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Table of Content

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Full Length Research Paper

Evaluation of bread wheat (*Triticum aestivum* L.) genotypes for resistance against stem rust (*Puccinia graminis* f. sp. *tritici*) diseases at seedling and adult stages

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Bread wheat is the most important food crops among cereals cultivated in the world and consume in various diets. However, the production of bread wheat majorly affected by fungal diseases especially rust diseases. Of the rust diseases, stem rust is the most destructive due to the frequent emerging of new races of the pathogen. The field experiment was conducted at Kulumsa Agricultural Research Debre Zeyit sub-center using 36 genotypes during 2015 cropping season, and the greenhouse experiment was carried out at Ambo Plant Protection Research Center to evaluate bread wheat genotypes for resistance/tolerance to wheat stem rust disease adult and seedling stages, respectively. For field experiment, the treatments were arranged in the randomized complete block design with three replications. Stem rust evaluations for Pgt races TTKSK, TKTTF, TRTTF and JRCQC were replicated so that a total of at least 20 seedlings from each cultivar were evaluated. At seedling stage, most of the genotypes confirm low IT ≤2 on four of the stem rust races indicating that they are resistance to the four stem rust races used. Out of these, nine of the gentypes namly genotype ETBW7178, ETBW7198, ETBW7236, ETBW7220, ETBW7161, ETBW7191 and one standard chick Dand'a has potential (IT≤1) to overcome stem rust races at sedling stage. On the experiment for adult stage, the only genotype showing strong resictance was genotype ETBW7178 (5R). The rest genotypes show moderately resistance, moderately susceptible and totally susceptible to stem rust inoculums used. These results can assist wheat breeders in Ethiopia for choosing parents for crossing in programs aimed at developing cultivars with desirable levels of stem rust resistance in collabration with International Maize and Wheat Improvement Center (CIMMYT) and will also facilitate stacking of resistance genes into advanced breeding lines.

Key words: Genotypes, inoculums, resistance, suscepitable.

INTRODUCTION

Ethiopia, with its range of altitudes, soils and climatic conditions provide ecological settings suitable for the cultivation of diverse species of wheat (Harlan, 1971). Durum wheat (*Triticum turgidum* Desf.) and bread wheat (*Triticum aestivum* L.) are, however, the two most important wheat species grown in the country although

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other species are also cultivated to a lesser extent (Amsal, 2001). Though bread wheat is believed to be a relatively recent introduction to Ethiopia (Hailu, 1991); it exhibits wider adaptation and higher yield potential than durum wheat (Amsal, 2001).

Approximately 630 million tons of wheat (*T. aestivum*) is produced in over 200 million ha of cultivation area globally (Rosegrant, 1995). Wheat contributes 21% of the food calories and 20% of the protein consumed by more than 4.5 billion people in 94 developing countries (Braun et al., 2010). However, wheat productivity is adversely affected by many biotic and abiotic stresses and rust diseases are of prime importance worldwide. Severe wheat yield losses due to stem rust, caused by *Puccinia graminis* f. sp. *tritici*, have been reported in Europe, Asia, Australia, and the United States before the middle of the 20th century (Roelfs, 1978; Saari and Prescott, 1985), making stem rust the most feared wheat disease (Singh et al., 2011). Since 1970, yield losses caused by stem rust have been minimal due to the successful deployment of stem rust resistance (*Sr*) genes (e.g., *Sr31*) in commercial wheat cultivars (Leonard and Szabo, 2005). A new virulent race of *P. graminis* f. sp. *tritici* was identified in Uganda wheat nurseries and was observed to be able to infect wheat lines carrying *Sr31*. This new race became popularly known as Ug99 after the country and year of discovery, and was reported to disperse over large distances due to windborne spores. Similarly, Ug99 was observed in 2001 in Kenya, in 2003 in Ethiopia, and in 2007 in Yemen and Iran. Therefore, stem rust is again becoming a threat to wheat production worldwide (Nazari et al., 2009).

Wheat stem rust, a devastating disease of wheat and barley caused by the fungal pathogen *Puccinia graminis* f. sp. tritici, was largely eradicated in Western world during the mid-to-late twentieth century. However, isolated outbreaks have occurred in recent years. Different scholars investigate whether a lack of resistance in modern varieties, increased presence of its alternate host barberry and changes in climatic conditions could be facilitating its resurgence. Climate changes over the past 25 years in Europe for example, suggest increasingly conducive conditions for infection. Furthermore, it was documented that the first occurrence in decades of *P. graminis* on barberry in the UK. The result from Clare et al. (2018) illustrated that wheat stem rust does occur in the UK and, when climatic conditions are conducive, could severely harm wheat and barley production. Stem rust is favored by humid conditions and warm temperatures of 15 to 35°C. The fear of black rust through history and today is understandable. Apparently, healthy crop three or four weeks before harvest can be reduced to a black tangle of broken stems and shriveled grain. Harvest losses of 100% can occur in susceptible crop varieties.

In the mid-twentieth century, devastation caused by stem rust spurred efforts to breed wheat strains that could resist the fungi. That research led by agronomist Norman Borlaug, famously led to the Green Revolution in agriculture, increasing crop yields around the world. But stem rust returned in the late 1990s and 2000s, with a variety called Ug99 that spread through Africa and parts of the Middle East. It ruined harvests and caused international concern because, Dusunceli says, more than 90% of wheat crops were susceptible to it. So far, however, it has not hit large wheat-producing regions such as Europe, China and North America. Researchers are developing resistant crops (Bhattacharya, 2017).

In Ethiopian highlands, bread wheat has been produced by small scale farmers since the introduction of the crop approximately about 5000 years ago but in recent years becuase of the emerging new races of stem rust and yellow rust, the production and productivity is highly reduced and in some case there is 100% yield losses. The highlands of western Ethiopia suitable for wheat production are in great problems due to lack of resistant varities with good yield and quality, since most of the adapated varieties became susceptible to the new emering races and reduction in productivity (Singh et al., 2015).

Strong messages emerge from the Ethiopian TKTTF experience. The presence of a race in one region and incursion into a new region can have a devastating impact. The speed with which a stem rust epidemic can develop and spread is incredibly fast. Effective control of stem rust, especially under small-holder farming systems, is virtually impossible in the absence of resistant varieties. The current stem rust situation in Ethiopia reinforces the need for effective global rust surveillance and monitoring, the critical need for the continued development and promotion of durable rust resistant varieties, and the diversification of varietal and cropping systems (Singh et al., 2015). Hence, there is a need for screening of genotypes aginst major disease and yield peroformance in order to come up with promising varieties which coud resist/tolerate the new races of stem rust pathogens with high grain yield. Therefore, the objectives of the project was to evaluate bread wheat genotypes for resistance/tolerance to wheat stem rust dieases.

MATERIALS AND METHODS

Site description

The experiments were conducted at Kulumsa Agricultural Research Debrezeyit sub-center and Ambo Plant Protection Resaerch Center during 2015 croping season for evaluation of bread wheat genotypes against stem rust races at adult and seedling stages, respectively. The sites ranged from mid to high altitude areas which favor the opportunity for different pests and diseases to occur and interact with genotypes. The annual rain fall distribution is 1800 to 2000 mm and the annual minimum and maximum temperature is 17 to 21°C. And have clay loam to loam soil types. The population of the area is engaged with mixed farming for both location (Tesfaya, 2001).

Experimental materials

Thirty six bread wheat genotypes including two standard checks selected from 121 first trial, preliminary yield trials at Shambu starting from 2012. The first 36 materials were originally obtained from Kulumsa Agricultural Research Centre National Wheat Research Coordination Centre and were promoted based on the yield and other agronomic performances in the season (Table 1).

Design and data management

At Debre-Zeyit, treatments were arranged in randomized complete block design with three replication on plot size of 5 rows × 1.2 m length × 20 cm between row spacing = 1 m^2 or on a 1.2×0.8 m area of land. The seed rate was 150 kg/ha. Treatments were subjected to grow on open field as the environment and the time of sowing used favours the infestation of stem rust in the area. At least six seedlings of each genotypes were grown in 10 × 10 cm^2 pots in Metro-Mix 200 vermiculite peat-perlite medium in a greenhouse with supplementary lighting to provide a 16 h photoperiod under controlled environment at Ambo Agricultural Research Center for seedling test against the reaction of the inoculated stem rust race.

Inoculums and inoculation

Inoculums' source and infection

Aeciospores from *Berberis vulgaris* are currently rare, but historically it was an important source of inoculums in Northern North America and Europe. Mycelium or uredinia on volunteer wheat are the most important source of inoculums in tropical and subtropical climates. Windblown urediniospores are usually from earlier maturing wheat from the south in the northern hemisphere, or from the north in the southern hemisphere. Urediniospores and aeciospore germinate when in contact with free water and infection by penetration through the stomata. Penetration requires at least a low light intensity. Germination optimum is 18°C, latent period varies from 10 to 15 days in the field with temperatures of 15 to 30°C (Berlin, 2017).

All isolates were derived from single pustule, increase in isolation, and stored at -80°C. Inoculation of *P. graminis* isolates was performed in an inoculation booth at Ambo Agricultural Research Center. Inoculum of four different races was used for stem rust inoculation. Isolates of Pgt races are described in Rouse et al. (2011). In addition, isolate 06YEM34-1 was used for race TRTTF. Inoculation and incubation were performed as described previously (Jin et al., 2007). *P. graminis* and *Puccinia triticina* urediniospores were retrieved from storage at -80°C and heat shocked at 45°C for 15 min. Spores were rehydrated by placing the capsules in an air-tight container at 80% humidity maintained by a KOH solution for 2 to 4 h. Urediniospores were then suspended in a light-weight mineral oil (Soltrol 70) and sprayed onto seedlings. Seedlings were inoculated when the first leaf was fully expanded with a suspension of urediniospores of single *P. triticina* and *P. graminis* races. The inoculation booth was washed with water between inoculations of plants with different *P. graminis* and *P. triticina* isolates in order to prevent contamination. For approximately 30 min, plants were under a fume hood for oil evaporating. Plants were kept in a 100% humidity chamber overnight and maintained in the greenhouse at 15 to 25°C with supplemental lighting after inoculation (Jin et al., 2007).

Disease assessment and data analysis

After dew chamber incubation, plants were kept in a greenhouse at the Ambo Agricultural Research Center, Cereal Disease Laboratory maintained at 18 ± 2°C for 14 days. Infection types (ITs) were classified on a 0-4 scale 12 to 14 days after inoculation on seedlings as described by Stakman et al. (1962): IT 0 = immune response, with no uredinia or necrosis; IT fleck (;) = necrotic flecks; IT1 = small uredinia surrounded by necrosis; IT2 = small uredinia surrounded by chlorosis; IT3 = moderate uredinia; IT 4 = large uredinia. Designations of + and - were added to indicate larger and smaller size of uredinia; X = a mesothetic response of flecks, small and large uredinia. Stem rust evaluations for Pgt races TTKSK, TKTTF, TRTTF and JRCQC were replicated so that a total of at least 20 seedlings from each cultivar were evaluated (Mohammadi et al., 2013).

Treatments use and experimental design for adult plant test

The experiment was arranged in RCBD with three replications. Plots having the size of 2 × 1 m was prepared. There are 10 rows per plot and the space between rows, plots and replications was 0.2, 0.5 and 1 m, respectively. To initiate sufficient disease development, known very susceptible bread wheat varieties (604) to rust was sown on the bordered of all plots. Seed of each variety was planted in each plot by hand drilling at the rate of 150 kg/ha, which was recommended for the area was used. Fertilizers at a rate of 46 kg/ha N and 46 kg/ha P_2O_5 was applied during planting. Weeds were controlled by hand weeding was carried out according the farmers' practices of the areas. Natural infection was used to initiate the epidemics of the disease.

Data collection

Diseases data

Disease incidence: Rust incidence was recorded on each experimental plot by counting number of diseased plants from 16 randomly taken and tagged plant/plot from eight central rows and calculated as the proportion of the diseased plants over the total stand count (16 plants) at 10 days interval.

Disease severity: Proportion of the stem and leaf of the plant affected by the disease, recorded using the modified Cobb's scale (Peterson et al., 1948). Starting from the appearance of the sign or symptoms, each plant within each plot was visually evaluated for percent foliar infection (severity) at 10 days interval.

RESULTS

The result of experimental analysis for seedling stage and adult stage was conducted separately and the result is shown in Tables 2 and 3, respectively.

The reaction of 36 wheat genotypes was determined, including cultivars currently and widely grown in Ethiopia, to four collected isolates of *P. graminis* f. sp. *tritici.* To combat the threat posed by Ug99, breeders require knowledge about existing sources of resistance to this race. Such information would enable wheat breeders to carefully design crosses to combine individual resistance sources into one breeding line and enhance germplasm for Ug99 resistance.

Infection types (ITs), described by Stakman et al. (1962), were assessed 14 days post-inoculation. From a practical point of view, seedling resistance genes can be

Table 1. List of bread wheat genotypes used in the study, and their pedigree and origion.

Entry	Genotype	Pedigree	Seed source
1	ETBW 7178	DVERD-2/AE.SQUARROSA(214)//2*ESDA/	IESRRL# 53
2	ETBW 7252	SAMAR-8/KAUZ'S'//CHAM-4/SHUHA'S'	IESRRL # 214
3	ETBW 7238	CROW 'S'/BOW'S' 3-1994/95//TEVEE'S'/T	IESRRL # 177
4	ETBW 7198	VAN'S/3/CNDR'S'/ANA//CNDR'S'/MUS'S'/	IESRRL# 84
5	Kubsa	Check	Breeder seed,2011
6	ETBW 7237	CROW 'S'/BOW'S' 3-1994/95//TEVEE'S'/T	IESRRL # 176
7	ETBW 7171	FOW'S'//NS732/HER/3/CHAM-6//GHURA	IESRRL# 43
8	ETBW 7208	CHAM-4/SHUHA'S'/6/2*SAKER/5/RBS/AN	IESRRL# 110
9	ETBW 7236	CROW 'S'/BOW'S' 3-1994/95//KATILA-11	IESRRL # 174
10	ETBW 7248	SAKER/5/RBS/ANZA/3/KVZ/HYS//YMH/TUL/	IESRRL # 209
11	ETBW 7173	FOW'S'//NS732/HER/3/CHAM-6//GHURA	IESRRL# 45
12	ETBW 7235	CROW 'S'/BOW'S'-1994/95//ASFOOR-5	IESRRL # 173
13	ETBW 7268	SOMAMA-9//SERI 82/SHUHA'S'	IESRRL # 272
14	ETBW 7174	CHAM-6/GHURAB'S'//JADIDA-2	IESRRL# 46
15	ETBW 7220	CHAM-4/SHUHA'S'/6/2*SAKER/5/RBS/AN	IESRRL# 135
16	ETBW 7221	DUCULA/KAUZ/3/KAUZ'S'//GLEN/PRL'S'/4	IESRRL# 142
17	ETBW 7227	IZAZ-2//TEVEE'S'/SHUHA'S'	IESRRL# 164
18	ETBW 7239	WEEBILL – 1/BOCRO-3	IESRRL # 178
19	ETBW 7160	CHAM-6/WW 1402	IESRRL# 29
20	ETBW 7161	CHAM-6/WW 1403	IESRRL# 30
21	ETBW 7191	BOCRO-4/3/MAYO'S'//CROW'S'/VEE'S'	IESRRL# 72
22	ETBW 7199	VAN'S/3/CNDR'S'/ANA//CNDR'S'/MUS'S'/	IESRRL# 85
23	ETBW 7182	CHIL-1//VEE'S'/SAKER'S'	IESRRL# 58
24	ETBW 7194	VAN'S/3/CNDR'S'/ANA//CNDR'S'/MUS'S'/	IESRRL# 76
25	ETBW 7204	SHA3/SERI//YANG87-142/3/2*TOWPE	IESRRL# 103
26	ETBW 7234	IRQIPAW 35 S5B-98/ABUZIG-4	IESRRL# 172
27	ETBW 7164	SHUHA-4//NS732/HER	IESRRL# 33
28	ETBW 7195	VAN'S/3/CNDR'S'/ANA//CNDR'S'/MUS'S'/	IESRRL# 78
29	ETBW 7244	ANDALIEB-5// TEVEE-1/SHUHA-6	IESRRL # 198
30	ETBW 7258	SABA/FLAG-1	IESRRL # 234
31	ETBW 7264	SERI 82/SHUHA'S'// SOMAMA-9	IESRRL # 268
32	ETBW 7215	CHAM-4/SHUHA'S'/6/2*SAKER/5/RBS/AN	IESRRL# 117
33	ETBW 7156	TAM200/TUI//MILAN/KAUZ/3/CROC-AB	IESRRL# 17
34	ETBW 7247	HD2206/HORK'S'/3/2*NS732/HER//KAUZ	IESRRL # 208
35	Danda'a	Check	Breeder seed,2011
36	ETBW 7175	CBME4SA#4/FOW-2	IESRRL# 47

useful in future selection processes. The information presented can be useful for wheat breeders contributing to a more efficient exchange of information and use of germ-plasm, but this research needs to be complemented with additional studies on adult plant resistance because some leaf rust resistance genes express resistance optimally in adult plants.

DISCUSSIONS

The emergence of virulence on *Sr31* in 1998 has increased the vulnerability of wheat to stem rust worldwide after decades of widespread use of this effective gene in global wheat-breeding programs. The occurrence of virulence to *Sr31* in Ethiopia has highlighted the need for germplasm enhancement to develop cultivars resistant to the Ug99 race of *P. graminis* f. sp. *tritici*. For seedling stage and most of the genotypes show low IT ≤2 on four of the stem rust races indicating that they are resistant to the four stem rust races used. Out of these, nine of the gentypes namly genotype ETBW7178, ETBW7198, ETBW7236, ETBW7220, ETBW7161, ETBW7191 and one standard chick Dand'a has potential (IT≤1) to overcome stem rust races at seedling stage. On the contrary, half of the

Table 2. Wheat germ plasm screened against four major stem rust races during seedling stage.

S/N	Code	TTKSK			TKTTF			TRTTF			JRCQC		
		1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
1	G-1	2	0	-	0	2	-	0	;	-	0	0	-
2	G-2	2	2+	-	;	0	-	;	;	-	;	0	-
3	G-3	3-	3-	-	2+	3-	-	2	;2-	-	;1	2+	-
4	G-4	;1	1	-	;1(c)	;1	-	;	;	-	;	;1	-
5	G-5	3-	3-	-	3-	2+,3-	-	;2-	;2-	-	0	3-	-
6	G-6(1)	2+	3-	-	2+	0	-	2	;1	-	;1	;1	-
7	G-6(2)	2+	2+,3-	-	2	3-	-	;2-	;2	-	0,2	2+	-
8	G-8	3-	3	-	2,2+	3-	-	2+	;2	-	;1	3-	-
9	G-9	;1	;1	-	;1(c)	;1	-	2-	;1+	-	;	;	-
10	G-10	2	1	-	;1	;1	-	2	;1	-	;1	1+	-
11	G-11	2+	;1	-	;1	3-	-	;1+	3-	-	;1	;1	-
12	G-12	3-	3-	-	2-	3-	-	2-	;1	-	;	3-	-
13	G-13	;1+	3-	-	;2+	;1	-	;1+	;2	-	;1	;1	-
14	G-14	2+,3-	3-	-	;1	;1	-	;1	;1	-	;1	;1	-
15	G-15	;1	;1	-	;1	0	-	;1	;	-	;1	1	-
16	G-16	2+,3-	2+	-	;2	2		2	3-	-	2-	2-	-
17	G-17	;1	2+	-	;	;1	-	;	;1+	-	;	;1	-
18	G-18	;	2	-	;	;1	-	;	;1+	-	;1	;1+	-
19	G-19	2-	2+	-	;1	;1	-	;	2-	-	;	;1+	-
20	G-20	;1	;1	-	;1	;1	-	;	;1+	-	;	;1	-
21	G-21	;1	;	-	;1	;1	-	;	;1	-	0	;	-
22	G-22	;1+	2	-	;1	2	-	;1	;1	-	;1	;1	-
23	G-23	;1,2+	3-	-	;1	1+	-	;1	;1+	-	;1	2-	-
24	G-24	2(c)	3-	-	;1	1+	-	;1	;1+	-	;	2	-
25	G-25	;1+	2-		0	1	-	;1	;1+	-	;1	;1	-
26	G26	3-	3-	-	;2	2	-	3-	3-	-	;	3-	-
27	G-27	2+	2+,3-	-	;1	;1	-	;1	;1+	-	;	;1	-
28	G-28	3-	3-	-	;2	3	-	2-	3-	-	;1	2+	-
29	G-29	2+	3-	-	;1	1+	-	;1	;2-	-	;1	;1	-
30	G-30	2+(c)	1	-	;1	1+	-	;1+	3-	-	0,1	0	-
31	G-31	;1+	;1	-	;1	;1	-	;1	;1	-	;1	;1	-
32	G-32	2-	2-	-	;1+	2	-	;1	;1	-	;1	;1	-
33	G-33	2-	2	-	;1+	2+	-	;1+	;1	-	;1	;1+	-
34	G-34	2,2+	3-	-	;1+	2-	-	;1	2,3-	-	;	;1+	-
35	G-35	;1	;1	-	0	0	-	0	0	-	0	;	-
36	G-36	;1	;1	-	;1	;1	-	;	0	-	0	;	-

[a]Infection types according to a 0 to 4 scale. Within line variation is indicated by '/'. [b]Races were represented by the following isolates: TTTTF 01MN84A-1-2, TTKSK 04KEN156/04, TTKST 06KEN19V3, TTKSF UVPgt55, TTKSP UVPgt59, PTKST UVPgt60.

materials used as ETBW7238, kubsa, ETBW7237, ETBW7171, ETBW7208, ETBW7182 and ETBW7804 show high infection type (IT) or are suscptability to stem rust races at seedling stage (Table 2).

On the experiment for addult stage, the only genotype showing strong resistance was genotype ETBW7178 (5R). The rest genotypes show moderatly resistance, moderately susceptable and totally susceptible to stem rust disease (Table 3). Genotypes ETBW7161, ETBW7227, ETBW7221, ETBW7174, ETBW7171, ETBW7198, ETBW7164 and ETBW7156 show MRMS. In countrast, genotypes ETBW7235, ETBW7204, 7234, ETBW7256 and ETBW7247 showed MSS and ETBW7182 showed SMS indicating highly susceptibility to stem rust at adult stage which can be used as border variety for infesting stem rust at field condition. The result was inline with that of Yu et al. (2010) which characterized resistance genotypes of a diverse and widely distributed collection of germplasm originating from the International Maize and Wheat Improvement Center (CIMMYT) and

Table 3. Severity of the tested wheat genotypes aginest stem rust at DebreZeit at adult stage at field condition.

S/N	Cultivar/Accession number	Terminal severity
1	ETBW 7178	5R
2	ETBW 7252	30MSMR
3	ETBW 7238	40MSS
4	ETBW 7198	30MRMS
5	Kubsa	40MRMS
6	ETBW 7237	40MSS
7	ETBW 7171	30MRMS
8	ETBW 7208	40MS
9	ETBW 7236	40MS
10	ETBW 7248	40SMS
11	ETBW 7173	40MRMS
12	ETBW 7235	50MSS
13	ETBW 7268	40MSS
14	ETBW 7174	30MRMS
15	ETBW 7220	30MS
16	ETBW 7221	30MRMS
17	ETBW 7227	30MRMS
18	ETBW 7239	40MSS
19	ETBW 7160	40MS
20	ETBW 7161	30MRMS
21	ETBW 7191	40MRMS
22	ETBW 7199	40MSS
23	ETBW 7182	50SMS
24	ETBW 7194	40MSS
25	ETBW 7204	50MSS
26	ETBW 7234	50MSS
27	ETBW 7164	30MRMS
28	ETBW 7195	40MSS
29	ETBW 7244	30MSMR
30	ETBW 7258	50MSS
31	ETBW 7264	30MSMR
32	ETBW 7215	40MSS
33	ETBW 7156	30MRMS
34	ETBW 7247	50MSS
35	Danda'a	40MSS
36	ETBW 7175	30MSS

IRs at the adult plant stage following the descriptions of Roelfs et al. (1992), where R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible.

obtained that most wheat cultivars lacks Sr. genes at adult stage.

Conclusion

Stem rust is caused by *P. graminis f.sp. tritici*. It is devastating fungal disease can cause 100% yield lose. Based on field and laboratory studies in Debrezyit and Ambo Plant Protection, Ethiopia, this report confirmed the broad virulence that race TTKS possesses, across most of the bread wheat genotypes used.

At seedling stage, most of the genotypes revealed that low infection type (IT ≤2) on four of the stem rust races indicating that they are resistant to the four stem rust inoculum used. Out of these, nine of the gentypes namey genotypes ETBW7178, ETBW7198, ETBW7236, ETBW7220, ETBW7161, ETBW7191 and one standard chick Dand'a has potential resistance (IT≤1) to overcome stem rust races at seedling stage. On the experiment for adult stage, the only genotype showing strong resictance was genotype ETBW7178 (5R). The rest genotypes show moderately resistance, moderately susceptible and totally susceptible to stem rust disease. Genotypes ETBW7161, ETBW7227, ETBW7221, ETBW7174, ETBW7171, ETBW7198, ETBW7164 and ETBW7156 show MRMS. In countrast, genotypes ETBW7235, ETBW7204, 7234, ETBW7256 and ETBW7247 showed MSS and ETBW7182 was the one showing SMS indicating highly susceptibility to stem rust at adult stage which can be used as border variety for infesting stem rust at field condition. These results can assist wheat breeders in Ethiopia for choosing parents for crossing in programs aimed at developing cultivars with desirable levels of stem rust resistance in collabration with International Maize and Wheat Improvement Center (CIMMYT) and will also facilitate stacking of resistance genes into advanced breeding lines. This requires extensive crossing of adapted germplasm with international cultivars and breeding materials that possess the effective resistance genes. Once crossed, procedures such as marker-assisted selection or marker-assisted backcross selection would be the methods of choice.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Variability of morpho-metric traits and oleaginous biofuel potential of *Jatropha curcas* L. (*Euphorbiaceae*) seeds in Burkina Faso

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In the recent decades, *Jatropha curcas* has received attention as a potential source of bio fuel production in many West African countries. To detect performant accessions for improvement and breeding programmes, the degree of variability of morphological traits and oil content of seeds in a large range of accessions were assessed in this study. The morphological traits and oil content of seed of 40 accessions of *J. curcas* in Burkina and their genetic values and correlation was calculated. The results show a high variability of morphological traits and oil content of seed. The accession has a significant effect on the morphological traits and oil content of seed whereas, phytogeographic zone has no significant effect. The results permitted identification of accessions with interesting morpho-metric parameters and oil content of seed. The results also show positive and significant correlations between seed width and all the morphological traits and the value of genetic parameters indicate that the high variability in seed traits and oil content can be explain by genetic factors. Also, the highest value of genetic gain was observed for the oil content. As a result, there are good opportunities for selection and improvement of traits and seed oil production through selection.

Key words: *Jatropha curcas*, Burkina, diversity, oil content, seed traits, heritability.

INTRODUCTION

Jatropha curcas is a shrub of *Euphorbiaceae* family and native to Central America and Mexico (Heller, 1996; Agnieszka et al., 2013; Sawadogo et al., 2015). It is well-known by African people, who use it for delimiting farms and concessions. In recent years, because of its biofuel potentials, the species is considered as a solution to deal with climate change, energy insecurity and to fight against poverty in rural areas by generating income especially for women (Terren et al., 2012; Guittet and Massimiliano, 2015).

In Burkina Faso, *J. curcas* plantations are widespread through out the country's phytogeographic zones (Bazongo et al., 2015); Tiendrebeogo et al., 2016a). However, the seed yields and seed oil content are variable and generally weak, reducing its economic potential and making its culture a risk company (Singh et al., 2010; Kumar and Singh, 2014). These constraints justify the need of a varietal improvement which require the identification of high oil content in local ecotypes in order to optimize profitability of the species and to boost the *J.curcas* sector (Guittet and Massimiliano, 2015). Unfortunately, data on the characterization of local varieties in Burkina Faso are limited. Yet, previous works in Burkina Faso (Sama et al., 2013; Tiendrebeogo et al., 2016b) and the world (Rao et al., 2007, 2011; Kaushik et al., 2007) have highlighted a wide range of variability in seed traits and oil content depending on phytogeographic conditions. Therefore, regarding the different phytogeographical areas in Burkina Faso, large variability of seed traits and oil content is expected and some accessions may have better oleaginous potential. Moreover, these studies have shown that there is a positive correlation between *J. curcas* seeds weight and its oil content. In addition, the authors reported that heavy *J. curcas* seeds have better vigor at emergence and the seedlings grown from heavy seeds have better growth. Thus, assessment of the natural variability of seed traits and oil content of the species could help in identifying accessions with better seed trait and would be an important step for improvement programs (Kumar and Singh, 2014; Tiendrebeogo et al., 2016b). In Burkina Faso, the lack of data on morphological variability and seed oil content of *J. curcas* strongly limits such perspectives. In fact, the level of seed oil production of local accessions of *J. curcas* is still poorly understood. Despite the considerable economic potential of the species, limited research has focused on the variability of seed traits and oil content of the *J. curcas* seeds in Burkina Faso. Researches on the species have focused on the species physiology and biology (Tiendrebeogo et al., 2016a). However, information regarding the extent and pattern of genetic variation in *J. curcas* of local accessions is limited barring a few recent studies undertaken by Sama et al. (2013) and Tiendrebeogo et al. (2016b). The present study aims at characterizing the morpho-metric traits and the oleaginous potential of seeds of the accessions of *J. curcas* in Burkina in order to identify the performant accessions. Such accessions would be very capital for improvement and breeding programmes for a durable production of biodiesel containing *J. curcas*.

MATERIALS AND METHODS

The study involved a total of 40 accessions of *J. curcas* L. collected in different sites of Burkina Faso. The characteristics of accessions are presented in Table 1. The collection was carried out based on a climatic gradient with a rainfall ranging from 500 to 1200 mm. The collection sites belong to southern Sudan, northern Sudan, sub-Sahelian and Sahelian zones (Figure 1). In each site, fruits were collected in plantations or hedges of *J. curcas* at least 5 years older, between July and September 2016. In each plantation or hedge, ripe fruits of ten (10) plants distant at least 10 m were randomly selected. All the fruits collected in the same plantation or hedge were gathered to form an accession. Afterward, fruits were dried under ventilation in the laboratory, decorticated manually and bagged in kraft envelopes without any pretreatment and stored at room temperature.

Characterization of traits variability and seed oil content

Variability of seed traits

For each accession, five batches of ten (10) seeds were randomly selected for the measurement of their morphological parameters (length, width and thickness) using an electronic Vernier caliper (precision of 0.01 mm) and ten seeds weight using an electronic balance (precision 0.01 g).

Oil content of the seeds

The seed oil content was determinate according AOAC 960.39 method described by Turinayo et al. (2015). Oil in seeds was extracted in with Soxhlet apparatus for 6 h, using petroleum ether (boiling point of 40 - 60°C) as an extraction solvent. The extracted oil is recovered by solvent evaporation using a rotavapor apparatus to remove the majority of the solvent by rotatory evaporation at 40°C under reduced pressure. The extracted seed oil was weighed. For each accession, the oil content of three samples was determined in triplicate tests in order to enhance accurate statistical inference. The amount of oil in seeds was calculated and expressed as percentage (%) by following formula:

$$\text{Oil content (\%)} = \frac{\text{Oil weight}}{\text{Sample weight}} \times 100$$

Statistical analysis

The investigated parameters were subjected to one-way analysis of variance at the 5% level. The effect of climatic zones was the first to be evaluated. The Sahelian zone was excluded from this analysis because of the small number of accessions; only two accessions belong to this zone. When there were no significant differences between climatic zones, the traits of the accessions were subjected to a one-way ANOVA. When the ANOVA test showed significant differences in seed traits, the Tukey test (at the 5% threshold) was performed for the ranking of averages. The Pearson test was performed at the 5% threshold to assess the correlation between seed traits. All these statistical analysis were carried out in the R. software. Ward's aggregation method was used to group the accessions. According to Vachon et al. (2005), it is a method of entities partitioning into classes according to their similarity traits. In this classification, each class is a group of entities in which the variance between members is relatively small. The ANOVA results were also used to calculate genetic parameters (variance and coefficient of genotypic and phenotypic variability, heritability of traits studied and expected genetic gain) according to Gbemavo et al. (2015).

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Table 1. Geographical coordinates of *J. curcas* plantations and hedges based on phytogeographic zones and age.

Zone climatic zone	Accessions	Age (years)	Geographical coordinates of accessions
Northern Sudan	J4	6	30P0502967 UTM 1416658
	J5	5	30P0510362 UTM 1416428
	J13	6	alt1:105 9 ft N: 12 47'08.5'' H 001 41'35.1''
	J14	5	30P049792 UTM 1361549
	J15	5	30P0440077 UTM 1364004
	J16	5	30P044351 UTM 1363920
	J22	5	alt1:220 9 ft N: 12 47'08.5'' H 001 41'35.1''
	J25	7	30P0658968 UTM 1488854
	J26	8	30P0447169 UTM 1259930
	J36	6	30P0510362 UTM 1408255
	J38	6	30P0447315 UTM1259885
	J40	7	alt:995ft N: 12 40'43.9 H:001 20'50.3
Sahelian	J17	6	30P067388 UTM 1529367
	J18	7	30P0697161 UTM 1531587
Sub- Sahelian	J6	5	30P075871 UTM 1485040
	J7	7	30 P0645225 UTM 1471332
	J10	8	31P0211417 UTM 1335233
	J11	7	31P0211366 UTM 1335283
	J12	5	31P0211805 UTM 1337988
Southern Sudan	J1	9	30P0338029 UTM 1242633
	J2	7	30P0336843 UTM 1242817
	J3	8	30P0423736 UTM 1286009
	J8	10	30P 0638098 UTM 1248194
	J9	10	30P0330932 UTM 1146119
	J19	10	30P0423736 UTM 1286009
	J20	5	30P0447169 UTM 1259930
	J21	6	30P0447315 UTM 1259885
	J23	6	30P0308695 UTM 1177542
	J24	8	30P0370952 UTM 1220292
	J27	6	30P 0601801 UTM 1232344
	J28	5	30P0699791 UTM 1491388
	J29	10	30P 0638087 UTM 1248075
	J30	8	30P0346283 UTM 1109579
	J31	6	30P 0585113 UTM 1221071
	J32	7	30P0345321 UTM 1116435
	J33	6	30P0347565 UTM 1108143
	J34	8	30P0342087 UTM 1108345
	J35	5	30P0402964 UTM2416446
	J37	7	30P0345762 UTM 1108143
	J39	10	30P 0601744 UTM 1232416

RESULTS

Distribution of accessions through phytogeographic zones of Burkina Faso

In Burkina Faso, *J. curcas* is found in all phytogeographic zones of the country mainly in the form of plantations. *J. curcas* hedgerows, used to delineate plots or to protect fields have also been encountered. However, plantations are more abundant in southern Sudan. In addition, older *J. curcas* plantations (more than ten years) are met in southern Sudan as compared to the other climatic zones

Figure 1. Phytogeographical zones in Burkina Faso (the collection sites represented by white circles).

where plantations are commonly below ten years old.

Morphological traits of seeds in relation to accession and the phytogeographic zone

The results of morphological seed traits showed that accession has a significant effect on seed traits ($P < 0.001$) in opposite to the phytogeographic zone that has no significant effect. Also, the results showed a very great variability of seed morphological traits from one accession to another. Seed length ranged from 17.40 ± 1.14 to 19.30 ± 0.98 mm with an average length of 18.55 mm. The shortest length was recorded in the J4 accession (North Soudan zone) and the highest value in the accession J13 (North Sudan zone). The width varied from 10.86 ± 0.46 mm for accession J4 (North Sudan zone) to 11.86 ± 0.43 mm for accession J31 (Sudanian zone) with an average value of 11.33 mm. The thickness ranged from 7.0 ± 0.17 mm for J18 accession (Sahelian zone) to 9.22 ± 0.22 mm for J35 accession (Sud Sudanian zone), while the weight of 10 seeds ranged from 5.88 ± 0.96 g for accession of J2 (Sudan zone) to 9.60 ± 0.96 g for accession J15 (Sudan North Area). Morphological traits of seeds were subjected to an Ascending Hierarchical Classification (CAH). This classification allowed the distribution of accessions into four classes and characteristics are presented in Table 2. Class IV is represented only by the J18 accession collected in the Sahelian zone, while the other classes include accessions from different phytogeographic zones. The highest values of traits are observed in class I while the lowest is in class IV.

Seed oil content in relation to the accession and the phytogeographical zone

Seed oil contents were expressed as a percentage of kernel dry weight and the corresponding accessions. Seed oil contents of the different accessions ranged from 33.83 ± 1.65 to 57.21 ± 2.44% respectively for J1 accession (South Sudan zone) and J7 (Sub-Sahelian zone). The results also show that accession has a significant effect on the seed oil content ($P < 0.001$), whereas phytogeographic zone has no significant effect. Table 3 shows different ranges of oil content as percentage of mass of dry seeds. The contents are stored in intervals of amplitude 5%.

Table 2. Classes of morphological traits of *J. curcas* seeds based on Ascending Hierarchical Classification (CAH) in relation to the accession and phytogeographic zone.

Classes	Climatic zone	Accession	Morphological traits of seeds			
			Length (mm)	Width (mm)	Thickness (mm)	Weight of 10 seeds (g)
I	Northern Sudan	J4, J13, J14, J26	19.100	11.640	9.020	8.32
	Southern Sudan	J1, J2, J7, J8, J9, J27, J28, J29, J32				
II	Northern Sudan	J15, J16, J22, J25, J36, J38, J40	18.400	11.240	8.800	7.36
	Sub-Sahelian	J5, J6, J10, J11,				
	Southern Sudan	J2, J19, J20, J21, J30, J31, J33, J34, J35, J37, J39				
	Sahelian	J17				
III	Northern Sudan	J3	17.740	10.920	8.600	6.10
	Sub-Sahelian	J12				
	Southern Sudan	J23, J24				
IV	Sahelian	J18	18.000	10.600	7.000	6.33

Table 3. Seed oil content in relation to the accession and phytogeographic zone.

Range of oil content percentage of mass of dry seeds (%)	Climatic zone	Accessions
[30; 35]	Northern Sudan	J16, J25
	Sub-Sahelian	J5
	Southern Sudan	J2
[35; 40]	Northern Sudan	J26, J40, J22, J15
	Sub-Sahelian	J11, J6,
	Southern Sudan	J9, J21, J39, J34,
[40; 45]	Northern Sudan	J3, J4, J14
	Sub-Sahelian	J19
	Southern Sudan	J31, J37
	Sahelian	J17
[45; 50]	Northern Sudan	J38
	Sub-Sahelian	J10, J12
	Southern Sudan	J1, J8, J23, J28, J30, J33, J35,
[50; 55]	Northern Sudan	J13, J36
	Southern Sudan	J20, J27, J29, J32
	Sahelian	J18
[55; 60]	Sahelian	J7

Genetic parameters

The genetic parameters have been calculated and results of calculate of genetic variables are reported in Table 4.

The highest values of phenotypic variation coefficient (CVP) (95.20%) and genotypic variation coefficient (CVG) (77.80%) were recorded for seed oil content. The lowest values of CVP (7.30%) and CGV (5.50%) were observed

Table 4. Genetic variables of seed traits of *J. curcas* accessions.

Seed source	Variance		Coefficient of variation		Heritability broad sense (H2B)	GA as % mean
	Phenotypic	Genotypic	Phenotypic	Genotypic		
Seed weight (g)	0.006	0.004	0.084	0.070	0.692	14.165
Length (mm)	0.191	0.087	0.101	0.069	0.464	2.249
Breadth (mm)	0.052	0.061	0.085	0.073	0.737	3.847
Thickness (mm)	0.048	0.027	0.073	0.055	0.560	2.856
Oil content (%)	39.162	26.108	0.952	0.778	0.667	19.929

Table 5. Pearson correlation coefficients between traits of *J. curcas* seeds.

Variables	Length	Width	Thickness	Weight
Length	**1**			
Width	0.453			
Thickness	0.424	**0.672**		
Weight	**0.652**	**0.642**	**0.539**	
Oil content	0.130	-0.001	-0.220	0.128

In bold, significant correlation.

with seed length. The lowest value of heritability in the broad sense (46.40%) was recorded in seed length while the other traits of the seeds showed heritability between 56.00 and 73.70%. The genetic gain ranged from 2.24 to 19.93% for the length and seed oil content, respectively.

Correlation between seed traits

The correlation matrix based on the Pearson coefficient correlation is presented in Table 5. These correlations are positive and significant between the thickness and the width and between the weight and the other morphological traits of the seeds. However, there is no correlation between seed oil content and seed traits.

Comparative analysis of accessions

The dendrogram plotted on the basis of the morphological features and the seed oil content using Ward's aggregation method regrouped the 40 accessions into three (03) clusters (I, II and III) (Figure 2) whose composition and average oil contents are shown in Table 6. In this classification, the average oil content of clusters I is 48.81% as compared to clusters II and III that have respectively 32.65 and 39.55%. Class I contains both accessions that have the high values of morphological traits and the highest oil content as compared to those of the other two classes.

DISCUSSION

J. curcas is a widespread species in the different phytogeographic zones of Burkina Faso. This broad distribution in addition to its adoption by the populations could reflect a considerable interest for the species.

The present study, focused on local *J. curcas* accessions in Burkina Faso, showed a high variability of seed traits. This high variability of seed traits constitutes an important input for the species improvement programs. Similar variations were reported by Ouattara et al. (2014) and Tiendrebeogo et al. (2016b) on *J. curcas* and by Govindaraj et al. (2011) and Basavaraj et al. (2017) on *Pennisetum glaucum* (L.) R. Br. Such variability of seed traits have been explained by the genetic diversity, and/or the environment effects or their interactions. Similar results were also reported by Subi and Idris (2013). However, the results show that the phytogeographic zone has no significant effect on morphological seed traits and oil content. This supposes that the variability would be related to the genetic factors. Indeed, Subi and Idris (2013) and Kumar and Singh (2014) reported that the genetic factors are factors influencing the features of seeds. In addition, Gbemavo et al. (2015) and Tiendrebeogo and al. (2016b) reported that cross fecundation (allogamy) of different genotypes in *J. curcas* could be a reason for the variability of seeds traits. Cross fecundation would support a natural genetic mixing leading to an important genetic diversity within the species.

The results showed positive and significant correlations between seed width and all the morphological traits. Such results are very interesting for breeding programs. Indeed, according to Freitas et al. (2011), knowledge of the magnitude of the correlation between characters is important in the choice of improvement methods and the formulation of strategies for the simultaneous selection

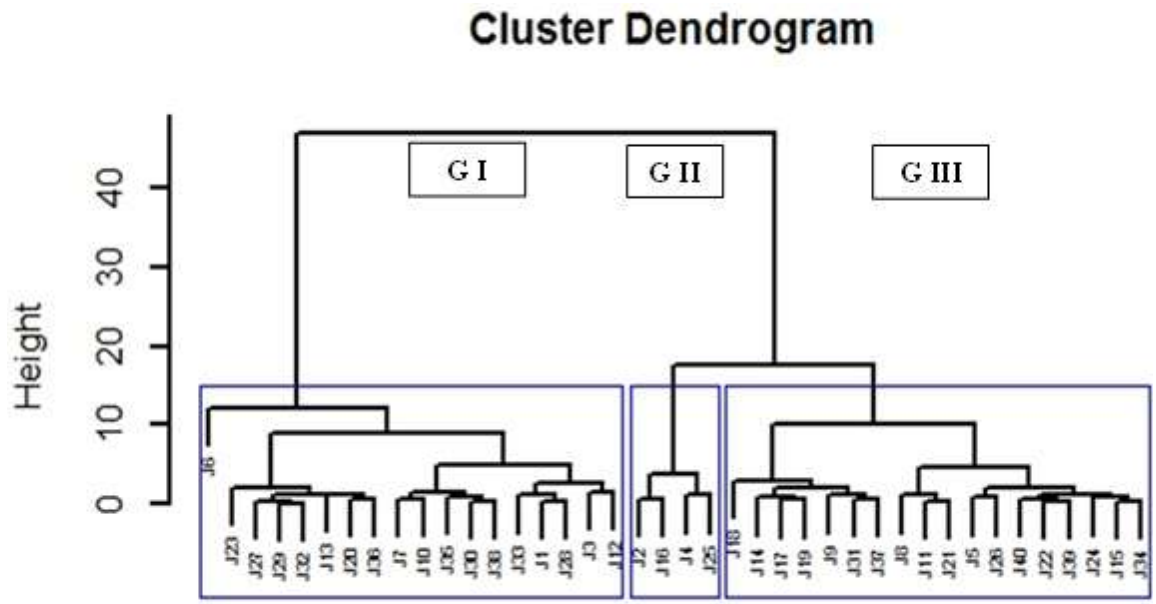

Figure 2. Dendrogram based on morphological traits and seed oil content of 40 Burkina Faso accessions.

Table 6. Composition and average oil content of the groups.

Classes	Accessions	Oil content (%)
I	J1, J3, J6, J7, J10, J12, J13, J20, J23, J27, J28, J29, J30, J32, J33, J35, J36, J38	48.808
II	J2, J4, J16, J25	32.650
III	J5, J8, J9, J11, J14, J15, J17, J18, J19, J21, J22, J24, J26, J31, J34, J37, J39, J40	39.551

for several desired characters.

Hierarchical ascending classification is a powerful tool used to assess the relative contribution of different traits to the total diversity and to quantify the degree of divergence or similarity between accessions. In this study, the Ascending Hierarchical Classification (CAH) based on the oil content and the morphological traits of the seeds showed a breakdown of the forty accessions into three groups. The constitution of groups is independent of the phytogeographic origin of the accessions. Similar results have been reported by Gbemavo et al. (2015) and Tiendrebeogo et al. (2016a). In this study, group I offers a possibility of varietal improvement that could fill the needs of setting up *J. curcas* plantations for biofuel production. Indeed, for the purpose of varietal improvement in order to establish plantations of *J. curcas* for biofuel purpose, accessions belonging to group I present the desired seed traits. Although, this study did not show a significant correlation between oil content and morphological traits of seeds, previous studies have shown that high weight seeds are oil-rich, have higher vigor at emergence, and better seedlings growth (Kaushik et al., 2007; Ouattara, 2013). In addition, these seedlings require little maintenance during the installation phase, which reduces the costs of setting up the plantations.

The genetic parameters presented in Table 4 show a small difference between the phenotypic and genotypic coefficients of variation. Similar results have been reported by Ouattara (2013) and Tiendrebeogo et al. (2016a). According to Basavaraj et al. (2017), a small difference between these two coefficients of variation indicates that the characters are not too influenced by the environment.

Indeed, this results show broad sense heritability values between 56.00 and 73.70%. Except the heritability of seed length, all the traits had high heritability values. According to Miftah (2016), heritability is high when its value is greater than 50%. These high values of heritability in the broad sense confirm the low influence of environmental factors on the expression of these characters. The determination of heritability shows that the observed variability is of genetic origin (Tiendrebeogo et al., 2016a). However, according to Kumar and Singh (2014), heritability and genetic gain must be considered together to predict the resulting effect in an improvement program. Indeed, high heritability (broad sense) may be due to the non-additive action of the gene and will only be reliable when accompanied by a high genetic gain (Kumar and Singh, 2014). The results show, in general, low genetic gain values (values between 2.55 and 19.93). The highest value (19.93) was observed for the oil content. This result suggests large potential for the improvement of seed oil content.

Conclusion

This study showed that plantations and hedgerows of *J. curcas* are widespread throughout the different climatic zones in Burkina Faso. The results show a very high variability of the morphological traits and the oil content of *J. curcas* seeds with certain accessions having good characteristics for these traits. The study also allowed the identification of genotypes of interest for their oleaginous potential that could serve as a basis for selection and breeding programs. The study of the determinism of this variability has revealed that the sources of this variability could be environmental and also genetic. As a result, there are good opportunities for selection and improvement of traits and seed oil production through selection. This study is an introduction of the identification of the best local genotypes with high oil potential.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Grass production Tifton 85 and nutrient extraction with swine wastewater doses

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Swine wastewater (SW) is considered as a source of nutrients for agriculture. The quantities and frequencies applied may vary according to the soil class, the nature and composition of the waste, the climatic conditions and the cultivated plant species. Therefore, this study aimed to evaluate the effects of application of five Swine wastewater doses (0, 500, 1000, 2000 and 2500 m³ ha^{-1}) in the production of components and grass nutrient extraction Tifton 85 and changes in soil chemical properties after the end of the last application of swine wastewater. To achieve these goals, we evaluated the quantities of extracted nutrients, density and height of the pre-grazing grass, forage accumulation rate and carrying capacity. The experimental design was a randomized block. The production of dry matter was 18159.80 kg ha^{-1}, and the average height of 85 Tifton was 34.83 cm. The higher carrying capacity, 10 AU ha^{-1} was obtained with the dosage of 2500 m³ ha^{-1}. While, the dose of 2000 m³ ha^{-1} supplied the nutritional needs of Tifton 85 in nitrogen, potassium, calcium, magnesium, copper, iron, zinc and boron. The quantities of extracted nutrients (kg ha^{-1}) at grass Tifton 85 grazed were: N = 405.14; P = 57.77; K^+ = 387.69; Ca^{2+} = 77.05; Mg^{2+} = 49.68; S-$SO4^{-2}$ = 31.48; B = 0.33; Cu^{2+} = 0.20; Fe = 2.88; Mn^{2+} = 4.61; Zn^{2+} = 2.86. The application of increasing doses of SW promoted a linear increase in grass production components Tifton 85, as well, promoted changes in soil chemical properties and quantities of extracted nutrients.

Key words: Pasture, liquid waste, nutrient extraction, plant growth.

INTRODUCTION

Brazilian swine production is increasing annually in order to meet both domestic and foreign market demand with regard to the quality of raw materials and environmental care (Pinto et al., 2014). Brazil is the fourth largest producer of pork in the world, this represents the equivalent of 3.7 million tonnes of pigs (EMPRAPA, 2017). The main problem of this activity, therefore, is the generation of enormous quantities of manure that can

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corroborate to the pollution of terrestrial and aquatic ecosystems (Segat et al., 2015).

The fertilization of crops with swine wastewater is a common practice and is attractive for the reduction of natural resources and environmental pollution control. The feasibility of such use is due to the large volume of waste generated and the amount of nutrients that are easily mineralized when applied in soil (Lucas et al., 2013).

In this sense, an alternative that has been highlighted in the pursuit of sustainability in the recovery of degraded cultivated soils and pastures is the use of organic fertilizers or cover crops because the mineral fertilizers in this context is rather low due to its high cost and due to the low purchase power of most producers. For instance, mineral fertilizers in Africa cost at the farm gate, two to six times as much as in Europe, North America, or Asia (Sanchez, 2002). Therefore, organic inputs are a viable alternative source of plant nutrients for resource-poor farmers. Application of organic input usually leads to increased crop yields and pasture (Ogundare et al., 2012; Matthews, 2017). The acceptance by local farmers of the benefits of compost to the soil and crops would be a significant incentive to reduce the expensive mineral fertilizers and optimize their use (Azim et al., 2017). Thus, the use of SW is available as an alternative to replace the mineral fertilizer, demonstrating its efficiency in dry matter production and nutrient availability (Zanine and Ferreira, 2015; Gomes et al., 2017; Lucas et al., 2013). However, there is little information regarding the use of this waste in areas with degraded pastures (Fogel et al., 2013).

The supply of nutrients in adequate amounts via SW can increase the nutritional quality of the forage, enhancing performance and/or animal productivity (Assmann et al., 2009). Therefore, the use of Swine wastewater (SW) as a source of nutrients in pasture areas is presented as an alternative to disposal of this waste (Assmann et al., 2009; Seidel et al., 2010). Camargo et al. (2011) observed the effects of different doses of swine manure on forage Tifton 85 and found that an increase in dry matter production and P content grew linearly with the doses, thus suggesting a dose of 100 m³ ha^{-1} to obtain about 3500 kg ha^{-1} dry weight in a period of 28 days. Serafim and Galbiatti (2012) discussed that an increase in the application of swine waste increased the supply of nitrogen and phosphorus to the soil, thus promoting plant growth and increasing the ratio of leaf/stem.

Thus, it is needed to find the proper management from a biophysical point of view to promote sustainable agriculture. However, it is also necessary that farmers accept new strategies that propose cultural and technical shifts (Cerdà et al., 2018a). The search for information and knowledge to clarify how to use the SW in pastures is growing. To have a correct land management in intensive systems, it is important to know the nutrient extraction capacity for forage to mitigate the negative environmental impacts on the ground and to define the best application rates.

In this way, there is a need to design proper policies to achieve sustainability, and for this, the scientific community should produce information in collaboration with land managers and other actors, which will guide policy makers to implement the most efficient managements and strategies (Cerdà et al., 2018b). This study aimed to evaluate the effects of application of five SW doses (0, 500, 1000, 2000 and 2500 m³ ha^{-1}) in the following production components: bearing capacity, dry matter yield, density, percentage of dry matter grass height, dry matter production per day and in grass nutrient extraction Tifton 85 grazed and changes in soil chemical properties.

MATERIALS AND METHODS

Study area

The study was conducted during the period from January to April, 2013, at the Bonsucesso farm, which is located in, Minas Gerais state, Brazil, at the geographical coordinates 19°05'17"S and 48°22'00"W, at an altitude of 820 m, at an dystrophic yellow Oxisol, according to Embrapa classification in 2006. According to the Köppen and Geiger (1928) system, the climate is characterized as Aw (typical tropical, with average rainfall around 1600 mm per year, with moderate water deficit in winter and excessive rain in summer). Before the experiment installation, it was determined that the chemical and physical soil characteristics of the area at different depths of 0.00-0.20 m and 0.20-0.40 m (Table 1). For this, 10 soil samples to form a composite sample were collected at random in the experimental area with a Dutch auger.

The study was conducted on grazing Tifton 85 and was installed five years ago. The experimental design was a randomized complete block design with five treatments and three replications. Plots were 3 x 3 m, amounting 9 m², 1 m boundary among plot. The treatments consisted of the following wastewater doses of swine (SW): control (without application of SW), 500, 1000, 2000 and 2500 m³ ha^{-1}. The waste used in the experiment comes from a swine production system in the finishing phase, handled with biodigester PVC blanket and stabilization pond, being stored for about 20 days. After this period, the SW is applied in the grazing areas. There was the uniform grass height of Tifton 85, 0.10 m tall, with hydraulic brush cutter before the start of the experiment. After the treatments (Table 2) and data collection (21 day cycle), the remaining forage of each cycle was quantified and grazed up to a height of 0.10 m.

The application doses of SW were performed manually with a 1-inch diameter hose and distributed evenly over each plot. The doses were calculated due to the application of time on each plot. Thus, the flow rate was set at 45 L min^{-1} and application times were 0 (no application), 2, 4, 8 and 10 min, respectively providing from the lower treatment dose to the higher dose. Each dose of SW was split into five applications, always at the beginning of each 21-day grazing cycle (Table 2). This installment was not to exceed the field capacity in a single application. In each application date, they were collected a sample of 600 mL of SW and stored in refrigerator. The five SW samples were homogenized, then pulled out a sample that was sent to Araxá Environmental laboratory for chemical characterization. The average levels of nutrients are SW: N = 823.60 mg L^{-1}; P = 20.46 mg L^{-1}; = K^{+} 509.40 mg L^{-1}; Ca^{2+} = 51.54 mg L^{-1}; Mg = 33.53 mg L^{-1}; organic matter = 331.80 mg L^{-1}, B = 0.55

Table 1. Chemical characteristics and grain size of the soil, in the depths studied the experimental area with the Tifton 85 grass before application of treatments.

Depth	pH	P resin	K^+	$S\text{-}SO_4^{2-}$	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al
(m)	(water)	...........mg dm^{-3}..............			.......................$cmol_c\ dm^{-3}$........................			
0.00-0.20	5.50	39.90	94.0	1.77	1.00	0.30	0.15	2.60
0.20-0.40	5.50	18.20	56.0	2.02	0.70	0.10	0.20	2.10

	B	Cu^{2+}	Fe	Mn^{2+}	Zn^{2+}	SB	t	T
	....mg dm^{-3}.................................					............$cmol_c\ dm^{-3}$..........		
0.00-0.20	0.11	4.00	91.00	6.30	8.10	1.54	1.69	4.14
0.20-0.40	0.28	1.60	28.00	2.40	2.00	0.94	1.14	3.04

	MO	V	m	Clay	Silt	Sand
	%	%	%	.... g kg^{-1}.................................		
0.00-0.20	2.3	37	09	153	25	822
0.20-0.40	1.6	30	17	165	17	818

Potassium (K) = (HCl 0.05 mol L^{-1} + H_2SO_4 0.0125 mol L^{-1}); available P (resin); Ca, Mg, Al, (KCl 1 mol L^{-1}); H + Al = (Buffer Solution - SMP pH 7.5); SB = Basic Sum; T = CEC at pH 7.0; V = Base saturation; m = saturation by aluminum; Organic matter (M.O.) = Colorimetric Method. Boron (B) = (0.0125% $BaCl_2.2H_2O$); Cu, Fe, Mn, Zn = (DTPA 0.005 mol L^{-1} + TEA 0.1 mol^{-1} $CaCl_2$ 0.01 mol L^{-1} a pH 7.3). Clay: pipette method. Chemical analysis carried out according to methodologies described by Embrapa (2009).

Table 2. Total dose of SW, portions of value applied to each treatment and dates of split applications in grass Tifton 85.

Dose total	05/01/13	26/01/13	16/02/13	09/03/13	30/03/13
	.... $m^3\ ha^{-1}$				
500	100	100	100	100	100
1000	200	200	200	200	200
2000	400	400	400	400	400
2500	500	500	500	500	500

mg L^{-1}; Cu^{2+} = 4.33 mg L^{-1}; Fe = 6.34 mg L^{-1}; Mn^{2+} = 0.91 mg L^{-1} and Zn^{2+} = 5.71 mg L^{-1} and pH $CaCl_2$ = 8.16. The methodologies used in determining the SW nutrients were based on Standard Methods for the Examination of Water and Wastewater (APHA, 2012). During the experiment, the rainfall and average temperature were measured daily and are displayed by means of each ten-day period (Figure 1). For evaluation of forage growth, five successive cuts were performed (over 0.10 m high) in 21-day intervals (cycles). To collect the sample mass of dry (DM) forage, the sampling method square template, proposed by Aguiar (2009), was used.

The forage harvested in the area sampled per plot were determined a fresh pasture mass. Subsequently, the identified sub-samples were dried in an oven with forced air circulation at 65°C for 72 h to determine the mass of dry matter over 0.10 m high (Gardner, 1986). The percentage of dry matter was then calculated and expressed in kg ha^{-1} DM. Then, soil samples were crushed (Willey mill) to determine the total content of nutrients. To determine the height of grass from the ground level to the highest part a top were used a scale and an x-ray paper were used to standardize the height of the plants, in 10 replications. The bulk density of forage was obtained by dividing the dry mass weight/height plants that was expressed in kg ha^{-1} cm^{-1}. The forage accumulation was calculated by subtracting the herbage mass in pre-grazing forage by the post-grazing mass. Forage accumulation rate (over 0.10 m) was expressed in kg ha^{-1} day^{-1} DM. It was calculated by dividing the accumulation of forage for 21 days grazing cycle by 21, the number of days in the cycle.

The pasture's carrying capacity was calculated considering an herbage allowance of 5 kg DM per 100 kg live weight. After the measurements of pasture, the cattle were put to graze, aimed at standardizing the grass height to 0.10 m. After 21 days of application of the last installment of SW, soil samples were taken at different soil depths such as 0.00-0.20 m and 0.20-0.40, originating six samples, randomly collected in the plot with a Dutch auger. The pH in water, exchangeable acidity (Al^{3+}), potential acidity (H + Al) and soil organic matter (OM), phosphorus (P), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}) copper (Cu^{2+}), zinc (Zn^{2+}), manganese (Mn^{2+}) and iron (Fe) was analyzed, according to the methodology described by EMBRAPA (2009).

The aerial parts of the plants were subjected to analysis of the N, P, K, Ca, Mg, S, Fe, Cu, Mn, Zn, B according to methodologies EMBRAPA (2009) after each 21-day cycle. With the nutrient content of aerial parts of each cycle, the accumulation of these nutrients and recovery efficiency of each of the plant were evaluated. The accumulation of nutrients in the aerial part of each cycle was then used to define the nutrient uptake of the grazed pasture (kg ha^{-1}) to 105 days of experiment. All results were analyzed using the Barttlet and Jarque-Bera test (Jarque and Bera, 1980) to check the homogeneity of variances conditions and normality, respectively. Analysis of variance and regression

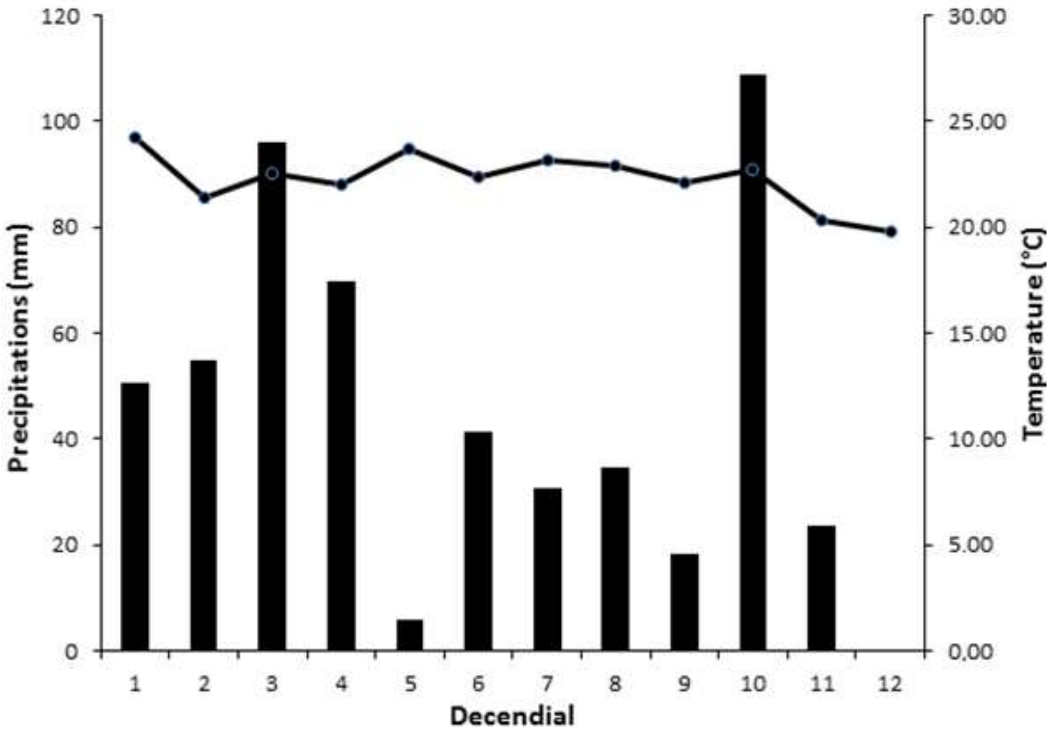

Figure 1. Storm Precipitation and Average Temperature during the period experiment.

analysis for the SW doses were carried out, to determine levels of significance, using the SAEG 9.1 program, 2007.

RESULTS AND DISCUSSION

The results of analysis of variance for the production of components are shown in Table 3. The application of different doses of SW provided a linear increase in all grass production components Tifton 85 studied (Figure 2). The average heights of Tifton 85 ranged between 31 and 39 cm with a 21-day cycle (Figure 2d). Results similar to the Aguiar (2009), an experiment in Uberaba, with intensive management, which found an average height in the spring/summer of 36.4 cm and an annual average of 29.7 cm.

The higher carrying capacity, 10 AU ha^{-1} was obtained with the dosage of 2500 m³ ha^{-1}, whereas grazing efficiency of 50% (Figure 2b). Lupatinie Hernandez (2006) showed, among various types of forage, the Tifton 85 grass with high fertilization responded better to irrigation, with a carrying capacity of up to 10 AU ha^{-1}. Which, showed the great forage production potential of grass associating intensive management, adequate fertilization and irrigation. The accumulation rate of dry matter per day obtained averages 138-219 kg ha^{-1} day^{-1} (Figure 2e). Aguiar et al. (2005) showed an average annual accumulation rate of forage of 172 kg ha^{-1} day^{-1}. Drummond et al. (2006) reached herbage accumulation rate of 148.2 kg ha^{-1} day^{-1} of dry matter in grazing Tifton 85 fertilized with swine waste in the region of Uberaba - MG. Research works on pastures have presented linear increases of forage dry matter using doses of wastewater (Orrico junior et al., 2013; Andrade et al., 2014; Homem et al., 2016), with average accumulation doses of up to 170 kg ha^{-1} day^{-1} of DM in Tifton 85 grass using swine wastewater (Andrade et al., 2014).

Vielmo et al. (2011) used SW at doses of 0 to 320 m^3 ha^{-1}, cycle of 28 days on Tifton 85, and verified a production of 151 kg ha^{-1} day^{-1} of dry matter at the highest dose. Gomes et al. (2018) obtained higher productivity, equal to 189 kg ha^{-1} day^{-1} of dry matter using dose of 300 m^3 ha^1. Andrade et al. (2012) in an experimental area on the campus of Rio Paranaiba UFV evaluated the forage accumulation and managed in the intensive irrigated system. The ideal point of grazing and the chemical composition of forage produced in summer and autumn in pasture managed in intensive system with Tifton 85 grass found herbage accumulation rates of 140.0 kg ha^{-1} day^{-1} of DM in the summer and 122.2 kg ha^{-1} day^{-1} of DM in the fall. The height of the ideal grass for grazing was 25.4 cm.

The highest production of dry matter was obtained with an average dose of 2500 m³ ha^{-1} (Figure 2a), which was 66.5% higher than the average control, in the period of 105 days. The linear increase of production and grass

Table 3. Averages of the components related to production Tifton 85 subjected to increasing doses of SW.

Components of production	Averages	p-valor	CV%
Carrying capacity (UA ha^{-1})	7.69	0.000	5.90
Production of dry matter (kg ha^{-1})	18159.80	0.000	5.90
Density (kg ha^{-1} cm^{-1} of DM)	109.40	0.008	7.08
Dry matter (%)	23.39	0.058	3.15
Grass height (cm)	34.83	0.000	2.05
Accumulation rate of dry matter per day (kg ha^{-1})	172.95	0.000	5.90

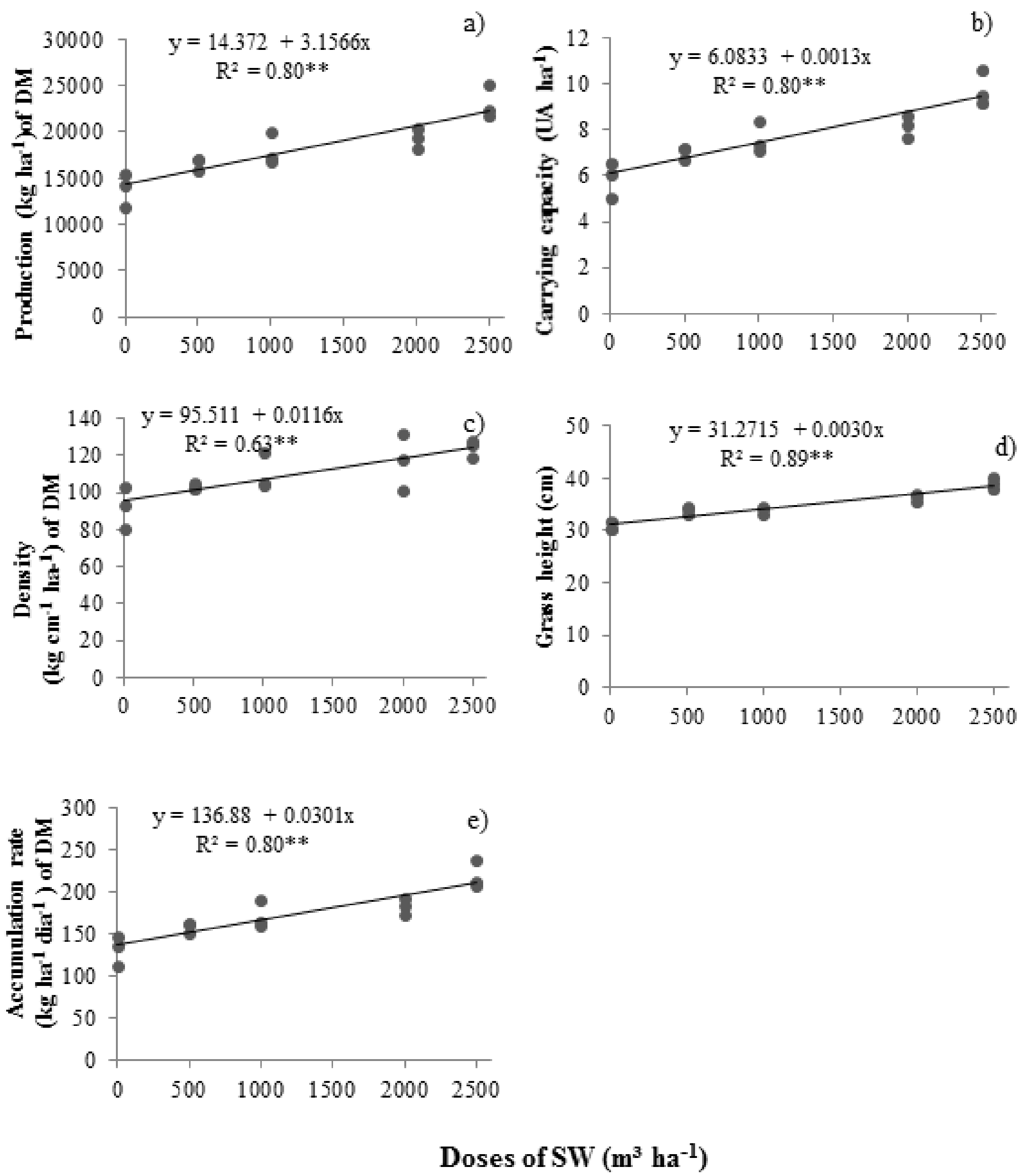

Figure 2. Components of production: production (kg ha^{-1}) **(a)** carrying capacity **(b),** density **(c)** of the grass height **(d)** and accumulation rate of dry matter per day **(e)** the trial period with the Tifton 85 grass, when subjected to increasing doses of SW. **: Significant at 1% probability.

Table 4. Average macro content, micronutrients and chemical characteristics of the soil subjected to increasing doses of SW.

Depth	Variable	P resin	K^+	$S\text{-}SO_4^{2-}$	Ca^{2+}	Mg^{2+}	SB	H+Al	T
		.... mg dm^{-3}			.... cmol$_c$ dm^{-3}				
0.00-0.20 m	Averages	31.27	101.00	3.67	0.64	0.44	1.34	2.29	3.63
	p-valor	0.385	0.468	0.011	0.230	0.034	0.092	0.594	0.344
	C.V. %	58.97	22.62	39.83	24.71	22.92	17.82	14.07	10.67
0.20-0.40 m	Averages	7.16	102.00	4.87	0.43	0.26	0.95	1.75	2.7
	p-valor	0.241	0.742	0.003	0.004	0.001	0.008	0.028	0.633
	C.V. %	57.79	27.81	26.66	16.85	13.14	14.94	10.95	8.12

Depth	Variable	pH	M.O.	V	B	Cu^{2+}	Fe	Mn^{2+}	Zn^{2+}
		(water)	%	%	.... mg dm^{-3}				
0.00-0.20 m	Averages	5.49	1.69	36.82	0.10	3.10	54.40	7.22	5.27
	p-valor	0.089	0.328	0.168	0.096	0.464	0.679	0.029	0.652
	C.V. %	3.44	15.64	14.91	15.18	26.26	42.83	23.21	42.75
0.20-0.40 m	Averages	5.61	1.05	35.09	0.9	1.89	18.87	4.52	1.5
	p-valor	0.003	0.906	0.003	0.977	0.725	0.313	0.015	0.463
	C.V. %	4.17	12.76	13.22	22.32	64.97	26.21	24.81	31.38

height influenced the dry matter density, which increased from 92.4 kg ha^{-1} cm^{-1} DM in witness to 125.2 kg ha^{-1} cm^{-1} DM at the highest dose (2500 m³ ha^{-1}), an increase of 34.5% in the grass density (Figure 2c). With increasing doses of the SW, the pH did not show any variation in the two depths (Tables 1 and 4), due to the alkaline characteristics of SW. According to the results this study, Silva et al. (2014) observed that soil attributes related to the acidity did not suffer influence of successive applications of SW. These differed from Queiroz et al. (2004) who observed a lowering of the pH with the application of SW. According to Bouwer (2000), in soils receiving wastewater, there may be a decrease in pH due to the mineralization of organic compounds of the SW, which facilitates the production of CO_2 and organic acids. Already, Lucas et al. (2013) observed the pH increased from 5.47 to 6.77 in the soil (0 to 0.60 m) at 1015 days of SW application. Increased pH values of soil were consistent with the high pH values of SW used, ranging from 7.08 to 7.70.

The available potassium content (K^+) in the soil was similar at both depths with 101.0 and 102.0 mg dm^{-3} levels (Table 4) and fall within the adequate availability class, according to CFSEMG Ribeiro et al. (1999). A significant increase in K^+ content at a depth of 0.20-0.40 m, indicated a percolation of K^+ for the deeper layers, because the soil characterization analysis before applying the SW doses (Table 1) the levels were 94.0 and 56.0 mg dm^{-3} in these depths. By being a monovalent, K^+ has low retention on soil colloids, being susceptible to being leached from the surface layers for the subsoil layers. Which with the application of SW doses, the amount of added K^+ was high than extracted by Tifton 85 (Figure 3c), favoring an increase in the levels of K^+ in the soil (Table 4).

The potassium content of the soil increased in this experiment because when compost is used, according to Scherer (2001), the potassium K^+ from the mineral and organic fertilizer are similar. Thus, there is no requirement to undergo any mineralization through the action of microorganisms.

According to Penha et al. (2015) long-term applications of pig slurry in a Brazilian Cerrado soil have shown to affect chemical characteristics of the soil. High pig slurry rates increased P contents only in the soil surface, while the contents of K increased throughout the soil profile. This fact shows the marked difference in terms of P and K behaviors in tropical soils, indicating that K is more prone to be leached in a Cerrado soil following successive applications of pig slurry. The phosphorus (P) showed a higher concentration in the surface layer (Table 4). This is because the positive organic radicals in SW adsorb P, favoring surface accumulation. Also the clay in these soils are sesquioxidic which feature high phosphorus adsorption. The P in Cerrado soils have low mobility focusing mainly in layers of 0.00 to 0.10 m. In a study on the use of swine wastewater in natural pasture, Ceretta et al. (2003) showed no changes in phosphorus concentration, with high concentration of P in the surface layer, with increases of 580% to 8.3 months and 6.710% at 48 months of application liquid effluent from pig farms. These studies indicate the importance of monitoring when performing constant application of SW in the same area.

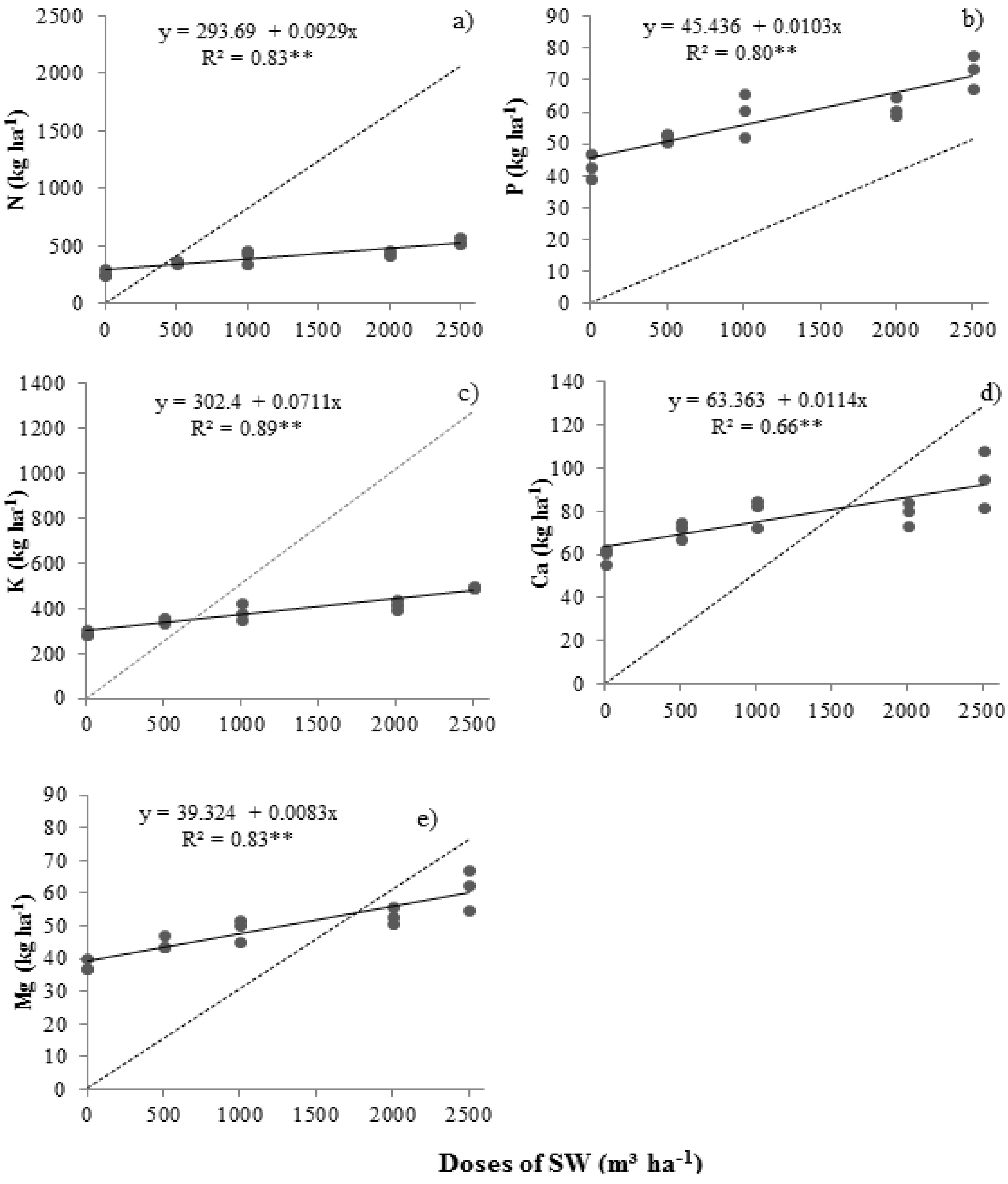

Figure 3. Extraction of the nutrients N (a), P (b), K (c), Ca (d) and Mg (e) by dry weight.
** and *: significant at 1 and 5% probability, respectively. The dotted line refers to the addition of nutrients to the soil by increasing doses of SW and the continuous line for extracting the forage.

However, Seganfredo et al. (2017), reports that the application of SW increases the ease of desorption of P and this makes it necessary to control its movement in the profile and transport through runoff. There was a significant effect of the SW application on the content of (Mg^{2+}) available in soil (Table 4). According to Ribeiro et al. (1999), the levels observed in two depths remained low (0.44 and 0.26 $cmol_c$ dm^{-3}). However, there was a significant increase in Mg^{2+}, in both evaluated depths, as in soil characterization analysis before applying the SW

Table 5. Medium of the leaf content of macro and micronutrients of Tifton 85 subjected to increasing doses of SW.

Element	N	P	K	S	Ca	Mg	B	Cu	Fe	Mn	Zn
	................................g kg^{-1}..............................						........................mg kg^{-1}........................				
Averages	22.62	3.12	21.28	1.69	4.18	2.66	18.11	11.19	151	242	144
p-valor	0.008	0.064	0.021	0.020	0.565	0.238	0.308	0.984	0.619	0.301	0.004
C.V. %	2.39	4.28	3.56	6.27	4.43	4.98	12.29	13.25	11.31	21.96	0.11

doses (Table 1), the determined values were 0.30 and 0.10 $cmol_c dm^{-3}$ at a depth of 0.00 to 0.20 and 0.20-0.40 m, respectively. With the application doses of the SW the added amount of Mg^{2+} was higher than that extracted by Tifton 85 (Figure 3e), thus favoring the increase in Mg^{2+} content in soil (Table 4). The content of calcium (Ca^{2+}) at a depth of 0.00 to 0.20 m is 0.64 $cmol_c dm^{-3}$, while the depth is 0.43 m 0.20-0.40 $cmol_c dm^{-3}$ (Table 4), and these levels considered low by Ribeiro et al. (1999). However, replacement of Ca^{2+} is less than the amount extracted at doses of 500 and 1.000 m³ ha^{-1} SW, however, higher in doses of 2000 and 2500 m³ ha^{-1} SW (Figure 3d), not favoring the accumulation of calcium in two depths assessed (Table 4). Queiroz et al. (2004) observed no changes in Ca^{2+} levels in soil with wastewater.

The base saturation (V) showed a significant increase in the depth of 0.20-0.40 m (35.09%) with the application of SW doses (Table 4) in relation to the initial content (30%) (Table 1). This result is related to the increase in potassium and magnesium content at this depth. With the application of SW, the levels of organic matter that were 2.3% at a depth of 0.00 to 0.20 and 1.6% in the depth 0.20-0.40 m (Table 1), did not show any increase due to the application of the treatments (Table 4), with no significant differences. Mattias (2006) did not observe an increase of organic matter in the application of liquid effluent from swine farms.

The lack of response to the increase of organic matter can be explained by Assmann et al. (2006) who observed no increase in organic matter content by applying liquid swine waste. For, according to the authors, they must be considered inherent characteristics of manure used, where the quality of the organic compounds may determine a greater or lesser accumulation in the soil. The organic compounds present in the liquid manure of pigs have been easily digested by oxidizing in a few days or weeks and are favored for higher microbial activity. The microbial biomass is considered a vital part of the organic matter, composed of micro-organisms (bacteria, fungi and actinomycetes), comprising 2 to 5% carbon and up to 5% of total nitrogen (Moreira and Siqueira, 2003). There were no significant differences among treatments in nutrient contents Fe, Cu^{2+} and Zn^{2+} (Table 4). However, for Lucas et al. (2013) and Rosa et al. (2017a, b), the applications of swine wastewater favored the accumulation of copper and zinc.

According to Girotto (2007), successive applications of SW to the soil cause accumulation of Zn^{2+} in the surface layers that were found significant for the accumulation of Zn^{2+} layer to the depth of 0.10 m. The Cu^{2} + and Zn^{2+} elements are used in animal feed as a supplement, without being fully absorbed and therefore excreted in high amounts, remaining in SW (Rosa et al., 2017b). The high capacity of Oxisol adsorbs Cu and Zn and low mobility of these elements could be verified by (Lopes et al., 2014). Gomes Filho et al. (2001) reported poor copper mobility in soil, stating that this element among the heavy metals is the most strongly adsorbed or complexed by the soil. Furthermore, according to Lopes et al. (2014) high adsorption of Cu^{2+} and Zn^{2+} may be the result of an increase in soluble organic compounds, which have the ability to complex these nutrients. The average leaf content of nutrients N, P, K, S and Zn differed due to the application of increasing doses of SW (Table 5). Thus, the application of different doses of SW influenced the foliar contents of these nutrients.

Knowledge of the leaf content of an intensive system of production, mainly in a very demanding nutritionally species, such as Tifton 85 grass, is very important for determining the amount of nutrient to be restored, reaching thus the mass production desired dry matter. Silva (1999) described the range of suitable concentrations for Tifton as follows, relative to macronutrients: C = 20.0-26.0; P = 1.5-3.0; K = 15.0-30.0; Ca = 3.0-8.0; Mg = 1.5-4.0; S = 1.5-3.0 g kg^{-1}. For optimal range micronutrient is: B = 5.0-30.0; Cu = 4.0-20.0; Fe = 50.0-200.0; Mn = Zn = 20.0-300.0 and 15.0-70.0 mg kg^{-1}.

In this experiment the nutrient contents fall within the proper range suggested by Silva (1999), except Zn, wherein the average of treatments was 144 mg kg^{-1}. The extraction of nutrients by dry weight of the shoot (above 10 cm) was high, demonstrating that the Tifton 85 grass has high nutrient extraction capacity (Table 6). The extraction of macro and micronutrients of shoots of grass Tifton 85 follows the following order: N> K> Ca> P> Mg> S> Mn> Fe> Zn> B> Cu (Table 6). As the DM output followed a linear growth projection (Figure 2a), all nutrients obtained the same trend (Figures 3 and 4).

The dose of 500 m³ SW ha^{-1} provided the soil 411.08 kg ha^{-1} of N and extraction was 358.01 kg ha^{-1} of N, which corresponds to 87.09% of the applied level. These results show that the synchronism between the availability of N from SW culture and demand generated

Table 6. Extraction of nutrients from the Tifton 85 grass (dry matter), subjected to increasing doses of SW.

Element	N	P	K	S	Ca	Mg	B	Cu	Fe	Mn	Zn
	..........kg ha^{-1}..........										
Averages	405.14	57.77	387.69	31.48	77.05	49.68	0.33	0.2	2.88	4.61	2.86
p-valor	0.001	0.001	0.000	0.005	0.017	0.008	0.057	0.025	0.007	0.078	0.050
C.V. %	7.73	6.25	5.34	11.23	9.65	8.98	10.50	13.30	8.49	27.09	15.36

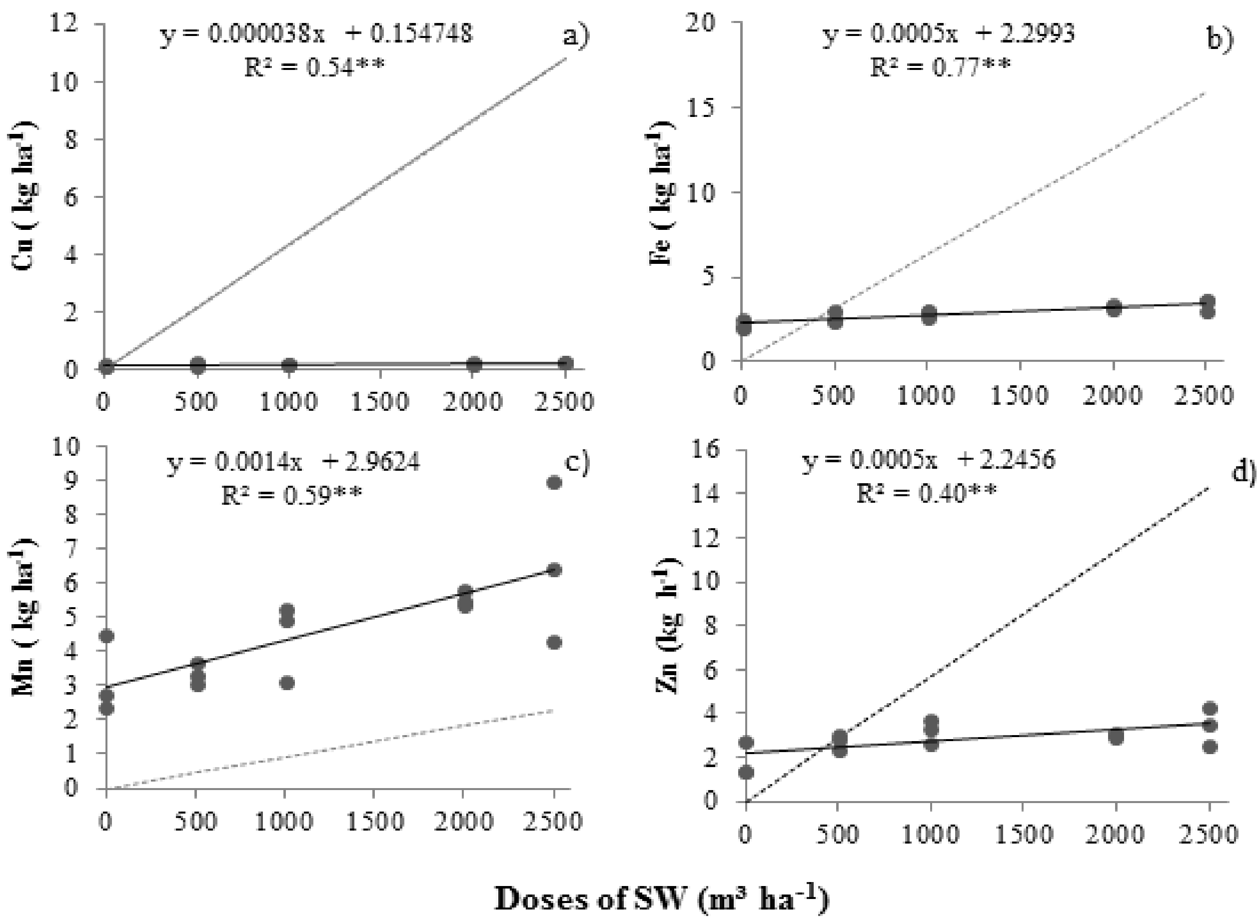

Figure 4. Extraction of micronutrients Cu **(a),** Fe **(b),** Mn **(c)** and Zn **(d)** by dry weight.
** Significant 10% probability. The dotted line refers to the addition of nutrients to the soil by increasing doses of SW and the continuous line for extracting the forage.

a beneficial effect on the nutrient recycling process. This balance can reduce the mineral N concentration in the soil solution, promoting the sustainability of the production system. Surely, this dose is the most appropriate in the environmental point of view with respect to N (Figure 3a). Sharpe and Harper (2002) further justify about 35% of N SW is lost in the form of NH_3 during application. The phosphorus provided by increasing doses of SW was lower than that extracted by the plant in all treatments (Figure 3b), since the nutrient content in this SW is low (20.46 mg L^{-1}). Silva et al. (2012) conducted an experiment on the Bonsucesso farm and found that the phosphorus content in SW was reduced by approximately 80% to pass the Biodigester and the lagoon decantation, thus justifying the low content in SW. According to Mattias (2006), the accumulation of P in soils treated with SW correlates with a lower N/P ratio of waste, since it meet the required amounts of N by plants it is necessary to simultaneously apply greater amounts of P resulting in accumulation and organic P moving in the environment. At a dose of 500 m³ ha^{-1} potassium applied via wastewater (254.69 kg ha^{-1})

was lower than that extracted by the plant (Figure 3c). Higher doses left nutrient residues in the soil (Figure 3c and Table 4).

The calcium extractions (Figure 3d) and magnesium (Figure 3e) by Tifton 85 grass were lower than the amount provided by the SW dose 2.000 m³ ha^{-1}. In this dose, the SW has provided the soil 103.8 kg ha^{-1} of calcium and magnesium 67.06 kg ha^{-1} and extracting the Tifton 85 was 86.16 kg ha^{-1} of calcium and 55.92 kg ha^{-1} magnesium, this being the SW dose sufficient to provide the amount extracted at 85 Tifton. There is a tendency to accumulate copper (Cu) in soil with SW (Figure 4a). Cu average extraction obtained in the dry matter of grass Tifton 85 was 0.2 kg ha^{-1}. The lowest dose of SW added tem times more than the Cu extracted by forage, while the highest dose added 40 more times. Figure 4 illustrates the low extraction by Cu Tifton 85 grass.

Lima and Miyada (2003) conducted an experiment with Cu in the form of cupric citrate, a more soluble source with greater utilization by the animal and less waste left in the excretions. Thus, they concluded that cupric citrate may replace the Cu sulfate, contributing to reducing this nutrient in the SW. SW in any of the doses could supply the manganese (Mn) needed for the production of Tifton 85 grass (Figure 4c). It is necessary to monitor this nutrient in the soil and the plant, as part of nutrients extracted by grass after being consumed by cattle and returned to the soil through animal waste. Zn supplied by the dose of 500 m³ ha^{-1} was 2.86 kg ha^{-1} approaches the extracted amount of and 2.72 kg ha^{-1} at Tifton 85 (Figure 4d). The view on organic waste recycling needs to be diversified, being recovery and recycling of nutrients from organic wastes are a possible solution. When organic waste recycling is complemented by nutrient extraction, some nutrient loops within society can be closes, enabling more sustainable agricultural production in future (Kirchmann et al., 2017).

Conclusions

The application of increasing doses of SW promoted a linear increase in carrying capacity, mass production of dry matter, density, height, and mass accumulation rate of dry matter per day of Tifton 85 grass. The wastewater doses promoted changes in soil chemical properties. The dose of 2.000 m³ ha^{-1} supplied the nutritional needs of the grass Tifton 85 in nitrogen, potassium, calcium, magnesium, copper, iron and zinc.

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CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Evaluation of improved chickpea varieties for resistance to Fusarium wilt (*Fusarium oxysporum)* under field condition in sick plot

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Chickpea Fusarium wilt caused by *Fusarium oxysporum f. sp ciceris* is a devastating disease of chickpea in Ethiopia. This study was done to evaluate the resistance of the improved chickpea varieties to fusarium wilt disease. About nine kabuli and ten desi types including one JG-62 differential were evaluated in RCBD Design with three replications in Debre Zeit sick plot in 2017. Results of the study showed that there are significant differences in reaction to fusarium wilt disease in both main and off-seasons. In main season, four kabuli chickpea varieties were susceptible and five were moderately resistant to wilt/root rot. The highest incidence (99.06%) was found in JG-62 differential followed by variety Yelbe (70.54%). In compression, three varieties were moderately resistant in off-season. Some of the promising varieties had lowest incidence of 13.92% followed by 16.39%. Fusarium wilt incidence was higher in main season than off-season. This is due to soil temperature and soil moisture is the indicator of variation in response to the same varieties. The majority of the varieties were specific adaptation and race specific resistance that break their source of resistance. Therefore, high genotypes released through phenotyping in pipeline at different fusarium wilt affected areas will be advisable. The reaction of variety or genotypes versus race using differential line under sick plot will be recommendable for non-races specific resistance source.

Key words: Chickpea**,** Fusarium wilt, incidence, phenotyping, races.

INTRODUCTION

Chickpea is attacked by a number of soil-borne and foliar diseases as well as field and storage insect pests. Among the soil-borne diseases Fusarium wilt (*Fusarium oxysporum f. sp ciceris)* is one of the most important root diseases affecting chickpea and wide spread in chickpea growing areas such as Asia, Africa and Southern Europe, where the chickpea-growing season is dry and warm (Meki et al., 2008; Sharma and Muehlbauer, 2007; Rafael et al., 2015). It is widely distributed and has been reported from at least 33 countries (Singh and Sharma, 2002; Dubey et al., 2007). The average yield reduction of chickpea due to Fusarium wilt globally varies from 10 to

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15% and can reach as high as 100% in some countries (Navas-Cortes et al., 2000; Sharma et al., 2005). Early wilting is reported to cause 77-94% yield loss (Haware and Nene, 1980). In Ethiopia, about 30% yield loss of chickpea due to chickpea wilt has been reported (Meki et al., 2008).

It is one of the major soil or seed borne disease of chickpea worldwide (Jalali and Chand, 1992). However, evolution of new races poses a serious threat to deployment of wilt resistance in chickpea. Wilt is more severe on sandy soil and less severe on clay loam soil. The pathogen appears to be highly variable (Jimenez-Diaz et al., 1993). Considering the nature of damage and survival ability of the fungus, management of the disease is difficult either through crop rotation or application of fungicides. Host plant resistance is an approach that gives systemic protection in crop plants for pest and diseases. The most practical and cost-efficient method for management of chickpea wilt is the use of resistant varieties (Bakhsh et al., 2007; Karuppaiah et al., 2013). Developing and releasing wilt/root rot-resistant cultivars is the major objective of the national chickpea improvement programme and chickpea varieties having resistance to wilt/root rot have been released for cultivation in Ethiopia (Geletu et al., 1996).

Majority of both kabuli and desi type of chickpea varieties had no wide adaptability to abiotic and biotic stress such as soil borne diseases that is wilt/root rot, dry/root rot and collar/root rot. Even the resistant varieties become susceptible as they break their source of resistance due to pathogenic variability and race specific reaction. Therefore, it is critical to evaluate their level of resistance and source for selection as breeding source of materials for essential trait and their inheritance resistance nature.

MATERIALS AND METHODS

Description of study area

Field experiment was conducted at Debre Zeit Agricultural Research Center in main and off-seasons irrigation based in 2017 cropping season. They were well established sick plot for wilt/root rot screening. Debre Zeit is located at latitude 8°44’ N, longitude 38°58’ E, altitude of 1900 m above sea level. It receives average annual rainfall of about 851 mm and the mean annual temperature of 28.3 and 8.9°C (maximum and minimum respectively); the soil type is black (verti sol) and is 47 Km away from South- east of Addis Ababa.

Experimental materials and design

A total of 19 varieties released (10 desi types and 9 kabuli chickpea varieties) were used in the study. JG-62 was used as differential check. The evaluation materials were obtained from Debre Zeit, Sirnka and Debre Berhan Agricultural Research Center in 2017 main and off cropping season (furrow irrigation based). The experiment was laid out as a randomized complete block design (RCBD) with three replications. The plot sizes were 4.0 m × 1.20 m. Seeds were planted in 30 cm × 10 cm between rows and seeds respectively.

Data collection and analysis

Disease incidence

Appearance of the disease in the experimental plots was recorded starting from disease onset from the given whole plot on susceptible check death.

$$\text{Mortality} = \frac{\text{No of wilted plants} \times 100}{\text{Total no. of the plants}}$$

The levels of resistance and susceptibility of infected plants were evaluated by using common ICARDA method (Nene et al., 1981): Highly resistant: Less than 2% plant are infected (HR); Resistant: 2-10% plants infected (R); Moderately resistant: 11-20% plant infected (MR); Moderately susceptible: 21-50% plant infected (MS); Susceptible: above 50% plant are infected (S).

Data analysis

Data on disease incidence were subjected to analysis of variance (ANOVA) using R statistics with General Linear Model. Mean separation was based on the LSD at the 5% probability level.

RESULTS AND DISCUSSION

The reaction of genotypes to fusarium wilt is presented in Table 1. The data revealed variation among chickpea varieties for Fusarium wilt disease resistance. There was highly significant difference in varieties ($P<0.001$)’s reaction Fusarium wilt in both main and off-seasons. Out of the nine chickpea varieties, four varieties (Yelbe, Arerti, Acos dubie and DZ-10-4) were susceptible whereas, five varieties (Habru, Shasho, Teji, Ejere and Chefe) were moderately resistant to Fusarium wilt. The highest incidence (99.06%) was in JG-62 differential followed by 70.54% in Yelbe and lowest incidence (26.5%) in Chefe variety. Meki et al. (2008) reported that out of the eight improved chickpea varieties released for cultivation in Ethiopia since 1970 only two varieties, namely DZ-10-4 and Arerti are kabuli types; they proved resistant in Debre Zeit sick plot which disagrees with our finding that both are susceptible to wilt/root rot.

Compared to the seasonal impact of disease severity, off-season six chickpea kabuli (Yelbe, Arerti, DZ-10-4, Shasho, Teji and Ejere) were moderately susceptible and three (Acos dubie, Habru and Chefe) were moderately resistant. The highest incidence (98.92%) was in JG-62 differential and lowest incidence (13.9%) was recorded in Chefe. The Fusarium wilt intensity was higher in main season compared to off season. Overall, three varieties, Shasho, Teji and Ejere with consistent reaction of improved chickpea both in main and off-season were moderately susceptible. Chaudhry et al. (2007) screened

Table 1. Mean of fusarium wilt incidence for kabuli chickpea varieties in 2017 both main and off-season in Debre Zeit sick plot.

Genotypes	Main season Mean ± SD	Reaction	Off Season Mean ± SD	Reaction
JG-62	99.06[a] ± 0.81	S	98.92[a] ± 0.38	S
Yelbe	70.54[b] ± 14.05	S	26.01[bcd] ± 2.74	MS
Arerti	52.73[bc] ± 20.24	S	37.62[b] ± 19.21	MS
Acosdubie	41.83[cd] ± 11.10	S	18.79[cd] ± 1.34	MR
DZ-10-4	41.19[cd]± 5.08	S	32.95[bs] ± 14.55	MS
Habru	38.36[cd] ± 2.86	MS	16.39[d]± 10.25	MR
Shasho	35.81[cd] ± 7.58	MS	26.53[bcd] ± 8.76	MS
Teji	35.08[cd] ± 8.47	MS	27.76[bcd] ± 9.40	MS
Ejere	32.16[d] ± 5.57	MS	22.43[bcd] ± 3.97	MS
Chefe	26.53[d] ± 18.5	MS	13.92[d] ± 4.58	MR
Grand mean	47.33		32.13	
CV (%)	24.29		28.37	
LSD (0.05)	19.64		15.58	
Error	132.18		83.16	

Means sharing similar letters do not different significantly at P<0.05. whereas; S= susceptible, MS= Moderately susceptible, MR= Moderately resistant and R= Resistant.

196 chickpea germplasm lines/cultivars for resistance to fusarium wilt disease in a wilt sick plot. None of the test line was found immune or highly resistant. These findings are quite in conformity with our results. The pathogen is highly variable in their nature. Iqbal et al. (2005) also report the sources of resistance to Fusarium wilt in chickpea germplasm originating from national and international research institutes. Delay in sowing can also help to escape disease from such severely affected areas. On the other hand, the varieties that showed resistance or tolerance are most suitable for exploitation in breeding programs or for direct sowing in wilt prone areas. This is concurrent with findings of Karimi et al. (2012). There was indication of season difference in kabuli type of chickpea varieties for their resistance to wilt/root rot. The reaction of genotypes is also influenced by various factors like soil temperature, soil moisture and depth of planting. High soil moisture and optimum soil temperature in main season account for high wilt/root rot incidence. Low level of soil moisture reduces plants mortality at 20°C (Sinha, 1973; Chi and Hanson, 1964). Increased percentage of infected plants, at reproductive compared to seedlings stage is due to increasing temperature (Haware et al., 1992; Mirzapour et al., 2014). It is also related to depth planting; seasonal difference of planting of seeds at proper depth (10-12cm) can reduce the disease incidence in off season (Singh and Sandhu, 1973), while shallow sown crop can be affected by the disease (Dahiya et al., 1988).

The interaction effects of main season by off season to Fusarium wilt revealed highly significant difference (P<0.001) in the genotypes. Three varieties (JG-62, Yelbe and Arerti) were susceptible and seven varieties (Acosdube, Shasho, Habru, Ejere, Teji and Chefe) were moderately susceptible during the seasons by variety. The highest incidence (98.99%) was recorded in JG-62 differential and lowest incidence (20.22%) was found in variety Chefe (Table 2). There was an indication of the pathogenic variability and soil factors such as moisture, temperature and inoculum load effects across season. Almost all of the varieties range from moderately susceptible to susceptible to wilt/root rot in kabuli type of chickpea in the same sick plot. Releasing one or two resistant varieties at a time and growing them in wider areas for long periods can cause new race development due to directional selection (Keller et al., 2000). Thus, it may be necessary to expand breeding work and increase varietal diversity to prevent development of new races through practicing different resistant gene management systems.

The present study results (Table 3) indicated that a highly significant effect (P<0.001) was noted against fusarium wilt in desi type of chickpea in main cropping season. Out of the ten desi types of chickpea one variety (Akaki) was susceptible and eight varieties (Worku, Natoli, DZ-10-11, Dalota, Teketay, Mariye, Mastewal and Dubie) were moderately susceptible in main season. Moreover, in off-season one variety was (Akaki) susceptible followed by five varieties (Worku, DZ-10-11, Dalota, Mastewal and Dubie) that were moderately susceptible and three varieties (Natoli, Tekatey and Minjar) that were moderately resistant and one variety (Mariye) that was resistant. A comparison of the result of the present study with a similar work by Haware and Nene (1982) and by Dolar (1997) indicates the presence of similar variability in isolates of *Fusarium oxyporum* f. sp. *ciceris* among the three countries (Ethiopia, India and Turkey). However, the susceptibility of some of the

Table 2. Combined mean of fusarium wilt incidence for kabuli chickpea varieties in 2017 in Debre Zeit sick plot.

Genotypes	Mean ± SD	Reaction
JG-62	98.99[a] ± 0.51	S
Yelbe	48.27[b] ± 26.01	S
Arerti	45.17[b] ± 1.00	S
Acosdubie	30.31[cd] ± 0.51	MS
DZ-10-4	37.07[bc] ± 1.05	MS
Habru	27.38[cd] ± 1.02	MS
Shasho	31.17[cd] ± 0.99	MS
Teji	31.42[cd] ± 1.33	MS
Ejere	27.29[bcd] ± 1.56	MS
Chefe	20.22d ± 0.98	MS
Grand mean	39.73	
CV (%)	25.92	
LSD (0.05)	12.01	
Error	106.10	

Means sharing similar letters do not different significantly at P<0.05. whereas; S= susceptible, MS= Moderately susceptible, MR= Moderately resistant and R= Resistant.

Table 3. Mean of fusarium wilt incidence for desi chickpea varieties in 2017 both main and off-season in Debre Zeit sick plot.

Genotypes	Main season Mean ± SD	Reaction	Off Season Mean ± SD	Reaction
JG-62	97.08[a] ± 1.56	S	98.92[a] ± 0.38	S
Akaki	56.15[b] ± 14.05	S	62.69[b] ± 16.90	S
Worku	40.60[bc] ± 11.35	MS	37.30[c] ± 2.93	MS
Natoli	38.30[bcd] ± 7.75	MS	15.67[ef] ± 8.52	MR
DZ-10-11	36.99[bcd]± 9.39	MS	26.01[cde] ± 15.82	MS
Dalota	35.87[bcd] ± 9.42	MS	20.86[def]± 11.18	MS
Teketay	34.06 [cd] ± 15.08	MS	19.05[def] ± 4.94	MR
Mariye	31.83[cd] ± 8.41	MS	8.98[f] ± 3.48	R
Mastewal	31.61[cd] ± 3.94	MS	25.86[cde] ± 7.43	MS
Dubie	25.27[cd] ± 9.71	MS	31.69[cd] ± 8.00	MS
Minjar	19.53[d] ± 7.74	MR	13.19 [ef] ± 2.59	MR
Grand mean	40.66		32.74	
CV (%)	29.61		26.84	
LSD (0.05)	20.45		14.92	
Error	145.04		77.29	

Means sharing similar letters do not different significantly at P<0.05. whereas; S= susceptible, MS= Moderately susceptible, MR= Moderately resistant and R= Resistant.

improved varieties indicates that the 'wilt sickplot' is perhaps dominated by a single race, and hence, the cultivars possibly escaped exposure to other virulent races (Meki et al., 2008). There are many sources with high resistance to Fusarium wilt available in desi type, while resistance sources in kabuli type are limited. The highest incidence (97.08%) was recorded in JG-62 differential and lowest (25.27%) was in the variety Dubie. Where as, in off season the highest incidence (98.92%) was in JG-62 and least incidence (8.98%) was found in Mariye followed by variety Minjar (13.19%) (Table 4).

Conclusion

So far different improved chickpea varieties were

Table 4. Combined mean of fusarium wilt incidence for desi chickpea varieties in 2017 in Debre Zeit sick plot.

Genotypes	Mean ± SD	Reaction
JG-62	98.00[a] ± 1.43	S
Akaki	59.42[b] ± 21.49	S
Worku	38.95[b] ± 7.63	MS
Natoli	26.99[cde] ± 14.37	MS
DZ-10-11	31.50[cd] ± 13.10	MS
Dalota	28.35[cde] ± 12.38	MS
Teketay	26.55[cde] ± 12.97	MS
Mariye	20.40[de] ± 13.77	MR
Mastewal	28.74[cde] ± 6.18	MS
Dubie	28.48[cde] ± 8.70	MS
Minjar	16.36[e] ± 6.22	MR
Grand mean	39.73	
CV (%)	25.92	
LSD (0.05)	12.01	
Error	106.10	

Means sharing similar letters do not different significantly at P<0.05. whereas; S= susceptible, MS= Moderately susceptible, MR= Moderately resistant and R= Resistant.

released at national and regional level with the main focus on yield attribute. But, there are few varieties that are resistance to diseases. Chickpea yield potential was constrained due to fusarium wilt disease. With this gap it is critical to evaluate the performance of improved chickpea varieties for their resistance. Among the varieties majority were susceptible to moderately susceptible in both main and off-seasons; whereas, few varieties were moderately resistant in off-season. The varietal resistance level was not consistent based on seasons and variety. The dominant race in wilt sick plot indicates variety resistance was specific resistance. There were no highly resistant varieties of chickpea. This implies that fusarium wilt incidence was higher in main season than off-season cropping. As preliminary study, this research contributes to visibility of wide range of race diversity and race specific resistance to improved varieties not evaluated for none-race specific before releasing at different testing location in chickpea growing areas. But, high varieties through phenotyping with different races in major affected areas and additional sick plot by fusarium wilt and exploring source resistance for non-race specific varieties will be highly useful. Thus, screening for source resistance will be continued as chickpea materials have narrow genetic base for breeding materials and introgression from wild types of chickpea.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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Full Length Research Paper

Host preference and performance of cabbage aphid *Brevicoryne brassicae* L. (Homoptera: Aphididae) on four different brassica species

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Host preference and performance of cabbage aphid *Brevicoryne brassicae* were studied on seven brassica varieties under Greenhouse. Nymphs, apterous and alate aphids were significantly different on day three and day 15 among tested varieties. Higher total number of aphids per plant was recorded on *Brassica carinata* and lower on *Brassica oleracea*. Alates of *B. brassicae* preferred more to feed and reproduce on *B. carinata* varieties than the other tested varieties. Developmental and reproduction periods, fecundity and longevity of *B. brassicae* were significantly different among tested varieties. Shortest and longest developmental period were recorded on Holeta-1 (6.4 days) and Kale (8.9 days), respectively. The aphid had the highest fecundity on *B. niger* (79.5 nymphs/adult) and the lowest on cabbage (62.4 nymphs/adult).The reproductive rate, intrinsic rate of increase, generation time, doubling time of *B. brassicae* were significantly different among tested varieties. The intrinsic rate of increase of *B. brassicae* was 0.337, 0.310, 0.286, 0.276, 0.262, 0.250 and 0.239 days^{-1} on Holeta-1, Yellow Dodola, Axana, Blinda, *B. niger*, Cabbage, and kale respectively. Varieties of *B. carinata* were more suitable for cabbage aphid feeding and reproduction than the other tested varieties.

Key words: *B. brassicae*, reproduction, development, intrinsic rate of increase, fecundity, Ethiopian mustard.

INTRODUCTION

Cabbage aphid *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) is a specialized aphid on brassica plants and creates problem worldwide with a significant negative impact on brassica crops (Pontoppidan et al., 2003; Bashir et al., 2013). Aphid cause direct damage by causing chlorosis and leaf curling on the leaves while disrupting the normal plant growth and development (Colette et al., 2008) and indirect damage through the transmission of viral diseases (Jahan et al., 2013). Brassicaceae plants are most popular vegetable crops consumed all over the world and good source of bioactive phytochemicals (Beekwilder et al., 2008) and they are

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characterized by their secondary metabolites (Glucosinolates) (Al-Gendy et al., 2010; Polat, 2010). The type and concentration of glucosinolates have been found to vary between *Brassica* species, cultivars and between the plant parts within the same plant (Rangkadilok et al*.,* 2002; Windsor et al*.,* 2005; Zytynska and Preziosi, 2011).Variation in plant quality such as physical, chemical or biological traits influences herbivores' performance such as growth rates, fecundity, and survivorship (Awmack and Leather, 2002; Zehnder and Hunter, 2008).

The cabbage aphid *B. brassicae* is a phloem sucking insects that have a strategy for manipulating toxic substance from Brassicaceae crops, which is harmful to other brassica insects (Rebecca et al., 2011). These substances are important as plant defense response and provoked consequently after insect herbivore feeding on the plant (Muhammad et al., 2009; Bones et al., 2011). Glucosinolates are strong stimulant substances for cabbage aphid, and used as signal to select its host plants (Gabrys and Tjallingii, 2002). The toxic effect of the plant substance on cabbage aphid *B. brassicae* is avoided by elimination, detoxification, sequestration methods and behavioral responses of the aphid (Hopkins et al., 2009). Mirmohammadi et al. (2009) and Aziz et al. (2016) studied the effect of *B. napas* and *B. oleracae* cultivars on the performance of cabbage aphid. However, the effect of Ethiopian mustard varieties on the performance of cabbage aphid has not been studied. The variation on quantity and quality of phytochemicals among plant genotypes, tissues and ontogenetic stages has an effect on insect performance (Ode, 2006).Therefore, the aim of the present study was to evaluate the effect of different brassica species on the preference and performance of cabbage aphid *B. brassicae* in Ethiopia.

MATERIALS AND METHODS

Host plant material

The preference and performance of cabbage aphid was studied on seven different brassica varieties; six varieties from three species (two varieties from each species) and one local variety of *B. niger* (red mustard) (Table 1). Seeds of the brassica were sown on plastic pots (15:15:10 cm height, bottom and top diameter respectively) containing 3:2:1 proportion of soil, sandy and manure, respectively to ensure the fertility of the soil for the plant growth and development. The pots were kept under the greenhouse of the, College of Agriculture, Hawassa University.

Aphid source and rearing

The aphid *B. brassicae* was collected from infected cabbage plants at Hawassa and introduced to each of the corresponding varieties planted on pot as stock culture. The plants were kept inside cages covered with fine net to prevent aphids from escaping and parasitoids and predators of the aphid from entering. The aphids were reared for two generations on respective host plants prior to the experiment. Aphids were transferred in to new potted plants when required to maintain the high aphid density for further experiment. The potted plants were kept under green house in ambient conditions at temperature of 28.86±0.82°C and 51.43±2 relative humidity.

Host preference of alate aphids of *B. brassicae*

The experiment was conducted in the greenhouse by placing the stock culture in one side and the aphid free plants in the opposite side direction two meters away. Forty two brassica plants; six plants for each of the seven varieties with high alate cabbage aphid densities on one side were used as aphid stock culture. Winged cabbage aphids were allowed to disperse and to feed on the healthy plants on another side. A total of twenty eight aphid free plants consisted of seven varieties replicated four times were used. Aphid free plants were approximately equal size with only five young, fully expanded leaves. The plants were labeled and randomly arranged on the opposite side in four rows. The number of nymphs, winged and non-winged adults on each plant were recorded three and fifteen days after the plants were exposed.

Performance of *B. brassicae*

Two seeds of each variety were sown on a plastic pot and thinned to one plants per pot two weeks after germination. To have a same age (<24 h) of the first nymphal stage; one young apterous adult was randomly collected from the stock culture and placed on the undersurface of the leaf on each plants. After 24 hours, one newborn nymph was left on the leaf and excess nymphs and adults were removed. Then all experimental pots were caged individually and arranged in completely randomized design with ten replications. Period for first, second, third and fourth instar, nymph developmental period, pre-reproductive, reproductive period, post-reproductive period, adult longevity, lifetime and fecundity were calculated.

Statistical analysis

Number of aphids per plant, developmental and reproduction period, intrinsic rate of natural increase, generation time, number of offspring per female and survivorship (%) on each tested varieties were subjected to PROC GLM analysis of variance procedure in SAS software version 9.0 (SAS Institute, 2002). Means were compared using Tukey's test at p=0.05.

Age specific life table were calculated for cabbage aphids on seven different brassica varieties. Parameters were calculated using *Euler–Lotka Equation* (Begon et al. 2006). The intrinsic rate of natural increase was calculated with the following equation.

$$\sum L_x m_x e^{-r_m x^*} = 1$$

$$L_x = \frac{l_x + l_{x+1}}{2} \quad \text{and} \quad x^* = \text{pivotal } x = \frac{x_l + x_{l+1}}{2}$$

Where, l_x is the fraction of individuals of the initial cohort alive at age x, m_x the number of female progeny produced per female in x and r_m the intrinsic rate of natural increase. The net reproductive rate ($R_o = \sum L_x m_x$), generation time ($T = \ln R_o / r_m$), population doubling time ($DT = \ln 2 / r_m$) and finite rate of increase ($\lambda = \exp.(r_m)$) were calculated.

Table 1. Brassica varieties used for the experiment and their sources.

Varieties	Species	Source
Yellow Dodolla	*B. carinata*	Holeta research Center
Holeta-1	*B. carinata*	Holeta research Center
Cabbage	*B. oleracea var. capitata*	Copenhagen Market
Kale	*B. oleracea var. Aciphala*	Local Market
Axana	*B. napus*	Kulumsa Research Center
Blinda	*B. napus*	Kulumsa Research Center
Red mustard	*B. niger*	Local Market

Table 2. Numbers of alate, nymph and apterae of cabbage aphid on seven brassica varieties after three and fifteen days of infestation.

Varieties	Number of aphids per plant						
	Day 3			Day 15			
	Alate	Nymph	Total	Alate	Apterae	Nymph	Total
Holeta-1	15.8^{a}	29.0^{a}	44.8^{a}	67.50^{a}	183.3^{a}	642.5^{a}	893.3^{a}
Yellow dodola	13.3^{b}	26.3^{a}	39.5^{b}	65.50^{a}	157.3^{ab}	568.0^{ab}	790.8^{ab}
Axana	10.5^{c}	17.0^{b}	27.5^{c}	49.50^{ab}	133.3^{bcd}	510.5^{bcd}	693.3^{bcd}
Blinda	9.3^{cd}	12.8^{c}	22.0^{d}	46.50^{b}	143.5^{abc}	538.5^{bc}	728.5^{bc}
Cabbage	8.3^{cd}	11.8^{c}	20.0^{d}	33.25^{bc}	108.8^{cd}	451.8^{cd}	593.8^{de}
Kale	7.8^{cd}	10.0^{c}	17.8^{d}	29.50^{c}	97.8^{d}	424.8^{d}	552.0^{e}
B. niger	8.0^{cd}	12.0^{c}	20.0^{d}	39.25^{bc}	112.3^{ab}	507.5^{bcd}	659.3^{cd}

Means in the same column followed by the same letters do not differ significantly at P=0.05 (Tukey's test).

RESULTS

Selection of brassicas by Alate *B. brassicae*

The number of cabbage aphid nymphs, winged adults and total aphids (nymph±winged adult) were significantly different on tested brassicae varieties on days three after infestation (Table 2).

Holeta-1 variety of *B. carinata* had the highest total number of alate aphids and nymphs (44.8 aphids/plant) and kale variety of *B. oleracae* the lowest (17.8 aphids/per plant) (Table 2). The number of alate, aptreous and nymphs on day 15 showed similar trend like that of the day three observation with the highest number of aphids on Holeta-1 (893.3 aphids/plant), the lowest on kale (552 aphid/plant) and the other varieties were in between (Table 2). Cabbage aphid preferred to feed on Holeta-1> followed by Yellow dodolla, Blinda, Axena, B. niger, cabbage and kale, in descending order of preference.

The day three pooled data of the varieties to species indicated that significantly more aphids were on *B. carinata* (44.38) than the remaining; *B. napus* (24.75), *B.* number of alates on *B. niger* and *B. oleracea* and the number of nymphs on *B. napus*, *B. niger* and *B. oleracea* were not significantly different. The trends were similar on day 15 and the highest number of aphids were on *B. carinata* (842 aphids/plant), the lowest on *B. oleracea* (572.9 aphids/ plant) and therewas no significant difference between *B. napus* and *B. niger* (Figure 1b).

Development and reproduction

The developmental times of the four instars and the total nymphal period of *B. brassicae* varied on the brassica varieties (Table 3). The longest development time for first and second instar was recorded on Blinda (1.53 days) and Holeta-1 (2.86 days), while the longest developmental time for third (2.26 days) and fourth (2.46days) instar were on kale. The first, third, and fourth instar aphids had the shortest period on Holeta-1 (Table 3). The total nymph period was significantly longer on Kale and there was no significant difference among the other brassicas. However, nymphal period were slightly higher on cabbage, Blinda and *B. niger* than Axana and *niger* (20) and *B. oleracea* (18.87) (Figure 1a). The Yellow dodolla. Adult aphids reared on Kale (2.1) had longer pre-reproductive period while those on Holeta-1(1.2 days) the shortest.

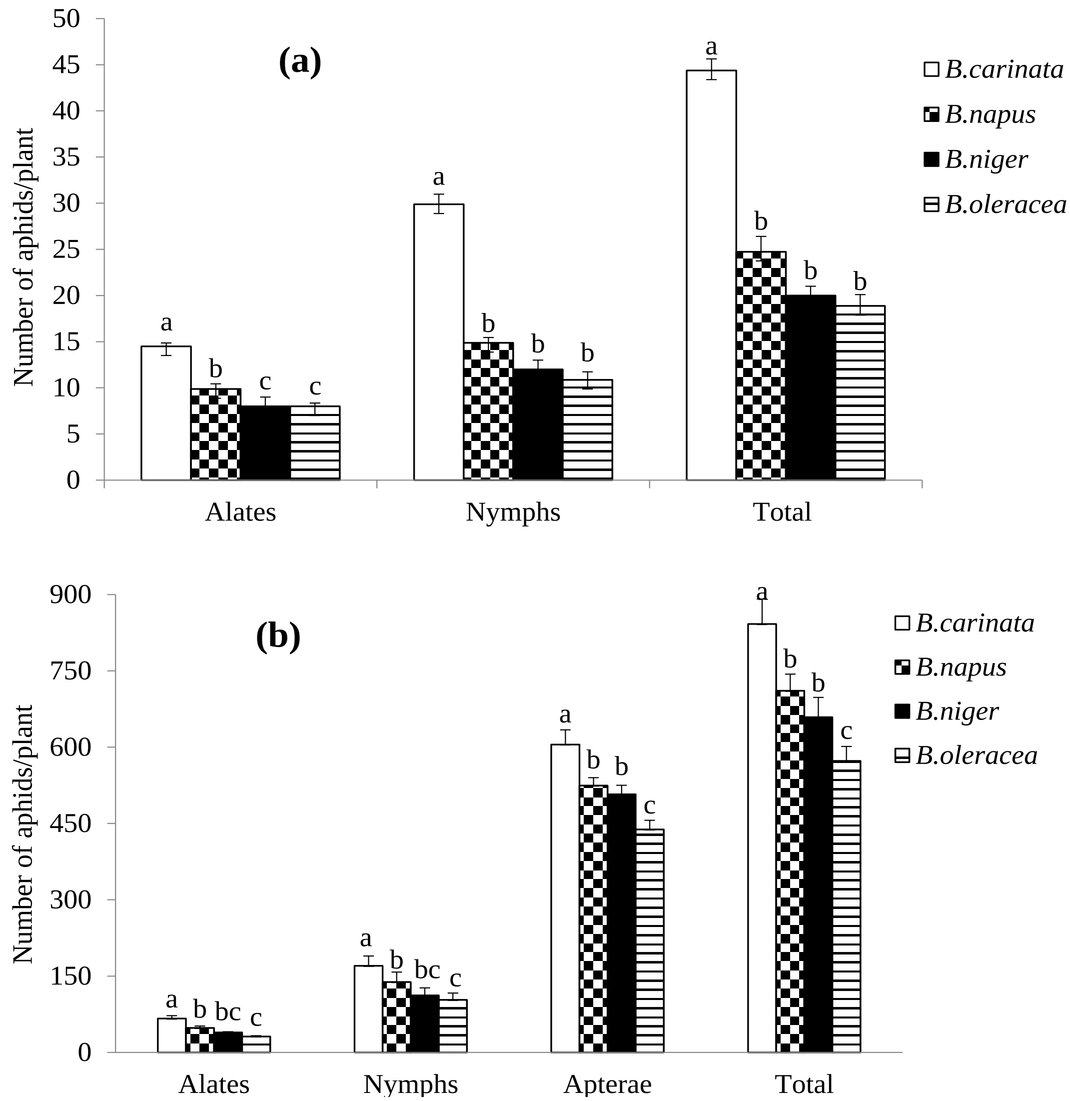

Figure 1. Number of nymphs, alate and apterae adults of *B. brassicae* on four brassica species on day three (a) and day fifteen (b) after infestation. Means ± SE in the same developmental stage (alate, apterae, nymphs) followed by the same letter do not differ significantly at P=0.05 (Tukey's test).

Table 3. Developmental period of nymphal instars of cabbage aphid, *B. brassicae*, on seven brassica varieties (Mean ±SE).

Varieties	Parameter					
	First instar	Second instar	Third instar	Fourth instar	Total Nymphal period	Adult Pre reproductive period
Yellow dodolla	1.20 ± 0.00^{ab}	2.40 ± 0.19^{ab}	1.6 ± 0.16^{b}	1.66 ± 0.18^{b}	6.67 ± 0.20^{b}	1.33 ± 0.21^{bc}
Holeta-1	1.00 ± 0.00^{b}	2.86 ± 0.00^{a}	1.53 ± 0.00^{b}	1.53 ± 0.16^{b}	6.40 ± 0.13^{b}	1.20 ± 0.16^{c}
Kale	1.46 ± 0.13^{ab}	1.60 ± 0.17^{c}	2.26 ± 0.13^{a}	2.46 ± 0.12^{a}	8.86 ± 0.55^{a}	2.06 ± 0.13^{a}
Cabbage	1.33 ± 0.00^{ab}	2.00 ± 0.06^{bc}	1.86 ± 0.16^{ab}	1.73 ± 0.15^{b}	7.00 ± 0.25^{b}	1.46 ± 0.13^{bc}
Axana	1.46 ± 0.10^{ab}	1.60 ± 0.06^{c}	1.80 ± 0.11^{ab}	1.80 ± 0.17^{ab}	6.73 ± 0.20^{b}	1.53 ± 0.13^{abc}
Blinda	1.53 ± 0.10^{a}	1.86 ± 0.15^{bc}	1.80 ± 0.12^{ab}	1.86 ± 0.16^{ab}	6.90 ± 0.19^{b}	1.80 ± 0.13^{ab}
B. niger	1.33 ± 0.00^{ab}	1.86 ± 0.09^{bc}	1.67 ± 0.06^{a}	1.66 ± 0.12^{b}	6.93 ± 0.16^{b}	1.26 ± 0^{bc}

Means in the same column followed by the same letters do not differ significantly at P=0.05 (Tukey's test).

Table 4. Biological parameters of *B. brassicae* on seven brassica varieties (Means± SE).

Varieties	Biological Parameter					
	Total pre reproductive period	Reproductive period	Post reproductive period	Adult longevity	Life span	Total fecundity
Yellow Dodolla	8.00±0.15^{bc}	18.83±1.37^{ab}	4.16±0.94^{a}	24.52±2.17^{ab}	30.90±1.94^{ab}	73.1±2.9^{ab}
Holeta-1	7.60±0.20^{c}	18.50±0.67^{b}	1.33±0.21^{b}	21.29±1.05^{b}	27.40±0.67^{b}	76.3±2.1^{ab}
Kale	10.93±0.18^{a}	20.66±1.56^{ab}	4.17±0.90^{a}	27.00±2.03^{a}	35.76±1.11^{a}	66.8±1.8^{ab}
Cabbage	8.40±0.23^{bc}	20.83±0.98^{ab}	2.67±0.98^{ab}	25.17±1.27^{ab}	31.90±1.58a	62.4±1.9^{b}
Axana	8.26±0.22^{bc}	15.66±1.52^{b}	1.20±0.00^{b}	18.56±1.76^{b}	25.12±1.54^{b}	64.6±5.6^{b}
Blinda	8.73±0.25^{b}	20.50±1.78^{ab}	3.33±0.76^{ab}	25.43±2.20^{ab}	32.56±2.00^{a}	72.2±2.9^{ab}
B. niger	8.20±0.16^{bc}	21.33±0.98^{a}	3.50±1.45^{ab}	25.66±0.98^{ab}	33.03±0.91^{a}	79.5±3.1^{a}

Means in the same column followed by the same letters do not differ significantly at P=0.05 (Tukey's test).

Table 5. Demographic parameters for cabbage aphid *B. Brassicae* on seven brassica varieties (Means ± SE).

Varieties	Ro	T	r_m	DT	λ
Yellow Dodola	45.70±1.30^{ab}	12.3±0.25^{de}	0.310±0.01^{ab}	2.23±0.1^{de}	1.362±0.01^{ab}
Holeta-1	47.75±2.87^{a}	11.45±0.29^{e}	0.337±0.02^{a}	2.05±0.1^{e}	1.399±0.02^{a}
Kale	37.70±4.60^{c}	15.2±0.16^{a}	0.239±0.006^{e}	2.9±0.08^{a}	1.269±0.01^{d}
Cabbage	38.25±2.19^{bc}	14.5±0.28^{ab}	0.25±0.01^{de}	2.77±0.03^{ab}	1.283±0.01^{cd}
Axana	41.07±3.15^{abc}	12.96±0.39^{cd}	0.286±0.01^{bc}	2.42±0.08^{cd}	1.329±0.01^{bc}
Blinda	39.7±1.36^{abc}	13.3±0.18^{cd}	0.276±0.01^{cd}	2.51±0.04^{bc}	1.316±0.01^{c}
B. niger	38.70±2.90^{bc}	13.9±0.24^{bc}	0.262±0.006^{cde}	2.65±0.07^{abc}	1.298±0.008^{cd}

The intrinsic rate of natural increase (r_m), net reproductive rate (R_0), finite rate of increase (λ), generation time (T) and doubling time (DT). Means followed by different letters within a column are significantly different at P=0.05 (Tukey's test).

The total pre reproductive, reproductive and post reproductive period of *B. brassicae* were significantly different among the brassica varieties (Table 4). The longest total pre reproductive was recorded on Kale (10.93 days) and shortest on Holeta-1 (7.6 days) (Table 4). Longest reproductive period was observed on *B. niger* (21.33 days) and the shortest period was on Axana (15.66 days). The adults lived longer on Kale (27days) and shorter on Axana (18.56 days). *Brevicoryne brassicae* had significantly different fecundity when reared on the seven varieties of brassica (Table 4). Fecundity was higher on *B. niger* (79.5 nymphs/female) and lower on cabbage (62.4 nymphs/female) and the other varieties were in between.

Life table parameters

The intrinsic rate of natural increase (r_m), net reproductive rate (Ro), population growth rate (λ), generation time (T) and doubling time (DT) of *B. brassicae* on seven brassica varieties were significantly different (Table 5).The aphids reared on Holeta-1 had the highest Ro (47.75), r_m (0.337days^{-1}), λ (1.39) and the shortest generation (11.45 days) and doubling times (2.05days). On the contrary, Ro, r_m and λ of cabbage aphids were the lowest and T and DT the longest on kale (Table 5).

Adult survivorship

The age-specific fecundity (mx) and survival of cabbage aphid reproduced on the seven varieties of brassica were significantly different (Figure 2). The aphids had higher survival on Holeta-1 and Yellow Dodola and followed by kale during the first ten days. On Blinda the aphids were surviving for four days and the decline started onwards. In all remaining hosts the decline in survival was at uniform rate. The highest age specific fecundity (mx) was observed on Blinda (14.18 nymphs/day) and followed by Holeta-1(12.5 nymphs/day), Axana (10.7 nymphs/day), *B. niger* (9.82 nymphs/day), kale (9 nymphs/day), Yellow Dodola (9 nymphs/day) and Cabbage (7.5 nymphs/day).

DISCUSSION

The host preference, biological and demographic

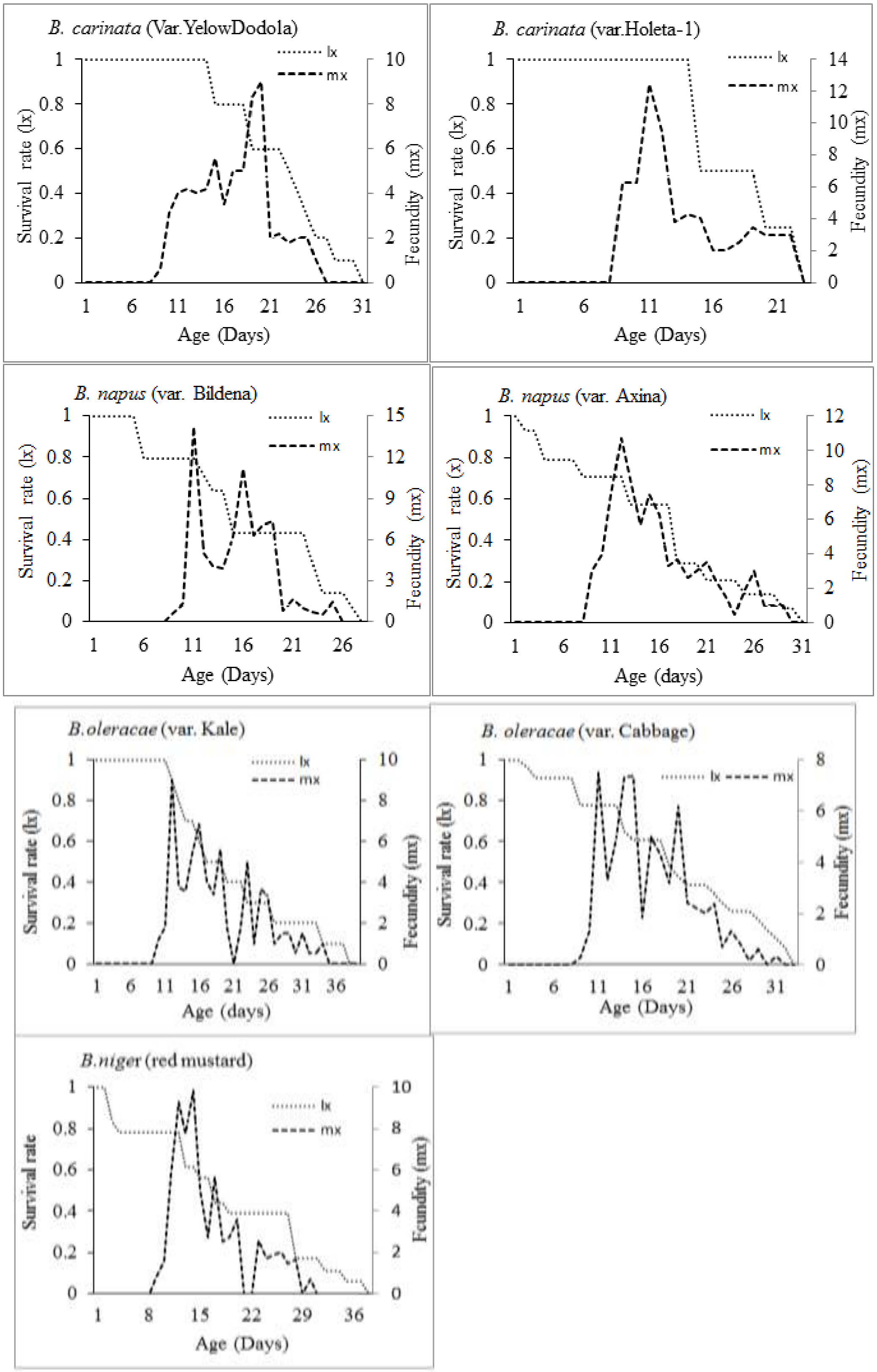

Figure 2. Age-specific survival rate (lx) and Age-specific fecundity (mx) of *B. brassicae* on seven brassica varieties.

parameters of cabbage aphid *B. brassicae* were significantly different on the seven brassica varieties. The aphid preferred to feed and reproduce on Holeta-1 more than the other tested varieties. As a result high number of nymph, apterae and alate aphids were recorded on this variety. The variation of insect performance on host plant could be described by the degree of plant resistance to a particular herbivore (Mirmohammadi et al., 2009). In the current study *B. carinata* variety Holeta-1 was found to be a suitable plant for aphid reproduction as compared to the other tested varieties.

The number of winged aphids varied among different species and between varieties of the same species. The variety Holeta-1 of *B. carinata* had significantly high number of winged aphids than Yellow Dodola. There was also more number of nymphs on Holeta-1 and Yellow Dodola than the other varieties. The higher number of nymphs could be due to the higher number of alates which preferred to feed on the host and the nutrition suitability of the host. In this study *B. carinata* was more suitable than the other tested species for the aphid reproduction. The variation on the number of aphid among the species could be due to the quality of the plant and the distribution of the different types of secondary metabolites among plant species. Quality variation in plant species influences the interactions between herbivore and its host plant and consequently its performance such as growth rates, fecundity, and survivorship (Zehnder and Hunter, 2008). The quality of the plants, such as physical, chemical or biological traits (for example size and structure, phenology, secondary compounds, and nutritional status) are important factor which influence the development, survival, longevity, and reproduction of herbivores (Mahdavi-Arab et al., 2014).

Nymphs of the cabbage aphid took longer developmental time on kale (*B. oleracae*) and faster on Holeta-1 (*B. carinata*). Different varieties of Brassicaceae family plants influence differently on the performance of brassica specialized insect pests (Sznajder and Harvey, 2003). Rebecca et al. (2011) reported that aphids reached reproductive maturity 14% faster when reared on *B. nigra* than *B. oleracea* plants which is in line with the current study. Recent study by Aziz et al. (2016) indicated that development time of cabbage aphid on different *B. oleracea* varieties were different and longer time was observed on KnolKhol (7.45 days) and followed by china cabbage (6.64 days) and cabbage (6.33 days). Study by Seyyed et al. (2014) on different varieties of *B. napus* showed that the developmental time of cabbage aphid varied from 8.38 to 9.16 days which is longer than the present study on the *B. napus* species (var. Axana and Blinda). Other studies by Jahan et al. (2014) on *B. oleracea* cauliflower cultivars the developmental time for immature of *B. brassicea* was ranged from 6.7 to 8.1 days which is in agreement with the current study on *B. oleracea* varieties. Studies by Bashir et al. (2013) on developmental time for immature of *B. brassicae* observed 7.94 days on cauliflower and 8.71 days on turnip. The variation on developmental time of the aphid could be due to the variation on nutritional quality of plant species, varieties and cultivars. Less sinigrin content in turnip leaves causes decline in the growth rate of *B. brassicae* and leads to longer nymphal development (Bashir et al., 2013).

The study also showed that the reproductive period, adult longevity and fecundity (number of nymphs per female) of *B. brassicae* significantly varied on tested varieties of brassica species. Cabbage aphid adults lived longer on *B. oleracae* (var. kale). Study by Rebecca et al. (2011) supports the current study and they found longer life span of *B. oleracae* when they reared on kale. Fecundity was highest on *B. niger*, followed by *B. carinata* (var. Holeta-1) and the lowest on *B. oleracea*. Studies by Jahen et al. (2014) on fecundity of *B. brassicae* on cauliflower cultivars indicated that, the fecundity was smaller than the current study on cabbage and kale and ranged from 30.9 to 58.6 nymphs per female.

Intrinsic rate of increase is most useful life table parameter to compare the fitness of populations of a specific species across different food resources (Ulusoy and Bayhan, 2006). The intrinsic rate of natural increase (r_m) was significantly different among tested brassica varieties. Intrinsic rate of increase (r_m) depends on the percentage of surviving nymph, developmental time, duration of nymph and fecundity of insect (Seyyed et al., 2014). The variety Holeta-1 had higher intrinsic rate of natural increase and kale the lowest on kale. Aziz et al. (2016) found that high intrinsic rate of increase on China cabbage (0.247) followed by Broccoli (0.218) and Cabbage (0.209), while the minimum on Knolkhol (0.104) which is comparable with the current study on *B. oleracae* varieties. Other studies by Ulusoy and Bayhan (2006) found that, the intrinsic rates of increase for cabbage aphid was 0.235, 0.201 and 0.197 on Cauliflower, Cabbage, and Broccoli, respectively which is smaller than the current study on cabbage. Study by Mirmohammadi et al. (2009) indicated that, the intrinsic rate of increase were higher on Hayola 401 and lower on Zarfam which is higher than the current result on two *B. napus* varieties (Axina and Bildena).

The capacity of plant resistance to a given insect pest could be measured by the variation of the pest performance on the host plants. The differences in the biological parameters can be attributed to the differences in the nutritional quality, physiology, morphology and chemistry of the host. Evaluating the antixenosis and antibiosis effect of different brassica crops for cabbage aphid *B. brassicae* has been employed to develop resistant varieties and include in integrated pest managements programs. The use of crop varieties that support only the low pest population growth or even of moderately resistant varieties is an important part of integrated pest management. Hence, our results may present valuable information for the management of the cabbage aphid *B. brassicae* in Ethiopia. They suggest that growing brassica varieties that are more or less tolerant to the *B. brassicae* population growth, may suppress or delay pest outbreaks and reduce the need for chemical control measures.

Conclusion

The knowledge on host plant effects on reproduction, development, survival and preference of insect pest is essential for developing pest management options. And the current study deals on the effect of seven different varieties on performance and preference of the specialist aphid *B. brassciae*. The aphid preferences to feed and to continue its reproduction on variety Holeta-1. However, the reproduce slower on variety Kale and the other varieties are in between. The study showed that cabbage aphid *B. brassicae* prefers to feed and reproduce more on *B. carinata* plants than *B. napus*, *B. niger* and *B. oleracae. B. oleracae* varieties are resistant to cabbage aphid *B. brassicae* compared to the other tested Brassicae species.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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