

COMPARATIVE EFFECTS OF THREE DIFFERENT POULTRY MANURES ON LENTIL *LENS CULINARIS*

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Abstract

This study was conducted to evaluate the effects of three different poultry manures on lentil growth, yield and prevalence of pathogens in manure and soil. For this purpose, a lentil (*Lens culinaris*) trait Punjab Masoor-2009 was cultivated in four different plots in triplicates namely negative control (NC); Control (C), plots treated with manure of the birds that used feed with no supplements, antibiotic (A), plots treated with manure of the birds that used neomycin as feed supplements; probiotic (P), plots treated with manure of the birds fed with feed supplemented with probiotic *Bacillus licheniformis* (Accession No. KT443923). The studied parameters were plant height (cm), number of branches per plant, number of pods per plant, number of seeds per pod, 1000-seeds weight (g), crop yield (kg) and prevalence of pathogens (*E. coli*, *Campylobacter* and *Salmonella spp*) in soil and poultry litter. Maximum crop yield and growth were observed in the crop plots treated with manure obtained from probiotic supplemented birds. Maximum plant height (49.93 ± 2.78 cm), number of branches per plant (16.68 ± 1.85), number of pods per plant (61.46 ± 2.73), number of seeds per pod (2.42 ± 0.59), 1000-seed weight (19.45 ± 0.83 g), crop yield (1243 ± 8.91 kg) was observed in plants from (P) group. Prevalence of *E. coli* was observed in poultry litter obtained from all groups of birds. Similarly *E. coli* was observed in soil samples from all groups of plots. However, prevalence of *Salmonella* and *Campylobacter* was detected in all plots except (P) group.

Key words: Probiotics, *Bacillus licheniformis*, Poultry manure, Crop growth and yield, *Lens culinaris*.

Introduction

Better yield of crop depends on many factors ranging from rainfall to usage of fertilizers (Wortmann & Ssali, 2001). Pakistan is an agricultural country and it is important to consider these factors especially the use of fertilizers. A high cost of chemical fertilizers is beyond purchasing power of the farmers and there is a decrease in its consumption which in turn decreases the crop yield. Mash and Masoor Lentils production was decreased by 12.7 and 5.8 percent, respectively (Pakistan Economic Survey, 2014-15). This has an adverse effect on our economy because a large population of our country depends upon agriculture. There are many causes of shrinkage of our agriculture and one main and important cause is the high costs of inorganic fertilizers. This problem can be solved by using the organic fertilizers. Organic fertilizers are environment friendly, low cost and enhance the soil fertility. The improvement of soil especially metal contaminated soils is essential for successful vegetation (Rotkittikhun *et al.*, 2007). The soil fertility is based on the presence of microbes and nutrients. Presently, inorganic fertilizers are mostly used which affects the soil health and decreases soil microbial diversity which results in decrease of crop production. The use of manure especially poultry litter helps in improvement of the crop productivity and soil fertility. Poultry manure is a good organic fertilizer; it contains phosphorus, high nitrogen, essential nutrients and potassium. Its usage is better than chemical fertilizer; it adds organic matter to soil which in turn improves soil structures, nutrient retention, aeration, soil moisture holding capacity and water infiltration (Deksissa *et al.*, 2008). According to the reports of some researchers, organic matters or manures in the form of organic fertilizer can increase the yield of the crops (Singh &

Bijayatt, 2006; Subavasugi *et al.*, 2008, Amanulla *et al.*, 2009). Since last decade, organic farming is growing very fast with annual growth of 20%. Statistically based analysis showed that 31 million hectares of land is cultivating by organic farming which in turn generate more than US \$ 26 billion worldwide as annual revenue (Ashraf *et al.*, 2016). The animal wastage is a good source of organic manure. Un-utilized organic wastage like animal and plants wastage cause pollution and its disposal is an expensive process for municipal corporations. Its usage as fertilizers can reduce a huge disposal cost and economic values can easily be achieved from the usage of these waste materials (Sim & Wu, 2010). It was estimated that one ton of droppings of poultry, sheep and cattle yield approximately 23 kg potassium (K), 10 kg nitrogen (N) and 16 kg phosphorus (P). Therefore, poultry manure can be used instead of an inorganic fertilizer which is expensive and dangerous for soil. Organic manure can improve the soil condition and especially the soil contaminated with potassium, nitrogen and phosphorous contents (Chiu *et al.*, 2006). Poultry manure is a complete fertilizer, rich in organic carbon and organic matter which increases the production of crop (Ramadan *et al.*, 2007). The growth of soil organisms can be increased by poultry manure which promotes soil's free mineral supply and for being evenly distributed in soil, poultry manure tends to stay longer and not leached out abruptly (Awodun, 2007). Poultry manure exerts beneficial effects on the crops and soil (Blay *et al.*, 2002). It increase aged soils fertility, improves the soil structure, facilitate the soil ventilation.

The use of poultry manure in Pakistan is increasing day by day due to its low costs and easy availability as compared to inorganic manure. In Pakistan, the available poultry manure can replace up to 101,000 tons of nitrogen, 58,000 tons of phosphorus and 26,000 tons of potassium in the form of chemical fertilizer which

reduces the input of farmers improve environmental issues and provide long term benefits to cultivated soils. Poultry litter is a useful and cheap source of soil fertility but it contains a high amount of human pathogens, such as *Campylobacter* spp., *Salmonella jejuni*, and *Listeria monocytogenes*, that can potentially contaminate environment and are responsible to the outbreak of food borne diseases (Wilkinson *et al.*, 2011, Chinivasagam *et al.*, 2010). Some pathogens are present in chicken litter or chicken litter based organic fertilizers, such as *Campylobacter*, *Salmonella*, *Escherichia coli*, *Bordetella*, *Clostridium*, *Corynebacterium*, *Globicatella*, *Listeria* and *Mycobacterium*, (Bolan *et al.*, 2010). For the treatment or reduction of pathogens many methods are adopted that includes physical, chemical, and biological methods. Among these methods, biological control techniques are best to control the pathogens because chemical and heating methods are expensive and also destroy beneficial microbes.

Some workers reported that lentil seed yield ranged from 1057-2880 kg ha⁻¹ (Sharaan *et al.*, 2003; Turan, 2003; Bicer & Sakar, 2010). The average value of the world lentil seed yield is 1095 kg ha⁻¹ (Anon., 2010). To achieve the maximum crop yield by using organic fertilizer we conducted a study where the effects of different types of organic fertilizers on crop growth, yield and prevention of pathogens like *E. coli*, *Campylobacter* and *Salmonella* were studied. Fertilizer was applied by soil as (Hamayun *et al.*, 2011) recommended that the most efficient way to apply fertilizer was by soil.

Material and Methods

Lentil trait: Punjab Masoor-2009 variety was used in the study to evaluate the effects of three different types of poultry manure.

Poultry manure: Poultry manure as an organic fertilizer was obtained from the droppings of three kinds of broiler birds. One was the control group of birds (C); the feed of birds had no additives, second was antibiotic group of birds (A); the birds were provided feed supplemented with commercially available neomycin, third was probiotic group of birds (P); the birds were given feed supplemented with *Bacillus licheniformis* probiotic produced at GC University, Lahore. Poultry manures of various types were carried from poultry farm to the fields in 20 kg sterile bags.

Usage of manure: Manure obtained from three different groups was added to the fields at the rate of 5 tons per hectare at the time of sowing.

Plots layout: Four agriculture plots were arranged in triplicates for lentil *Lens culinaris* cultivation. One was negative control plot (NC) in which poultry manure was not added; second was control plot in which manure obtained from control group of birds was added; third was antibiotic plot (A) which was provided manure obtained from antibiotic group of birds and fourth was probiotic

plot (P) in which manure obtained from probiotic group of birds was added.

The size of each plot was 2 x 8 m (Zafar *et al.*, 2003) and the distance between agricultural plots was 2.5 m. Crop was sown in various plots with a distance of 30 cm between each plot. The frequency of seeds used in each plot was 20 kg per hectare. The crop was sown in the last week of October with the help of single row hand drill. The field was irrigated 15 days before planting lentil *Lens culinaris* and plowed at a time when the field was in proper moisture condition. Various parameters were studied during the cultivation of plants that includes height of mature plant (cm), number of branches per plant, number of pods per plant, number of seeds per pod, weight of 1000 seeds (g) and seed yield per hectare (kg).

Prevalence of pathogens in poultry manure and soil:

To evaluate prevalence of *Salmonella*, *E. coli* and *Campylobacter* in manure and soil, poultry litter samples were collected from the experimental farms and soil samples were collected from the plots in sterile polythene bags. Total 12 samples from soil and 9 samples from poultry litter were sent to the lab to confirm the presence of pathogens.

Statistical analysis: The statistical analysis was conducted according to randomized complete block design (RCBD). Collected data was analyzed by using analysis of variance (ANOVA) techniques (Steel *et al.*, 1997) using SAS, 9.1 (2002-03) portable software. The means were compared by using least significant difference (LSD) (Steel & Torrie, 1981).

Results

Lentil (*Lens culinaris*) crop was sown on specified land in October, 2014. After addition of various types of poultry manures, lentil crop was harvested after five and half months in April, 2014. Various parameters like growth of plants, seed yield and prevalence of pathogens that are mentioned in material and methods were analyzed.

The height of plant: The maximum average height of mature lentil plant was observed to be 49.93±2.78 cm in P plots. The average height of 44.90±2.17 cm was observed in A plots. Similarly, average height of 39.60±1.43 cm and 35.63±1.29 cm of lentil plant was observed in C and NC plots respectively (Table 1). The average maximum difference in height of plants was observed between plots P and NC which was calculated to be 14.3 cm and average minimum difference was observed to be 3.97 cm between plots P and A.

Number of branches per plant: The maximum number of branches per plant were (16.68±1.85) observed in P plots. However, number of branches per plant in plot A was observed to be 12.81±1.20. Less number of branches were observed in plants from C and NC plots which were 9.33±0.89 and 6.57±0.64 respectively (Table 1). Maximum difference in number of branches per plant was 10.11 between P and NC plots and minimum difference in number of branches per plant was 2.76 between C and NC plots.

Table 1. Efficacy of three different poultry manures (P, A and C) on lentil plant growth and Seed yield in comparison with negative control.

Treatments	Manure (Ton ha ⁻¹)	Height of plant (cm)	No. of branches per plant	No. of pods per plant	No of seeds per pod	1000-seeds weight (g)	Seed yield (kg ha ⁻¹)
NC	5.0 ± 0	35.63 ± 1.29 ^d	6.57 ± 0.64 ^c	36.76 ± 0.89 ^c	1.07 ± 0.25 ^b	14.62 ± 0.47 ^b	183.70 ± 8.29 ^d
C	5.0 ± 0	39.60 ± 1.43 ^c	9.33 ± 0.89 ^{bc}	39.71 ± 1.77 ^c	1.33 ± 0.37 ^b	17.27 ± 0.51 ^{ab}	910.50 ± 6.38 ^c
A	5.0 ± 0	44.90 ± 2.17 ^b	12.81 ± 1.20 ^b	54.27 ± 1.15 ^b	1.61 ± 0.45 ^{ab}	18.30 ± 0.62 ^a	1133.37 ± 31.64 ^b
P	5.0 ± 0	49.93 ± 2.78 ^a	16.68 ± 1.85 ^a	61.46 ± 1.73 ^a	2.42 ± 0.59 ^a	19.45 ± 0.83 ^a	1243.93 ± 8.91 ^a

where 'NC' stands for negative control, 'C' for control, 'A' for antibiotic and 'P' for probiotic

Table 2. Prevalence of pathogens in poultry litter.

Pathogens	Control			Antibiotic			Probiotic		
	<i>S</i>	<i>E.c</i>	<i>C</i>	<i>S</i>	<i>E.c</i>	<i>C</i>	<i>S</i>	<i>E.c</i>	<i>C</i>
Replicate 1	×	×	×	×	×	×	×	×	×
Replicate 2	×	×	×	×	×	×	×	×	×
Replicate 3	×	√	×	×	√	×	×	×	×

where 'S' stands for *Salmonella*, 'E.c' for *E.coli* and 'C' for *Campylobacter*

The signs √ represents the presence and × represents the absence of the pathogens

Table 3. Prevalence of pathogens in soil samples.

Pathogens	Negative Control			Control			Antibiotic			Probiotic		
	<i>S</i>	<i>E.c</i>	<i>C</i>	<i>S</i>	<i>E.c</i>	<i>C</i>	<i>S</i>	<i>E.c</i>	<i>C</i>	<i>S</i>	<i>E.c</i>	<i>C</i>
Replicate 1	×	×	×	×	×	√	×	×	×	×	×	×
Replicate 2	×	×	×	√	×	×	×	×	×	×	×	×
Replicate 3	√	√	√	√	√	√	√	×	√	×	×	×

where 'S' stands for *Salmonella*, 'E.c' for *E.coli* and 'C' for *Campylobacter*

The signs √ represents the presence and × represents the absence of the pathogens

Number of pods per plant: The effect of manures obtained from various groups of broilers on the number of pods was studied. The manure obtained from broilers fed with probiotic supplemented feed has a pronounced effect on the number of pods of the plants and was observed to be 61.46±1.73 and 54.27±1.15 in the P and A plots respectively. Least number of pods was obtained in plants that were grown in C and NC plots and was calculated to be 39.71±1.77 and 36.76±0.89 pods per plant respectively (Table 1). The maximum difference in number of pods was 24.70 between P and NC plots and minimum difference was observed between C and NC plots that were observed to be 2.95.

Number of seeds per pod: The average maximum number of seeds per pod was 2.42±0.59 in P plots. However, number of seeds per pod in plants belong to A and C plots were 1.61±0.45 and 1.38±0.37 respectively. Minimum number of seeds per pod was observed in plants of NC plots and observed to be 1.07±0.25 (Table 1). Maximum difference in number of seeds was observed between plants of P and CN plots (1.35) and minimum difference (0.31) was observed in plants that were planted in plots A and C and in plots C and NC plots.

Weight of 1000 seeds: The average maximum weight of 1000 seeds was (19.45±0.83 g) observed in P plot. A weight of 18.30±0.62 g was in A plot while 17.27±0.51 g was obtained from C plot and average minimum weight was 14.62±0.47 g obtained from plot NC (Table 1). Maximum difference of 1000 seeds was (4.83) observed between P and NC plots and minimum difference was (1.03 g) observed between A and C plots.

Yield per hectare: In this study average maximum seed yield per hectare was 1243.93±8.91 observed in P plot. However, significantly less yield of 1133.37±31.64 kg was observed in A plot, while even lesser yield was obtained from lentil crop grown in C plot that was observed to be 910.50±6.38 kg. Average minimum or fewer yields obtained was 183.70±8.29 of NC plot (Table 1). Maximum difference was 1060.23 kg between P and NC plots and minimum difference was 110.56 kg between P and A plots.

Prevalence of pathogens in litter and soil: In this study, pathogens *Salmonella*, *E. coli* and *Campylobacter* were detected as these are known for their disease causing ability and also for causing food borne diseases. During the crop management, these pathogens can affect the farmers or the farmers can act as vector. It was observed that *E. coli* was present in the litter of all three groups but varies in occurrence. In the litter of (C) control and (A) antibiotics groups' existence of *E. coli* was observed. However, *E. coli* was in negligible amount in the litter samples obtained from poultry birds of (P) probiotic group. *Campylobacter* and *Salmonella* were absent in all groups (Table 2). In case of soil, the presence of pathogens are much higher that can survive for longer periods of times. However, when soils from various plots were tested, negligible presence of *Salmonella* was observed but *E. coli* and *Campylobacter* were completely absent in the soils of (P) plot. On the other hand *Salmonella*, *Campylobacter* and *E. coli* were observed in A, C and NC plots (Table 3).

Discussion

The use of poultry manure is being used for the increased production of various vegetables, pulses and other crops. Many reports have shown the significant increase in the production of lady finger (*Abelmoschus esculentus*), cucumber (*Cucumis sativus*), maize (*Zea mays*), Lentil after adding poultry manure in the fields (Farhad *et al.*, 2009; Islam *et al.*, 2014; Hadiuzamman *et al.*, 2015; Maqshoof *et al.*, 2015). In this study, the effect of poultry manure obtained from birds fed with probiotic supplemented feed in comparison to poultry manure obtained from birds fed with feed supplemented with antibiotic was studied on the growth and production of lentil (*Lens culinaris*). The results of this study clearly show the efficacy of poultry manure on soil fertility, plant growth and yield per hector as compared to negative control. In this study, maximum crop yield obtained was 1243.93 kg per hector of (P) plot. These results are similar to the work that was reported earlier which showed the increase in yield of lentil crop after addition of poultry manure to soil and the increase of lentil ranged between 1057 kg to 2880 kg per hectare (Sharaan *et al.*, 2003; Turan, 2003; Bicer & Sakar, 2010).

High yield per hector was (1243.93 kg) of lentil *Lens culinaris* cultivated in (P) plots that was provided with poultry manure from birds provided with feed supplemented with probiotic (*Bacillus licheniformis*) and this yield was highest among all plots in which various types of poultry manures were added this result was in line of Tamoor Ul Hassan and Asghari Bano (2016) who concluded in their study that the PGPR application to crop can effect beneficially on plant's biochemical and physiological parameters. Plot (A) also yields reasonable production per hector of lentil which was (1110.56 kg). However, significantly fewer yields per hector were obtained from plot (C) (333.45 kg), although manure was also added to these plots. The higher yield in plot (P) was due to the type of manure containing *B. licheniformis* spores. It has been shown in a recent study that *Bacillus* species have direct effect on the growth of plant and can be used as biofertilizers which synthesize plant growth hormones (Amer & Utkhede, 2007). Similarly, height of the plants grown in the plot (P) was high as compared to plants grown in other plots including negative control. This observation is in accordance with the report of Arshad & Frankenberger (1998) which states that the plant growth promoting bacteria including *Bacillus* species affect the plant growth positively. In a similar report, it was proposed that bacteria from genus *Bacillus* provide nutrients to the crops, enhance growth of plants through plant hormones, restrain the activity of plant pathogens, improve soil structure and plant stress control (Davison, 1988). However, it has been proposed that apart from other bacteria, *Bacillus* species also control stress of plant that leads to the better plant growth and high production (Brierley, 1985). One interesting report has demonstrated that presence of *B. licheniformis* is very helpful in protecting plants against various diseases and therefore, can be safely used as a biocontrol agent. The central mechanisms of growth promotion include solubilization, mobilization of phosphate, production of growth

stimulating phytohormones, siderophore production, antibiosis i.e., production of antibiotics, inhibition of plant ethylene synthesis and induction of plant systemic resistance to pathogens (Gutierrez-Manero *et al.*, 2001; Idris *et al.*, 2007).

Maximum number of branches were (16.33±1.85) observed in plants that were cultivated in plot (P). It is evident that number of pods was increased due to the normal functions of plant hormones. Increased number of branches in lentil plants was due to the ability of *B. licheniformis* to stimulate the hormone production in plants. *B. licheniformis* are involved in the production of gibberellins hormone in seeds and vegetative body of plant. Gibberellins are plant hormones which affect various developmental processes and regulate the plant growth. Due to increased hormone synthesis, additional branches are sprout from the plant. In recent study, Jamil *et al.* (2013) showed that *Bacillus licheniformis* had the ability to produce gibberellins and hence more branches are produced in the plants. In other study, it is reported that plant growth promoting bacteria *Bacillus licheniformis* and *Bacillus pumilus* make physiologically active gibberellins in a high amount (Gutierrez-Manero *et al.*, 2001). Previously, it has been shown that plant growth promoting bacteria enhance the plant growth through plant hormones (Davison, 1988). The growth of plants and increase in yield is due to the production of gibberellins and Indole Acetic Acid by *B. licheniformis* (Kang *et al.*, 2015; Idris *et al.*, 2007; Bottini *et al.*, 2004). Similarly, auxin like activity was reported by *Bacillus* spp. (Idris *et al.*, 2004). The mechanism by which plant growth hormone activates plant growth involves seed germination by stimulating enzymes that are essential for food production. In some plants, aleurone layer wraps the endosperm tissues. The gibberellins and other growth factors produced by *Bacillus* spp., are transported to the aleurone layer and helped in the production of enzymes that break down reserve food in the endosperm. The food available after enzymatic action is made available to the growing seed (Grennan, 2006). Gibberellins also cause increase in internodes length and are involved in rapid and increased cell division (Tasai *et al.*, 1997).

The number of seeds per pod was also increased due to the increased activity of hormones in (probiotic plots) plants treated with the manure obtained from the birds provided with feed supplemented with probiotics as compared to negative control. One reason for high weight gain by seeds and seed yield per hector in (P) plots was due to prevention of the plants from pathogens, normal hormonal function and availability of required nutrients, good soil structure and stress less environment where plants were grown (Davison 1988).

In this study, pathogens *Salmonella*, *E. coli* and *Campylobacter* were detected as these strains are known for their disease causing ability and also for causing food borne diseases. During the crop management, these pathogens can affect the farmers or the farmers can act as vector. It was observed that *E. coli* was present in the litter of (C) and (A) groups but varies in occurrence but not observed in (P) group the same results we found in case of *Campylobacter* and *Salmonella* which were

observed in (C) and (A) groups but not in (P) group. The absence or less presence of pathogens in the poultry litter was due to the anti bacterial activity of *Bacillus licheniformis* against pathogens. In case of soil, the presence of pathogens are much higher and includes *Salmonella*, *E. coli*, *Campylobacter* and other pathogens that can survive for longer periods of times (USFDA, 2013). However, when soils from various plots were tested, no pathogenic bacteria was found in probiotic (P) plots except *Salmonella* which was presence in negligible amount. However *Salmonella* and *Campylobacter* were observed in (A), (C) and (NC) plots as well as *E.coli* was observed in (C) and (NC) plots but not in (A) plot . It has been shown that *Bacillus* species express antagonistic activities by suppressing the pathogens (Arrebola *et al.*, 2010). Choudhary & Johri (2008) have reviewed ISR by *Bacillus* spp. In relation to crop plants and emphasized on the mechanisms and possible applications of ISR in the biological control of pathogenic microbes.

Conclusion

It was concluded that probiotics especially *Bacillus* species like *Bacillus licheniformis* recommended for commercial poultry industry as feed additive. The farmers were suggested to use organic fertilizer of poultry origin. The manure obtained from poultry birds used spore forming probiotics supplemented feed has dual benefits of organic and biofertilizers. This fertilizer in comparison with in organic fertilizer is low cost, anti pathogen, soil, plant, environment, animal and human friendly. We recommend our *Bacillus licheniformis* probiotic to poultry industrialist made at GC University Lahore, a cheap product as compare to commercial products.

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