



Effect of Lime Application for Neutralizing Fertilizer Acidity on Growth and Yield of Maize in Alfisols

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Authors' contributions

This work was carried out in collaboration among all authors. Author KD carried out this research and interpreted the results as a part of MSc. (Agri.) degree and wrote manuscript under the guidance of Author PSS. All authors read and approved the final manuscript.

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ABSTRACT

A field experiment was conducted at College of Agriculture, V. C. Farm, Mandya during *Kharif* 2021-22 to evaluate the effect of lime application for neutralizing fertilizer acidity on growth and yield of maize in *Alfisols*. There were eight treatments that include 500 kg lime (T_3) and 250 kg

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granular dolomite (T_4) + FYM and recommended dose of fertilizers (RDF), 100% RDF and FYM + 100 and 50% fertilizer acidity neutralization with lime (T_5 and T_6) and granular dolomite (T_7 and T_8), absolute control (T_1) and FYM + 100 % RDF (T_2). The experiment was laid out in Randomized Complete Block Design with three replications. The results revealed that application of lime @ equivalent to 100% neutralization of fertilizer acidity with 100% RDF and FYM (T_5) recorded significantly higher plant height (221.56 cm), number of leaves (13.01 plant $^{-1}$), leaf area (6389 cm 2 plant $^{-1}$) and total dry matter accumulation plant $^{-1}$ (168.74 g plant $^{-1}$) which was on par with T_8 , T_6 , T_7 and T_4 . Similarly, significantly higher yield parameters like cob length (21.98 cm), cob girth (20.01 cm), number of rows cob $^{-1}$ (16.86), number of kernels row $^{-1}$ (36.98) and test weight (35.98 g) were recorded in T_5 when compared to T_2 , T_3 and T_1 . Kernel and stover yield (81.57 and 90.67 q ha $^{-1}$, respectively) was observed to be significantly higher in T_5 treatment which was on par with T_6 which clearly indicated that 100% neutralization of fertilizer acidity with lime along with 100% recommended dose of fertilizer and FYM is necessary for obtaining higher growth and yield of maize.

Keywords: Fertilizer acidity; lime; granular dolomite; neutralization; growth; yield.

1. INTRODUCTION

Soil acidity is a major constraint affecting agricultural productivity in over 25 per cent of the total geographical area of the country. The most important factor that is responsible for soil acidification in agricultural land is the application of acidifying fertilizers such as elemental sulphur (S), urea or ammonium (NH $_4^+$) salt and SSP. The poor growth of crops in acid soils is due to the presence of elements like hydrogen, aluminium, iron and manganese in toxic concentration and decreased availability of other essential nutrient elements. Besides, its effect on essential soil biological functions like nitrogen fixation, shift in the dominance of microbial community, lower rate of organic matter decomposition [1] also contribute to lower productivity of crops in acid soils.

Continuous use of acid forming fertilizers are causing the reduction in soil pH and leaching of nitrate and sulphate along with removes some base cations and this results in soil acidification. Results of long-term fertilizer experiments revealed that continuous application of N alone had the most acidifying effect with soil pH value declining from 5.80 to 4.49 in 42nd cropping cycle and the grain yield of wheat in 100% N had declined to 0.0 q ha $^{-1}$. This might be due to the increased soil acidity and deteriorated soil quality with the continuous use of N over a period of 42 years [2].

Application of lime to neutralize exchangeable Al $^{3+}$ and H $^+$ helps in raising the pH of acid soils thereby improving availability of plant nutrients, biological functions of the soil and reducing toxicity of aluminium, iron etc. Lime, when

applied to acidic soils, either in the form of oxide, hydroxide or carbonate, reacts with carbon dioxide and water to form bicarbonates. These liming materials, on reaction with soil colloids, replace H $^+$ and Al $^{3+}$ ions from the colloidal phase to the soil solution. Hence, the liming material is required to neutralize the physiological acidity created by acid forming fertilizers. Fertilizer use in acid soil regions is low, which needs to be stepped up. Lime plus recommended NPK application increases crop yield by 50 to 100 per cent compared to lime alone. Farmers have to be conscious of the fact that the continuous use of acid forming fertilizers (ammonium sulphate, urea, DAP, SSP etc.) has an adverse impact on soil health.

When ammonium sulphate added to soil, it decreases soil pH or either increases soil acidity. One molecule of (NH $_4$) $_2$ SO $_4$ yields 2 atoms of Ca that can be lost by leaching. Therefore, 100 kg of (NH $_4$) $_2$ SO $_4$ causes a loss of 45.5 kg of Ca. To compensate the loss of Ca, and to neutralize the soil acidity produced by 100 kg of (NH $_4$) $_2$ SO $_4$, 110 kg of pure CaCO $_3$ is needed and for application of 100 kg urea, 83.12 kg of pure CaCO $_3$ need to be applied [3].

Maize (*Zea mays L.*) is one of the most versatile emerging crops, cultivated in a wide range of agro-climatic conditions. Globally, ranked 4th among cereal crops in terms of area under cultivation, nearly 1162.35 million tonnes of maize are being produced together by over 170 countries from an area of 201.98 m ha with an average productivity of 5.75 t ha $^{-1}$ [4]. In India, it is grown under diverse agro climatic situations covering an area of 9.96 million hectares with a production of 33.73 million tonnes and

productivity 3.39 t ha⁻¹. In Karnataka, maize is cultivated over an area of 1.72 million hectare with a production of 6.36 million tonnes and productivity of 3.42 t ha⁻¹ [5]. In Mandya district of Karnataka, maize is grown in an area of 3,903 ha with a production of 15,978 t and productivity of 4.308 kg ha⁻¹. Hence considering the above facts, an attempt has been made to evaluate the effect of liming materials applied to neutralize the physiological acidity created by acid forming fertilizers in agriculture by using maize as a test crop in *Alfisols*.

2. MATERIALS AND METHODS

The field experiment was conducted at College of Agriculture, V.C. Farm, Mandya which comes under the Region III and Agro Climatic Zone VI (Southern Dry Zone) of Karnataka, which is situated at 12° 34' 03" North latitude and 76° 49' 08" East longitude with an altitude of 705 meters above mean sea level. The normal rainfall of the V. C. Farm, Mandya during crop growing period (September – January) was 344.0 mm. The major part of the rainfall was noticed in September (154.4 mm). The normal mean monthly maximum air temperature ranged from 27°C to 31.9 °C. The highest mean maximum air temperature was observed during the month of September (31.3 °C). The normal mean monthly minimum air temperature ranged from 17.4°C to 19.8 °C. The lowest mean minimum temperature was observed during December (16.0°C). The mean sunshine hours varied from 5.8 to 6.8 hours during September 2021 to January 2022. (Table 4).

The actual rainfall received during the present research investigation period at V. C. Farm, Mandya was 572.9 mm. The major part of the rainfall was received in the month of October (307.4 mm). The mean maximum air temperature varied from 28.2°C to 29.9°C. The highest mean maximum air temperature was noticed during September (30.0°C). The mean minimum air temperature ranged from 17.1°C to 19.6°C. The lowest mean minimum temperature recorded during December (28.7°C). The mean bright sunshine hours varied from 2.6 to 4.8 hours during September to January. The relative humidity varied from 89 to 93% and 64 to 85% during morning and afternoon hours, respectively during the crop growing period of 2021-22.

The experiment was conducted at College of Agriculture, Vishweshwaraiah Canal Farm, Mandya, India during Kharif 2021. Soil of the

experimental site was classified as *Alfisols* with red loamy sand with neutral soil reaction (7.19), electrical conductivity (0.14 dSm⁻¹) and organic carbon content (4.84 g kg⁻¹) was found to be low. The available nitrogen (241.06 kg ha⁻¹), phosphorus (25.56 kg P₂O₅ ha⁻¹) and potassium (182.45 kg K₂O ha⁻¹) was medium. The investigation was carried out in Randomized Complete Block Design with eight treatments and replicated thrice. The treatments comprised of application of 500 kg lime (T₃) and 250 kg granular dolomite (T₄) along with FYM and recommended dose of fertilizers, 100 and 50% neutralization with lime (T₅ and T₆) and granular dolomite (T₇ and T₈) along with FYM and RDF, absolute control (T₁) and FYM + 100 % RDF (T₂). In the present experiment, maize (*Zea mays* L.) variety MAH-14-05 was grown as the test crop. The land was prepared by ploughing with tractor drawn disc plough followed by disc harrowing and passing cultivator twice to bring the soil to fine tilth.

Layout of the experiment was done with gross plot and net plot size of 5.1 m × 3.6 m and 3.9 m × 2.4 m, respectively. A distance of 0.5 m between two plots and 0.6 m was set to differentiate the replications. The bund height of 30 cm was raised in the space available between replications and plots. The recommended FYM (10 t ha⁻¹) was applied uniformly to all the treatments two weeks before sowing except for absolute control plot. After layout of experiment, recommended quantity of zinc sulphate (10 kg ha⁻¹) were applied. Furrows at an interval of 60 cm were opened using furrow openers attached to bullock pair. Basal dose of recommended fertilizers (50 per cent N and 100 per cent P and K) were applied treatment wise to each plot and mixed with soil at the base of seed row. Urea, Di Ammonium Phosphate (DAP) and Muriate of potash (MOP) were used as sources of N, P and K. For treatments T₃ to T₈ the liming materials were applied 15 days before sowing and proper moisture was maintained by irrigating. Treatments T₃ and T₄ received calculated quantity of 500 kg lime and 250 kg granular dolomite respectively. For treatments T₅ and T₇ calculated quantity of lime and granular dolomite @ equivalent to 100% neutralization of fertilizer acidity and for treatments T₆ and T₈ calculated quantity of lime and granular dolomite @ equivalent to 50% neutralization of fertilizer acidity were broadcasted plot wise and thoroughly mixed with soil. Seeds were dibbled at 30 cm spacing (2 seeds per hill). The remaining 50 per cent dose of nitrogen was top

dressed in two equal splits, one at 30 DAS and another at 45 DAS in the form of urea. First irrigation was given on the day of sowing and subsequent irrigations were given as and when required by the crop using ridges and furrow method.

Weed management was done through application of Atrazine 50% WP @ 6.5 g L⁻¹ at two days after planting. Inter-cultivation practice was done at 35 and 60 DAS with the help of bullock drawn harrow. Hand weeding was done at 30 and 60 DAS to keep the plots devoid of weeds. Five plants were randomly selected from each net plot and labelled to take growth parameters at different growth stages viz., 30, 60, 90 DAS and at harvest of the crop. After reaching the physiological maturity the crop was harvested according to the treatments from all the plots. From the net plot the cobs and stover were harvested

separately from each plot. Kernel and stover were sun dried and weighed separately after threshing. The yields obtained were expressed in quintals ha⁻¹. The data was statistically analysed by following the method of Gomez and Gomez, [6].

2.1 Methods for Measuring Fertilizer Acidity

Potential acidity of fertilizers is estimated using Pierre's method (PM) expressed in calcium carbonate equivalents (CCE) per unit weight of fertilizer. It refers to the CCE required to neutralize the acidity resulting from application of the fertilizer [7]. To calculate the acidity of the individual salts, the following equations were used:

$$\frac{\text{Molecular weight of element} \times \text{equivalent acidity for element} \times 1000 \text{ kg}}{\text{Molecular weight of salt}}$$

= kg of CaCO₃ acidity for element per 1000 kg metric tonne of fertilizer salt.

2.2 Liming Materials Applied for Neutralizing Fertilizer Acidity

Two different sources of liming materials such as lime and granulated dolomite were applied to soil 15 days prior to sowing of maize as per treatment. Quantity of liming material applied for 100 and 50% neutralization with lime (342 and 171 kg ha⁻¹, respectively) and granular dolomite (372 and 186 kg ha⁻¹, respectively).

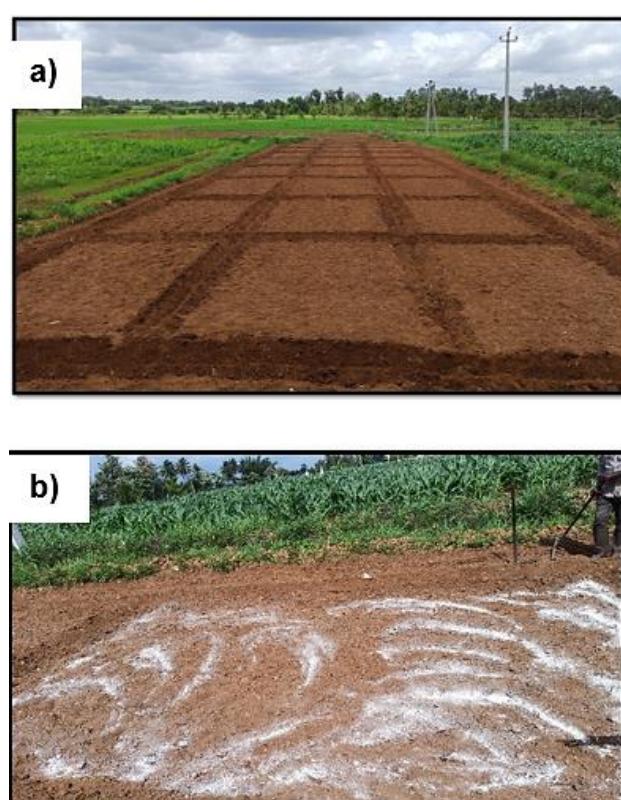


Fig. 1. a) Layout of the experimental site, b) application of liming material to plot

3. RESULTS AND DISCUSSION

3.1 Plant Height

Application of liming material for neutralizing fertilizer acidity showed significant effect on plant height (Table 1). At harvest, application of lime @ equivalent to 100% neutralization of fertilizer acidity with 100% RDF and FYM recorded significantly higher plant height (T_5 – 221.56 cm) than T_1 (68.58 cm), T_2 (183.68 cm) and T_3 (192.23 cm) treatments but it was statistically at par with rest of the treatments.

3.2 Number of Leaves Plant⁻¹

According to results obtained, application of liming material for neutralizing fertilizer acidity could improve the number of leaves in plant, maximum number of leaves were observed in T_5 (13.01 plant⁻¹) which was significant over T_2 (10.98 plant⁻¹) and T_3 (11.43 plant⁻¹) and the least leaf count was noticed in absolute control (8.28 plant⁻¹) (Table 1).

3.3 Leaf Area Per Plant (cm² Plant⁻¹)

Leaf area was also significantly influenced by the application of liming materials (Table 1). At harvest, significantly higher leaf area was recorded in 100 percent RDF and FYM + application of lime @ equivalent to 100% neutralization of fertilizer acidity (T_5 – 6389 cm² plant⁻¹) and was on par with 100% RDF and FYM + 50% acid neutralization with GD (T_8 – 6389 cm² plant⁻¹), 100% RDF and FYM + application of lime @ equivalent to 50% neutralization of

fertilizer acidity (T_6 – 6161 cm² plant⁻¹), 100% neutralization with GD + 100% RDF and FYM (T_7 – 6099 cm² plant⁻¹), 250 kg GD and 100% RDF + FYM (T_4 – 5689 cm² plant⁻¹). Significantly lower leaf area was observed in control (T_1 – 3519 cm² plant⁻¹).

3.4 Total Dry Matter Accumulation (g Plant⁻¹)

Treatment receiving 100 percent RDF and FYM + application of lime @ equivalent to 100% neutralization of fertilizer acidity (T_5 -168.74 g plant⁻¹) recorded significantly higher dry matter production compared to control (81.05 g plant⁻¹), T_2 (144.47 g plant⁻¹) and T_3 (147.11 g plant⁻¹) and was on par with rest of the treatments (Table 1).

Neutralization of acidity has positive effect on microbial population which may also has contributed to the enhanced supply of nutrients [8]. The application of lime along with acid forming fertilizers, apart from neutralizing the acidity, also serves as source of calcium for plant growth and, the slow solubility of granulated dolomite, which is helpful in neutralizing the soil acidity throughout the crop growth besides, serving as both Ca and Mg source to plant. The lower growth parameters in control, clearly indicate that the nutrient status of soil was not able to meet the nutrient requirement of the crop, as a result the growth was stunted with pale yellow colour (Plate 1d). The above findings are in accordance with Talashilkar et al. [9], Rajashree and Pillai [10], Oluwatoyinbo et al. [11] and Brandon et al. [12].

Table 1. Effect of liming material on growth parameters of maize

Treatment	Plant height (cm)	No. of leaves plant ⁻¹	Leaf area (cm ² plant ⁻¹)	Total dry matter accumulation (g plant ⁻¹)
T_1	68.58	8.28	3519	81.05
T_2	183.68	10.98	5389	144.47
T_3	192.23	11.43	5471	147.11
T_4	199.56	11.99	5689	151.87
T_5	221.56	13.01	6389	168.74
T_6	209.98	12.68	6161	161.33
T_7	207.55	12.57	6099	158.65
T_8	216.31	12.99	6389	165.88
S.Em±	7.59	0.49	232.78	6.03
CD @ 5 %	23.04	1.48	706.14	18.28

Treatment details

T_1 : Absolute control
 T_2 : 100 % RDF + FYM (150:75:40 NPK kg ha⁻¹)
 T_3 : T_2 + 500 kg lime
 T_4 : T_2 + 250 kg GD

T_5 : T_2 + 100 % neutralization with lime
 T_6 : T_2 + 50 % neutralization with lime
 T_7 : T_2 + 100 % neutralization with GD
 T_8 : T_2 + 50 % neutralization with GD

Note: RDF: Recommended dose of fertilizers, FYM: Farm Yard Manure, GD: Granular dolomite

3.5 Yield Parameters

3.5.1 Cob length

Application of lime @ equivalent to 100% neutralization of fertilizer acidity with 100% RDF and FYM (T_5) recorded significantly higher cob length of 21.98 cm and was on par with (T_8 - 21.79 cm), (T_6 - 20.87 cm), (T_7 - 20.65 cm) and (T_4 - 19.97 cm). Results revealed that application of 500 kg lime along with 100% RDF and FYM (T_3 - 18.29 cm) was on par with T_2 (18.01 cm). Lowest cob length was noticed in T_1 (7.80 cm) (Table 2 and Fig. 2).

3.5.2 Cob girth

Application of liming material for neutralizing fertilizer acidity revealed that cob girth recorded significantly higher value of 20.01 cm with application of 100 per cent RDF and FYM + application of lime @ equivalent to 100% neutralization of fertilizer acidity (T_5) (Table 2 and Fig. 2) and was on par with T_8 , T_6 , T_7 and T_4 . On the other hand, T_2 was observed to be on par with T_3 . Absolute control (T_1) recorded significantly lowest cob girth with no fertilizer applied.

3.5.3 Number of rows per cob

Least number of rows per cob was recorded in control (10.83) which increased significantly to 16.86 in T_5 due to application of 100 per cent RDF and FYM + application of lime @ equivalent to 100% neutralization of fertilizer acidity (Table 2 and Fig. 2). But the number of rows per cob recorded in T_5 was statistically on par with T_8 , T_6 , T_7 and T_4 and significant with T_2 and T_3 .

3.5.4 Number of kernels per row

Significantly higher number of kernels per row was recorded in T_5 (36.98) which received 100 per cent RDF and FYM + application of lime @ equivalent to 100% acid neutralization and was on par with treatments receiving T_2 + application of GD @ equivalent to 50% acid neutralization, T_2 + application of lime @ equivalent to 50% acid neutralization, 100 per cent RDF and FYM + application of GD @ equivalent to 100% acid neutralization and 250 kg GD along with 100% RDF and FYM and i.e., T_8 (36.10), T_6 (35.76), T_7 (34.50) and T_4 (33.62) respectively (Table 2 and Fig. 2). Lower number of kernels per row of 13.25 was recorded in T_1 (absolute control).

3.5.5 Test weight

Application of lime @ equivalent to 100% neutralization of fertilizer acidity with 100% RDF and FYM (T_5) recorded significantly highest test weight of 35.98 g and was observed to be on par with treatment receiving T_2 + application of GD @ equivalent to 50% acid neutralization (T_8 - 35.71 g), T_2 + application of lime @ equivalent to 50% acid neutralization (T_6 - 34.91 g), T_2 + application of GD @ equivalent to 100% acid neutralization (T_7 - 33.99 g) and 100 per cent RDF and FYM + 250 kg GD (T_4 - 32.54 g). Control registered significantly lowest test weight (T_1 - 19.43 g) (Table 2 and Fig. 2).

Application of liming material along with 100% RDF and FYM increased yield parameters which might be attributed to the ameliorating effect of lime by increasing the pH from its initial value, thereby increasing the availability of nutrients to plants use these nutrients for cell division and multiplication that in turn resulted in increased yield and quality of crops [8]. The application of lime and GD enhanced metabolic activity of plants that helps in flower initiation in tassel, silk initiation in maize and also overcoming the soil fertility constraints with increased availability of nutrients over a longer period of time for crop uptake [13]. Similar results were also found by Antonio et al. [14] that, 100% NPK + lime produced the same results of maximum number of grains per spike and 1000 grain weight compared to unlimed treatment.

3.5.6 Kernel and stover yield

Kernel and stover yield of maize varied significantly with the application of liming material along with recommended dose of fertilizer and FYM (Table 3 and Fig 3). The results indicate that, kernel yield (81.57 q ha^{-1}) and stover yield (90.67 q ha^{-1}) recorded in T_5 treatment (100% RDF + FYM + 100% acid neutralization with lime) was significantly higher than that recorded in T_2 (69.65 q ha^{-1}) and T_3 (72.54 q ha^{-1}) and was statistically on par with rest of the treatments. Lower kernel and stover yield were noticed in absolute control (T_1 - 24.35 and 39.95 q ha^{-1}) which received no fertilizers.

The increase in yield of maize might be attributed to improvement in growth and yield parameters. The higher yield in the treatment that received lime or GD at different rates than the application of only RDF could be attributed to an increase in the availability of Ca and Mg, as well as an

increase in the availability of other essential nutrients. Crusciol et al. [15] also reported that liming improved nutrient absorption, particularly for K, Ca, and Mg uptake by plants. The surface application of lime resulted in a significant increase in cereal yield components and kernel/grain yield. Dolomite also outperformed

the control in terms of kernel yield, which could be attributed to the magnesium content. Granulated dolomite has a slow solubility, which aids in neutralising soil acidity throughout crop growth besides, serving as both Ca and Mg source to plants. Similar result was also reported by Raboin et al. [16] and The et al. [17]

Table 2. Effect of liming material on yield parameters of maize

Treatments	Cob length (cm)	Cob girth (cm)	No. of rows cob ⁻¹	No. of kernels row ⁻¹	Test weight (g) per 100 seeds
T ₁	7.80	12.32	10.83	13.25	19.43
T ₂	18.01	17.01	13.01	30.67	30.01
T ₃	18.29	17.51	13.70	31.03	31.00
T ₄	19.97	18.76	15.23	33.62	32.54
T ₅	21.98	20.01	16.86	36.98	35.98
T ₆	20.87	19.76	15.48	35.76	34.91
T ₇	20.65	19.43	15.36	34.50	33.99
T ₈	21.79	19.98	16.35	36.10	35.71
S.Em±	0.76	0.75	0.61	1.28	1.30
CD @ 5 %	2.30	2.28	1.84	3.87	3.95

Treatment details as indicated under Table 1

Table 3. Effect of liming material on kernel yield and stover yield of maize

Treatments	Kernel yield (q ha ⁻¹)	Stover yield (q ha ⁻¹)
T ₁	24.35	39.95
T ₂	69.65	77.89
T ₃	72.54	80.13
T ₄	78.33	85.28
T ₅	81.57	90.67
T ₆	79.86	89.45
T ₇	79.00	88.91
T ₈	80.76	89.76
S.Em±	2.87	3.27
CD @ 5%	8.70	9.93

Treatment details as indicated under Table 1.

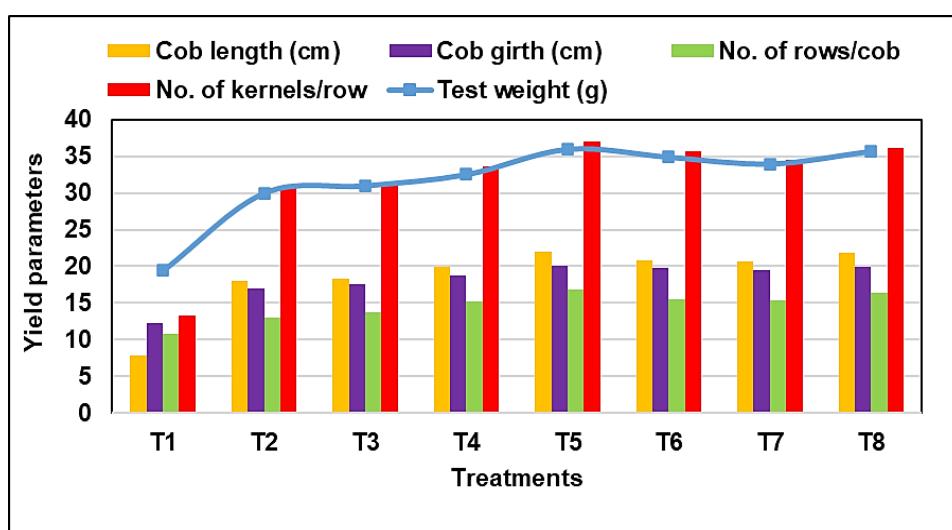


Fig. 2. Effect of liming material on yield parameters of maize

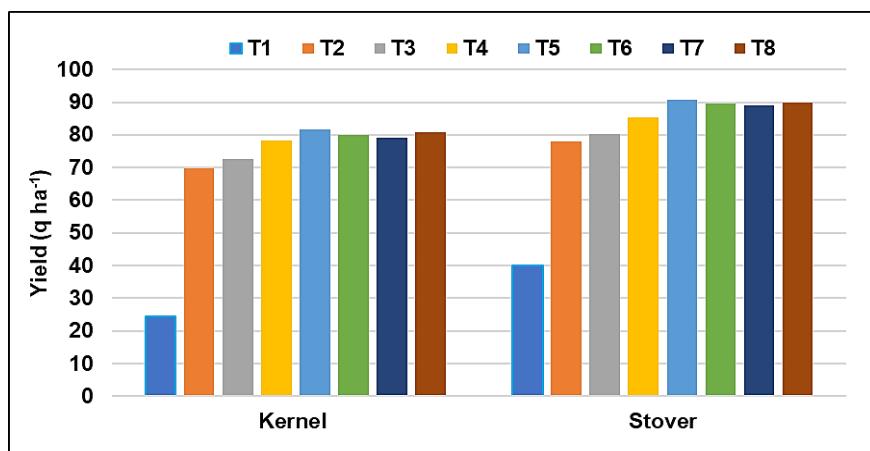


Fig. 3. Effect of liming material on kernel and stover yield of maize
Treatment details as indicated under Table 1.

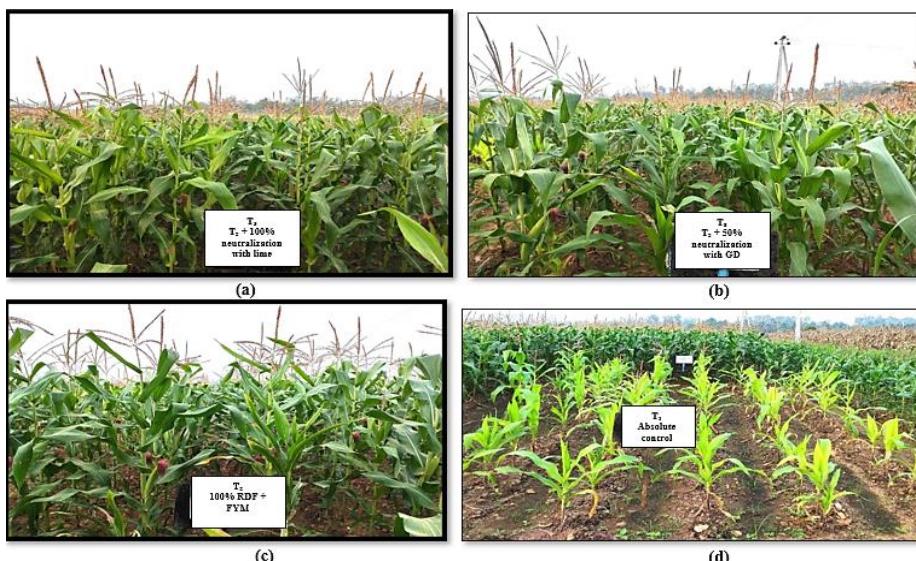


Plate 1. Effect of application of liming material on maize crop at 60 DAS

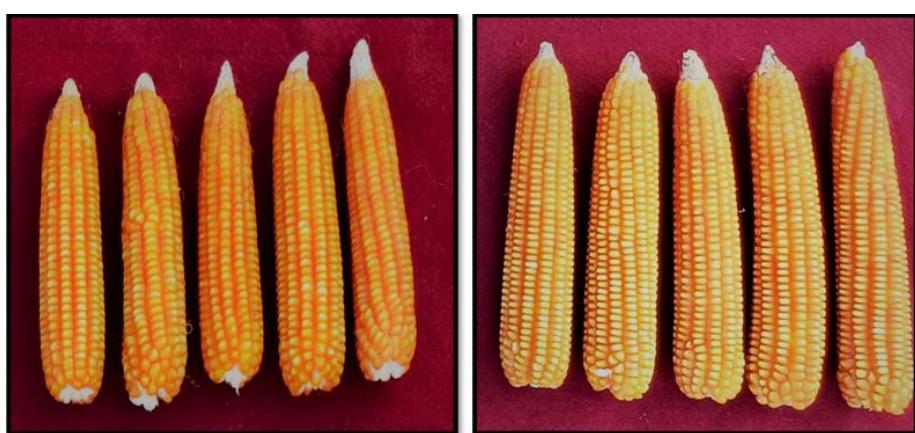


Plate 2. Effect of application of liming material on cob length and girth of the maize crop

Table 4. Meteorological data of the weather parameters during the experimental period 2021-22

Month	Max (°C)			Min (°C)			RHI (%)			RHII (%)			SSH (hours)			RF (mm)			Rainy days			
	A	N	D	A	N	D	A	N	D	A	N	D	A	N	D	A	N	D	A	N	D	
September	30.0	31.3	-1.3	19.4	19.0	0.4	88	91.0	-0.3	62	78.8	-16.8	4.6	4.5	-0.1	54.9	133.7	-78.8	3	6	-3	
October	29.9	30.7	0.8	19.7	20.7	-1.0	88	92.0	-4	62	76.9	-14.9	5.0	7.1	-2	307.4	141.1	166.0	14	15	-1	
November	28.8	30.9	-2.1	17.8	18.2	-0.4	90	90	0	69	69.3	-0.3	2.6	6.8	-4.2	161.2	53.2	108	9	1	8	
December	28.7	27.5	1.2	16.5	16.0	0.5	87.2	81.2	6	58.5	66.9	-8.4	6.1	6.9	-9.2	49.4	14.3	108	9	1	8	
January	29.8	30.5	-0.7	16.2	17.0	-0.8	87	90.9	-3.9	57	78.2	-21.2	8.3	6.8	1.5	0	1.74	-1.74	0	0	0	0
Total																572.9	344.0					

A- Actual N- Normal (1973-2021) D- Deviation from Normal

Max – Maximum Min – Minimum RH – Relative Humidity SSH – Sunshine Hours RF - Rainfall

4. CONCLUSION

The present study highlighted the application of liming materials along with recommended dose of fertilizers and evaluating their effect on growth and yield by using maize as a test crop. Among the different sources of liming material, application of 100% neutralization with lime along with 100% RDF and FYM recorded higher growth and yield parameters when compared to rest of the treatments. Moreover, application of 50% neutralization with granular dolomite along with RDF and FYM was next best treatment in terms of growth, yield attributes and yield of maize. Lime application with 50% neutralization and granular dolomite with 100% neutralization of fertilizer acidity has performed better than the blanket recommendation of lime and granular dolomite. Thus, application of liming materials is superior over unlimed treatments in achieving higher growth and yield parameters in maize. Hence, the liming material is required to neutralize the physiological acidity created by acid forming fertilizers.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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