude of 2450 m.a.s.l. and average annual rainfall of about 900 mm and the mean annual maximum and minimum temperatures of 26.0 and 7.0°C. Both sites have vertisol with waterlogging problem. The experiment was laid out in split plot design (seedbed types as main plot and lentil genotypes as sub-plot) with three replications. The plot size was 3.2 m^2 (4 rows per plot) with 4 m row length. The seed rate was 800 seeds per plot. Lentil seeds were drilled by hand at a depth of 3 to 3.5 cm. Four lentil genotypes (ILL-590, Alemaya, Denbi and Derash) with varying levels of resistance and four seedbed types (flat, open raised, farmer's practice and tie-ridge seedbed) were used in the study. Alemaya, Derash and Denbi are moderately resistant varieties, and ILL-590 (susceptible check).

Data collection and analyses

Disease incidence was recorded four times at every fifteen days starting from the first appearance of disease symptoms. Complete or partial wilting plants were considered as wilted and staked to avoid double counting in subsequent assessments. Percent of wilt incidence was calculated on the basis of initial plant count and total number of diseases plants in each plot using the following formula (Chavdarov, 2006). Data were analyzed using the SAS system and means were compared using least significant difference (LSD) (SAS, 2002). Disease incidence data were transformed using monomolecular, ln (1/1-y) transformation (Campbell and Madden, 1990). Transformed data were subjected to linear regression to determine disease progress rate. Disease progress rate was analyzed using the statistical software called Minitab, version or release 15.0 for windows®, 2007. Area under progress curve (AUDPC) was calculated for each treatment from the assessment of disease incidence using the formula:

$$AUDPC = \Sigma\ [(1/2(xi+xi+1)]\ [ti+1-ti]$$

where xi = disease incidence in percentage at i^{th} assessment, ti = time of the i^{th} assessment in days from the first assessment date (Campbell and Madden, 1990).

RESULTS AND DISCUSSIONS

Significant differences (P≤0.05) were observed among varieties and seedbed preparation methods on disease mean incidence percent (Table 1). The first lentil Fusarium wilt symptom was appeared at 25 days after planting (DAP). The performance of lentil genotypes resistance to Fusarium wilt in field test with the integration of seedbed types was varied. The final wilt incidence ranged from about 20.4% for variety Derash and to 57.6% for the susceptible check (ILL 590) and wilt incidence of 23.8% was recorded for cultivar Alemaya. Wilt incidence was significantly (P≤0.05) lower on all improved lentil varieties than the wilt susceptible lentil line. Cultivars Alemaya and Derash showed significantly (P≤0.05) lower amount of infection than cultivar Denbi and susceptible check, ILL-590 (Table 1). This finding is similar to the observation by Taylor et al. (2007) and Pouralibaba et al. (2015) who found that the use of resistant varieties showed the most effective, economical and environment-friendly method to manage lentil Fusarium wilt. Teklu et al. (2006) reported that seedbed type induced the highest surface runoff as compared to farmers' practice and flat seedbed for vertisol in the central highlands of Ethiopia. This result was in accordance with the investigations of Cowie et al. (1996) who observed that persistence of wetness within rooting zone adversely affected the crop growth since legumes were too sensitive to high soil moisture.

Significant differences (P≤0.05) were observed in interaction of variety x seed bed preparation method in diseases incidence (Table 2). The highest (67.5%) final Fusarium wilt incidence was obtained by planting susceptible check, ILL-590 on flat seedbed (Table 2). This might be excess soil moisture condition predisposes resistant varieties to be easily attacked by pathogens, which are not problems during normal growing seasons and facilitating spore germination and penetration into the host by the pathogen. Similar results were reported by Isleib (2014) and Binagwa et al. (2016) who also observed higher population of Fusarium wilt fungus that can also be explained by presence of high soil moisture, poor drainage of excess soil moisture and soil compaction that favors the pathogen development.

The lowest 8.8% final Fusarium wilt incidence was obtained in planting variety Derash on the raised seedbed type (Table 2). This indicated that integration of Derash variety with raised seedbed type resulted lower wilt incidence than susceptible check with planting on the flat seedbed. The reason for such variation might be the removal of excess water from the raised beds that might have helped the drained plots to produce higher yield than the flat seedbed and enhanced the movement of required soil moisture through the root system. This result agrees with the observations made by other researchers (Srivastava et al., 2000) who indicated that many attempts were made to manage this disease using cultural, varietal, biological and chemical methods.

Area under disease progress curve (AUDPC)

The highest (899.4% - days) AUDPC values were found from the integration of flat seedbed and ILL-590, while the lowest (114.0% - days) in AUDPC values were noted in the integration of raised seedbed with the variety Derash (Table 2). The overall results indicated that integrated resistance and /moderately resistance variety and raised seedbed type practice was effective to slow down the epidemics of Fusarium wilt and to sustain lentil production and productivity which confirmed with the finding of Negussie et al. (2006) and Palti and Katan (1997) they reported that substantial reductions in plant mortality with wilt/root rots were recorded when a combination of moderately resistant varieties and drainage methods that was used in raised seedbed type.
Similarly, Merkuz and Getachew (2012) reported that growing resistant and moderately resistant varieties on raised seedbed that drained excess water with recommended seeding rate to reduce plant mortality in case of by chickpea wilt. Fusarium wilt pressure on the susceptible line and high inoculum presence exhibited major influence on disease development and reproduction in conformity with the reports of de Jensen et al. (2002) and Abawi and Ludwig (2005). They observed that root diseases were most severe in susceptible crop varieties, because the pathogen inoculum could build up quickly when favourable conditions were conducive for disease development. Hence, the present study indicated that Fusarium wilt incidence might be minimized by careful selection of resistant lentil genotypes and raised seedbed type that enforced as the most important agronomic factors to increase lentil productivity.

Disease progress rate and curve

The disease progress rate was significantly differed among varieties and seedbed practiced (Table 3). The

Table 1. Main effects of seedbed type and varieties on the lentil Fusarium wilt incidence (%) at Chefe Donsa, Ethiopia, in 2017/2018 main cropping season.

Factor		Percent of disease incidence at 15 days interval after the disease onset			
		First score	Second score	Third score	Fourth score
		DAP 25	DAP 40	DAP 55	DAP 70
Variety	ILL-590	13.2^{c}	25.1^{c}	40.4^{c}	57.6^{c}
	Alemaya	6.5^{a}	10.9^{a}	16.1^{a}	23.8^{a}
	Denbi	9.1^{b}	16.8^{b}	23.5^{b}	31.6^{b}
	Derash	7.4^{a}	12.4^{a}	15.6^{a}	20.4^{a}
Mean		7.3	13.1	25.6	28.5
LSD (0.05)		1.5	2.0	2.3	4.0
CV (%)		7.4	11.8	11.4	12.0
Bed type	Flat bed	16.4^{b}	23.3^{c}	30.9^{c}	46.0^{c}
	Raised bed	10.7^{a}	10.8^{a}	17.7^{a}	23.8^{a}
	Farmers' practice	15.8^{b}	17.1^{b}	25.4^{b}	38.0^{b}
	Tied ridge bed	11.8^{a}	14.0^{ab}	21.9^{ab}	32.8^{b}
Mean		13.7	16.3	23.9	33.6
LSD (0.05)		1.7	3.8	4.7	7.9
CV (%)		19.5	14.5	11.5	16.5

Table 2. Interaction effects of lentil variety and seedbed type on Fusarium wilt (*F.oxysporium* f.sp. *lentis*) of final disease incidence (%) at Chefe Donsa, Ethiopia, in 2017/2018 main cropping season.

Treatment combinations		Final percent of disease incidence at every 15 days interval at Chefe Donsa (Var)				
		ILL-590	Alemaya	Denbi	Derash	Mean
	Var.	PDI$_{f}^{1}$				
Bed type	Flat bed	67.5 ^{g}	35.1^{de}	39.8^{e}	31.2^{d}	67.5 ^{g}
	Raised bed	52.5 ^{f}	11.4^{a}	20.5^{bc}	8.8 ^{a}	52.5 ^{f}
	Farmers' practice	51.1 ^{f}	31.6^{de}	36.3^{de}	29.0^{cd}	51.1 ^{f}
	Tied ridge bed	59.8^{fg}	16.8^{ab}	30.1^{cd}	15.6^{ab}	59.8^{fg}
Mean		63.37	24.40	28.0	20.0	24.40
LSD (0.05)				4.9		
CV (%)				15.2		

PDI$_{f}$ ^{1} = final percent of disease incidence of lentil Fusarium wilt at every 15 days interval; LSD = Least significant difference at P ≤ 0.05; CV= Coefficient of variation; Means followed by same letter(s) within a column are not significantly different at 5% level of significance.

disease progress rates on flat seedbed, raised seedbed, tie-ridge and farmers' practice were 0.0249, 0.0107, 0.0103, and 0.0.0134 units per day for the variety ILL-590; 0.0.00699, 0.0.00400, 0.00565, and 0.00483 units per day for Derash; 0.00776, 0.0044, 0.00469 and 0.00512 units per day for Alemaya; 0.00821, 0.00432, 0.00499 and 0.0061 for Denbi respectively (Table 3).

The disease progress rate was faster (0.315) on the susceptible check, ILL-590 than on other varieties in all seedbed types. The disease progress curves of Fusarium wilt (incidence versus DAP) were sketched separately for each lentil variety (Figure 1). Fusarium wilt incidence progressively increased for each curve of all lentil varieties starting from the first typical wilt symptom appeared to the final wilt incidence recorded during the period of assessment. However, the increasing trend in the raised seedbed method was comparatively lower than in the other tested seedbed types (Figure 1).

Effect of seedbed types and varieties on lentil aboveground biomass

Aboveground biomass showed highly significant (P≤0.01) difference in the main effects and interaction effects of seedbed types and lentil varieties (Table 4). Higher

Table 3. Interaction effects of seedbed type and lentil variety on Fusarium wilt (*F.oxysporium* f.sp. *lentis*) area under disease progress curve (%-days) at Chefe Donsa, Ethiopia, during the 2017/2018 main cropping season.

Treatment combinations		Area under disease progress curve (%-days) at Chefe Donsa (Var.)				
	Var.	ILL-590	Alemaya	Denbi	Derash	Mean
Seedbed type	Flat bed	899.4^{h}	396.0^{cd}	518.0^{e}	415.2^{d}	561
	Raised bed	679.0^{fg}	155.5^{a}	295.0^{bc}	114.0^{a}	326
	Farmers' practice	679.2^{f}	423.0^{de}	446.0^{de}	352.0^{cd}	374
	Tied ridge bed	752.6^{g}	207.0^{ab}	397.0^{cd}	215.0^{ab}	376
Mean		738.6	278.9	379.9	238.9	
LSD (0.05)				62.9		
CV (%)				11.0		

LSD = Least significant difference at P ≤ 0.05; CV= Coefficient of variation; Means followed by same letter(s) within a column are not significantly different at 5% level of significance.

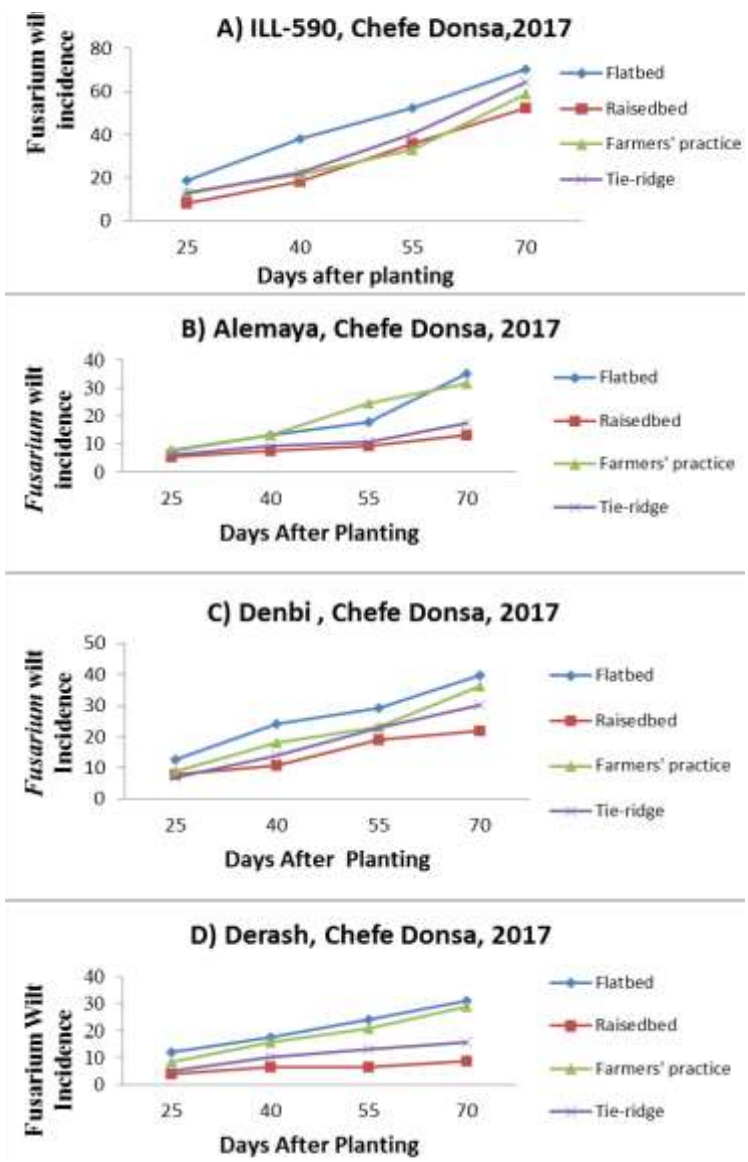

Figure 1. Fusarium wilt progress curves as influenced by seedbed types and lentil varieties at Chefe Donsa.

Table 4. Disease progress rates on lentil varieties with seedbed types at Chefe Donsa in 2017/2018 main cropping season.

Treatments		Variety	Intercept	SE of Intercept	Progress Rate	R^2
Seedbed type	Planting on the open raised bed type	ILL-590	- 0.254	0.15569	0.0107	89.9%
		Derash	- 0.0515	0.0387780	0.00400	71.3%
		Denbi	- 0.0420	0.0966788	0.00432	96.1%
		Alemaya	- 0.0367	0.086691	0.00440	72.1%
	Planting on the farmer practice seedbed type	Denbi	- 0.0755	0.0715654	0.00616	94.3%
		ILL-590	- 0.064	0.190048	0.01340	63.0%
		Derash	- 0.107	0.15111	0.00483	79.1%
		Alemaya	- 0.137	0.18332	0.00512	71.5%
	Planting on the flat seedbed type	Denbi	- 0.0526	0.141774	0.00821	64.6%
		Alemaya	- 0.159	0.14171	0.00776	81.3%
		ILL-590	- 0.253	0.31489	0.0249	84.2%
		Derash	-0.035	0.089524	0.00699	80.6%
	Planting on the tie- ridge bed type	Derash	- 0.0101	0.111725	0.00565	77.3%
		Alemaya	- 0.152	0.322408	0.00469	65.4%
		Denbi	- 0.113	0.274120	0.00499	63.3%
		ILL-590	- 0.114	0.0931541	0.0103	75.6%

aboveground biomass (1767.0 g per plot) was obtained by the integration of raised seedbed with Derash variety (Table 4). Relative to the flat seedbed, aboveground biomass yield was increased by 40.16% in the raised bed. The lowest 196.67 g per plot aboveground biomass weights of lentil were obtained from the plots planted with ILL-590 genotype on the flat seedbed (Tables 4).

Effect of seedbed types and lentil varieties on the mean grain yield

Highly significant ($P \leq 0.01$) differences were obtained in the main effects and the interaction effects of seed bed type practiced and lentil varieties at Chefe Donsa (Table 5).

The highest (3827.0 kg ha^{-1}) mean lentil grain yield was observed from plots where Derash variety was planted on raised seedbed type but, the lowest 68.0 kg ha^{-1} mean grain yield of lentil was found from the integration of ILL-590, susceptible line with flat seedbed type (Table 5). Derash variety showed better performance on all seedbed types than other varieties. Similarly, growing lentil crops on raised seedbed produced significantly superior agronomic characteristics; yield attribute traits, seed and straw yields as compared to the flat bed sown crop (Rathore et al., 2010). Absolutely this interpretation indicated that the best management option to reduce the wilt problem was approached to use improved varieties with resistance to wilt. Potential mean grain yields (3827.0, 3268.0 and 2893.0 kg ha^{-1}) of lentil were obtained when the varieties Derash, Alemaya, and Denbi were integrated with raised seedbed type respectively (Table 5). This result is in agreement with the results of Schulthess et al. (1997) who reported that significant increase in lentil grain yield vertisol with the appropriate seedbed type and improved variety were used. Similarly, Abate et al. (1993) reported 58% yield increase in durum wheat and 106% in chickpea and lentil were obtained when planted on raised bed over planting on flatbed.

Correlations of disease incidence and AUDPC with growth and yield

There was highly significant ($P \leq 0.01$) and negatively correlated between disease incidence with seed grain yield and biomass yield of lentil.

Table 5. Interaction effects of seedbed types and lentil varieties on aboveground biomass at Chefe Donsa in 2017/2018 main cropping season.

Treatment combinations	Aboveground biomass weight of lentil (gram per plot) at Chefe Donsa				
	ILL-590	Alemaya	Denbi	Derash	Mean
Flat bed	326.67[c]	1000.0[a]	1053.3[a]	1167.0[a]	854.2
Raised bed	623.0[a]	1367.0[ab]	1433.0[ab]	1767.0[b]	1197.4
Farmers' practice	423.33[a]	1200.0[a]	1216.7[a]	1500.0[ab]	1085.0
Tie ridge bed	760.0[a]	1200.0[a]	1100.0[a]	1467.0[ab]	1006.7
Mean	275.75	1191.67	1200.83	1475.0	
LSD (0.05)			228.2		
CV (%)			15.5		

LSD =Least significant difference at P ≤ 0.05; CV-Coefficient of variation; Means followed by same letter (s) within a column are not significantly different at 5% level of significance.

This indicated that the higher wilt incidence resulted in the lower lentil aboveground biomass and seed grain yield. Similarly, there was a strong negative correlation between Fusarium wilt incidence and seed grain yield, which was estimated at 8.8% yield loss for every 10% Fusarium wilt incidence (Erskine and Bayaa, 1996). It is also true for area under disease progress curve that exhibited highly significant (P≤0.01) and negatively correlated with biomass and seed grain yield. Positive association was calculated between days to 90% physiological maturity and seed yield. These results were in agreement with findings of Anjam et al. (2005) who reported that the increase in biomass would have a positive and significant effect on grain yield. Similarly, Singh et al. (1999) reported that plant height, plant biomass, branches/plant, and days to maturity resulted in significant positive correlation with grain yield.

Conclusions

Lentil is one of the most important leguminous crops widely grown in central highlands of Ethiopia. It is also a major cash crop and farmers earn high income. In Ethiopia, lentil is produced under a wide range of altitude from 1600 to 2700 m.a.s.l. mainly in main season. Due to several biotic and abiotic factors, lentil production and productivity has been low in Ethiopia. Of all constraints, lentil wilt caused by *Fusarium oxysporum,* was one of the most economically important that tackle the lentil farming systems in growing areas.

The interactions effects of varieties by seedbed types showed significant (P<0.05) difference. The highest (67.5%) final Fusarium wilt incidence was obtained by planting susceptible check, ILL-590 on flat seedbed type, while the lowest 8.8% final wilt incidence was obtained from the integration of variety Derash with raised seedbed. The highest (736.2% - days) AUDPC was recorded on the susceptible genotype, ILL-590, followed by Denbi variety (414.0% - days). AUDPC value clearly indicated that varietal difference among the treatments. Fusarium wilt disease rate progressed rapidly on genotype ILL-590 with flatbed than the others. Association of disease incidence with yield and yield components were negatively correlated and significant and inverse relationship.

For Chefe Donsa Derash with raised bed was suggested and the uses of raised seedbed type integrated with improved variety (Derash) will be comprehensive to improve lentil production and reduce the lentil wilt disease. Thus, planting improved lentil variety on the raised seedbed type to reduced Fusarium wilt should be regarded as one facet of the integrated control program rather than used alone. In this current study, it was observed that moderately resistance variety and raised seedbed type reduced disease parameters of lentil Fusarium wilt.

CONFLICT OF INTERESTS

The author has not declared any conflict of interest.

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