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Effect of Planting Space and Dose of Phosphate Fertilizer on the Development and Production of White Sorghum (*Sorghum bicolor* L. Moench) Samurai-2 Variety

Amran Jaenudin[1]✉, Dukat Dukat[1], Utyta Alaydrus[1], Maryuliyanna Maryuliyanna[2]

[1]Program Studi Agronomi, Sekolah Pascasarjana, Universitas Swadaya Gunung Jati, Cirebon. INDONESIA
[2] Sekolah Pascasarjana, Universitas Swadaya Gunung Jati, Cirebon. INDONESIA.

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✉Corresponding Author:
amranjaenudin57@gmail.com

ABSTRACT

White sorghum is utilized for food, beverage, fodder, and other industrial purposes. Spacing and fertilization are among problems for the development of sorghum plants. The aim of this study was to evaluate the interaction effect of planting space and phosphate fertilizer treatments on the development and production of white sorghum (Sorghum bicolor L. Moench) varity of Samurai-2, and to determine the best results from those treatments as well. This research was performed at the experimental field of Al-Zaitun Islamic Boarding School located in Mekarjaya Village, Subdistrict of Gantar, Indramayu Regency, West Java. A factorial randomized block design was employed with planting space treatment of PS30 (70 cm × 30 cm), PS40 (70 cm × 40 cm), and PS50 (70 cm × 50 cm) and phosphate fertilizer treatment of PF50 (50 kg/ha), PF100 (100 kg/ha), and PF150 (150 kg/ha). Response parameters consisted of plant heigh, number of leaves, leaf area index (LAI), stem diameter, root volume, plant growth rate, and crop yield (weight of 1000 grains and yield per plot). Results of this study showed no interaction in all treatments, and the PS30 with crop spacing of 70 cm x 30 cm resulted the best yield on weight per plot of 5.08 kg/plot equivalent to 8.5 ton/ha.

1. INTRODUCTION

Sorghum (*Sorghum bicolor* L. Moench) was native to tropical Ethiopia in East Africa around 7000 years ago, and the highlands of Ethiopia are considered to be a major center of sorghum domestication. This plant has long been known as a producer of food and is cultivated in dry areas in several African countries. From the African continent, sorghum then spread to tropical and subtropical regions such as India, China, Myanmar, Southeast Asia, and Indonesia (Sumarno *et al*., 2013). Sorghum has many benefits in both the food and feed industries. As a food ingredient, sorghum has a high nutritional content.

Sorghum production in Indonesia has not been fully able to meet domestic and export needs. According to the Sorghum Roadmap (2022-2024), the main target of this crop is food, feed, and fuel (bioethanol). In 2022, an area of 15,000 ha was allocated for sorghum planting, but an Automatic Adjustment (AA) occurred so that the allocation was changed to only 4,600 ha. For the upcoming 2023, the government has proposed an allocation of 100,000 ha through ABT 2023 and a regular area of 15,000 ha (Directorate General of Food Crops, 2022).

Planting space influence the efficiency of light absorption, competition between plants in the utilization of nutrients and water, which ultimately will determine yields. There is a tendency to decrease yields due to high populations, increased competition between the plants themselves in obtaining nutrients, water and sunlight. Planting space that is too wide reduce the number of plant populations. Wide planting space also decrease the utilization of nutrients and sunshine by crops. Some nutrients will be lost due to evaporation and leaching, while some light will reach empty area on the soil surface (Suminar *et al*., 2017).

The study of Dudato *et al*. (2020) showed that a planting space of 70 cm × 40 cm produced better sorghum performance in term of plant height, number of leaves, leaf width, and leaf length as compared to other planting spaces (50 cm 30 cm, 30 cm × 20 cm, and 10 cm × 10 cm). It was inferenced that 70 cm × 40 cm is the planting spacing with the uppermost agronomic characteristics for white sorghum variety Samurai 2.

One of the problems for the development of sorghum plants is fertilization, because it is closely related to the planting medium (Selvia *et al*., 2014). In addition, sorghum plants are known to be very responsive to fertilization, especially P and K fertilizers. This is very related because the element P has an important role in the formation of seed protein, as a source of energy and can stimulate the process of plant development and rooting. In the process of growth and yield of sorghum plants requires sufficient phosphate elements. Phosphate fertilizer plays important roles during plant development, including: (1) nucleic acids formation, (2) photosynthesis and respiration, (3) stimulating the development of root that plants are more resilient to drought, (4) plant seeds formation and fruit manufacturer, and (5) accelerating the maturity that minimize the threat of harvesting postponement (Sofian *et al*., 2019).

Based on the foregoing discussion, it is deemed important to conduct an experiment on the effect of phosphate fertilizer and planting space on the development and production of white sorghum of Samurai 2 variety. Therefore, the objective of this research was: 1) to appraise the effect of interaction between planting space and phosphate fertilizer treatment on the development and yield of white sorghum variety Samurai 2, and 2) knowing the best results from spacing treatment and phosphate fertilizer on white sorghum variety Samurai 2.

2. MATERIALS AND METHODS

2.1. Location and Design of Experiment

This experiment was carried out at the Al-Zaitun Islamic Boarding School experimental field located in Mekarjaya Village, Gantar District, Indramayu Regency, West Java. The field was characterized with Latosol soil at an altitude of 50 m asl, and rainfall of D type according to Schmidt & Fergusson (1952). Based on the analysis, the soil had nutrient content with low phosphate and a neutral pH, while the soil had a clayey texture. The experiment was designed with factorial Randomized Block Design (RBD). The treatment consisted of two factors, namely planting space consisted of PS30 (70 cm × 30 cm), PS40 (70 cm × 40 cm), and PS50 (70 cm × 50 cm), and the second factor was dose of phosphate fertilizer, namely FP50 (50 kg/ha), FP100 (100 kg/ha), and FP150 (150 kg/ ha). This study was performed with 9 plots sizing of 2 m × 3 m to execute 9 treatment combinations of planting spaces and phosphate fertilizer doses. The number of sample plants per plot was 10 plants.

Experimental data on the main observations were processed using statistical tests with a linear model proposed by Hanafiah (2001) as follows:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \rho_k + \varepsilon_{ijk} \quad (1)$$

where Y_{ijk} is the observation on factor A level i^{th}, factor B level j^{th}, and the k^{th} group, μ is the general mean value, α_i and β_j is respectively the main effect of factor A level i^{th} and factor B level j^{th}, $(\alpha\beta)_{ij}$ is the interaction effect of factor A level i^{th} and factor B level j^{th}, ρ_k is the effect of k^{th} group, and ε_{ijk} is the random effect on factor A at the i^{th}-level, factor B at the j^{th}-level and k^{th} group.

2.2. Measurement and Analysis

Observations of plant growth parameters were carried out on 14, 28, and 42 DAP (days after planting) including plant height, number of leaves, leaf area index, stem diameter, plant growth rate, and root volume. While observations of crop yields, namely weight per 1000 grains, were carried out after harvest.

Observational data were analyzed using ANOVA and was continued with the DMRT test (Duncan Multiple Range Test) at a = 5%.

3. RESULTS AND DISCUSSION

3.1. Growth Observation

3.1.1. Plant Height

Measurement of plant height at different stages (14, 28, and 42 DAP) can be seen in Table 1 and Table 2. Based on the ANOVA results, it can be showed that interaction effect between the planting space and phosphate fertilizer on the average plant height of white sorghum was not significant. The average plant height of sorghum observed at 14 DAP was not significantly different on all treatments applied. This is possible because at age 14 DAP sorghum plants are still in their early stage of growth and did not need space and a large amount of nutrients so that the need for nutrients and space to grow can be fulfilled. Nevertheless, there was a statisticaly different plant height among the treatments carried out at 28 DAP and 42 DAP (Table 2).

Table 1. Observation of plant height (cm) of white sorghum at age 14 DAP

Treatment	Plant height (cm)
PS30 x PF50	23.40 a
PS30 x PF100	24.07 a
PS30 x PF150	**24.93 a**
PS40 x PF50	22.80 a
PS40 x PF100	23.33 a
PS40 x PF150	22.20 a
PS50 x PF50	22.80 a
PS50 x PF100	22.93 a
PS50 x PF150	20.33 a

Note: The mean values followed by the same lowercase is not significant according to the Duncan Multiple Range Test (DMRT) at a = 5% level.

Table 2. Effect of treatment on the plant height (cm) of white sorghum at age 28 and 42 DAP

Treatment	Plant heigh (cm)	
	28 DAP	42 DAP
Plant spacing		
PS30	**83.71 b**	**125.53 b**
PS40	76.09 a	**127.20 b**
PS50	73.11 a	114.51 a
Phosphate fertilizer dose		
FP50	74.71 a	118.29 a
FP100	80.42 a	**129.29 b**
FP150	77.77 a	119.67 ab

Note: The mean values followed by the same lowercase in the same column is not significant according to the Duncan Multiple Range Test (DMRT) at a = 5% level.

At the age of 28 DAP and 42 DAP there was an independent significant effect on the spacing treatment, and at 42 DAP the independent effect was also found in the treatment of phosphate fertilizers. At sorghum age of 28 DAP, treatment PS30 (planting space of 70 cm x 30 cm) produced the highest average plant height (83.71 cm), which was significantly different than those of other treatments. At the age of 42 DAP the treatment with a spacing of 70 cm x 30 cm and 70 cm x 40 cm had a significantly higher plant height than the other spacing treatments, whereas the 100 kg/ha phosphate fertilizer treatment had a significant effect on plant height than other phosphate fertilizer dosages. This shows that the narrower the plant spacing affects the average plant height and the more dose of phosphate fertilizer given also affects the average plant height according to the results of the research that has been done (Table 2).

Spacing that is too far also does not get the best results. This is because the soil becomes dry in the sun and evaporation occurs on the surface of the soil which is greater which causes the plants to lack water (Simanjuntak *et al*., 2016). This is in accordance with Purba (2020) which states that the use of spacing that is too tight between the leaves of fellow plants covers each other as a result, the growth of plants will be tall and elongated because they compete for light, which will hinder the photosynthesis process and plant production is not optimal.

The addition of phosphate fertilizers also affects the average plant height, this is in a good agreement with the study by Sihaloho & Situmeang (2021) that the increasing dose of phosphorus fertilizer given will increase vegetative growth. Then the availability of phosphorus can stimulate root development which in turn can affect all the performance of plant development, such as plant height, because increasing the dose of phosphate can affect plant nutrient uptake (Ayub *et. al*., 2002).

3.1.2. Number of Leaves

Number of leaves is among the determinant factors of the increase in plant growth. Based on the results of ANOVA that was carried out on the number of leaves at the age of 14 and 28 DAP, the number of leaves of white sorghum were not significantly different for all treatment combinations (Table 3). As for the 42 DAP observations, the phosphate fertilizer treatment had an effect on the average number of leaves (Table 4). The phosphate fertilizer dose of 50 kg/ha and 100 kg/ha produced an average yield of more leaves than that of 150 kg/ha phosphate fertilizer treatments.

Table 3. Effect of treatment on the number of leaves (strands) of white sorghum at age 14 and 28 DAP

Treatment	Number of leaves (strands)	
	14 DAP	28 DAP
PS30 x PF50	4.73 a	7.80 a
PS30 x PF100	5.00 a	8.53 a
PS30 x PF150	5.00 a	7.60 a
PS40 x PF50	4.80 a	7.60 a
PS40 x PF100	4.80 a	7.60 a
PS40 x PF150	4.87 a	7.60 a
PS50 x PF50	4.73 a	7.13 a
PS50 x PF100	4.73 a	7.80 a
PS50 x PF150	4.60 a	6.73 a

Note: The mean number followed by the same lowercase in the same column is not significant according to the Duncan Multiple Range Test (DMRT) at a = 5% level.

Table 4. Effect of treatment on the number of leaves (strands) of white sorghum at age 42 DAP

Treatment	Number of leaves (strands)
Plant spacing	
PS30	9.33 a
PS40	9.44 a
PS50	9.29 a
Phosphate fertilizer dose	
FP50	9.16 ab
FP100	**9.82 b**
FP150	9.09 a

Note: The mean values followed by the same lowercase is not significant according to the Duncan Multiple Range Test (DMRT) at a = 5% level.

In the observation variable number of leaves, spacing treatment and doses of phosphate fertilizers did not significantly influence number of leaves. This is probably because the number of leaves is subjective to genetic factors. This result is in agreement with Novri *et al.* (2015) that genetic factors are one of the determinants of growth and yield in sorghum plants. Each different genotype will have different advantages in utilizing environmental factors such as water, light, and nutrients so that they can affect plant development and yield.

3.1.3. Leaf Area Index

Leaf area index (LAI) is a parameter of plant growth measured through leaf area per unit area of experimental land or experimental plots. Based on the ANOVA at the age of 14, 28, and 42 DAP, the *p* value was more than 0.05 meaning the LAI of white sorghum was not significantly different as shown in Table 5. This shows that there is no interaction between planting space and phosphate fertilizer treatment on the LAI of white sorghum. This is because ILD is directly influenced by the number of leaves and as previously discussed this parameter is not significantly different. The more the number of leaves, the greater the ILD (Rahmawati *et al.*, 2016) and more photosynthate is produced. Therefore, the increasing the leaf area index is an implication of plant adaptation so that a lot of solar radiation can be intercepted for photosynthesis in shaded conditions (Suwarto, 2013).

Table 5. Effect of treatment on the leaf area index of white sorghum at age 14, 28, and 42 DAP

Treatment	Leaf area index		
	14 DAP	28 DAP	42 DAP
PS30 x PF50	28.43 a	239.03 a	239.03 a
PS30 x PF100	27.43 a	297.96 a	297.97 a
PS30 x PF150	31.99 a	268.42 a	268.42 a
PS40 x PF50	28.40 a	211.70 a	211.71 a
PS40 x PF100	28.46 a	245.80 a	245.80 a
PS40 x PF150	26.40 a	228.06 a	228.06 a
PS50 x PF50	27.20 a	223.74 a	223.76 a
PS50 x PF100	29.19 a	301.46 a	301.46 a
PS50 x PF150	23.34 a	213.61 a	213.61 a

Note: The mean values followed by the same lowercase in the same column is not significant according to the Duncan Multiple Range Test (DMRT) at a = 5% level.

3.1.4. Stem Diameter

Based on the analysis results presented in Table 6, stem diameter measured at the age of 14 and 42 DAP was not statistticaly different. This shows that there is a significant genetic role in the size of the stem diameter of sorghum cultivars, each variety is a genetic population that has a different growth pattern (Khasanah *et al*., 2016).

Table 6. Effect of treatment on the stem diameter (cm) of white sorghum at age 14 and 42 DAP

Treatment	Stem diameter (cm)	
	14 DAP	42 DAP
PS30 x PF50	0,84 a	2,28 a
PS30 x PF100	0,87 a	2,67 a
PS30 x PF150	0,89 a	2,37 a
PS40 x PF50	0,85 a	2,20 a
PS40 x PF100	0,85 a	2,37 a
PS40 x PF150	0,85 a	2,70 a
PS50 x PF50	0,86 a	2,35 a
PS50 x PF100	0,85 a	2,71 a
PS50 x PF150	0,81 a	2,01 a

Note: The mean values followed by the same lowercase in the same column is not significant according to the Duncan Multiple Range Test (DMRT) at a = 5% level.

At the age of 28 DAP there was an independent effect of phosphate fertilizer treatment. At dose of 100 kg/ha and 150 kg/ha white sorghum had stem diameter that is statisticaly higher than that of fertilizer dose of 50 kg/ha (Table 7). The study by Gondal *et al*., (2017) showed that planting space was not significantly influence the stem diameter, number of heads per area, and the weight of 1000 grains. Plant characters such as stem diameter and plant height are influenced by the plant population. Increase in plant population adversely affected stem diameter. Meanwhile, the increase in seed number planted positively affected plant height. In high plant population, however, the increase in plant height is not matched by an increase in stem diameter so that the plant will be more susceptible to overturning.

Table 7. Effect of treatment on the stem diameter (cm) of white sorghum at age 28 DAP

Treatment	Stem diameter (cm)
Plant spacing	
PS30	1,41 a
PS40	1,51 a
PS50	1,44 a
Phosphate fertilizer dose	
FP50	1,32 a
FP100	**1,55 b**
FP150	1,48 ab

Note: The mean number followed by the same lowercase is not significant according to the Duncan Multiple Range Test (DMRT) at a = 5% level.

3.1.5. Plant Growth Rate

Table 8 below shows the results of insignificant variance in the observations of plant growth rate (PGR) at age 14 to 28 DAP and ages 28 to 42 DAP. There was no interaction in the treatment of spacing with phosphate fertilizers. The PGR is closely related to plant spacing, with wide spacing so that the competitiveness of plants in obtaining nutrients, water, light is not so high that the plant's need for vase growth can be fulfilled. This is evidenced by the results of research that has been conducted that the highest PGR is found at wide spacing.

Table 8. Effect of treatment on the growth rate of white sorghum at age 14-28 DAP and 28-42 DAP

Treatment	PGR ($g.m^{-2}.^{-d}$)	
	14-28 DAP	28-42 DAP
PS30 x PF50	0,20 a	1,44 a
PS30 x PF100	0,31 a	2,21 a
PS30 x PF150	0,19 a	1,37 a
PS40 x PF50	0,35 a	1,21 a
PS40 x PF100	0,30 a	2,22 a
PS40 x PF150	0,26 a	**2,36 a**
PS50 x PF50	0,24 a	1,32 a
PS50 x PF100	**0,53 a**	1,62 a
PS50 x PF150	0,40 a	1,15 a

Note: The mean number followed by the same lowercase in the same column is not significant according to the Duncan Multiple Range Test (DMRT) at a = 5% level.

Photosynthesis, which is sourced from nutrients absorbed by the roots, is processed in the leaves with the help of sunlight, known as photosynthate results, which are an indication of the rate of plant growth. While photosynthesis itself occurs in the leaves where the number of leaves and leaf area greatly affect the results of plant photosynthesis. The width, length and number of leaves affect the area of the field so that photosynthesis will affect biomass production (Hajar *et al.*, 2019). The process of photosynthesis increases as seen from the area and width of the leaves (Li *et al.*, 2015). Based on this description, the results of observing the number of leaves and LAI that were not statisticaly different would result in the PGR which was neither different significantly.

3.1.6. Root Volume

Based on table 9 at the age of observation 14, 28, and 42 DAP there was no significant effect on all treatments. Then based on the results of observations when viewed from the treatment of phosphate fertilizers the addition of phosphate fertilizers can increase root volume. This proves that the addition of nutrients, especially phosphate fertilizers, can help the development of roots in the soil, although it does not show significant results. The addition of phosphate fertilizers has an effect on root volume, such as research conducted by Ratnawulan *et al*. (2013) applying P fertilizer doses of up to 120 kg SP-36/ha, can provide a large volume of rice roots, but with additional doses of up to 150 kg SP-36/ha, did not have a good effect on the root volume of rice plants, even reducing root volume. The functions of element P include stimulating root development, so that plants will be more resistant to drought, speeding up the harvest period and adding to the nutritional value of seeds (Sumbayak & Gultom, 2020). The research on phosphate fertilizers showed that there was no significant effect on observing root volume in black rice plants (Jaenudin *et al*., 2021).

Table 9. Effect of treatment on the root volume (ml) of white sorghum at age 28 and 42 DAP

Treatment	Root volume (ml)		
	14 DAP	28 DAP	42 DAP
PS30 x PF50	2,33 a	6,17 a	16,33 a
PS30 x PF100	2,70 a	6,83 a	23,67 a
PS30 x PF150	2,73 a	5,67 a	17,67 a
PS40 x PF50	2,17 a	5,50 a	16,00 a
PS40 x PF100	2,50 a	5,17 a	19,00 a
PS40 x PF150	2,03 a	6,00 a	20,73 a
PS50 x PF50	2,33 a	5,50 a	17,33 a
PS50 x PF100	2,27 a	6,50 a	14,17 a
PS50 x PF150	2,20 a	5,67 a	9,00 a

Note: The mean number followed by the same lowercase in the same column is not significant according to the Duncan Multiple Range Test (DMRT) at a = 5% level.

3.2. Yield

Table 10 below illustrates the homogeneous values in the treatment carried out on the observation of dry seed weight of 1000 grains and per plant cluster, so that the results were not significantly different and there was no interaction in all treatments. This shows that the panicle weight per hill is strongly influenced by the plant variety or genotype. In this study, the same varieties were used so that the panicle weight per clump was the same.

In addition to genetic factors, environmental factors and water availability also greatly affect the weight of 1,000 sorghum grains. This is in agreement with the research conducted by Fernandez *et al*. (2012) in dry land conditions, where in the second year there was no significant difference in the weight of 1000 seeds, both with narrow and wide spacing. Under better irrigation conditions, however, crop population of 124,000–160,000 plants/ha under single row planting produced the highest weights. Seed weight is more influenced by the physical shape of the seeds related to seed size. Seed size in plants is more influenced by genetic factors. According to Marlina *et al.* (2015) seed size for certain plants is generally not too much influenced by the environment but seed size is more controlled by the plant's own genetic factors. This is in line with Sitepu *et al*. (2015) where the height and low weight of the seeds depending on the amount or amount of dry matter contained in the seeds, the shape of the seeds and the size of the seeds are influenced by plant genetics.

Table 10. Effect of treatment on the dry seed weight of 1000 grains and dry seed weight per clump

Treatment	Weight od dry seed (g)	
	1000 grains	Per clump
PS30 x PF50	27,13 a	762,00 a
PS30 x PF100	27,73 a	971,00 a
PS30 x PF150	27,03 a	430,67 a
PS40 x PF50	27,23 a	352,33 a
PS40 x PF100	27,20 a	453,00 a
PS40 x PF150	27,43 a	283,00 a
PS50 x PF50	27,40 a	365,33 a
PS50 x PF100	27,30 a	666,00 a
PS50 x PF150	29,13 a	882,33 a

Note: The mean number followed by the same lowercase in the same column is not significant according to the Duncan Multiple Range Test (DMRT) at a = 5% level.

Based on the results of ANOVA on the weight of dry grains per plot, it was revealed that there was no significant interaction. Nonetheless, there was an independent effect on the 70 cm x 30 cm planting space treatment which was statistically higher than those of other spacing treatments. The phosphate fertilizer dosages of 100 kg/ha and 150 kg/ha produced significantly better grain weight as compared to that of 50 kg/ha phosphate fertilizers dose (Table 11).

Table 11. Effect of treatment on the dry seed weight per plot (kg) of white sorghum

Treatment	Dry seed weight (kg/plot)
Plant spacing	
PS30	**5,08 b**
PS40	4,03 a
PS50	3,88 a
Phosphate fertilizer dose	
FP50	3,77 a
FP100	**4,87 b**
FP150	4,35 ab

Note: The mean number followed by the same lowercase is not significant according to the Duncan Multiple Range Test (DMRT) at a = 5% level.

A large plant population can produce the highest seed weight per plot if it is supported by the availability of sufficient nutrients. The denser plant density affected the total plant population per plot so that the panicle weight per plot also had a significant effect on the large plant population, this was also because the panicle weight per clump did not show a significant difference. Then the addition of phosphate fertilizers affected the panicle weight per plot. As it is known that the high production is due to the good adaptability of these varieties to the environment in which they live. This is also possibly due to the fact that the element phosphate is a very important part of the cell nucleus in cell division and for the development of meristem tissue, thus phosphate can stimulate the growth of roots and young plants, thus increasing the absorption of nutrients. Increased nutrient uptake causes metabolic processes to run optimally which will increase the formation of proteins, carbohydrates and starch which will be translocated to food reserves, namely panicles, as a result the panicles that are formed have a greater weight (Nuryan & Haryono, 2019).

This is in a good agreement with the research conducted by Cavalaris *et al*. (2017) in Thessaly, Central Greece that narrow spacing resulted in not only higher plant populations, but also crop productivity in terms of total sugar and dry matter yield in the first year. In addition, narrower row spacing (0.75 m x 0.375 m) results in higher quality products. Low seeding rate (5 kg/ha) in narrow spacing (30 cm) has resulted in consistent maximum grain yields for both years. Larger spacing (60 cm) and higher seed rates (7.5 to 15.0 kg/ha), conversely, produced lower yields. The greater number of seeds in a hole and narrower row spacing causes morphological changes that cause plants to fall over (Gondal *et al*., 2017).

The addition of phosphate fertilizers affected the dry weight of sorghum seeds per plot. This is in agreement with research conducted by Silveira *et al*. (2018) where higher fertilization dose natural phosphate improved the dry matter production of sorghum and the efficiency index of plant growth.

4. CONCLUSIONS AND RECOMMENDATIONS

Based on the discussion that has been described, it can be concluded that in all growth and yield observations, there was no interaction in all spacing treatments with phosphate fertilizers. The highest increase in sorghum yields occurred when the plant spacing was 70 x 30 cm and the phosphate fertilizer treatment was 100 kg/ha.

Based on these conclusions, it can be suggested to improve growth and increase the yield of sorghum plants, it is recommended to use a combination of spacing of 70 cm X 30 cm and 100 kg/ha of phosphate fertilizer with the same soil and climate conditions as the experiment. In addition, further investigation on various soil conditions and different experimental locations and followed by analysis of plants and soil fertility after the experiment need to be carried out.

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