

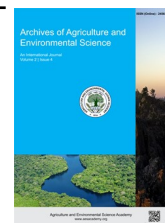


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ORIGINAL RESEARCH ARTICLE



Graded level of nitrogen and mulching effect on growth and yield parameters of tomato in Arghakhanchi, Nepal

Aakriti Kafle^{1*} , Sushil Khatri¹, Bibek Budhathoki¹, Bipana KC¹ and Tej Narayan Bhusal²

¹Faculty of Agriculture, Agriculture and Forestry University, Rampur, Chitwan, NEPAL

²Assistant Professor, Department of Genetics and Plant Breeding, Agriculture and Forestry University, Rampur, Chitwan, NEPAL

*Corresponding author's E-mail: aakritikafle1@gmail.com

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ABSTRACT

A field experiment was carried out to find out the effect of different levels of nitrogen and mulching on growth and yield parameters of tomato (*Solanum lycopersicum* Mill.) var. VL443 at commercial tomato farm in Sandhikharkha, Arghakhanchi from February – June 2022. The eight treatments were laid out in two factorial RCBD with three replications. The treatment combinations were T1 (Non-mulching with 0 kgha⁻¹), T2 (Non-mulching with 50 kgha⁻¹), T3 (Non-mulching with 100 kgha⁻¹), T4 (Non-mulching with 150 kgha⁻¹), T5 (Mulching with 0 kgha⁻¹), T6 (Mulching with 50 kgha⁻¹), T7 (Mulching with 100 kgha⁻¹), and T8 (Mulching with 150 kgha⁻¹). Growth parameters, yield, and yield attributing traits were recorded. The result indicated that the 150 kgha⁻¹ dose of N application contributes to the higher plant height (178.13 cm), the number of leaves (47.83), fruit length (72.50 mm), fruit diameter (58.83 mm), Individual fruit weight (71.67 g) and yield (2.51 kg/ plant). Similarly, plastic mulch contributes significantly higher plant height (173.6 cm), the number of leaves (47.30), fruit length (68.84 mm), fruit diameter (54.20 mm), Individual fruit weight (72.52 g) and yield (2.53 kg/ plant) as compared to non-mulched condition. Furthermore, fruit yield per hectare in mulching with 150 kg⁻¹ plot was significantly higher in comparison to non-mulching with 0 kgha⁻¹ plot. So, the application of 150 kgha⁻¹ nitrogen along with plastic mulching is recommended to increase the yield of tomatoes under the plastic tunnels in Arghakhanchi.

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INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely grown vegetables in the world which is consumed as salads, cooked vegetables, and in processing products like canned tomatoes, tomato juice, ketchup, and “sun-dried” tomatoes or dehydrated pulp (Ghimire *et al.*, 2017). Tomato is the third most important vegetable crop in Nepal after cauliflower and cabbage in terms of production area and production with 21,747 ha area and 4,13,761 metric tons of production. The productivity of tomato in Nepal is 19.03 mt ha⁻¹ which is significantly lower than the global productivity (MoALD, 2021). In Nepal, the average annual tomato consumption is 11.97 kilograms per person

(Ghimire *et al.*, 2017). Tomato production is peak in May-September during the summer season in the hill, which becomes offseason in terai. And cultivated during Nov-Mar in Terai, inner Terai, and foothills as an open-field crop. (Khadka and Adhikari, 2021).

Nitrogen is essential for vegetative growth, flowering, fruit set, and proper growth and yield (Bose and Som, 1990). Phosphorus is known as the “key of life” for the plants. It is because of its direct involvement in most life processes (Amapu, 1998). Phosphorus is a constituent of nucleoprotein, which assist in photosynthesis, cell division, and tissue formation (Singh, 2000). Tomato is one of the most important horticultural crops which requires a high amount of nitrogenous fertilizer because tomato

removes a large amount of N from the soil (Badr et al., 2008). Tomato is also known as a heavy feeder crop because of its huge nutrient uptake from soil (Shrestha et al., 2018). Nitrogen fertilizer affects the growth, yield, and fruit quality of tomatoes. Nitrogenous fertilizer promotes excessive vegetative growth, flowering, and fruit set. It also influences plant height, leaf number per plant, leaf colors, fruit number per plant, fruit mean weight, and total yield per plant in tomato crops (Direkvandi et al., 2008). Management of N fertilizer along with its rate, type of N fertilizer, and application time is very important.

Plastic mulching can be used in either open field or inside tunnel house. Application of mulching inside tunnel house significantly influences tomato plant growth, fruit yield and root zone soil temperature (Tegen et al., 2016). It maintains optimum moisture condition, reduce evaporation, regulate temperature, increases soil microbial biomass, reduces fertilizers leaching as well as soil salinity, and increase nutrient availability whereas indirectly favoring the growth of plant by reducing pathogen and weed number, increasing photosynthesis and root biomass (Joshi et al., 2019). Plastic house technology is one of the viable alternatives for quality tomato production in the high hills (Chapagain et al., 2011). High rainfall and blight incidence during flowering stage limit tomato cultivation in open field condition of high hills because of low temperature (Pandey and Chaudhary, 2004). The practices of open field cultivation of tomatoes with heavy input of fungicides to control diseases and pest becomes eco-unfriendly and create serious health hazards to the human beings. Due to all of these factors, plastic house tomato production technology is getting popularity among the farmers. High quality product possesses better export potentiality in international market (Chapagain et al., 2011). Several independent studies have been conducted to examine the effects of mulching and N, P, and K fertilizer on tomato yield and quality in various regions of Nepal. There haven't been many studies,

nevertheless, that try to figure out the right nitrogen dose for growing tomatoes in diverse mulching circumstances. Hence, a key component of increasing tomato production in Nepal is determining the proper nitrogen dosage for tomato cultivation with regard to mulching methods.

MATERIALS AND METHODS

The field experiment entitled "Graded level of nitrogen and mulching effect on growth and yield parameters of tomato in Arghakhanchi, Nepal" was conducted from Falgun 2078 to Ashad, 2079. The details of materials, method, and methodology used in the study are described on following headings:

Site description

The research was conducted at Sandhikharka-1, Arghakhanchi. The experimental field were located in the humid sub-tropical region having elevation of 960 masl. Arghakhanchi is a district of Lumbini province located in the mid hills between 28° N and 29° N latitude to 81° E and 83° E longitude having an area of 1193 sq.km. The district ranges from 305-2575 m in altitude with 40% forested area. Due to variation in altitude, temperature differences can be seen. The average annual rainfall is approximately 1600 mm. The map showing study area is illustrated in Figure 1.

Climatic condition during experiment

The meteorological data regarding rainfall, temperature, and humidity during the experimental period (15th February to 30th June, 2022) were recorded and is presented in Figure 2. Highest amount of rainfall was occurred in June of 364.23 mm and lowest rainfall occurred during March of 2.57 mm. Highest temperature was recorded in Jun 26 °C and lowest temperature recorded was 16 °C in March. Highest humidity occurred in July which is 81.5 % and lowest humidity was recorded 53.16 % in April.

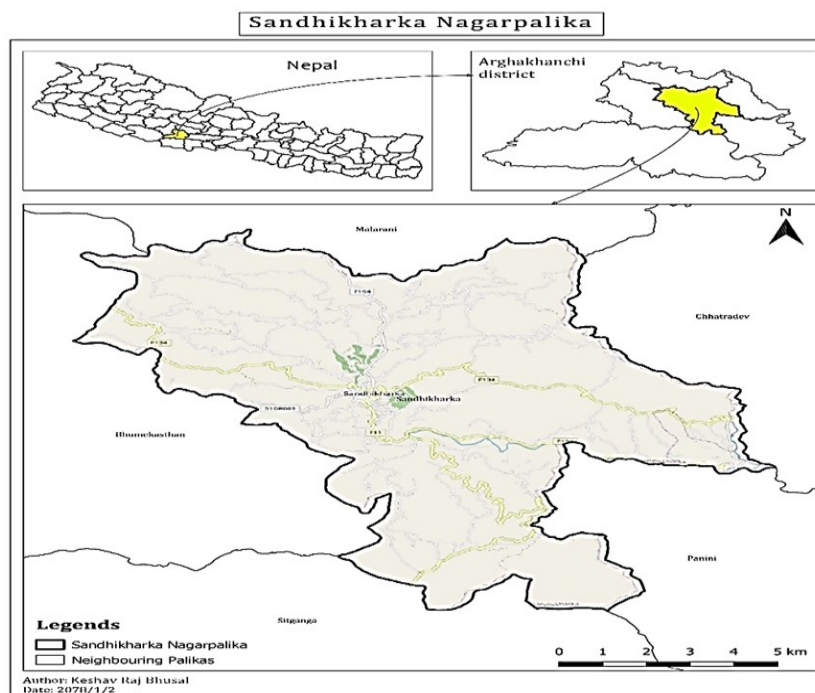


Figure 1. Study area map, Sandhikharka, Arghakhanchi, Nepal.

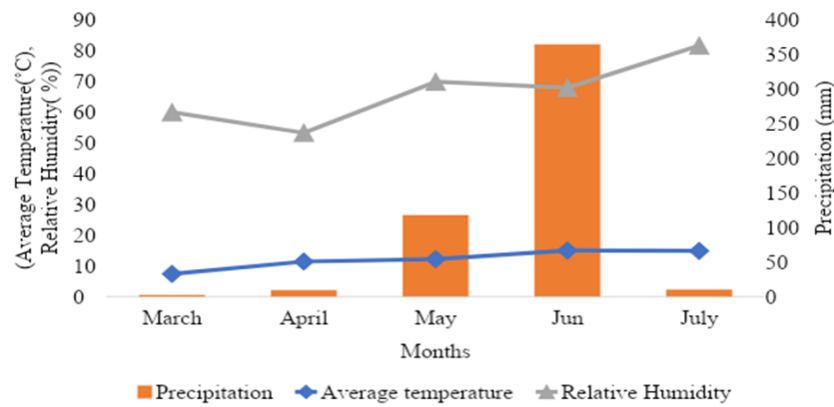


Figure 2. Weather condition of experimental period of research site Source: (NASA Power, 2022)

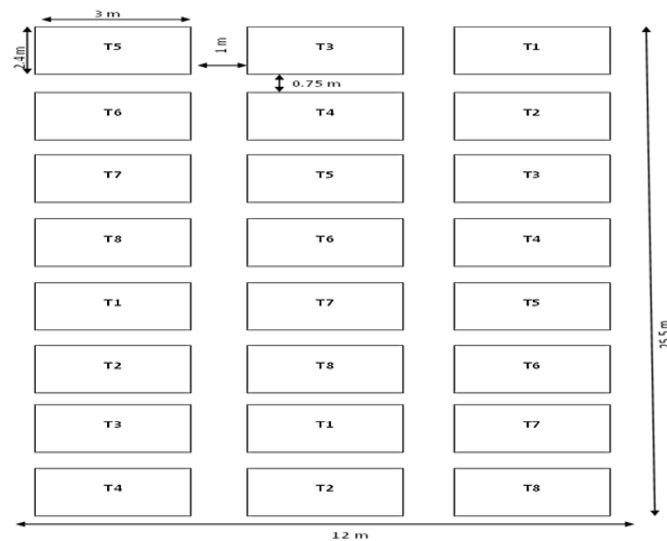


Figure 3. Layout of experimental plot.

Experimental design

The experiment was conducted in a two factor Randomized Complete Block Design (RCBD). The one factor was different doses of nitrogen, and another was mulching practices. There was a total of 8 treatment and replications of 3 times with total of 24 plots having individual area of 7.2 m² each. 24 plants were planted per plot with spacing of 50*80 cm². Treatment consists of four different doses of nitrogen: 0 kg ha⁻¹, 50 kg ha⁻¹, 100 kg ha⁻¹, and 150 kg ha⁻¹, and two different mulching condition: plastic mulching and non-mulching condition.

Treatment details

The treatment combinations were T1 (Non-mulching with 0 kg ha⁻¹), T2 (Non-mulching with 50 kg ha⁻¹), T3 (Non-mulching with 100 kg ha⁻¹), T4 (Non-mulching with 150 kg ha⁻¹), T5 (Mulching with 0 kg ha⁻¹), T6 (Mulching with 50 kg ha⁻¹), T7 (Mulching with 100 kg ha⁻¹), and T8 (Mulching with 150 kg ha⁻¹).

General cultural practices

The tomato seeds were sown on 4th Falgun on a nursery bed and seedlings was transplanted in 1st week of Chaitra. Seedlings were raised in a tray filled with a mixture of cocopeat and peat moss. Seeds were sown in depth of 2 cm. After sowing, seed were covered by cocopeat mixture and kept in a gumlose. After 25-30 days, it was transplanted in the main field at 3-4 leaf stage

of the growing seedlings. Seedlings were transplanted after 4 weeks of sowing under plastic tunnel at a distance of plant to plant 50 cm and row to row at 80 cm. FYM was applied as main source of organic fertilizer in the field @ 3 mt ha⁻¹. It was applied in all experimental plots and was uniformly incorporated into the soil during land preparation. Urea, SSP, and MOP were used as sources of fertilizers. The recommended dose of 85kg ha⁻¹ of phosphorus and 50 kg ha⁻¹ of potassium were applied as basal in all plots at the time of transplanting. The recommended doses of nitrogen were 125 kg ha⁻¹. ½ dose of N, full dose of Phosphorus, and Potassium were applied as basal dose, ¼ dose of N was top dressed at 30 DAT and remaining ¼ dose of N was applied at 45 DAT. 234g, 156g, and 78g of urea as a nitrogen source was applied in each plot as per treatment.

Statistical data analysis

All the recorded data were arranged systematically under three replications based on various parameters. Experimental data were tabulated in MS-Excel software and data was analyzed by R-studio software and treatment means were separated using Duncan's Multiple Range Test (DMRT) at a 5% level of significance. Analysis of variance (ANOVA) was used to test differences between treatment means. A simple correlation and regression analysis was done between the yield and yield attributing characters.

RESULTS AND DISCUSSION

The result of Field experiment “Graded level of nitrogen and mulching effects on growth and yield parameters of tomato in Arghakhanchi, Nepal” were analyzed and presented in this chapter with necessary figures and tables. The obtained results were explained with available evidence as far as possible for recorded variations in mentioned parameters.

Effect of mulching and nitrogen dose on height of tomato

The effect of nitrogen and mulching on plant height was analyzed and presented in Table (1) which shows mulching has significant effect on increment of plant height, and N at 150 kg ha^{-1} gives higher height of tomato vines. Among different nitrogen level plant height was found highest at 150 kg ha^{-1} of N at 45 DAT (93.66 cm), 60 DAT (142.16 cm), and 75DAT (178.13 cm). Nitrogen dose of 150 kg ha^{-1} and 50 kg ha^{-1} were significantly at par having height of 178.13 cm and 169.73 cm respectively. The result was found in agreement with the previous investigation performed by Basha et al. (2022). In mulching Significantly higher height was found in 30 DAT (56.91 cm), 45 DAT (92.50 cm), 60 DAT (141.35 cm), and 75 DAT (173.6 cm). Hamed et al. (2022) revealed that plastic mulching increases vegetative growth and maximizes plant height. There were several reports which showed the plastic mulching had increased soil temperature and early shoot growth in tomato (Qasem, 2019). The CV shows highest variability of plant height of tomato at 30 DAT while lower variability at 60 DAT.

Effect of mulching and nitrogen dose on the number of leaves of tomato

The effect of nitrogen and mulching on numbers of leaves of tomato plant was analyzed and presented in Table (2), which shows mulching has significant effect on increment of leaves

number, and N at 150 kg ha^{-1} gives higher leaves number. Among different nitrogen levels, the number of leaves was found significantly highest at 150 kg ha^{-1} of nitrogen at 45 DAT (26.90), 60 DAT (36.83), and 75 DAT (47.83). The number of leaves per plant increases as the nitrogen level increases. It is because nitrogen is associated with photosynthetic activity and vigorous vegetative growth (Hariyadi et al., 2019). This result is in line with the result obtained Basha et al. (2022), who reported that leaf number increased as nitrogen level was increased during tomato growth. In mulching significantly higher number of leaves per plant was found in 30 DAT (18.45), 45 DAT (24.83), 60 DAT (34.46), and 75 DAT (47.30) than in non-mulching. The microclimate created by mulching favors for a greater number of leaves production. This study supports the findings of Goel et al. (2020), who showed the maximum number of leaves per plant was found in mulch condition.

Effect of mulching and nitrogen dose on days to first flowering and total number of flowers per plant of tomato

The effect of nitrogen and mulching on days to first flowering and total numbers of flowers per plant was analyzed and presented in Table (3). At 150 kg ha^{-1} of N, days to first flowering is significantly earlier (31.83 days). Days to first flowering is significantly higher in 0 kg ha^{-1} (37.86 days) which is statistically par at with 50 kg ha^{-1} (37.53 days) and 100 kg ha^{-1} (36.43 days). Days to first flowering was found earlier with increased level of nitrogen as nitrogen accelerate protein synthesis, photosynthesis and carbohydrate production which promotes earlier floral primordia development (Dhiman et al., 2018). The earliness is due to faster enhancement of vegetative growth and storing reserved food materials for differentiation of buds into flowers buds (Khan et al., 2020). In mulching, days to first flowering were significantly lower (33.13 days) while that of non-mulching was significantly higher (38.70 days).

Table 1. Effect of mulching and nitrogen dose on height at different growth stages of tomato in Arghakhanchi, Nepal during 2022.

Treatments	Plant height (cm)			
	30 DAT	45 DAT	60 DAT	75 DAT
Mulching				
Mulching	56.91 ^a	92.50 ^a	141.35 ^a	173.6 ^a
Non-Mulching	44.20 ^b	78.16 ^b	128.53 ^b	161.7 ^b
SE(±)d	1.50	1.94	2.70	3.77
F-value	71.17 ^{**}	62.14 ^{**}	22.48 ^{**}	9.95 ^{**}
CV(%)	7.30	5.55	4.90	5.51
Nitrogen Dose				
0 kg ha^{-1}	49.60	82.40 ^b	128.43 ^c	161.10 ^b
50 kg ha^{-1}	52.60	83.36 ^b	132.50 ^{bc}	169.73 ^{ab}
100 kg ha^{-1}	52.10	83.90 ^b	136.66 ^{ab}	161.63 ^b
150 kg ha^{-1}	47.93	93.66 ^a	142.16 ^a	178.13 ^a
SE(±)d	2.13	2.75	3.82	5.33
F-value	2.10 ^{ns}	7.31 ^{**}	4.72 [*]	4.53 [*]
CV(%)	7.30	5.55	4.90	5.51
Grand Mean	50.55	85.83	134.94	167.65

*Treatment mean followed by common letter (s) are not significantly different from each other based on DMRT at 5%, * significant at 0.05 p level, **Significant at 0.01 P level. DAT= Days after Transplanting, ns= non-significant, SE(±)d, Standard error of difference, CV= Coefficient of variation

Table 2. Effect of mulching and nitrogen dose on number of leaves per plant of tomato at different growth stages in Arghakhanchi, Nepal during 2022.

Treatments	Number of leaves per plant			
	30 DAT	45 DAT	60 DAT	75 DAT
Mulching				
Mulching	18.45 ^a	24.83 ^a	34.46 ^a	47.30 ^a
Non-Mulching	13.65 ^b	21.00 ^b	30.46 ^b	42.78 ^b
SE(±)d	0.85	1.04	1.17	1.24
F-value	31.39**	13.49**	11.61**	13.25**
CV(%)	13.07	11.15	8.85	6.74
Nitrogen Dose				
0 kgha ⁻¹	15.33	20.93 ^b	30.13 ^b	45.20 ^a
50 kgha ⁻¹	17.66	21.66 ^b	31.53 ^b	45.83 ^a
100 kgha ⁻¹	16.33	22.16 ^b	31.36 ^b	41.30 ^b
150 kgha ⁻¹	14.86	26.90 ^a	36.83 ^a	47.83 ^a
SE(±)d	1.2	1.47	1.66	1.75
F-value	2.09 ^{ns}	6.71**	6.43**	4.86*
CV(%)	13.07	22.91	8.85	6.74
Grand Mean	16.05	22.91	32.46	45.04

*Treatment mean followed by common letter (s) are not significantly different from each other based on DMRT at 5%, * significant at 0.05 p level, **Significant at 0.01 P level. DAT= Days after Transplanting, ns= non-significant, SE(±)d, Standard error of difference, CV= Coefficient of variation.

Table 3. Effect of mulching and nitrogen dose in days to first flowering and total flowering of tomato in Arghakhanchi, Nepal during 2022.

Treatment	Days to first flowering	Total Number of flowers per plant
Mulching		
Mulching	33.13 ^b	67.92 ^a
Non-Mulching	38.70 ^a	39.84 ^b
SE(±)d	1.02	2.71
F-value	29.77**	107.07**
CV(%)	6.95	12.33
Nitrogen Dose		
0 kgha ⁻¹	37.86 ^a	34.45 ^d
50 kgha ⁻¹	37.53 ^a	50.04 ^c
100 kgha ⁻¹	36.43 ^a	61.04 ^b
150 kgha ⁻¹	31.83 ^b	70.00 ^a
SE(±)d	1.44	3.83
F-value	7.48**	31.84**
CV(%)	6.95	12.33
Grand Mean	35.91	53.88

*Treatment mean followed by common letter (s) are not significantly different from each other based on DMRT at 5%, * significant at 0.05 p level, **Significant at 0.01 P level. DAT= Days after Transplanting, SE(±)d, Standard error of difference, CV= Coefficient of variation.

Total numbers of flowers were significantly different at different levels of nitrogen as shown in Table (3). In 150 kgha⁻¹ of N, total number of flowers was significantly higher (70.00) and at 0 kgha⁻¹ total number of flowers were significantly lower (34.45) respectively. Mulching show significantly higher number of flowers (67.92) and non-mulching show significantly lower number of flowers (39.84). Increased rate of nitrogen promotes vegetative growth which help in production of higher assimilates leading to formation of active sink like flowers and fruit (Pawar and Rana, 2019). Improvement of Vegetative growth in plastic mulching with moisture conservation and availability ultimately led to plant growth.

Effect of mulching and nitrogen dose on fruit length (mm) and fruit diameter (mm) of tomato

The effect of nitrogen and mulching on Fruit length (mm) and Fruit diameter (mm) were analyzed and presented in Table (4) which shows mulching and N at 150 kgha⁻¹ has significant effect

on increment fruit length and fruit diameter. Among different nitrogen levels, the fruit length (mm) was found significantly highest (72.50 mm) at 150kgha⁻¹ of N which is statistically par at with 50 kgha⁻¹ of nitrogen (65.84mm). The result agrees with the findings of Okunlola et al. (2018). Significantly highest fruit length was found in mulching (68.84mm) and significantly lowest fruit length was found in no mulching (61.05mm). Similarly, the fruit diameter (mm) was found significantly highest (58.83mm) at 150kgha⁻¹ of N. The smallest fruit diameter (40.43 mm) was found at 0kgha⁻¹ of N. Okunlola et al. (2018) found the result which shows the higher dose of nitrogen increases fruit diameter. Significantly highest fruit diameter was found in mulching (54.20mm) and significantly lowest fruit diameter was found in no mulching (46.77mm). There was significant increase in the diameter and length of tomato due to mulching (Zhang et al., 2019). This result is in line with the result obtained Karear et al. (2020), who reported that fruit diameter increased using mulching in tomato.

Table 4. Effect of mulching and nitrogen dose in Fruit length (mm) and fruit diameter (mm) of tomato in Arghakhanchi, Nepal during 2022.

Treatments	Fruit length and Fruit diameter (mm)	
	Fruit length	Fruit diameter
Mulching		
Mulching	68.84 ^a	54.20 ^a
Non-Mulching	61.05 ^b	46.77 ^b
SE(±)d	2.61	1.63
F-value	8.89**	20.52**
CV(%)	9.84	7.95
Nitrogen Dose		
0 kgha ⁻¹	57.7 ^c	40.43 ^c
50 kgha ⁻¹	65.84 ^{ab}	49.61 ^b
100 kgha ⁻¹	63.70 ^{bc}	53.08 ^b
150 kgha ⁻¹	72.50 ^a	58.83 ^a
SE(±)d	3.69	2.31
F-value	5.45*	22.12**
CV(%)	9.84	7.95
Grand Mean	6.49	50.4

*Treatment mean followed by common letter (s) are not significantly different from each other based on DMRT at 5%, * significant at 0.05 p level, **Significant at 0.01 P level. DAT= Days after Transplanting, SE(±)d, Standard error of difference, CV= Coefficient of variation.

Table 5. Effect of mulching and nitrogen dose in fruit per plant, individual fruit weight (g), and yield per plant of tomato in Arghakhanchi, Nepal during 2022.

Treatment	Fruit per plant	Individual fruit weight (g)	Yield per plant (kg)	Yield ha ⁻¹ (mt ha ⁻¹)
Mulching				
Mulching	34.85 ^a	72.52 ^a	2.53 ^a	55.73 ^a
Non-Mulching	25.66 ^b	60.75 ^b	1.57 ^b	34.58 ^b
SE(±)d	0.95	3.33	0.10	2.1
F-value	92.58**	12.43**	93.51**	93.51**
CV(%)	7.72	12.26	11.86	11.86
Nitrogen Dose				
0 kgha ⁻¹	27.13 ^c	57.66 ^b	1.59 ^c	35.08 ^c
50 kgha ⁻¹	28.50 ^{bc}	68.92 ^a	1.96 ^b	43.20 ^b
100 kgha ⁻¹	30.36 ^b	68.28 ^a	2.13 ^b	46.96 ^b
150 kgha ⁻¹	35.03 ^a	71.67 ^a	2.51 ^a	55.38 ^a
SE(±)d	1.35	4.72	0.14	3.09
F-value	13.05**	3.40*	14.85**	14.85**
CV(%)	7.72	12.26	11.86	11.86
Grand Mean	30.25	66.63	2.05	45.15

*Treatment mean followed by common letter (s) are not significantly different from each other based on DMRT at 5%, * significant at 0.05 p level, **Significant at 0.01 P level. DAT= Days after Transplanting, SE(±)d, Standard error of difference, CV= Coefficient of variation.

Effect of mulching and nitrogen dose on fruit per plant, individual fruit weight (g), yield per plot and yield per hectare of tomato

The effect of nitrogen and mulching on Fruit per plant, Individual fruit weight (g), yield per plot, and yield per hectare were analyzed and presented in Table (5). Among different nitrogen level fruit per plant was found highest (35.033) at 150 kgha⁻¹ of N. The fruit per plant was found significantly lowest (27.133) at 0 kgha⁻¹ of N. Islam et al. (2018) found that increasing nitrogen doses increases fruit number. Fruit per plant was found significantly highest (34.85) at mulching and lowest (25.66) at non-mulching. Similarly, Mendonca et al. (2021) reported greater number of fruits per plant in mulched condition.

Individual fruit weight was found significantly highest (71.67 g) at 150 kgha⁻¹ of N. The individual fruit weight was found significantly lowest (57.66 g) at 0 kgha⁻¹ of N. Individual fruit weight was found significantly highest (72.52g) at mulching and lowest (60.75) at non-mulching. Yield per plant was found significantly highest (2.519kg) at 150 kgha⁻¹ of N. And found significantly lower (1.59 kgha⁻¹) at 0 kgha⁻¹ of N. Yield per plant was found

significantly highest (2.53 kg) at mulching and lowest (1.57 kg) at non-mulching. Application of black plastic mulches in tomato increased the yield per plant and total yield compared to non-mulched condition (Sarmah et al., 2022). Our results on the effect of black plastic mulch on total yield are similar to those reported by (Mendonca et al., 2021).

The yield ha⁻¹ was significantly different with varying levels of nitrogen. Yield (mt ha⁻¹) was found significantly highest (55.38 mt ha⁻¹) at 150 kgha⁻¹ of N and significantly lowest (35.08 mt ha⁻¹) at 0 kgha⁻¹ of N. Yield ha⁻¹ was found significantly highest (55.73 mt ha⁻¹) at mulching and lowest (34.58 mt ha⁻¹) at non-mulching. Nitrogen levels had significant influence on total yield increase as dose increases which in lines with the result obtained by Kaniszewski et al. (2019). Mulching significantly improved the tomato plant growth and increase in fruit yield (Mendonca et al., 2021). Higher yield in mulch treatment might be due to its soil temperature, conserve soil moisture and weed suppression (Sarmah et al., 2022)

Conclusion

At various nitrogen levels and mulching condition, the yield and yield attributing parameters, such as plant height, leaves number, earlier days to first flowering, individual fruit weight (g), fruit length (mm), fruit diameter (mm), fruit per plant, yield per hectare under plastic tunnel were significantly different. Fruit per plant was found statistically higher in mulching with 150 kg ha⁻¹ of N and lower in non-mulching with 0 kg ha⁻¹ of N. Likewise, individual fruit weight (g) was significantly higher in mulching and at nitrogen dose of 150 kg ha⁻¹. Also, nitrogen dose of 150 kg ha⁻¹ was found to be effective to increase the growth and yield of tomato. Based on the results, it can be said that a nitrogen level of 150 kg/ha along with plastic mulching is ideal because it results in a higher yield and this combination is recommended to the farmers to get higher productivity and profitability under similar climatic condition in Arghakhanchi, Nepal.

Conflict of interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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