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Integration of Cow Urine with Mineral Nitrogen Fertilizer to Enhance Sustainable Production of Wheat in the Central High Land *Nitisol* of Welmera District, Ethiopia

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Abstract

The prevalence of challenges on mineral fertilizer such as escalating cost in Ethiopia urged to explore and exploit alternative organic nutrient sources like cow urine. Hence, the current study was initiated to understand the composition dynamics across bred types and storage conditions of Cow urine, and evaluate its effect on wheat growth, yield, and quality and soil properties. The composition dynamics phase was dealt in laboratory and had 30 factorial combined treatments of bred type (local and cross breed), temperature (4, 21 and 38°C) and time (0, 24, 48, 72 and 120 hours) laid in triplicate and completely randomized design. The crop response evaluations had treatments of factorial combined urea: Cow urine integration and urine concentration with respective levels of (0:0, 100:0, 0:100, 50:50 and 75:25) and (15, 25, 35, 50, 75and 100%) for the pot, and (0:0, 100:0, 25:75, 50:50 and 75:25) and (0, 15 and 50%) for the field. The result showed that storage time and breed type highly influenced the total nitrogen and total phosphorus composition of Cow urine as compared to 0hr. Total phosphorus at 120 hour and total nitrogen at 48 hour were the highest records. Likewise, local bred total nitrogen and total phosphorus were superior to cross breeds with records of 0.9%, and 22%, respectively. Supplementation of 75% Recommended nitrogen from urea with 25% Recommended from Cow urine and 50% Recommended nitrogen from urea with 50% Nitrogen from Cow urine exhibited the highest biomass and grain yield of wheat irrespective of the farm typology and statistically at par with the full Recommended from urea (positive control). Further validation of the output is essential to confirm the effectiveness of the technology.

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Cow urine, Integration, Mineral fertilizer, Welmera, Wheat, Organic fertilizers.

Introduction

Though undeniable cereal production revolution has come via chemical fertilizer for over five decades in Ethiopia, its escalating cost, less accessibility and high application inefficiency has posed a threat to its sustainable use. The prevalence of such challenges often leads to the exploitation and exploration of new frontiers in research (Devasenaa and Sangeetha, 2022) and hence, organic fertilizer sources received the priority in substituting or supplementing potential to the chemical fertilizer. Organic fertilizers are known enhance crop growth, yield and quality; improve soil health and fertility, and reduce greenhouse gas emission. Cow urine (CU), the main constituent of organic fertilizer, could thus be best complimentary as it is locally available, cheaper and eco-friendly (Pradhan *et al.,* 2018). Unlike other organic fertilizer forms such as yard manure (FYM), compost, and vermicompost, very little or no attention has been given to collecting and utilizing Cow urine for agronomic purposes in Ethiopia. Cows expel dominant proportion (52%) dietary N intake in the urine while 28% is in the dung (Devasenaa and Sangeetha, 2022). According to Janjal *et al.,* (2021), CU contains 95% water, 2.5% urea, and 2.5% others (mineral salts, hormones, and enzymes). Sandukhan *et al.,* (2018) disclosed that total N in the Cow urine ranged from 6.8-21.1 g N L-1 of which on average 69% was urea, 73% allantoin, 5.8% uric acid, and 0.5% xanthin plus hypoxanthin, 1.3% free amino acid nitrogen and 2.8% on ammonia. These reports confirmed that Cow urine could be a potential biofertilizer, biopesticide and bioenhancer organic product.

The beneficial effect of Cow urine application, alongside or in combination with chemical fertilizers, has been reported on several crops such as on mustard (Gupta, 2005; Meena *et al.,* 2013 and Pradhan *et al.,* 2016), Maize (Devakumar *et al.,* 2014), and Sweet corn (Pande *et al.,* 2015), and on vegetables such as on Watermelon (Burubhai and Eribo, 2012), Chilli (Keduka *et al.,* 2014) and Lablab bean (Maheshari *et al.,* 2017). Nevertheless, wheat is produced across a wide range of agro ecological and crop management regime in Ethiopia, the national average productivity is 2.11 t/ha (CSA, 2013). This is far below the average of Africa and the world and is attributed highly to disease (Zegeye *et al.,* 2001). On the other hand, organic inputs are often proposed as good supplement to mineral fertilizers for intensified agricultural practices. Verma *et al.,* (2013) reported that integrated application of organic and inorganic fertilizers provided plants with balanced nutrients, guarantee organic produce and save reasonable hard currency. On top of that, this research paper is done to analyze the effect of storage conditions over time on the major selected chemical properties of cow urine and assess the effect of cattle urine rate integrated with mineral fertilizer and its concentration on growth, yield, and nutrient uptake of wheat and soil properties.

Materials and Methods

Study area description

The pot culture observation study was carried out at screen house found in Holeta Agricultural Research Center. The field trial was conducted on two farms at Sedemo Kebele, Wolmera district on altitudes of 2366 masl. The growing season had favorable average maximum and minimum To of 24.7 and 9.4OC, respectively (Fig 1). Farm 1 was located at 09o03.158' latitude and 038o 31.288' longitude whereas farm 209o03.158' latitude and 038o 31.288' coordinates during 2022 main growing season. *Danda'a* variety of wheat was the planting material used after two times ploughing. The average pre planting composite soil sample tests confirmed that the fields were slightly acidic (0.2-0.4 meq/100gm soil), very low available P (11ppm), high TN (0.21%) and medium OC (1.8%).

Cow Urine Collection and Test

Collection of CU for the study was made manually during the early awake period using bail. CU obtained from local (Sedamo kebele) and breed cows (HARC) during April, 2022 was used for the preliminary characterization. Subsample were taken to test pH, EC(dS/m), OC(%), total N(%), and total P(%) at Holeta Agricultural Research Center based on the established protocol. The Cow urine for the field and pot culture trials were collected from local cows successively during June to August, 2022 and September 2023, respectively. Every time the Cow urine was filtered through neat cotton sheet to get rid of debris and precipitated material and stored in air tight sterile plastic container at 4OC. In the pot culture, the urine was collected a week before the 1st application whereas for the field trials, the required amount was higher and collections were made 3 weeks before each split application.

Experimental Setup and Management

The pot culture observation trial was conducted using 4kg surface (oven dry weight base) slightly acidic Nitisol. The potted soil was brought to field capacity just before planting. Danda'a variety was planted and thinned to 5 after two weeks. The urea N and phosphate doses were computed using 60 kg N and 69 kg P2O5 ha-1 recommendations, i.e. equivalent for 2,200,000 kg soil. The Cow urine doses for same quantity of soil was computed based on the average TN (%) of local cows (0.92%) as shown in fig 3. Accordingly, 270mg TSP was basal dressed to each pot while 240mg urea and 12ml urine were equally applied in 3 rounds according to the treatment. The structure of the treatment was factorial combination of Urea: Cow urine integration having 5 levels (0:0, 100:0, 0:100, 50:50 and 75:25) and urine concentration with 6 levels (15, 25, 35, 50, 75and 100%). The treatments were laid out in a randomized complete block design with three replications. Both urea and Cow urine were applied on soil and foliar mode, respectively in 3 equal splits on the 2nd, 4th and 7th weeks after emerging. Watering was done in each other day. Growth data were taken on the 60th day after planting.

For the field study, the treatment arrangement was factorial combination of urea: Cow urine integration factor having five levels (0:0, 100:0, 25:75, 50:50 and 75:25) on N equivalence base and urine concentration factor having 3 levels (0, 15 and 50%). By taking 60 kg N /ha in to account, 40g urea or 2000 ml Cow urine were determined to be the full N doses per plot having an area of 3m2. The urea dose was applied on 2 equal splits (planting and tillering) while Cow urine was applied foliar/spray undiluted or diluted with water in 3 equal splits (2nd, 4th and 7th week after emerging). All plots received 50g TSP as basal application. All the treatments were replicated three times and laid in RCBD. Spacing between blocks and plots was 1m and 0.5 m, respectively while the inter-row spacing was 20cm. At physiological maturity agronomic and soil data were collected on plot and treatment basis, respectively.

Data Management

To measure the response of wheat to Cow urine integration with chemical fertilizers on the pot culture study, growth parameters like dry weight, plant height, etc were measured during biomass harvesting on booting stage. Mean separation was made in the presence of statistical significance variation at 5% significance level. In the field case, yield, yield component, and growth data were collected on plot base at physiological maturity.

Both study data were subjected to two-way ANOVA procedure with urea: Cow urine integration and Cow urine concentration as fixed effects using statistics 10 (just after grain moisture adjustment to 12.5% for the field one). Post-harvest surface (0-20cm) soil samples were also collected on treatment base.

Results and Discussion

Effect of source and storage condition on Cow urine composition dynamics

To understand the compositional dynamics of Cow urine across temperature and time, samples were taken from local and cross bred cows and pH, TN, TP, and EC were measured at Holeta Soil Chemistry Laboratory. As depicted in Table 1, time, bred type but temperature affected TP and TN of cow urine significantly ($p \geq 0.05$).

In addition, the interaction effect of Time * Bred type, Temperature * Time and Temperature * Bred type did not show significantly different TP and TN.

Only storage temperature was able to affect the pH of cow urine. Unfortunately, none of these main and interaction effects were able show significant differences on Electrical conductivity of cow urine.

The mean value comparison of the different properties of cow urine against time of storage is shown in Figure 2. TP displayed linear increase with storage durations (times). Thus, 120hr gave the highest record (23%) followed by 72 hrs (22%).

The trend of TN dynamic across time of storage depicted concave pattern; lower in both extremes and higher in the middle. The mean value of TN at 48 and 24 were 0.74 and 0.73%, respectively. The effect of time on pH appeared to follow convex pattern, just the opposite of total nitrogen. Maximum measurement was taken at 72hr (8.69) and 120hr (8.67).

The comparison of means of the different properties of cow urine total nitrogen and total phosphorus was also analyzed in bred type of the cows (Figure 3). Accordingly, pH and EC were not significantly affected by bred type. However, local bred cows could give very highly significant ($P \leq 0.01$) different total nitrogen and total Phosphorus as compared the cross bred cows. About 22% TP and 0.9% TN were the recoded values, which was double of that of the respective records of the cross bred cows.

The composition variations observed between the cow urine of local and cross bred cows is in line with other studies and could be attributed to the diet, physiology, cattle breed and storage conditions. The difference in composition of Cow urine at 0 hr might have come from the difference in genetic make-up of cows, diets or physiological conditions.

The change in records between the fresh cow urine (0 hr) and stored cow urine (> 24hr) is expected as chemical reaction starts to proceed in the presence of urease enzyme that hydrolyze urea (69% of CU N) to hydroxide and free ammonia despite its rate is relied on specific environmental conditions.

$$NH_2(CO)NH_2 + 3H_2O \text{ --------->} 2NH_4^+ + HCO_3^- OH^-$$

As the reaction results in ionic species and hydroxyl ion, associated rise of EC and pH in the aqueous solution of

Cow urine is expected. Ray *et al.,* (2018) reported that pH ranging up to 9 is a useful measurement for testing if urea hydrolysis is occurring and whether or not the urine is fresh or partially hydrolyzed because of its ability to be buffered while EC is an effective measurement for tracking the progression of the reaction. In the contrary, particularly total nitrogen showed a gradual decrease after 24 hours regardless of temperature and bred.

This depicts that the reaction was fast enough to remove N from the system probably in ammonia form. Precipitation of phosphorus at the bottom of the container through time would contribute for the sharp decrease of TP in local cows.

Response of wheat to integrated urine application in potted soil

In the potted soil factorial experiment results depicted that the interaction and concentration main factor had no statistically significant plant height, root length, shoot dry weight and root dry weight responses (P>0.05). However, the Urea: Cow urine main factor significantly affected plant height, shoot dry biomass, and root dry weight responses of wheat (Table 2).

Accordingly, 50%RN from Urea and 50%RN from cow urine showed statistically significant plant height and shoot dry biomass over the full RN from urea (sole urea) and the absolute control. The relative biomass increase to sole urea and absolute control were 24 and 29%, respectively.

The result confirmed that bread wheat above ground biomass is responsive to cow urine integration. The existence of statically significant difference responses between urea: cow urine integration and the full RN from urea (having same amount of total N) has two possible implications.

The Cow urine as an organic fertilizer might enhanced nutrient (N) use efficiency and correct micronutrient deficiency (Pradhan *et al.,* 2018) and the hormones and enzymes may hasten growth and development of leaves and stems (Kgasudi and Modiri, 2020). Janjal *et al.,* (2021) reported a similar finding that 50% RN from urea + 50%N from cow urine and 75% RN from Urea+ 25%N from cow urine applications produced the highest plant height and dry biomass of forage maize, respectively. They obtained about 137 and 434% relative biomass yield increment over the RN from urea and the absolute control, respectively.

Growth and yield response of wheat to integrated urine application in field

The ANOVA showed that plant height, grain yield, biomass yield, thousands seed weight, seed number per spike and spike # m-2 were not significantly affected by the interaction effect (Urea-cow urine integration* cow urine concentration) and the main effect of cow urine concentration (Table 3) as compared to the absolute control on both farms. Nevertheless, the urea-cow urine integration main effect revealed that the application of urea N and cow urine under different combination showed very highly significant improvement ($p < 0.001$) on the mentioned parameters except thousands seed weight on farm 1 and spike # m-2 on farm 2.

Urea-Cow urine integrated application (main effect) on wheat showed positive plant height response regardless of farm typology difference (Table 4). On farm 1, only the absolute control measured significantly lower ($P<0.01$) plant height to the rest of the combination levels.

On farm 2, however, 75:25 and 50:50 urea: Cow urine combination levels recorded significantly superior ($P < 0.01$) to the absolute control and the 25:75 combination. Relative to the sole urea (full RN level), 75:25 produced remarkably superior plant height. In the case of seed number per spike, 75:25 produced statistically the highest record (70 seeds) at both farms as compared to the absolute control and the 25:75 integration level. For growth and seed/spike parameters, the75:25 and 50:50 levels remained superior and statistically at par to each other.

Grain yield of wheat varied considerably with graded urea-Cow urine integration levels (Table 4). Application of 75:25 and 50:50 urea: Cow urine integration enhanced grain yield by 144 and 138% on ferm1 and 61 and 42 % on farm 2, respectively as compared to absolute control.

Though these levels exhibited slight numerical superiority on sole urea on farm 2, they were statistically indifferent. Likewise, the same integration levels revealed 64 and 60% on farm 1 and 61 and 45% higher biomass yield over the absolute control, respectively. The 75:25 and 50:50 integrations produced statistically similar biomass yield with the sole urea (Recommended Nitrogen).

Table.1 ANOVA table for Cow urine selected parameters as affected by storage condition and bred type during 2022

Variables	TP(%)	TN(%)	pH	EC (dSm^{-1})
Temperature (^{O}C)	ns	ns	ns	**ns**
Time (hr)	**	***	*	**ns**
Bred type	***	***	ns	**ns**
Temperature * Time	ns	ns	ns	**ns**
Temperature * Bred type	ns	ns	ns	**ns**
Time * Bred type	ns	ns	ns	**ns**
Mean	16.5	0.67	8.5	**28**
CV (%)	**35.0**	**1.21**	**2.28**	**14**

Table.2 Growth response of bread wheat to urea: Cow urine integration on pot culture during 2022

SN	Urea: CU integration	Plant height (cm)	Shoot dry weight (gm/3pls)	Root dry weight(gm/3 pls)
1	Full RN from urea	42.89B	2.39B	**2.82A**
2	75%RN from Urea and 25%N from CU	51A	2.82A	**2.08B**
3	50%RN from Urea and 50%RN from CU	51.19A	2.97A	**2.15B**
4	25RN from Urea and 75%RN from CU	45AB	2.64AB	**1.98B**
5	Absolute control	44.56B	2.31B	**2.41AB**
	Mean	47	0.41	**2.3**
	LSD ($P \geq 0.05\%$)	6	0.41	**0.46**
	CV (%)	**19**	**23**	**30**

Table.3 ANOVA table for growth and yield response of wheat to urea and CU integrated application on field during 2022

Source of variation	Farm 1						Farm 2					
	B Y	GY	TS	Pl	See	Spi	B Y	GY	TS	Pl	See	**Spi**
Urea-CU integration	***	***	NS	***	***	***	***	***	***	***	**	**NS**
CU concentration	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	Ns	**NS**
Urea-CU integration* CU conc.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**NS**
Grand mean	6.41	3383	46	84	56	524	10.1	4748	45	91	65	**281**
CV(%)	**22**	**16**	**6**	**3.4**	**8**	**14**	**26**	**28**	**4.5**	**5.5**	**8**	**19**

Note: BY= biomass yield (t ha^{-1}); GY = grain yield (t ha^{-1}); TSW = thousands seed weight (g); PlHt = plant height (cm); seed ((# $spike^{-1}$); Spike (# m^{-2})

Table.4 Mean of growth and yield parameters of wheat to urea and Cow urine integrated application response on field condition during 2022

Urea-CU integration	Farm 1					Farm 2				
	BY	GY	PlHt	Seed/ spike	Spike	BY	GY	TSW	PlHt	Seed /spike
Absolute control	4.5B	1.6C	71 B	51C	332B	5.18C	2.45 C	42.7 B	82.3C	**64B**
Full N from urea	6.4A	4.2A	89 A	60A	540A	7.73AB	3.33 AB	45.7 A	92.3B	**64B**
75%N (Urea) + 25%N (CU)	7.4A	3.9A B	88 A	70.7A	596A	8.35A	3.94 A	45.9 A	97.2A	**70A**
50% N (Urea) + 50%N (CU)	7.2A	3.8A B	86 A	57AB	572A	7.52AB	3.47 AB	45.3	94AB	**66AB**
25%N (Urea) + 75%N (CU)	6.5A	3.5B	86 A	55B	582A	6.07BC	2.92 BC	45.6 A	91.1B	**61.4B**
LSD(%)	**1.4**	**537**	**2.8**	**4**	**73**	**1729.8**	**881.3**	**1.96**	**4.8**	**5.2**

Note: BY = biomass yield (t ha^{-1}); GY = grain yield (t ha^{-1}); TSW = thousands seed weight (g); PlHt = plant height (cm); seed ((# $spike^{-1}$); Spike (# m^{-2})

Fig.1

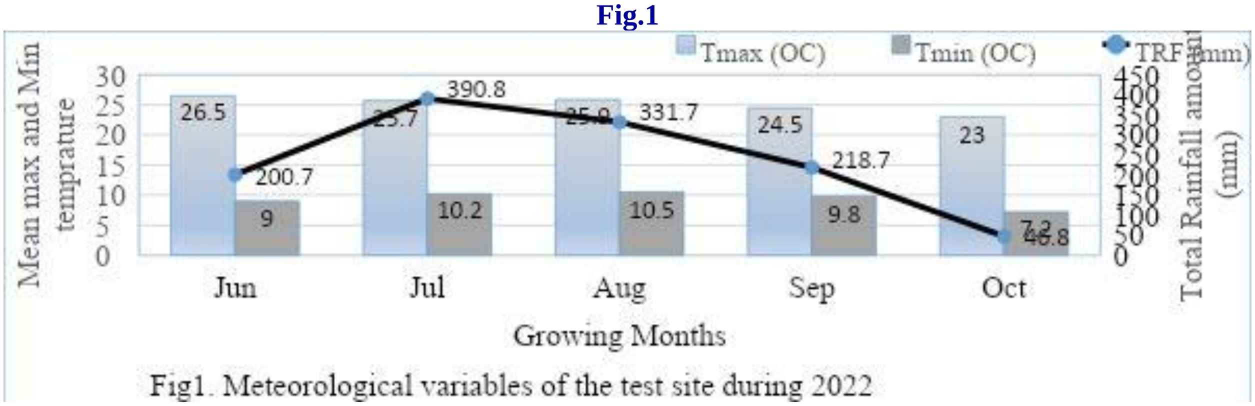


Fig1. Meteorological variables of the test site during 2022

Fig.2 The effect of storage time on different cow urine properties (LSD = 12, 0.016 and 0.39 for TP, TN and pH)

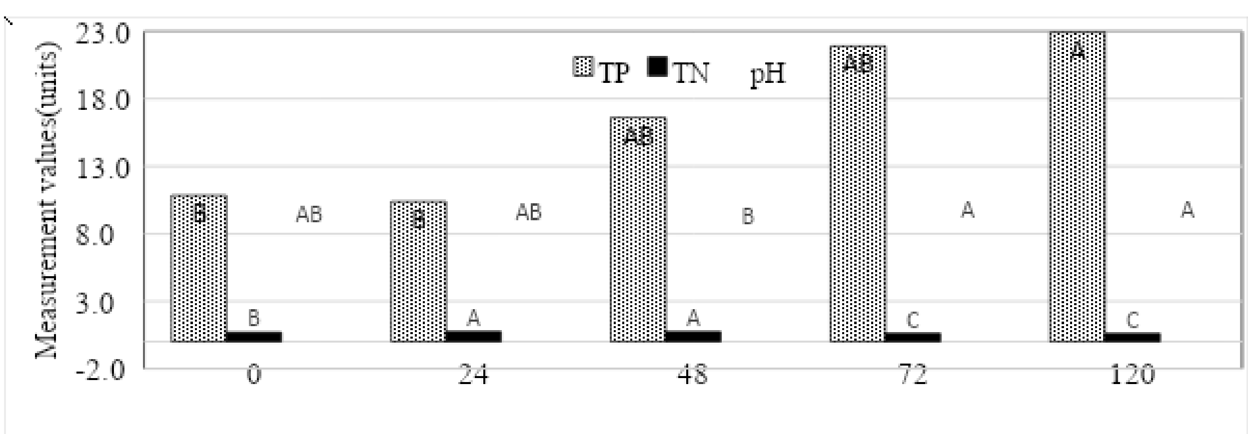
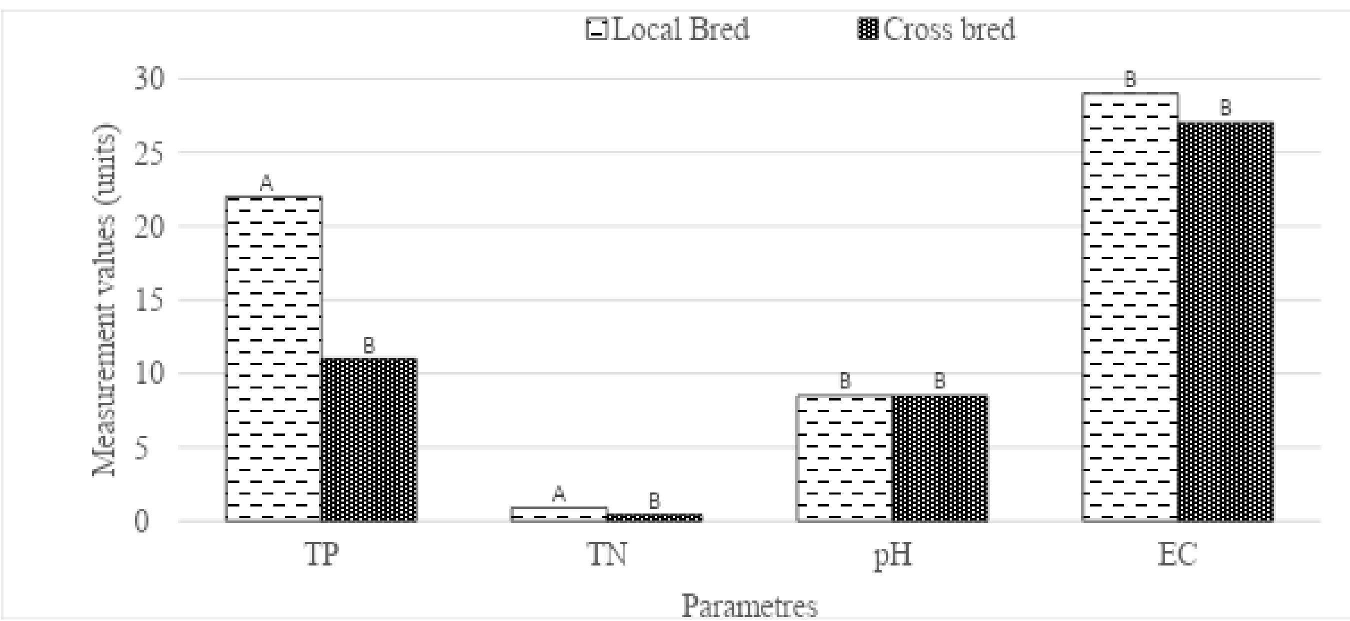


Table.5 Post-harvest major surface soil test results of both farms during 2022

Treatment	Farm 1					Farm 2				
	pH	Ex. A	Av .P	TN	OC	pH	EX A	Av. P	TN	**OC**
Absolute control	5.87	0.6	7.26	0.1 5	1.84	5.60		13.2	0.19	**2.0 1**
Full RN from urea	5.93	0.8	9.06	0.1 7	1.83	5.75	0.97	13.1 9	0.22	**2.0 6**
75%RN (Urea) + 25%RN (CU)	5.87	0.55	8.18	0.1 7	1.86	5.56	0.97	12.5 2	0.19	**1.9 5**
50%RN (Urea)+ 50%RN	5.91	0.45	10.38	0.1 7	1.82	5.57	1.20	14.5 2	0.20	**1.9 9**
25% RN (Urea) + 75%RN (CU)	5.90	0.58	10.00	0.1 7	1.89	5.59	1.10	14.9 2	0.20	**1.9 1**
Mean	5.8	0.5	9.3	0.1 7	1.8	5.56	1.04	13.8 4	0.2	**1.9 7**
Standard deviation	0.05	0.08	1.69	0.0 1	0.06	0.11	0.18	2.71	0.01	**0.1 5**
Test Method	**1:2.5 water**	**Van.**	**Bray 2**	**Kjel**	**W&B-wet**					

Hint: ExA: exchangeable acidity (meq/100gm); AvP: available phosphorus (ppm); TN: total nitrogen (%); OC : Organic carbon (%).

Fig.3 The effect of bred type on different Cow urine properties (LSD = 4.88 and 0.0.007 for TP and TN)



The treatment of cow urine to the crop plant growth showed higher N uptake in wheat grain and straw (Karale *et al.*, 2020). Thus, supplementation of 75% RN with 25% N from cow urine, and 50% RN with 50% N from cow urine exhibited the highest grain yield of wheat irrespective of the farm typology. They earned 144 and

138, and 61 and 42% grain yield increment over the control on Farm 1 and Farm 2, respectively. These grain yield advantages are superior to what was obtained by Sadhukha *et al.,* (2018). This author reported a 2.69%, 18.01% and 27.21% higher grain yield of wheat through application of 50%, 75% and 100% cow urine spray, respectively over control. The high bioavailability of nutrients in the mineral fertilizer and cow urine enhances the uptake of nutrients and in turn it results in higher crop growth and grain yield (Arif *et al.,* 2006; Pandey *et al.,* 2009).

Effect of cow urine on selected soil chemical properties

The soil pH, TN, OC and available phosphorus of all treatments in both farms were found in moderately acidic (Murphy, 1968), high (Debele, 1980), moderate (Debele, 1980) and very low (Cottenie, 1980) ratings, respectively. On the other hand, these post-harvest ratings were not different from that of the pre-planting soil test results of the fields.

The cow urine sprayed treatments did not show pH, TN, OC and available P improvements in the current study. The current observation is in contrary to the finding of Sakhare *et al.,* (2022) that reported improvement of TN, Avail P, OC and pH via soil application of 100% RN through cattle urine on potted laterite soils. The foliar application modality of the cow urine might be one possible reason for the absence of consistent and reliable improvements. The 2nd and 3rd split applications were in particular made when wheat canopy was closed and no chance of cow urine and contact.

Recommendation

The core message of the current study is that direct use of local cow urine improves productivity of wheat in the Nitisol areas of Wolmera when used in integrated manner with mineral nitrogen fertilizer (urea).

A well collected and stored (≥ 3 weeks) cow urine co-applied in spray mode, 3 equal splits, foliar mode and at a rate of 3333L/ha (to supply half of the 60kg N/ha) with 65kg urea/ha or 5000L/ha (to supply 75% of 60 kg N/ha) with 32.5kg urea/ha gave statistically similar wheat grain and biomass yield response to that of the mineral nitrogen fertilizer. This implies that cow urine can replace urea consumption by 25-50%. Dilution of cow urine with water is not required from seedling burn or shock point of view if collected clean and stored for over 3 weeks properly.

But, dilution in 15% water might be helpful for uniform distribution of the urine. In addition, foliar application of cow urine at once or split and dilute or undiluted did not improved selected soil chemical characteristics. The current pot and field studies disclosed the fertilizer prospect of local cow urine in terms of wheat yield improvement despite data were derived from single year on two farms.

We suggest the implementation of subsequent validation of these findings at on-farm level to build confidence. Moreover, detailed characterization and diversified crop responses studies need to be properly supported, designed and implemented to valorize cow urine organic fertilizer and bio-control value, and reduce mineral fertilizer expenditures at large.

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