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Twice Applications of Laying Hens Manure Produced the Highest Yield of Organic Okra Pods (*Abelmoschus esculentus* L. Moench.)

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Abstract

The optimal okra cultivation using organic fertilizers remains a subject of limited information. This study aimed to assess the impact of applying 18 t.ha^{-1} of laying hens organic fertilizer on the morphological and physiological traits of the "Naila IPB" green okra variety. The study was conducted between September and December 2022 at the IPB organic experimental field at Cikarawang, Bogor, West Java, Indonesia. A randomized complete block design with a single factor namely frequency of organic fertilizer application, was employed. The factor consisted of four levels: no organic fertilization, 1-, 2-, and 3-times applications. The results demonstrated that a single application of organic fertilizer led to an increase in plant height at 5, 7, and 9 weeks after planting (WAP), leaf number at 5 WAP, root dry weight at 4 WAP, stem dry weight at 10 WAP, and an acceleration in the onset of flowering. This resulted in a flowering period lasting 29.6 days, with a total flowering duration of 69.33 days. In contrast, a two-times application of organic fertilizer resulted in elevated plant height at 11 WAP, an increased leaf number at 11 WAP, heightened leaf potassium content, a greater number of pods per plant, and a higher yield of young pod okra. These findings contribute to our understanding of the effects of organic fertilizer application frequency on the growth and yield of young pod okra, aiding in the formulation of improved cultivation strategies.

Keywords: flowering age, gradual fertilization, green okra, "Naila IPB", young pods.

Introduction

Okra (*Abelmoschus esculentus* L. Moench) represents a functional vegetable presently under development across numerous tropical and subtropical regions (Lim, 2012). This annual fruit-bearing crop holds significant agricultural importance within tropical and warmer climatic zones worldwide (Patil et al., 2015). The conspicuous prevalence of dietary fiber within okra fruit arises from its abundant mucilage content. The constituents of fiber in okra encompass α-cellulose, hemicellulose, lignin, pectin compounds, gums, and mucilage (Camciuc et al., 1998; Jain et al., 2012; Kumar, 2014). The mucus extracted from okra serves diverse roles in the realms of food and pharmaceutical industries, operating as a binding agent, emulsifier, and suspending agent (Lim et al., 2015). The dietary fiber present in okra fruit significantly contributes to the stabilization of blood glucose levels by modulating sugar absorption within the intestinal tract (Jain et al., 2012)

Organic cultivation of okra has the potential to add value of this functional vegetable. However, proper guidance for organic fertilization is necessary due to the asynchronous development of okra flowers and fruits. Existing guidelines recommend a gradual application of inorganic fertilizers for okra plants (Rokhmah et al. 2019). Nonetheless, organic fertilizers, which have distinct characteristics in nutrient provision, require specific investigation into gradual organic fertilization techniques.

As organic product demand continues to increase (Ali et al., 2019), farmers tend to involve themselves in organic farming as long as it is environmentally friendly and produces healthy and safe products. Gosling et al. (2006) characterize organic farming as a cultivation system reliant on organic fertilizers alongside a limited range of naturally derived chemicals. Organic fertilizers serve as substitutes for their inorganic counterparts, promoting food safety and enhancing the availability of soil water and nutrients

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(Wang et al., 2020). One of the challenges facing organic fertilizer is its large quantities of applications to satisfy the crop's needs. As a form of organic fertilizer, poultry manure is generally rich in mineral nutrient composition. Despite its large composition of nutrients, poultry manure has a slow rate of nutrient mineralization, requiring the need to apply it in large quantities to meet the crop's demands.

Among the various types of organic fertilizers, chicken manure emerges as a beneficial resource for crop cultivation. Prior studies by Fauziah (2008) demonstrated the positive impact of applying 10 t.ha^{-1} of chicken manure on Aloe vera leaf width, leaf thickness, leaf number, and fresh weight. Similarly, Hariyadi (2019) reported the growth acceleration of corn through chicken manure application. Furthermore, the combined utilization of inorganic fertilizers and chicken manure at a rate of 20 t.ha^{-1} for hybrid tomato yields noteworthy outcomes, including a fruit weight of 17.41 kg per plot and a projected maximum fruit weight of 22.79 t.ha^{-1} (Luthfyrakhman and Susila, 2013). Application of the poultry organic matter in split rates across the entire corn silage crop assures technical and environmental sustainability during wet periods. On the other hand, reapplication of this organic fertilizer only at pre-sowing can increase fresh matter yields and protein quality of organic whole-plant corn silage cultivated in on inceptisol in the dry season (Yagi et al., 2020).

The study of young harvest okra cultivation with frequently applied fertilizers has been carried out to test the most important nutrients for okra growth (Rokhmah et al., 2019). A study involving undissolved goat manure as supplementary fertilizer failed to enhance okra fruit growth and production (Panjaitan, 2021). Consequently, refining manure application techniques through frequency adjustments assumes critical importance. Lack of information about relevant previous studies and limited publications about the effect of frequency of fertilizing on okra highlights the significance of this approach. This approach is expected to enhance okra cultivation technology and optimize fruit quality. By determining the appropriate frequency of laying hen manure application, desirable morphological and physiological traits can be obtained, supporting optimal okra productivity.

Material and Methods

The study was conducted from September to December 2022, involving the transplantation of okra seedlings in September 2022. The experiment was conducted at the IPB organic experimental farm in Cikarawang, Darmaga, Bogor. The materials used were green okra seeds of the "Naila IPB' variety and laying hen manure, with a dosage of 18 t.ha^{-1} for each treatment, as per the reference provided by Mangana (2020). The chicken manure contains 2.24, 8.86, and 2.22% of N, P, and K, respectively. The tools used included plastic mulch, a seedling tray, an oven, and a UV-vis spectrophotometer.

The experiment used a Randomized Complete Block Design with okra fertilization frequency as a single factor consisting of four levels, with a total dose of 18 t.ha^{-1}:

P0 = no fertilization (control)
P1 = fertilization of the entire dose at 2 weeks before planting
P2 = fertilization at 2 weeks before planting and 4 weeks after transplanting.
P3 = fertilization at 2 weeks before planting, 4 and 8 weeks after transplanting.

Okra seeds were sown in trays before being planted in the field. The planting of okra seedlings was done by planting one seedling in each hole in experimental plots with a size of 1.2 m x 5 m and a planting distance of 60 cm x 25 cm, a distance between plots of 100 cm, and a distance between replicates of 200 cm. Each experimental unit consisted of 40 plants. Seedlings meeting the criteria of being visually healthy and disease-free were the only ones transplanted 13 days after sowing (DAS). These seedlings were characterized by a height of 10 cm and 2 leaves.

Maintenance of okra plants involves essential tasks such as watering and manual weeding, performed as required. Organic fertilization was executed following the guidelines outlined in Table 1. The second and third doses of additional fertilizer were applied with blending the manure and 20 L of water, then pour them around the plant base. The harvesting of okra was undertaken at approximately 9 weeks after transplanting (WAP), with the actual harvesting process occurring 6 days after anthesis (DAA), as detailed in Aplugi et al. (2019). Time of anthesis was used to determine the optimal harvest age. Harvested okra fruits met specific criteria, including a length of approximately 7 cm, a diameter of 1.2 cm, and a smooth green exterior.

Measurements were made on 10 plant samples per treatment in each experiment on variables in the vegetative phase; plant height (cm), number of leaves, dry weight (g), leaf area (cm^2), relative growth rate (g per day), net assimilation rate / unit leaf rate (g.cm^{-2} per day), leaf area index, and leaf nutrient content. Measurements on the generative phase include flowering age and length of flowering period, while

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production variables are the number of pods per plant, the number of pods per plot (g), and productivity (kg. ha^{-1}). Quantitative data were analyzed using ANOVA and further tested using Duncan's Multiple Range Test at the level of α = 5% using Microsoft Excel and SPSS software. The amount of organic fertilizer applied to the plants at various times is presented based on dosage of 18 $t.ha^{-1}$ for each treatment (Table 1). The highest average temperature in October 2022 the highest rainfall and rainy days in October 2022 during the study were recorded (Table 2).

Leaf number

The average number of leaves was significantly different between treatments at 5 and 11 WAP (Table 3). At 5 WAP, the once-fertilization frequency treatment (where all organic fertilizers doses were applied 2 weeks prior to planting) recorded the highest number of leaves, showing a 30.9% increase compared to the control. Similarly, at 11 WAP, the twice-fertilization-frequency treatment recorded the highest number of leaves, with a 48% increase compared to the control.

Table 1. Okra fertilization schedule

Fertilization frequency	Weeks after planting (WAP)		
	-2	4	8
Control	0%	0%	0%
Once	100%		
Two times	50%	50%	
Three times	33.3%	33.3%	33.3%

Tabel 2. Temperature, rainfall and rainy days during the study

Month	Temperature (^{0}C)			Rainfall (mm)	Rainy days (day)
	Average	Max	Min		
Sep 2022	26.0	34.0	20.2	344.2	25
Oct 2022	26.4	34.1	20.8	495.6	25
Nov 2022	26.0	33.4	20.8	321.0	23
Des 2022	25.7	33.4	20.0	213.8	25

Source: BMKG (2022).

Result and Discussion

Okra Vegetative Phase

Plant height

The average plant height at 5 and 7 weeks after planting (WAP) following a single fertilization exhibited increases of 30.7% and 34.3% ($p < 0.05$) respectively, surpassing the control group's results (Table 3). Similarly, at 9 and 11 WAP, both one- and two-times fertilizations increased plant height by 32.3% and 33.4% ($p < 0.01$) respectively, when compared to the control group. However, the effect of fertilization frequency treatments did not yield statistically significant differences. Table 1 outlines the quantity of organic fertilizers administered in accordance with the specific frequency of fertilization during each observation period.

The provision of adequate nutrients for plant growth was initially achieved with the once-fertilization frequency, resulting in the earlier attainment of a higher number of leaves compared to the two or three fertilization frequencies. The availability of sufficient nitrogen (N) and phosphorus (P) promotes the growth of plant organs, including the process of leaf formation (Maghfoer et al., 2013). These results were supported earlier by Mohammedaltom and Dagash (2017) who found that the increase in leaves number due to the application of organic components had stimulatory effects on cell division and enlargement, protein and nucleic acid synthesis.

Table 3 also reveals that in the once-fertilization treatment, the number of leaves started to decrease at 11 WAP, while in the two and three fertilization treatments, the number of leaves continued to increase at this stage (The difference in leaves can be seen in Figure 1). The decrease in leaf count was

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Table 3. Effect of fertilization frequency on okra height and leaf number at 3-11 weeks after planting

Fertilization frequency	Weeks after planting									
	3		5		7		9		11	
	Height (cm)	Leaf no	Height (cm)	Leaf no	Height (cm)	Leaf no	Height (cm)	Leaf no	Height (cm)	Leaf no
Control	24.5	10.8	38.9b	15.2b	62.9b	20.9	87.4	22.7	108.2b	20.0b
Once	28.2	13.8	50.9a	19.9a	84.5a	23.1	115.7	26.7	144.5a	25.6 ab
Two times	26.2	11.7	47.5a	18.2ab	80.6a	22.5	112.7	29.2	148.0a	29.6a
Three times	27.7	11.6	46.2a	18.7a	78.7a	23.1	111.4	27.4	145.3a	29.4 a
	ns	ns	*	*	*	ns	**	ns	*	*

Note: values followed by the same letters in a column are not significantly different according to DMRT; ns = not significant; * = significant at α=0.05; ** = significant at α=0.01.

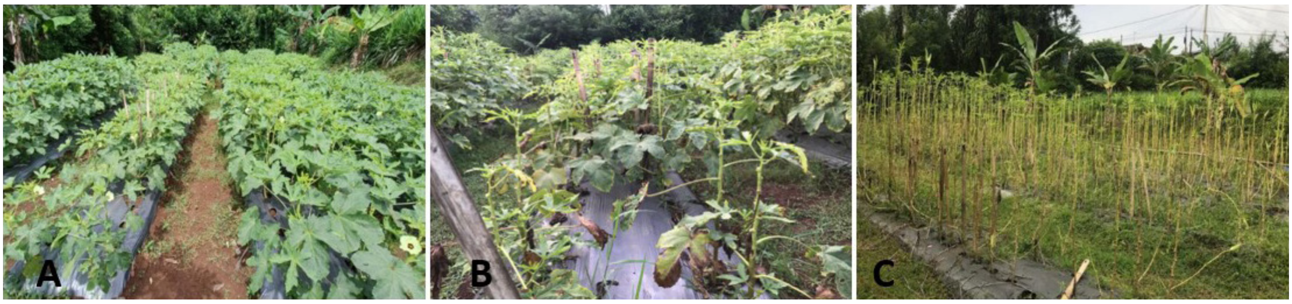

Figure 1. Okra at 7 (a), 10 (b) and 14 (c) weeks after planting.

attributed to the shedding of lower leaves caused by drying. The leaf cycle pattern was also reported by Priatna (2020), who observed an initial increase in the number of leaves in okra plants followed by a decrease in the middle stage of plant growth.

Leaf dry weight

The application of an entire dose of organic fertilizer before planting (one-time organic fertilizer frequency), resulted in a significantly higher leaf dry weight at 4 weeks after planting (WAP), with a 56.38% increase compared to the control (Table 4) ($p < 0.05$). On the other hand, the three-times organic fertilizer frequency led to a 62.68% increase in leaf dry weight at 10 WAP ($p < 0.01$) compared to the control. However, there was no significant difference observed between the organic fertilizer frequency treatments in terms of their effect.

Stem dry weight

One-time fertilization frequency caused a higher stem dry weight at 4 WAP ($p < 0.01$) by 78.33% and at 10 WAP ($p < 0.05$) by 131.77% compared to the control, but the effect between fertilization frequency treatments was not significant (Table 4). With the fertilization applied once, the entire dose of organic fertilizer was applied before planting. The plants grew quickly afterwards and the results of photosynthesis were more quickly obtained and directly allocated to all plant organs. The faster the nutrient sufficiency in the plant, the greater the biomass of the plant (Firdaus et al., 2013).

On the other hand, when the organic fertilizer is applied three times, with the complete application at 10 WAP, it stimulates the source to be more productive and transferred the assimilates to the sink.

Root dry weight

Applying the entire dose of organic fertilizer before planting (one-time organic fertilizer frequency) resulted in a significantly higher root dry weight at 4 weeks after planting, showing a 96.1% increase ($p < 0.05$) compared to the control (Table 5). However, there was no significant difference in the organic fertilizer frequency treatment at both 4 and 10 WAP.

According to Nurmayulis et al. (2014), the increase in plant biomass is attributed to enhanced absorption of water and nutrients. Nutrients stimulate the development of plant parts such as roots, enabling greater nutrient and water absorption. This, in turn, increases photosynthetic activity and contributes to an overall increase in plant dry weight.

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Leaf area

The differences in the okra leaf area due to fertilization frequency treatments occurred at 10 WAP ($p < 0.05$); the largest leaf size with three split fertilizers was 244.1% of the control, whereas at 4 and 7 WAP it was not significantly different (Table 5). The widest leaf area of one plant with one fertilization was only 64.3% and 30.3% larger compared to the control (Table 5).

Relative growth rate and net assimilation rate

The relative growth rate of okra increased by 16.70% ($p < 0.01$) when organic fertilization was applied three times between 7 and 10 weeks after planting (WAP), in comparison to the control group (Table 6). However, there were no significant differences observed among the treatments in terms of relative growth rate during the 4–7 WAP period and the net assimilation rate of plants aged 4–10 WAP.

Leaf area index

The frequency of organic fertilization had a significant impact on the leaf area index (LAI) at 10 weeks after planting (WAP) ($p < 0.05$, Table 7). The highest LAI at 10 WAP was observed in the treatment with three rounds of organic fertilization, with an 84.61% increase compared to the control, 41.17% higher than the one-time fertilization, and 18.81% higher than the two-time fertilization treatment. When considering the LAI at 4 and 7 WAP, the treatment with a single fertilization frequency showed the highest values ($p > 0.05$), indicating no significant difference from other treatments. However, at 10 WAP, the LAI was significantly higher ($p < 0.05$) in the treatment with a frequency of three times fertilization compared to the control treatment, while there was no significant difference compared to the other treatments.

Table 4. Effect of fertilization frequency on the dry weight of okra leaves and stems

Fertilization frequency	Weeks after planting (WAP)					
	Leaf dry weight (g)			Stem dry weight (g)		
	4	7	10	4	7	10
Control	2.27b	16.35	24.17b	1.80b	24.82	40.35b
Once	3.55a	25.63	36.50a	3.21a	51.25	93.52a
Two times	2.75b	22.00	38.85a	2.23b	35.32	90.50a
Three times	2.76a	18.73	39.32a	2.30b	24.18	89.03a
Significance	*	ns	*	**	ns	*

Note: values followed by the same letters in a column are not significantly different according to DMRT; ns = not significant; * = significant at α=0.05; ** = significant at α=0.01.

Table 5. Effect of fertilization frequency on average root dry weight of okra plants and leaf area of okra plants

Fertilization frequency	Weeks after planting (WAP)					
	Root dry weight (g)			Total leaf area (cm^2)		
	4	7	10	4	7	10
Control	1.31b	3.05	4.82	884.81	1547.77	637.13b
Once	2.57a	5.23	6.56	1453.77	2017.96	1121.23ab
Two times	1.70b	3.47	6.80	821.81	1799.31	1576.88ab
Three times	1.62b	3.33	7.07	921.38	1668.14	2193.00a
Significance	*	ns	ns	ns	ns	*

Note: values followed by the same letters in a column are not significantly different according to DMRT; ns = not significant; * = significant at α=0.05; ** = significant at α=0.01.

treatments, three applications led to the lowest leaf K nutrient levels. This shows that the same dose of organic fertilizer but applied with a higher frequency causes nutrient insufficiency for the early growth of okra plants.

Based on Rogeri et al. (2016), when higher rates of poultry litter (PL) were applied, along with any rate of PL solely at pre-sowing, it is suggested that the NH_{4+}-N nitrification process might have led to the generation of protons, thereby helping to mitigate soil acidity. Additionally, the linear increase in potassium

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Table 6. Effect of fertilization frequency on okra relative growth rate and net assimilation rate

Fertilization frequency	Relative growth rate ($g.g^{-1}$ per day)		Net assimilation rate ($g.cm^{-2}$ per day)	
	4-7 WAP	7-10 WAP	4-7 WAP	7-10 WAP
Control	3.80	4.31b	0.80	0.78
Once	4.48	4.97a	1.53	1.49
Two times	4.06	4.99a	1.07	2.28
Three times	3.80	5.03a	0.75	2.66
Significance	ns	**	ns	ns

Note: values followed by the same letters in a column are not significantly different according to DMRT; ns = not significant; * = significant at α=0.05; ** = significant at α=0.01. WAP = weeks after planting.

Table 7. Effect of fertilization frequency on okra leaf area index

Fertilization frequency	Weeks after planting (WAP)		
	4	7	10
Control	0.75	1.00	0.65b
Once	0.98	1.14	0.85ab
Two times	0.71	1.05	1.01ab
Three times	0.78	1.03	1.20a
Significance	ns	ns	*

Note: values followed by the same letters in a column are not significantly different according to DMRT; ns = not significant; * = significant at α=0.05.

When considering the age of the plants, the leaf area index (LAI) displayed a decrease in integrated leaf development (ILD) at the 10-week mark after planting (WAP) for treatments without fertilization, as well as those with one and two fertilizations. Intriguingly, the frequency of fertilization occurring three times demonstrated a contrary trend, with ILD exhibiting an increase. This can be attributed to the fact that the nutrient intake frequency of three times remained adequate, maintaining sufficient nutrient availability at the 10-week stage. In contrast, treatments without fertilization and with one or two fertilizations experienced reduced nutrient availability by this point. It is worth noting that plants that received a complete dose of fertilizer prior to planting exhibited accelerated leaf formation. However, these plants subsequently shed older leaves, leading to a reduction in leaf count by the 10-week mark (Table 3).

Leaf nutrient content

The frequency of organic fertilizer had no significant effect on N and P nutrient levels in the early leaf generative phase but on leaf K levels (Table 8). The addition of organic fertilizers increases the K content of the leaves. The K nutrient content of okra plant leaves was high in the gradual treatment frequency of two fertilizations ($p < 0.05$) of 104.49% compared to the control. Among the three fertilization frequency (K) levels was observed only in the treatment involving split applications of PL rates, which could indicate that fewer nutrients were lost through this fertilization method during the rainy year. Considering these results, it can be inferred that the efficiency of split PL fertilization might have increased, leading to a higher nitrogen-to-phosphorus (N:P) ratio. This, in turn, reduced the accumulation of phosphorus (P) in comparison to the fertilization method involving PL only at corn pre-sowing, as discussed by Yagi et al. (2020).

Okra Generative Phase

The flower emergence is a transition process from the vegetative phase to the generative phase. There is a difference in flowering age between treatments of fertilization frequency, but there is no difference in the length of the flowering period. Flowering age was significantly different ($p < 0.01$) due to the frequency of fertilization (Table 9).

Flowering age

Okra exhibited earlier flowering when subjected to different fertilization frequencies. The treatment with once fertilization frequency resulted in the earliest flowering, followed by twice and three times fertilization, while the plants without fertilization

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Table 8. Effect of fertilization frequency on leaf nutrient content of okra plants at 4 WAP

Fertilization frequency	Leaf nutrition content (%)			
	N	P	K	Organic C
Control	4.15	0.37	0.89c	32.74
Once	4.36	0.33	1.67ab	32.66
Two times	4.29	0.37	1.82a	32.91
Three times	3.99	0.39	1.36b	32.99
Significance	ns	ns	*	ns

Note: values followed by the same letters in a column are not significantly different according to DMRT at α=0.05. ns = not significant; * = significant at α=0.05

flowered last. Specifically, the okra plants with one-time fertilization frequency flowered 9 days earlier than the control group and 7 days earlier than the plants with three times fertilization frequency. According to Aplugi (2019), on average, okra typically start flowering around 36-38 days after transplanting, with 50% flowering occurring at 7 weeks after planting (WAP) and peak flowering at 8 WAP.

The delayed flowering in a slow growing crop like okra is a result of the utilization of photosynthates for fulfilling their vegetative cycle. Consequently, in okra without fertilization as well as in those subjected to gradually decreasing fertilization frequencies of three times and twice, the onset of the generative phase is extended. This delay in flowering can also lead to a longer duration for the formation of okra fruits. According to Mal et al. (2013), nutrient deficiencies can cause plants to exhibit late flowering due to a longer time required for the initiation of generative growth.

Okra Productivity

The utilization of organic fertilizers has been shown to have a positive impact on both the quantity and size of fruits (Table 10). Among the various fertilization frequencies studied, the most notable increase in okra plant productivity (within an area of 5.1 m^2) was recorded with a two-stage fertilization schedule, with 106.6% improvement over the control group. While there was no significant difference in the average number of pods per plant and per plot across different fertilization frequencies, the mean values were consistently higher with a two-stage fertilization schedule, demonstrating a 101.9% and 75.4% enhancement, respectively, compared to the control group (Table 10).

Giving all doses of organic fertilizer before planting can support vegetative growth because when plants begin to grow, nutrients are already available to plants earlier, thus supporting plants to growth better than those fertilized two or three times. Application of a gradual, two-stage fertilization approach, administered two weeks before planting and four weeks after transplanting, has been shown to yield the highest productivity. This method ensures an ample supply of nutrients during the vegetative growth phase and seamlessly transitions into the generative phase as the plants mature.

The nutrients made available during the second feeding, occurring four weeks after transplanting, play a crucial role in promoting plant development during the generative phase, thereby optimizing fruit production compared to alternative treatments. According to Atsari and Suntari (2018) findings, there exists a strong positive correlation between the quantity of fruits and the overall yield of okra plants. This implies that an increase in the number of fruits produced directly corresponds to a yield increase in okra. Organic fertilizer can increase the soil's organic matter content, resulting in an increased

Table 9. Effect of fertilization frequency on flowering age and flowering duration

Fertilization frequency	Flowering age 50%	Flowering duration (days)
Control	38.3d	65.00
Once	29.6a	69.33
Two times	32.6b	69.67
Three times	36.3c	69.33
Significance	**	ns

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Table 10. Effect of fertilization frequency on okra pod number and productivity

Fertilization frequency	Number of pods per plant	Number of pods per plot (34 plants)	Weights per pod	Productivity (kg.ha^{-1})
Control	15.6b	582.0b	11.8	313.9c
Once	29.5a	889.0a	12.4	502.1b
Two times	31.5a	1020.7a	14.0	648.7a
Three times	28.6a	974.7a	12.6	560.7ab
Significance	**	**	ns	**

Note: Values followed by the same letters in a column are not significantly different according to DMRT. ns = not significant; ** = significant at α=0.01.

yield and quality of okra fruit. The experiment done by Adekiya et al. (2018) reported a decreased bulk density and increased organic matter content of the soil treated with green manure, while the soil treated with NPK fertilizer showed no increase in the organic matter. It was also reported that among the green manure used, Moringa (*Moringa oleifera*) performed well in increasing the quality, minerals, and vitamin C content while mesquite (*Prosopis africana*) performed well in increasing the yield parameters of an okra fruits. Organic fertilizers show a positive effect on the growth parameters of okra. According to Núñez-colima and Pedroza-sandoval (2018), organic fertilizer application can bring similar impacts as those earned by the single application of Nitrogen fertilizers. All biofertilizer treatments combined with organic fertilizer (cow dung) gave similar results as 60% of nitrogen fertilizer.

Conclusion

The results of this study show a notable impact resulting from varying frequencies of laying hen manure application on the growth and productivity of Naila IPB okra. Application of laying hen manure at 18 t.ha^{-1}, through two fertilization steps: first, two weeks preceding planting, followed by a second application four weeks after planting resulted the highest okra productivity.

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