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SHORT COMMUNICATION

A solution at the doorsteps: Improved biofertilizer enhances soil fertility and yield of Chili (*Capsicum annuum* L.) in Bhutan

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Abstract

Purpose Two field experiments were conducted to evaluate the effects of locally prepared biofertilizers (Jholmal 1 and Jholmal 2) on soil properties and chili yield (*Capsicum annuum* L.).

Method The experimental site was in west-central Bhutan. The experiments were Randomized Complete Block Designs (RCBD) consisting of three treatments (cattle dung/ urine: Jholmal 1, cattle urine: Jholmal 2, and Control), and each treatment was replicated four times. The individual plot size of each treatment was 1 m × 3 m in total the experiment occupied a total area of 36 m^2. The effects of biofertilizers on soil properties and chili yield were compared before and after application.

Results Overall soil pH was within the suitable range (pH 6-7) for chili cultivation, although soil pH declined significantly in Jhomal 2 and control treatments. The soil C:N ratio increased significantly in all plots after application. Plots receiving Jholmal 2 showed the highest increase in the C:N ratio (2.30%; $p<0.01$) after the application. Available soil P increased after the application and was recorded highest (21.02 mg/kg; $p<0.05$) in plots amended with Jholmal 1. Exchangeable K declined significantly in all treatments after the application and the decline was highest (56.00 mg/kg; $p<0.05$) in the plots amended with Jholmal 1. The plots amended with Jholmal 1 also gave the highest chili yield in both experiments (≈13 t/ha in experiment I and ≈15 t/ha in experiment II).

Conclusion Jholmal 1 has better effects on soil fertility and chili yield and could be a prospective organic biofertilizer to use in organic farms in Bhutan.

Keywords Cattle Dung, Cattle Urine, Chili Crop, Effective Microorganism, Organic Farming

Introduction

Organic farming is lauded for its ability to sustain and enhance the health of the ecosystem and humans by producing high-quality and nutritious food (Murmu 2018; Meemken and Qaim 2018). It has garnered global attention and is viewed as a nature-based solution to mitigate climate change effects (Eco Voice 2021). With more studies emerging, it has become clear in recent years that organic practices can support agricultural production, confer resilience, reduce climate change impacts (Wani et al. 2013), and enhance nature and biodiversity (Tuck et al. 2013). In the Hindu Kush Himalaya (HKH), climate change can negatively impact crop production. The rising temperature is predicted to expand the geographic

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range of pests and diseases (Skendžić et al. 2021). The higher temperature is reported to cause a decline in soil moisture and affect soil fertility (Ostle et al. 2009). Therefore, the changing climate can exacerbate the pest, disease, and soil fertility problems, which could hinder accomplishing the United Nation's SDG 2 on achieving food security and promoting sustainable agriculture. However, organic farming is a climate-smart practice (Hamidov et al. 2018) and organic products command premium prices over conventional products and have good markets (Jeong and Jang 2019; Carlson and Jaenicke 2016). Hence, organic farming also plays a crucial role in reducing poverty (Anderberg 2020), prompting developing countries to emphasize organic practices in recent times. Nonetheless, compared to conventional farming, one of the main challenges of organic farming is limited options to manage pests and diseases (El-Shafie 2019) and improve the nutrient status of infertile soils (Nandwani and Nwosisi 2016). Moreover, organic farming needs to balance a healthy ecosystem with affordable organic products (Meemken and Qaim 2018). Such a scenario demands a low-cost organic production system, possible only by using low-cost organic inputs.

Jholmal, a homemade biofertilizer as well as pesticide, is a proven and successful technology in Nepal (Subedi et al. 2019). It is a low-cost and effective organic input that has helped smallholder farmers in Nepal maintain high agricultural productivity at low costs (Bhusal and Udas 2020). Jholmal is prepared by mixing in a defined ratio and fermenting farmyard manure (FYM), animal urine, water, and plants having insect repellent properties (Bhusal and Udas 2020; Subedi 2016). It is a good practice that could benefit countries like Bhutan considering its aggressive aspiration to pursue organic agriculture and become a fully organic country by 2035 (Kuensel 2020). Bhutan has a majority of its rural population engaged in cattle rearing (Wangchuk and Dorji 2008) and dung production for manuring is one of the objectives of cattle rearing in rural Bhutan. Although cattle dung is produced in huge quantities, Bhutanese farmers generally lack the knowledge of using it as a biofertilizer. The promotion of Jholmal in Bhutan is important because it could help diversify and optimize the use of cattle manure. Moreover, it supports the national objective to promote affordable technologies and bio inputs for organic agriculture in Bhutan (Department of Agriculture 2019).

In this paper, we present the findings of field experiments in Bhutan where two different types of Jholmal biofertilizers were evaluated on chili (*Capsicum annuum* L.) crops. Chili was selected as an important ingredient in Bhutanese dishes and annually it is imported on a large scale to meet the domestic demand. Therefore, the objective of the study was to assess the effects of Jholmal biofertilizers on soil properties and chili yield.

Materials and methods

Study site

Two field experiments were conducted at the College of Natural Resources in Punakha District, Bhutan, where the second experiment was conducted to validate the results of the first experiment. The experimental site was in west-central Bhutan (27°29'47.4"N 89°53'07.0"E), about 112 km (87 miles) east of the Paro international airport. The district has an elevation range from 1400 to 1800 meters above sea level and receives an annual rainfall of about 500 mm in winter and 1500 mm in summer with the annual temperature ranging from 5-30^{o}C (Punakha Dzongkhag Administration 2021). It lies in the subtropical region and experiences hot and dry summers with moderate winters. Paddy is the principal cereal crop in the area, followed by spring wheat. Chili production is also increasing in the district and is cultivated both in the dryland and wetland.

Experimental design and treatments

Two separate experiments were conducted. The first experiment was carried out in 2020 from May to October and the second experiment was in 2021 from March to August. Both experiments were carried out at the same site. The experiments were laid out in Randomized Complete Block Design (RCBD). There were three treatments and each treatment was replicated four times. Due to the lack of sufficient flat land on the sloping terrain, the individual plot size was maintained at 1 m × 3 m. Treatments were randomly allocated to the experimental plots. Three plots and four repetitions occupied a total area of 36 m^2. Seedbeds were raised to a height of 15 cm. A space of 35 cm was maintained between seedbeds as a buffer zone and also to allow for intercultural practices. The three treatments were Jholmal 1 (T1), Jholmal 2 (T2), and Control (T3). Jholmal 1 is used as a biofertilizer and Jholmal 2 is used as both biofertilizer and biopesticide. Control treatment did not receive fertilizer. Intercultural management was uniform for all plots.

Chili (*Capsicum annuum* L.) variety *Sha Ema* was chosen as a test crop because it is popular among Bhutanese and forms the main ingredient of the national dish in Bhutan. Healthy chili seedlings at the two-three-leaf stage were transplanted at a recommended planting distance of 40 cm × 40 cm row to row and plant to plant. Therefore, each plot (seedbed) had 14 plants and a total of 168 plants in 12 plots.

Jholmal ingredients, preparation method, and application

Jholmal 1 and Jholmal 2 were liquid solutions, prepared following the procedures of Bhusal and Udas (2020) but with slight modifications in the quantities and ingredients used. Cattle dung was included in Jholmal 1 and not in Jholmal 2. Effective Microorganisms (EM) were included in both Jholmal treatments. EM is a mixed culture of beneficial naturally-occurring organisms that is applied as an inoculant. The ingredients and chemical properties of Jholmal 1 and Jholmal 2 are presented in Table 1 and Table 2.

All ingredients were mixed thoroughly. The mixture was left to ferment for two weeks at 15-30 °C in an air-tight container. During the fermentation period, the mixture was stirred once every day both clockwise and anti-clockwise. After two weeks, the Jholmal biofertilizers were considered ready for use when the odor of urine disappeared and green color appeared at the top of the liquid.

Table 1 Constituents of Jholmal 1 and Jholmal 2 biofertilizers

Constituent	Jholmal 1	Jholmal 2
Water	16 liter	18.75 liter
Cattle dung	17 kg	-
Cattle urine	16 liter	16 liter
Effective microorganism (EM)	1 liter EM mixed thoroughly in a 1000 liter water	1 liter EM mixed thoroughly in a 1000 liter water

Table 2 Chemical properties of Jholmal 1 and Jholmal 2 (Source: Subedi et al. 2019)

Fertilizer type	pH	Organic carbon (%)	N (mg/g)	P (mg/g)	K (mg/g)
Jholmal 1	8.10	1.70	1.30	0.10	0.20
Jholmal 2	7.70	0.10	0.30	0.10	0.10

Jholmal treatments were diluted with water in a ratio of 1: 1000. Just before transplanting chili seedlings, respective treatment plots were drenched with Jholmal 1 and Jholmal 2 at the rate of 1667 l/ha (0.50 l/plot).

After transplanting, the foliar application of both Jholmal treatments was followed at the rate of 23333 l/ha (0.50 l/plant) per application. A higher amount of diluted solution was used for foliar application to ensure that the plants are not scorched. The two foliar applications were carried out at three weekly intervals. In total, there were three applications viz. first application on the soil at the base of each plant and the second and third applications on the individual plants (drenching). Soon after transplanting, all plots were irrigated. Irrigation was done once every two days until the seedlings were established. Thereafter, the irrigation frequency was reduced to once in three-four days depending on the weather condition. Irrigation was discontinued about two weeks before harvest. Manual weeding was carried out twice during the cropping period. The first weeding was done two weeks after transplanting and the second three to four weeks after the first weeding.

Soil sampling

Soil samples were collected twice only in the second experiment, before and after the experiment. The first sampling was carried out in March, a week before transplanting seedlings and the second sampling was in August after harvest. Soil samples were taken at 20 cm depth (Adetunji 2008) from 10 locations scattered randomly at the four corners and the center of the plot. The collected soil samples from each plot were mixed thoroughly in a clean container and three subsamples per plot were taken and preserved in labeled plastic bags. After harvest, a similar collection procedure was followed for the second soil samples collection. Twelve composite soil samples (three samples from each plot) were collected before and after the experiments. The samples were air-dried and sieved using a 2 mm sieve. The samples were analyzed for total nitrogen (N%), available phosphorus (P mg/kg), exchangeable potassium (K mg/kg), soil organic carbon (C%), and pH. The analytical methods used were micro-Kjeldahl digestion and calorimetry method for total N (AOAC 1990), Bray and Kurtz method for available phosphorus P (Bray and Kurtz 1945), and Leaching method for exchangeable K (Carson 1980). Organic C was measured using the Walkley-black titration and colorimetric method (FAO 2019) and soil pH with a pH meter.

Field measurements

Seven plants in each treatment were tagged randomly for measuring yield. To eliminate the border effect, plants along the plot edge were excluded from data collection. The matured fruits were harvested and weighed. The yield per plant was used to estimate the total yield per hectare of land as given below.

$$\text{Chili yield (t/ha)} = \frac{Number\ of\ plants\ per\ ha\ \times Yield\ per\ plant\ (kg)}{1000}$$

The number of plants per ha was estimated as follows.

$$Number\ of\ plants/ha = \frac{10000\ \text{sq. m}\ \times \text{number of plants per plot}}{\text{Area of a plot (sq. m)}}$$

Statistical analysis

Data analysis was carried out in SPSS version 25 (IBM 2004). Data sets on soil and chili yield were checked for the normal distribution and homogeneity of variances. Two sets of Multivariate Analysis of Variance (MANOVA) tests were performed before and after the experiment to investigate differences in soil properties among treatments. Paired samples t-tests were conducted to investigate differences between soil variables before and after the experiment. Two separate One-way ANOVA tests were performed for the first and second experiments to investigate yield differences among treatments. Tukey pairwise comparisons were performed to check significant differences between the two treatments. Differences in variable means were considered statistically significant when the statistical *p*-values were smaller than 0.05.

Results and discussion

Soil properties

All treatments differed significantly in soil properties except for K before the start of the experiment (Table 3), showing the soil heterogeneity in the experimental fields at the College of Natural Resources. On the contrary, there were no significant differences in soil properties among treatments after the experiment. Chilies grow well under a wide range of soil pH from 5.5 to 7.0 (Dorji et al. 2009). In this study, the overall soil pH was within the suitable range (pH 6-7) for chili cultivation (Chatterjee et al. 2012). Soil pH was insignificant at the start of the experiment although it declined in all treatments after the experiment. The decline was significant for Jholmal 2 and control treatments. Roy and Kashem (2014) reported similar results on the decline in pH of soils amended with organic manures. However, the decline in soil pH contradicts the result of Wang et al. (2019) who reported a slight increase in soil pH after the application of organic manures. Nonetheless, the nonsignificant decline in soil pH of Jholmol 1 treatment plots is likely due to its alkaline pH (Subedi et al. 2019) and the presence of cow dung which is known to reduce soil acidification (Roy and Kashem 2014; Williams et al. 1995). The soil C:N ratio increased significantly in all treatments after the experiment (Table 3). Plots treated with Jholmal 2 showed the highest increase in C:N ratio after application. The final C:N ratios of all treatments were around 20. The soil organic C:N ratio of around 20 is generally considered a threshold point (Bengtson et al. 2003). Higher C:N ratios exceeding the threshold level are found to affect the microbial activity and could cause N immobilization as compared to a low C:N ratio (Haney et al. 2012). The greater increase in C:N ratio in plots amended with Jholmal 2 (increase by 348.5%) suggests that the long-term applications of Jholmal 2 could escalate the C:N ratio beyond the threshold level and may affect N mineralization. However, it should be noted that this result is from two short-term experiments. A long-term study is needed to ascertain the long-term effect of Jholmal 2 on the C:N ratio. All treatments showed an increase in available soil P after the experiment. However, the increase was greater for plots treated with Jholmal 1. The amount of P in the Jhomal 1 treated plots was on the higher side of the critical levels (10.9 mg/kg to 21.4 mg/kg) of soil Olsen-P necessary for optimal crop yield (Bai et al. 2013). The soil P increase is consistent with the results reported by Sanni (2016) and concurs with the finding of Parham et al. (2002) that the long-term application of cattle manure increases soil P. This is most likely the combined effect of cattle manure and EM. Microorganisms in cattle dung are known to solubilize insoluble phosphorous and make it available to the plants (Arcand and Schneider 2006), which partly explains the higher level of available P in plots treated with Jholmal 1. In the mountain soils of Bhutan where crop production is often limited by the low levels of P (Parham et al. 2002; Roder et al. 2001), Jholmal 1 application may partly address this limitation. Exchangeable K declined significantly in all treatments after the experiment and the decline was higher in the plots amended with Jholmal 1. The decline is explained by the fact that K is an element that is taken up by plants in luxurious amounts under favorable conditions (Brady 1995). Therefore, without external application, a sharp fall in K levels is expected (Cayley et al. 2002).

Chili yield

The plots amended with Jholmal 1 gave the highest chili yield in both experiments. The yields were about 13 t/ha and 15 t/ha in experiment I and experiment II, respectively (Fig. 1). The yields are much higher than Bhutan's national chili production of 9.33 t/ha in 2020 (MoAF 2020) but comparable with ≈15 t/ha reported earlier by Dorji et al. (2009) under high application of farmyard manure and chemical fertilizers. Subedi et al. (2019) reported a yield increase in rice following the applications of Jholmal solutions. Mabuza et al. (2019) also reported an increase in chili yield because of applying greater amounts of cattle dung. This is because the application of a greater amount of cattle manure creates a favorable soil environment and enhances water-holding capacity and soil nutrient availability to the plants, leading to enhanced growth and yield (Rashid et al. 2013).

Table 3 Differences in soil nutrient variables among the three treatments before and after application of Jholmal treatments in the field experiment II

Soil variables	Treatments	Before experiment (March 2021)	After experiment (August 2021)	Significance
Soil pH	Jholmal 1	6.63 ± 0.00	6.40 ± 0.07	ns
	Jholmal 2	6.85 ± 0.00	6.44 ± 0.02	*
	Control	6.77 ± 0.00	6.48 ± 0.02	*
	Significance	ns	ns	
Organic Carbon (C%)	Jholmal 1	1.05 ± 0.01	2.51 ± 0.09	*
	Jholmal 2	0.12 ± 0.00	2.30 ± 0.02	**
	Control	1.56 ± 0.01	2.35 ± 0.05	*
	Significance	***	ns	
Total Nitrogen (N%)	Jholmal 1	0.09 ± 0.00	0.13 ± 0.00	*
	Jholmal 2	0.02 ± 0.01	0.11 ± 0.00	*
	Control	0.13 ± 0.00	0.12 ± 0.00	ns
	Significance	***	ns	
C:N ratio	Jholmal 1	11.67 ± 0.00	19.31 ± 0.00	*
	Jholmal 2	6.00 ± 0.00	20.91 ± 0.00	**
	Control	12.00 ± 0.00	19.58 ± 0.00	*
	Significance	***	ns	
Phosphorus (P mg/kg)	Jholmal 1	14.55 ± 0.05	21.02 ± 3.72	ns
	Jholmal 2	13.35 ± 0.05	16.81 ± 0.62	ns
	Control	12.75 ± 0.05	16.30 ± 1.82	*
	Significance	***	ns	
Potassium (K mg/kg)	Jholmal 1	101.45 ± 0.05	56.00 ± 4.20	*
	Jholmal 2	102.90 ± 0.10	72.10 ± 3.50	*
	Control	103.10 ± 0.10	66.30 ± 3.50	*
	Significance	ns	ns	

*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ns-nonsignificant

In this study, the positive effect of Jholmal 1 on yield could be attributed to the combined effects of cattle dung and effective microorganisms. Since integrated use of EM stimulates quick decomposition of organic materials and the mineralization of nutrients (Fatunbi and Ncube 2009; Daly and Stewart 1999), EM appears to have enhanced the nutrient release efficiency of cattle dung in Jholmal 1. The rapid proliferation of effective and beneficial microorganism content within the soil system results in the consumption of C, N, and other nutrient elements by the microbes and their subsequent release for plant use (Fatunbi and Ncube 2009). Moreover, Dung N is mainly of bacterial origin and less hydrolyzable, much of which is incorporated into the soil organic matter by soil microbes (Deenen and Middelkoop 1992). Zhang et al. (2020) reported higher diversity of bacterial communities in soils fertilized with cow manure than in the soils fertilized with urea. Hence, Deenen and Middelkoop (1992) observed a greater effect of dung N on the yield of moderately fertilized pasture swards in comparison with the effects of urine N. On the other hand, these activities seemed to have not occurred in Jholmal 2 where cattle dung was absent despite having the EM. Increased soil P as a result of the Jholmal 1 application also partly explains the increase in chili yield.

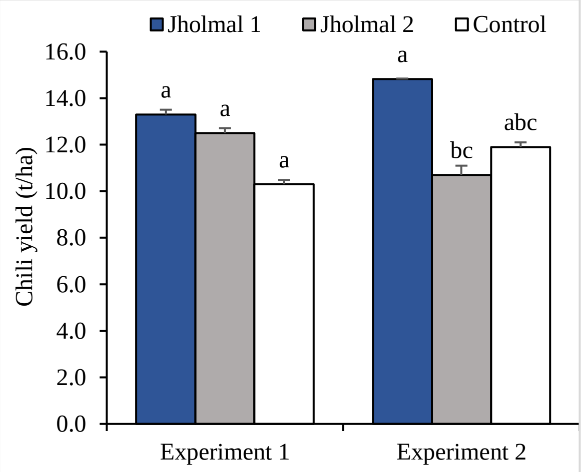

Fig. 1 Differences in chili yield among three treatments in the first and second experiments

Means with different letters indicate significant differences among treatments

Significance of Jholmal biofertilizer

This study demonstrates that a simple solution, to address farm issues such as soil fertility, can be found within a farm. Creating biofertilizers on-farm is not only an economical and sustainable option for managing farm waste but is also an environmentally friendly technique to convert organic waste into useful products. Jholmal biofertilizer is a simple example of an organic solution that can be easily prepared on-farm and from locally available raw materials. This is the primary reason why Jholmal adoption reduces farm expenditure by 50% (Subedi 2016). It also offers hope to counteract declines in organic matter content of soils due to repeated cultivation and erosion, a major threat to sustainable agriculture farming in Bhutan. As Bhutan embarks on organic agriculture, Jholmal 1 as a low-cost alternative could maintain or increase soil organic matter content desirable for better soil fertility and crop productivity in the mountain environment. The promotion of Jholmal biofertilizers can also ensure that the food items produced are local and free of harmful chemicals. Hence, Jholmal-based farming not only supports sustainable agriculture in Bhutan but also contributes to achieving the United Nations' Sustainable Development Goal on good health and well-being.

Conclusion

Jholmal 1 performs better than Jholmal 2 in enhancing soil fertility and yield of chili crops in Bhutan. As Bhutan embraces organic agriculture, Jholmal 1 could be a cheap source of organic nutrients and biopesticides. The preparation of Jholmal biofertilizer is simple with materials readily available on the farm, hence, Jholmal 1 is likely to be welcomed by farmers who have always opted for good and affordable practices to maintain soil health and productivity. However, it is important to try out Jholmal biofertilizers on other commercial vegetables. Further, there is also an opportunity to enhance the efficacy of Jholmal by improvising it with other locally available ingredients.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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