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REHMAN, A.¹
QAMAR, R.^{1*} 
SAFDAR, M.E.¹
JAVEED, H.M.R.²
SHEHZAD, M.³
ALI, M.²
ASIF, M.¹
TARAR, Z.H.⁴
AHMED, A.³
JAMIL, M.³

* Corresponding author:
<rafi.qamar@uos.edu.pk>

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UNDERSTANDING THE COMPETITIVE EFFECTS OF BLESSED MILKTHISTLE DENSITIES ON WHEAT

Entendendo os Efeitos Competitivos das Densidades de Cardo-mariano sobre o Trigo

ABSTRACT - Weed-induced yield loss in wheat crop is a great threat to food security in Pakistan. A comprehensive understanding of weed-crop competition is very important to develop sustainable and cost-effective weed management. For this purpose, two-year field studies were conducted to determine the effect of different blessed milkthistle densities on the phenology and yield of wheat crop in a rice-wheat cropping scheme in Sargodha, Pakistan during 2013-2014 and 2014-2015. The experiment comprised seven treatments: control (weed free), weedy check (weedy without any control) and blessed milkthistle densities of 5, 10, 15, 20 and 25 plants m⁻². In response to increasing weed density, a gradual reduction in yield and yield-related traits of wheat was noted. Compared to the weed-free control, a significant reduction in number of productive tillers m⁻² (20% and 18%), plant height (15% and 18%), spike length (19% and 26%), number of grains spike⁻¹ (23% and 26%), 1000 grain weight (28% and 28%), grain (29% and 30%) and biological (20% and 24%) yields of wheat occurred at and beyond blessed milkthistle density of 5 plants m⁻² during 2013-2014 and 2014-2015 respectively. It can be concluded that blessed milkthistle weed must be controlled if its population density reaches 5 plants m⁻² in order to avoid significant grain yield losses in wheat.

Keywords: blessed milkthistle, density, phenology, rice-wheat cropping systems, yield.

RESUMO - A perda de rendimento induzida por plantas daninhas na cultura do trigo é uma grande ameaça à segurança alimentar no Paquistão. Uma compreensão abrangente da competição de culturas de plantas daninhas é muito importante para desenvolver um manejo sustentável e econômico dessas plantas. Para esse propósito, foram conduzidos estudos de campo de dois anos para determinar o efeito de diferentes populações de cardo-mariano na fenologia e rendimento da safra de trigo no esquema de cultivo de trigo de arroz de Sargodha, Paquistão, durante 2013-2014 e 2014-2015. O experimento consistiu de sete tratamentos: controle (livre de plantas daninhas), teste de plantas daninhas (sem controle) e densidade populacional do cardo-mariano de 5, 10, 15, 20 e 25 plantas m⁻². Em resposta ao aumento da densidade de plantas daninhas, observou-se redução gradual no rendimento e nas características relacionadas com o rendimento do trigo. Comparado ao controle livre de plantas daninhas, reduções significativas em número de perfilhos produtivos m⁻² (20% e 18%), altura de plantas (15% e 18%), comprimento de espiga (19% e 26%), número de grãos espigão⁻¹ (23% e 26%), peso de mil grãos (28% e 28%), grãos (29% e 30%) e rendimento biológico (20% e 24%) de trigo ocorreram em e além da densidade populacional de cardo-mariano de 5 plantas m⁻² durante 2013-2014 e 2014-2015, respectivamente.

¹ Department of Agronomy, College of Agriculture, University of Sargodha, Pakistan; ² Department of Environmental Sciences, COMSATS University Islamabad, Vehari Campus, Pakistan; ³ Department of Agronomy, Faculty of Agriculture, University of The Poonch, Rawalakot, AJK, Pakistan; ⁴ Soil and Water Testing Laboratory, Mandi Bahaudin, Punjab, Pakistan.

Pode-se concluir que a planta daninha Cardo-mariano deve ser controlada se sua densidade populacional atingir 5 plantas m⁻², a fim de evitar perdas significativas no rendimento de grãos do trigo.

Palavras-chave: cardo-mariano, densidade, fenologia, sistemas de cultivo de trigo do arroz, rendimento.

INTRODUCTION

Wheat is a dominant winter season cereal crop, and is a major staple food of people in Pakistan, providing basic nutrients like protein and carbohydrate (Govt. of Pakistan, 2012). Total production in the country is 25.5 million tons and consumption is estimated to rise by 1.31% with every passing year, whereby total production is still less than local demand. Its share in agriculture is 9.1% while in terms of gross domestic product (GDP) of country wheat represents 1.7% (Govt. of Pakistan, 2017-2018). In Pakistan, the shortfall in production is currently attributed to a decline in the area sown, a delayed and prolonged sugarcane crushing season, acute water shortages, fog and smog, and weed infestation (Pakistan, 2017-18).

Blessed milkthistle (*Silybum marianum* L.), rather than having any medicinal value, is becoming an insidious weed and has infested the considerable crop area throughout the country. It has moved from road sides to agricultural lands, thus restricting the agricultural land operations and affecting crop growth and development. Its plants have a tendency to grow in patches that affect other plants by imposing shade and competition for resources. In several other countries, it is grown for medicinal purposes as well as for biofuel production (Sulas et al., 2008). Additionally, it has a variable concentration of bioactive compounds and antioxidants in different plant parts at different growth stages (Sulas et al., 2016). In Mediterranean pastures, blessed milkthistle is consumed by small and large grazing animals due to its spontaneous growth, while ruminants prefer it as hay or silage (Carpino et al., 2003). Grazing of blessed milkthistle by goats and sheep has been recommended so as to decrease its biomass in uncultivated areas (Khan et al., 2009). However, in winter legumes and wheat, it is classified as a weed (Sulas et al., 2008) that causes a significant reduction in yield (Khan et al., 2009).

Blessed milkthistle has a seed size approximately equal to wheat seed, and therefore easily disperses with it. It can be suppressed by using a high seed rate of crop that decreases its density and competition duration. So, the highly dense crop can inhibit the weed growth by imposing severe interspecific competition (Mamollos and Kalburtji, 2001; Weiner et al., 2001). Blessed milkthistle infestation of about 15-20 plants m⁻² can cause reduction in wheat yield of up to 36% (Darwent et al., 2006), while at 12 m⁻² plant density a 47% reduction in wheat yield has also been reported by Yenish et al. (1997). Crop density and weed management strategies determine the build-up of crop canopy and light penetration, and thus improve the yield attributes (Turk et al., 2002). However, there is a linear relationship between weed density and weed dry matter m⁻² (Moore et al., 2004).

Integrated weed control measures are being proposed to reduce the use of herbicides in the ecosystem (Sanyal and Shrestha, 2009). Weed threshold level is an important principle of integrated weed control, which provides assistance to the farmer regarding herbicide use (Fleck et al., 2002). Baloch (2002) reported that interspecific competition in weed and crop is a composite phenomenon that depends on many factors, i.e. species of both the crop and weed, their population, time of appearance, period of weed competition, efficacy of herbicides and climate factors. Higher crop populations have the potential to suppress weeds due to greater intra-specific than interspecific competition (Weiner et al., 2001). An acceptable weed control measure to reduce blessed milkthistle in a crop could not be practiced without comprehensive knowledge of the weed-crop competition. Information about critical blessed milkthistle density is vital for this purpose (Park et al., 2003).

Studies related to wheat yield losses due to blessed milkthistle infestation have been conducted in different countries. However, there is a lack of information about such studies in Pakistan. Furthermore, an estimate of the critical density of blessed milkthistle-in wheat crop has never been made before. Therefore, studies were planned with the aim of estimating the

probable grain yield losses in wheat due to blessed milkthistle infestation, and the critical density of this weed in wheat to avoid significant yield losses under agro-ecological conditions in a semi-arid area of Pakistan. It has been speculated that by increasing the population density of blessed milkthistle weed in wheat, the grain yield losses also increase.

MATERIALS AND METHODS

The current study was conducted at the Agronomic Research Area, College of Agriculture, University of Sargodha in a rice-wheat cropping scheme under agro-ecological conditions in Sargodha-Punjab, Pakistan during the winter of 2013-2014 and 2014-2015. The Agronomy Research Area is situated at 32.08° N, 72.67° E at an altitude of 193 m. In the Sargodha region, climatic conditions are subtropical semi-arid with annual rainfall of 400±5 mm. Almost 70% of the total rainfall is concentrated during the monsoon season between July and September (Source: Agro-Metrological Lab, University of Sargodha). Average minimum temperatures are about 10 °C in the winter. The summary of weather data during the whole crop growth period is shown in Figure 1 and 2 (2013-2014 and 2014-2015). In 2014-2015, the mean maximum and minimum temperatures at the time of plant germination and tilling during December were 7% and 11% lower than the mean maximum and minimum temperatures in 2013-2014. Relative humidity in December was 1% higher in 2014-2015, which favors plant growth. In 2014-2015, the mean maximum temperature in January was 15% lower than the mean maximum temperature in the respective month of 2013-2014 while the mean minimum temperature remained the same. Furthermore, relative humidity in January was 11% higher in 2014-2015. During the crop season, monthly rainfall was lower in 2013-2014 (795 mm) compared to 2014-2015 (1138 mm). Most of the rain fell during the months of January, February, and March in 2015 compared to February-March only in 2013-2014. Maximum rainfall was recorded in March 2014-2015 (974 mm) and April 2013-2014 (343 mm). Average relative humidity was lower during 2013-2014 than in 2014-2015.

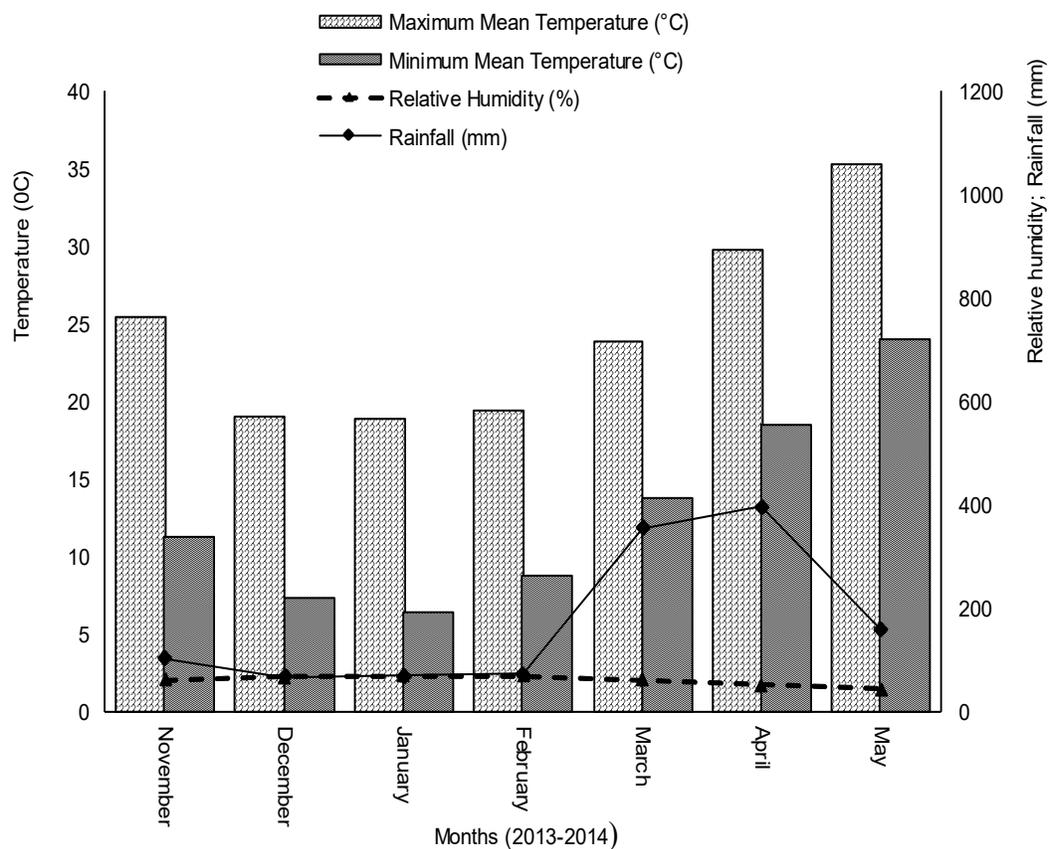


Figure 1 - Meteorological data recorded at the College of Agriculture, University of Sargodha, Sargodha, Punjab, Pakistan, during 2013-2014.

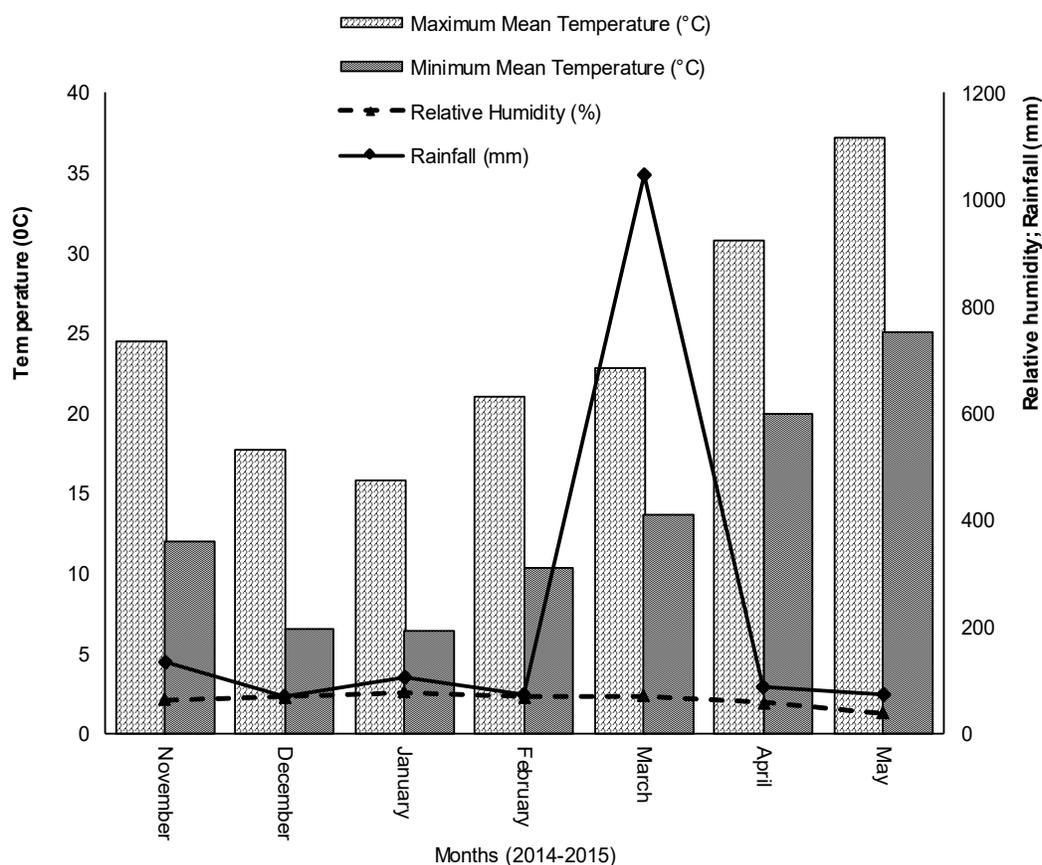


Figure 2 - Meteorological data recorded at the College of Agriculture, University of Sargodha, Sargodha, Punjab, Pakistan, during 2014-2015.

The soil series of this region is Hafizabad and the soil texture is loam-clay loam (Khan, 1986). Other pre-sowing physio-chemical properties of the soil were recorded as EC 2.19 ± 0.3 dS m^{-1} (Conductivity bridge from 1:2:5 soil water ratio), pH 7.8 ± 0.1 (Beckman's Glass electrode pH meter by Jackson, 1973), organic matter 0.72% (Walkley and Black method by Piper, 1966), total N 600 mg kg^{-1} (Modified Kjeldahl Method by Piper, 1966), available P 60 mg kg^{-1} (Olsen's Method by Jackson, 1973) and exchangeable K 80 mg kg^{-1} (Flame photometric by Jackson, 1973)).

The experimental units were arranged according to randomized complete block design with three replications and net plot size of 7 m \times 3 m with ten wheat lines in each plot. Wheat variety (CV. *Punjab-2011*) was sown on November 05, 2013-14 and 2014-2015 using a hand drill with seed rate of 100 kg ha^{-1} keeping row-to-row distance at 30 cm. The recommended fertilizer rate was applied to the wheat crop, i.e. $120:100:60$ kg ha^{-1} N:P:K with urea, diammonium phosphate, and muriate of potash as fertilizer sources, respectively. A full dose of phosphorus and potassium and a one-third dose of nitrogen were applied at the time of sowing. The remaining nitrogen was applied in two equal doses at stem elongation and booting stages. The experiment was comprised of seven treatments: weed free (control), weedy check, 5, 10, 15, 20 and 25 blessed milkthistle plants m^{-2} . The crop was harvested at full maturity on April 29 in both years. All weeds other than blessed milkthistle were removed manually to maintain the prescribed density. All the other cultural operations were kept normal and uniform.

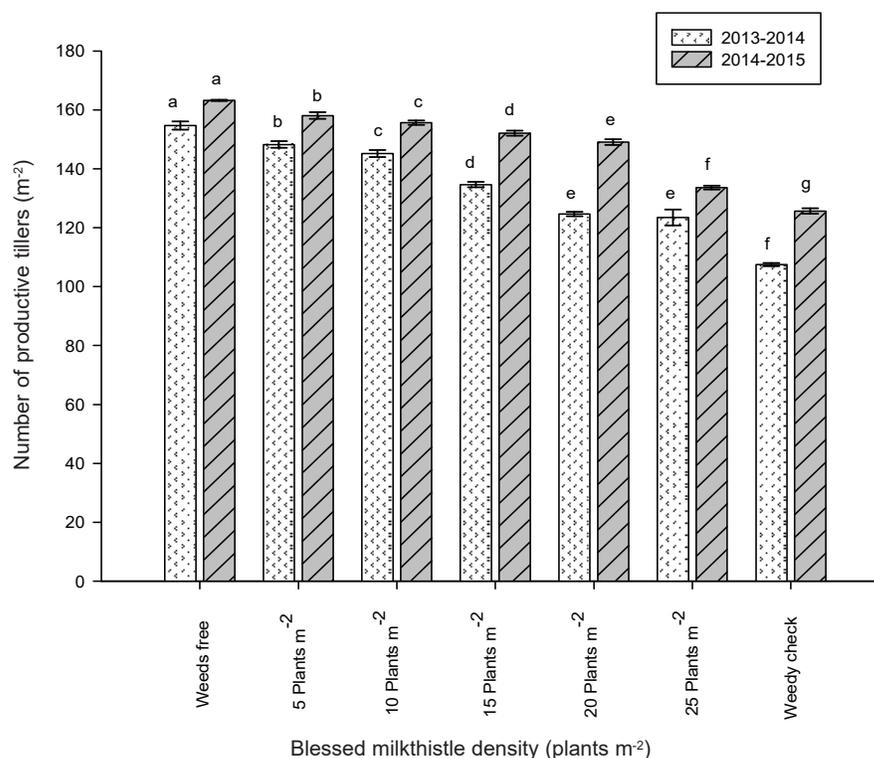
At maturity, the number of fertile tillers was counted in four adjacent lines to one m length. Height of plants was measured using a meter rod. Spike length was measured when head reached maturity using a meter rod. From these spikes, grains per spike were counted and then their average was calculated. From each experimental unit a sub-sample of 1000 grain was taken and weighed using an analytical set of scales (Model Number: HC2204) after oven-drying at 70 °C for 24 hours until a constant weight was obtained. At physiological maturity, the wheat crop was harvested from each experimental unit, tied up in bundles and tagged accordingly. To determine the biological yield of each experimental unit, these bundles were weighed using

bench scales (Model Number: TCS-602). All bundles were threshed separately and grain yield was weighed and converted into Mg (mega gram) per hectare.

The data regarding all the variables were statistically analyzed by Fisher's analysis of variance technique, and the variation among the treatment averages was compared using LSD at 5% probability (Steel et al., 1997). The MSTAT-C statistical package (MSTAT-C, 1998) was used to analyze two-year (2013-2014 and 2014-2015) data by randomized complete block design. As the effects in different years were found to be significant, as shown by year by weed density, the data are presented for individual years. Graphical presentation of data and linear regression equations were calculated using Sigma Plot software (SigmaPlot, 2008).

RESULTS AND DISCUSSION

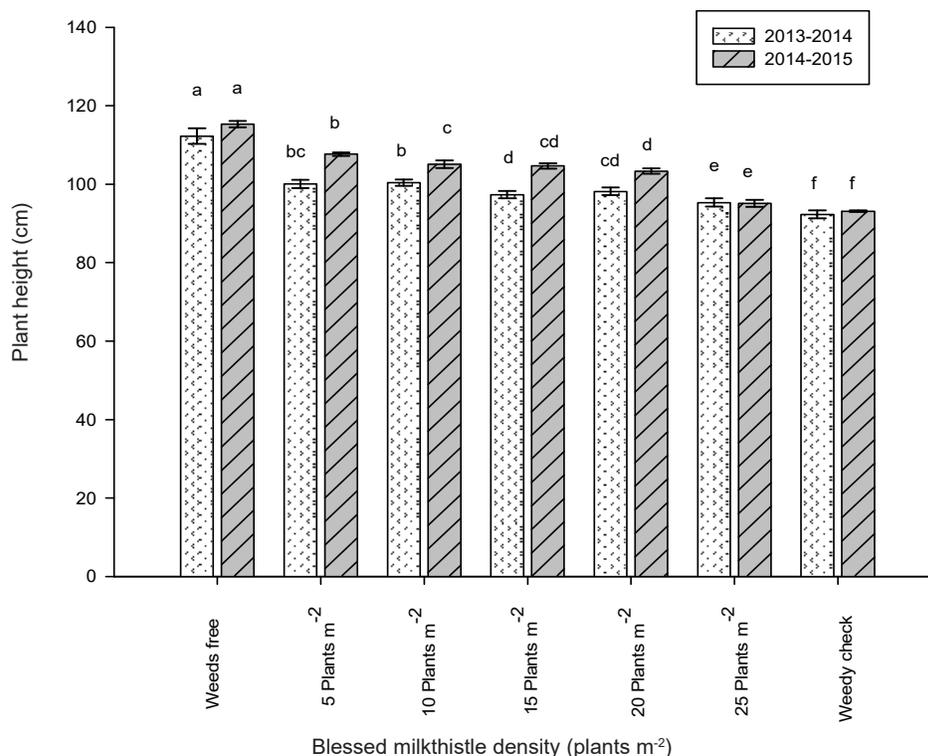
The number of productive wheat tillers was affected by different densities of blessed milkthistle (Figure 3). The results highlighted that this number decreased gradually with the increase in blessed milkthistle density during both years. Significantly, there was a higher number of productive tillers (31% and 23% in 2013-2014 and 2014-2015, respectively) in weed-free treatment than in the weedy check. A significant decline in this parameter started to occur with 5 plants m^{-2} of blessed milkthistle. The minimum number (108 m^{-2} and 126 m^{-2}) of productive tillers was noted in weedy check during years 2013-2014 and 2014-2015, respectively. The intensification in weed population progressively reduced the number of productive wheat tillers (Karim et al., 2002). Competition between crop plants and weeds for water, nutrients, space and light deprived plants of the necessary inputs to produce higher tillers (Spink, 2000).



Means separated by lowercase letters in each bar are significantly different among density at $P \leq 0.05$.

Figure 3 - Number of productive tillers m^{-2} as influenced by different blessed milkthistle density at 5% probability.

Plant height is a dominant component of crop growth which is determined by plant genome, seed vigor and the prevailing climatic conditions of an agro-ecological zone. Plant height of wheat was affected by various blessed milkthistle plant populations during both years of study (Figure 4). Statistically, the taller wheat plants (18% and 19% in 2013-2014 and 2014-2015, respectively higher than weedy check) were noted in weed-free treatments. A significant

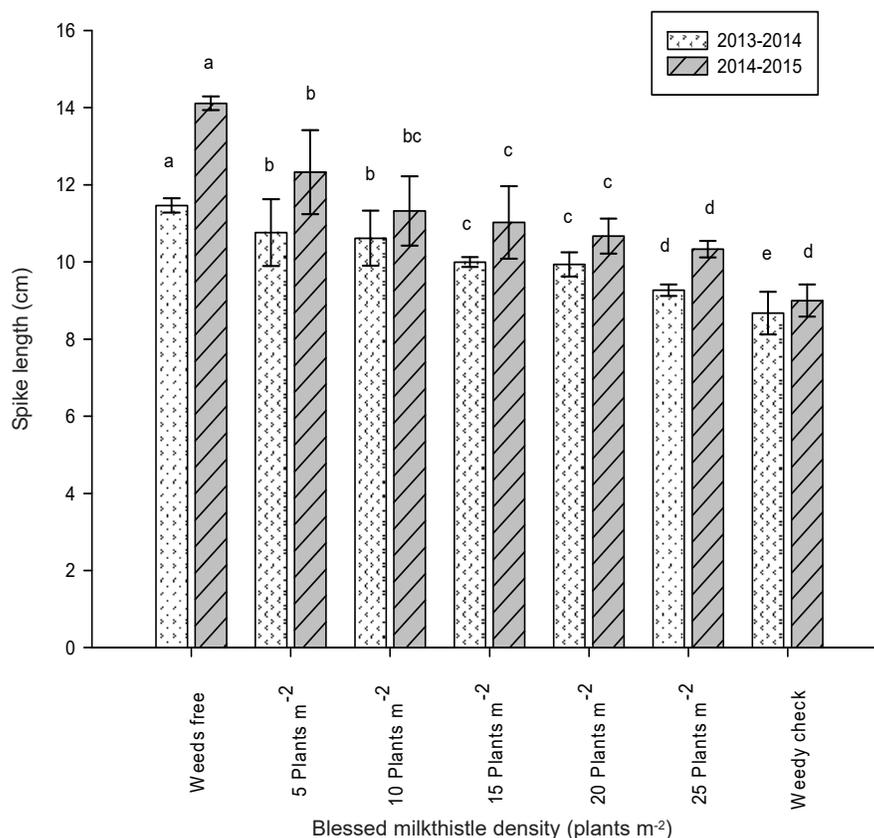


Means separated by lowercase letters in each bar are significantly different among density at $P \leq 0.05$.

Figure 4 - Plant height (cm) of wheat as influenced by different blessed milkthistle density at 5% probability.

reduction in wheat plant height was noted at and beyond blessed milkthistle density of 15 and 25 plants m^{-2} in years 2013-14 and 2014-15, respectively. Consequently, the shorter (92 cm and 93 cm) wheat plants were noted in weedy check during 2013-2014 and 2014-2015, respectively. In weed-free treatments, wheat plants utilized all input and other growth factors without any weed competition, which favored their growth and development. There is a gradual decrease in plant height with the increase in weed density that reduced the availability of nutrients, space and water (Irshad, 2000). Results illustrated that maximum plant height in the weed-free plot as compared to the weedy check plot might be due to the healthy initial growth of plants and ease of uptake of the nutrients and water from the soil. The major morphological character is plant height, which mainly depends upon nutrients, water and environmental stresses. Poor plant height represents the hampered vegetative growth of the crop plant and vice versa. Increase in densities of blessed milkthistle decreased the wheat crop height due to competition (intraspecific and interspecific), and weed canopy cover over the wheat preventing sunlight reaching the crop plant (Mishra et al., 2006). The taller plants were noted in 2014-2015 as compared to the 2013-2014 crop growing season. A possible cause may be higher rainfall and lower mean maximum and minimum temperatures during 2014-2015 in the month of January (Figures 1 and 2) compared to 2013-2014. Our results are in conflict with the findings of Alford et al. (2004) who stated that plant height was not affected by weed competition and density. The probable reasoning behind this statement is that crop plants sometimes grow higher in order to reach light due to the shading effect of weeds.

Longer spike length results in a greater number of spikelets per spike and ultimately higher grain yield and vice versa. Data presented in Figure 5 represent the effect of different blessed milkthistle densities on spike length of wheat. Data indicate that spike length decreased significantly with the increase in blessed milkthistle density during both years of study. Statistically, there were longer spikes (24% and 36% in 2013-2014 and 2014-2015, respectively) in the weed-free treatment than the weedy check, these were lower (6% and 13%) at 5 plants m^{-2} of blessed milkthistle during 2013-2014 and 2014-2015 respectively. The minimum (9 cm and 9 cm) spike length was noted in weedy check during 2013-2014 and 2014-2015,



Means separated by lowercase letters in each bar are significantly different among density at $P \leq 0.05$.

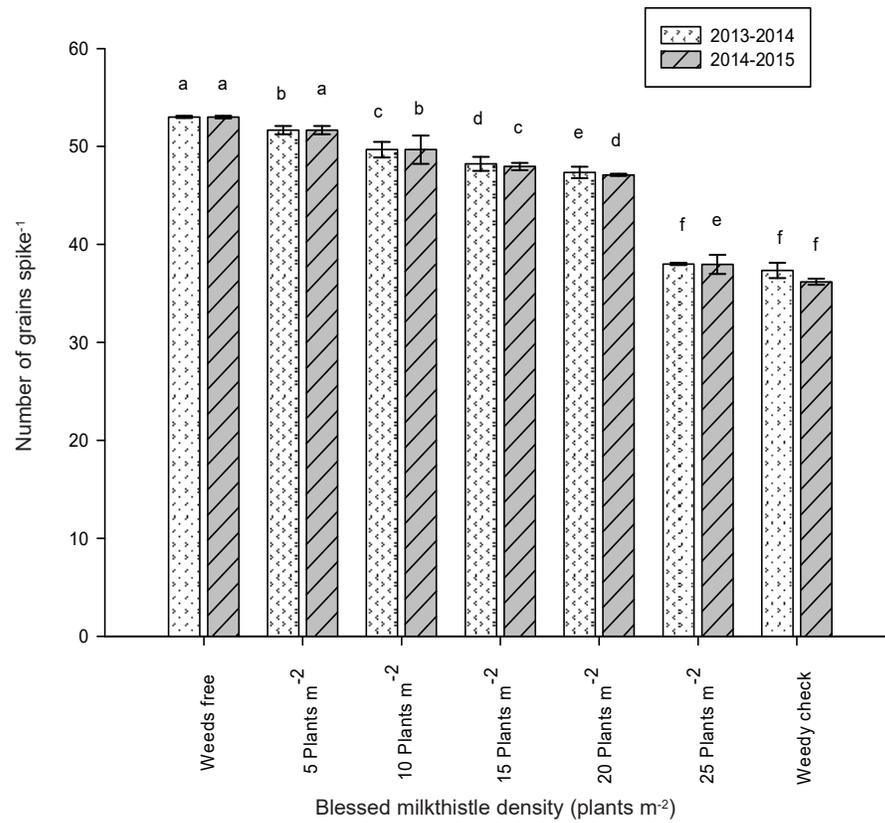
Figure 5 - Spike length (cm) of wheat as influenced by different blessed milkthistle density at 5% probability.

respectively. Continuously favorable temperatures and rainfall (Figure 2) during 2014-2015 might have improved spike development and thus spike length compared to 2013-2014 (Figure 1).

Grains spike⁻¹ of wheat were significantly affected by blessed milkthistle plant populations (Figure 6) during both years. The maximum grains spike⁻¹ (29% and 31%) were obtained from weed-free plots during 2013-2014 and 2014-2015. A significant decline in number of grains spike⁻¹ (22% and 20%) was recorded at 25 plants m⁻² in years 2013-2014 and 2014-2015, respectively. Ultimately, the lowest number of grains spike⁻¹ (37 and 33) was recorded in weedy check during the year 2013-2014 and 2014-2015, respectively. The optimum number of grains spike⁻¹ of wheat in weedy free plots might be due to efficient dry matter production during grain development. However, an inverse relationship has been reported between number of grains spike⁻¹ and population densities of weeds m⁻² (Karimmojeni et al., 2010). Khan et al. (2008) found that number of grains spike⁻¹ decreased with increasing weed density.

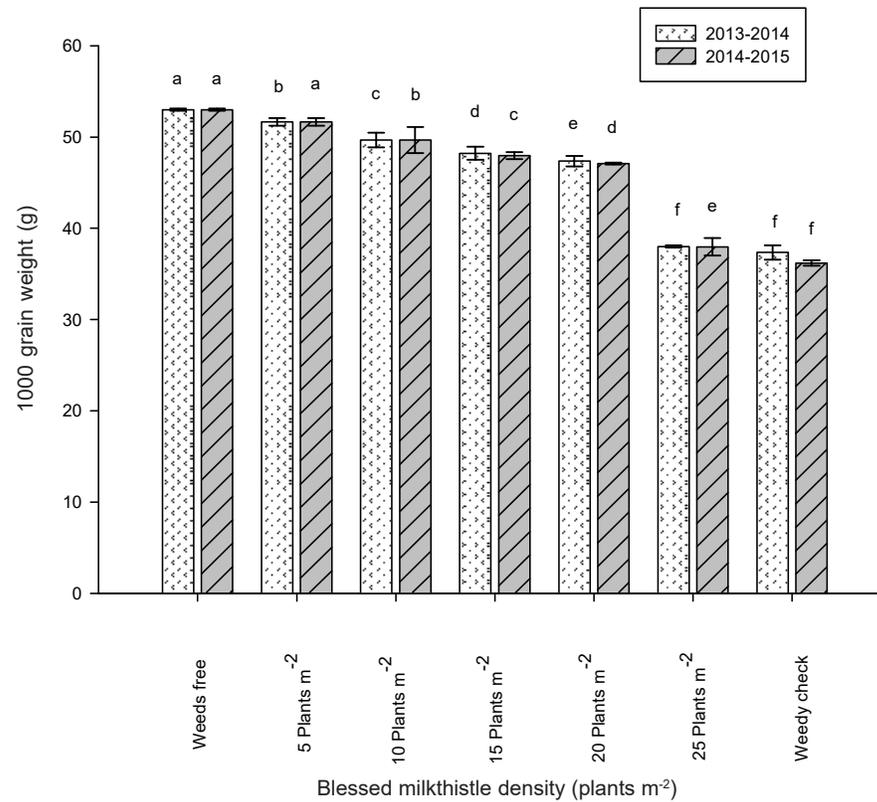
Grain yield is directly influenced by the change in 1000 grain weight. Thousand-grain weight of wheat was significantly affected by various plant populations of blessed milkthistle (Figure 7). Significantly, it was highest (53 g and 53 g during 2013-2014 and 2014-2015 respectively) in weed-free treatment, which was 30% and 32% higher than weedy check during 2013-2014 and 2014-2015, respectively. Grain weight of wheat decreased significantly with increase in blessed milkthistle density and suffered a significant decrease due to the presence of blessed milkthistle density at and above 5 plants m⁻² during both years. The decrease in 1000 grain weight of wheat upon increase in blessed milkthistle density might be due to the increased severity of crop-weed competition at the grain filling phase. Oad et al. (2007) reported that higher densities of various weeds individually and combined considerably decreased 1000 grain weight of wheat.

Grain yield is the combination of various yield attributes like productive tillers, grains spike⁻¹ and 1000 grain weight, which exhibit variations due to growing conditions and crop management practices. Grain yield was affected by different population densities of blessed milkthistle during



Means separated by lowercase letters in each bar are significantly different among density at P<0.05.

Figure 6 - Number of grains per spike of wheat as influenced by different blessed milkthistle density at 5% probability.



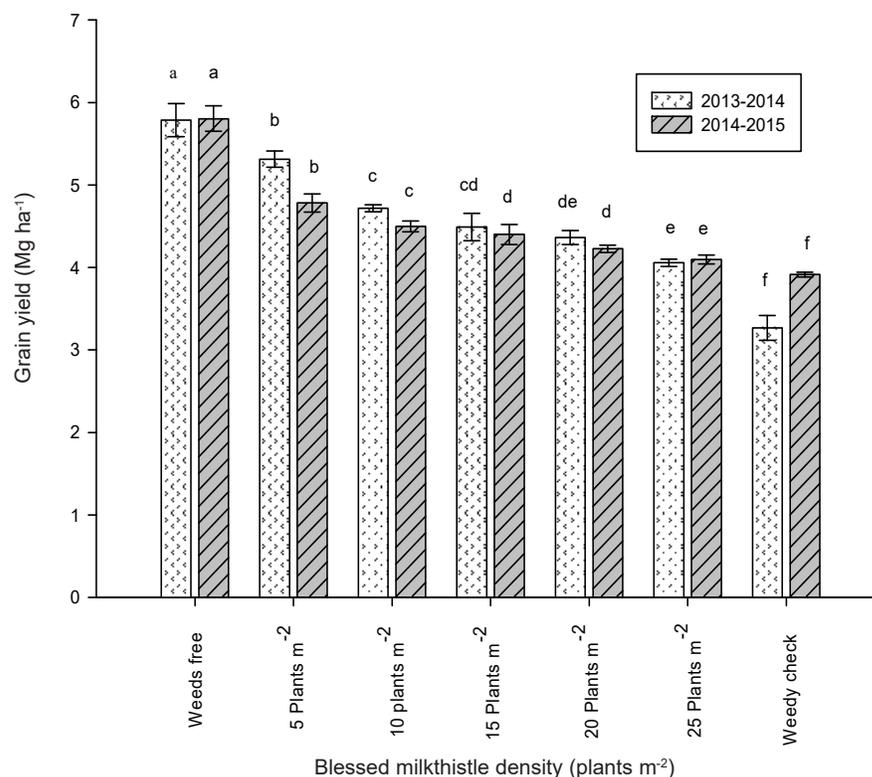
Means separated by lowercase letters in each bar are significantly different among density at P<0.05.

Figure 7 – 1000 grain weight (g) of wheat as influenced by different blessed milkthistle density at 5% probability.

both years (Figure 8). Weed-free treatment produced significantly the maximum grain yields of wheat (6 Mg ha^{-1} and 6 Mg ha^{-1} during 2013-2014 and 2014-2015, respectively), which were 33% and 44% higher than those harvested from weedy check plots during 2013-2014 and 2014-2015, respectively. Moreover, the significant reduction in grain yield of wheat started to occur when blessed milkthistle density was at and beyond 5 plants per m^2 . Minimum grain yield (4 Mg ha^{-1} and 3 Mg ha^{-1}) was noted in weedy check during 2013-2014 and 2014-2015, respectively. The decrease in wheat grain yield was probably due to a significant reduction in productive tillers, grains spike $^{-1}$ and 1000 grain weight (Figures 3, 6 and 7) because of increased blessed milkthistle density.

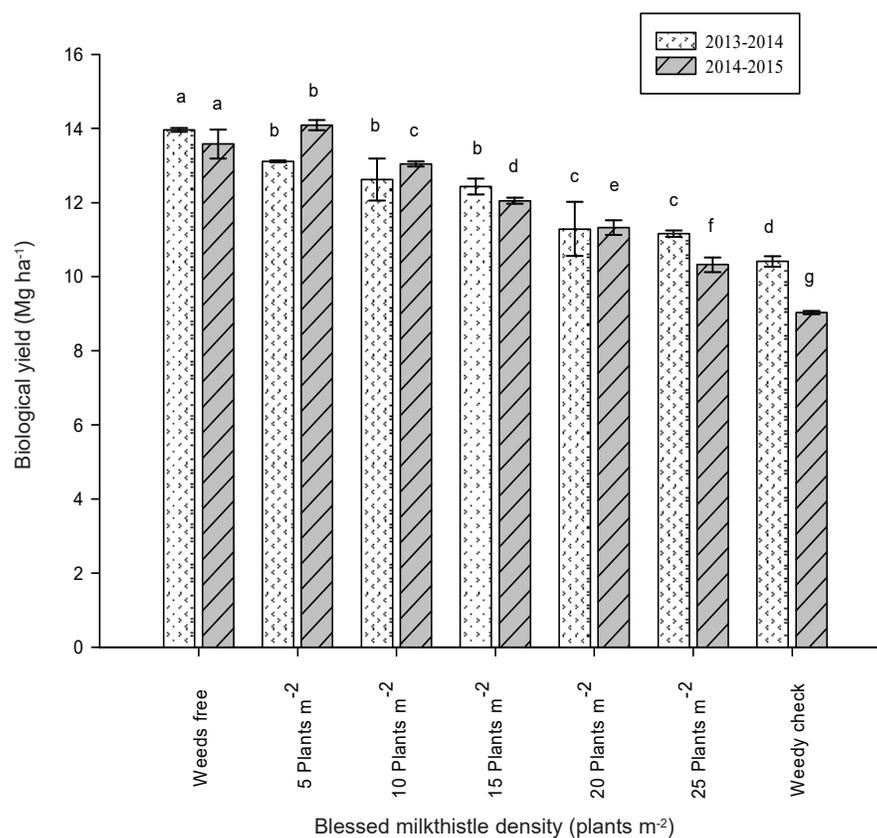
Similarly, in both years, maximum biological yields (14 Mg ha^{-1} and 14 Mg ha^{-1} during 2013-2014 and 2014-2015, respectively) were harvested from weed-free plots that were 25% and 34% higher than those noted from weedy check treatment during 2013-2014 and 2014-2015, respectively (Figure 9). Moreover, by increasing blessed milkthistle plant densities up to and more than 5 plants m^{-2} , a significant reduction in biological yield of wheat was noted. These results support the findings of Cerrudo et al. (2012) who stated that the decrease in grain yield was the result of a decrease in yield-contributing attributes due to crop weed competition. Blessed milkthistle demonstrates vigorous growth in the early growth phases that results in a bigger canopy than crop plants and results in increased competition for sunlight, soil nutrients, and water (Mamolos and Kalburtji, 2001). Blessed milkthistle's density at more than 5 plants m^{-2} can cause a considerable reduction in wheat yield (Darwent et al., 2006). Sunlight plays a significant role in the crop production system (Ballaré and Casal, 2000) and, since it is a taller plant, blessed milkthistle impaired light penetration (Rohrig and Stutzel, 2001).

A linear regression equation of biological yield (dependent) with yield attributes was calculated during both years of study. In the case of individual regression equation (Table 1), biological yield showed a significant dependence on the number of productive tillers, number of grains spike $^{-1}$, 1000 grain weight and grain yield; while in the combined regression equation biological yield was significantly dependent on the number of productive tillers, plant height, spike length,



Means separated by lowercase letters in each bar are significantly different among density at $P \leq 0.05$.

Figure 8 - Grain yield (Mg ha^{-1}) of wheat as influenced by different blessed milkthistle density at 5% probability.



Means separated by lowercase letters in each bar are significantly different among density at $P \leq 0.05$.

Figure 9 - Biological yield (Mg ha^{-1}) of wheat as influenced by different blessed milkthistle density at 5% probability.

Table 1 - Linear regression equation of different wheat yield traits on biological yield as affected by weed density during 2013-2014

Regression equation	Adj. (R^2) (%)	R^2 (%)	Number of productive tillers m^{-2}	Plant height (cm)	Spike length (cm)	Number of grains spike ⁻¹	1000-grain weight (g)	Grain yield (Mg ha^{-1})
$\text{BY} = -8.30 + 0.15 \times \text{NPT}$	95.1	95.4	**					
$\text{BY} = -19.26 + 0.32 \times \text{PH}$	40.2	43.2		ns				
$\text{BY} = 5.40 + 0.62 \times \text{SL}$	66.6	68.3			ns			
$\text{BY} = -10.13 + 0.54 \times \text{NGS}$	92.0	92.4				**		
$\text{BY} = -10.52 + 0.52 \times \text{GW}$	78.7	79.7					**	
$\text{BY} = -5.57 + 3.86 \times \text{GY}$	95.5	95.7						**
$\text{BY} = -4.36 + 0.08 \times \text{NPT} - 0.09 \times \text{PH} - 0.05 \times \text{SL} + 0.25 \times \text{NGS} + 0.02 \times \text{GW} + 0.72 \times \text{GY}$	99.2	99.4	**	**	**	**	**	**

BY = Biological yield, NPT = Number of productive tillers, PH = Plant height, SL = Spike length, NGS = Number of grains spike⁻¹, GW = 1000-grain weight, GY = Grain yield. ** = Highly significant, ns = Not significant.

number of grains spike⁻¹, 1000 grain weight and grain yield during 2013-2014. During the second year of study, biological yield was significantly dependent on all yield attributes in the individual and combined regression equation (Table 2). The consolidated results of two years' research showed that blessed milkthistle can cause reductions of up to 16% and 26% in the grain yields of wheat. Moreover, a population density of 5 blessed milkthistle plants m^{-2} is the critical density of this weed in wheat. It is therefore concluded that control measures against blessed milkthistle should be adopted at a density of 5 plants m^{-2} of this weed to prevent substantial grain yield reduction. The yield losses and critical thresholds of this weed in other winter crops should also be measured.

Table 2 - Linear regression equation of different wheat yield traits on biological yield as affected by weed density during 2014-2015

Regression equation	Adj. (R ²) (%)	R ²	Number of productive tillers m ⁻²	Plant height (cm)	Spike length (cm)	Number of grains spike ⁻¹	1000-grain weight (g)	Grain yield (Mg ha ⁻¹)
BY = -30.73+0.31xNPT	91.2	91.6	**					
BY = -26.01+0.37xPH	93.8	94.1		**				
BY = 0.76+1.02xSL	83.6	84.4			**			
BY = -9.72+0.47xNGS	90.8	91.3				**		
BY = -6.77+0.40xGW	97.9	98.0					**	
BY = -8.55+4.71xGY	76.6	77.8						**
BY = -7.26-0.07xNPT+0.14xPH+0.07xSL -0.07xNGS+0.39xGW-0.12xGY	99.2	99.4	**	**	**	**	**	**

BY = Biological yield, NPT = Number of productive tillers, PH = Plant height, SL = Spike length, NGS = Number of grains spike⁻¹, GW = 1000-grain weight, GY = Grain yield. ** = Highly significant.

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