

Seed Yield and Nutritional Content of *Mucuna pruriens* in Different Doses of NPK Fertiliser and Plant Density

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ABSTRACT

Mucuna pruriens is a member of Leguminosae family with the potential to be developed into a source of vegetable protein. Despite the significant potential, plant commonly has not been cultivated by good agriculture practices. Therefore, this study aimed to analyze the effect of different doses of NPK fertilizer and plant density on yield, nutritional, and bioactive content of *M. pruriens* seed. The experiment was conducted in Kuningan Regency, West Java, Indonesia (6°48'0.31"S, 108°28'17.5"E), from June to December 2024. A randomized complete block design was used, with treatments containing the combination of NPK fertilizer doses and plant density. The NPK fertilizer doses used consisted of four levels, namely 0, 0.5, 1, and 1.5 recommended doses. The recommended doses for N, P₂O₅, and K₂O were 112.5, 90, and 108 kg/ha, respectively, with plant densities of 1, 2, and 3 seeds per planting hole. There were 12 treatment combinations, and each was repeated three times. The results showed that the treatment had a significant effect on *M. pruriens* yield, sugar, and starch content, as well as antioxidant activity (P<0.05). The combination of 1.5 recommended doses (168.75 kg N/ha, 135 kg P₂O₅/ha, and 162 kg K₂O/ha) and 1 seed per hole gave a higher number of pods per plant, pod weight per plant, and seed weight per plant although not significantly

different from 1 and 0.5 recommended doses with the same plant density. This combination of treatments also produced higher starch and sugar levels. Seed protein content was not affected by the treatment, including total phenol and flavonoid levels. Furthermore, the highest antioxidant activity was obtained in the combination of 1.5 recommended doses and 3 seeds per hole.

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INTRODUCTION

Mucuna pruriens is one type of plant from the Leguminosae family that is still underutilized. This plant is known as velvet bean and “koro benguk” in Indonesia, which is rarely consumed as food compared to another legume plant like soybeans with potential as raw materials for making tofu, tempeh, and soy sauce. The use of *M. pruriens* seed includes as raw materials for making tempeh and tofu (Gravitiani et al., 2022), although the level of public preference for koro tempeh is still lower than soybean tempeh (Suwasono et al., 2022).

In addition to serving as a source of vegetable protein, *M. pruriens* seed has significant medicinal properties, including antidiabetic, aphrodisiac, antimicrobial, and anti-inflammatory (Rai et al., 2020). The use of *M. pruriens* seed as a medicinal plant and a source of protein is due to the presence of bioactive content such as alkaloids, flavonoids, tannins, saponins, and phenols (Jadhav et al., 2022). This bioactive content shows potential of *M. pruriens* as a source of antioxidants that are beneficial for human health (Jimoh et al., 2020). Due to its bioactive contents, *M. pruriens* can be classified as functional food. There were some definitions of functional food by the expert. In general, functional foods contain ingredients that provide health benefits (Baker et al., 2022).

Due to the potential of *M. pruriens* as a functional food which is still underutilized, several efforts are needed to increase the yield and seed quality. One important aspect of cultivation is fertilization, which should be performed in appropriate dose to enhance plant growth and optimal production. Fertilizer provides important nutrients for plants such as N, P, and K which are essential macronutrients. The availability of these nutrients in sufficient quantities can support plant growth and yield. The functions of the three nutrients include supporting vegetative growth, increasing CO₂ assimilation and photosynthesis, and playing a role in seed formation (Zewdie & Hassen, 2021). However, excessive application can affect content and quality of seed. Previous studies on wheat reported that increasing nitrogen level caused a rise in seed protein content (Nasiroleslami et al., 2021). Excess of nitrogen will reduce bioactive content and antioxidant capacity, affecting the composition of fatty acid in seed (Elhanaf et al., 2019). The decrease in bioactive content and antioxidant activity is often caused by increasing levels of nutrients, reducing the activity of the PAL enzyme which catalyzes the formation of phenolic compound precursors (Li et al., 2021).

High crop yields can be obtained by increasing the number of plant populations per unit area to a certain limit. However, increasing the population or high density reduced plant biomass, yield caused by a lower photosynthetic rate and low nutrients (Postma et al., 2021). Previous studies have shown that increasing the population of soybean plant due to increasing the number of seed per hole can reduce the number of pods and the weight of soybean seed (Xu et al., 2021). This showed the need to determine the right population to optimize the yield of *M. pruriens* by regulating the number of seed per hole.

MATERIALS AND METHODS

Study Area

The study was conducted at Kuningan District, West Java, Indonesia at coordinates 6°48'0.31" S, 108°28'17.5" E from June to December 2024. This location has an altitude of 296 m above sea level. Laboratory analysis was carried out at the Laboratory of Department of Agronomy and Horticulture IPB University (Seed nutrient, starch, sugar, protein, phenolic, and flavonoid content), Integrated Laboratory IPB (Fatty acid content), Food and Biochemistry Laboratory Universitas Negeri Sebelas Maret (Fiber content and antioxidant activity), and Laboratory of Plant Physiology, Universitas Swadaya Gunung Jati (Sample preparation).

Materials

The material used were *M. pruriens* seed obtained from wild *M. pruriens* plant at Banyumas District, Central Java, Indonesia. Fertilizer as source of nitrogen, phosphorus, and potassium used urea, SP-36, and KCl, respectively. Furthermore, the bamboo used as a pole to support growth of plant.

Procedures

The experiment used a randomized complete block design, with treatment containing the combination of NPK fertilizer dose and plant density. Dose of fertilizer consisted of four levels, namely 0, 0.5, 1, and 1.5 recommended dose. Moreover, the recommended doses for N, P₂O₅, and K₂O were 112.5, 90, and 108 kg ha⁻¹, respectively, based on doses of NPK fertilizer that was used in cowpea (Gustiningsih et al., 2023). Urea, SP-36, and KCl were used as source of nutrient with rates 250, 250, and 180 kg ha⁻¹, respectively. Plant density consisted of three levels, namely 1, 2, and 3 seeds per planting hole and each treatment combination was repeated three times. There were 36 experimental units and each unit was a plot of 2 m × 3 m. Furthermore, 12, 24, and 36 plants were put in the treatment 1, 2, and 3 seeds per planting hole, respectively.

Seed was planted with a spacing of 100 cm × 50 cm. Phosphorus and potassium fertilizer were applied at 3 weeks after planting. Nitrogen fertilizer was applied twice at 3 and 5 weeks after planting with 50% dose in each application.

The pods were harvested starting at 18 weeks after planting and harvested four times. Subsequently, the criteria of pods that could be harvested was brownish colored pods. The yield observation included pods number per plant, pods weight per plant, and seed weight per plant, as well as weight of 100 seeds. These pods were harvested from all plants in each plot.

The observation of nutritional and bioactive content in *M. pruriens* seed included nutrient content (N (titrimetric), P (spectrophotometric), and K (AAS)), protein (Calculation by 5.30 as factor) (Mariotti et al., 2008), crude fat (AOAC 2005: 4.5.06),

starch (spectrophotometric), sugar (spectrophotometric), and crude fiber (gravimetric). Other contents included fatty acid profile (AOAC (2012): 969.33), total phenolic (Folin-Ciocalteau), and total flavonoid (Aluminum chloride colorimetric) (Vongsak et al., 2013), and antioxidant activity (DPPH).

Data Analysis

The data were analyzed using analysis of variance (ANOVA) at $\alpha = 5\%$. When treatment had a significant effect, the analysis continued with DMRT post hoc with $\alpha = 5\%$, although fatty acid profile was not analyzed statistically.

RESULTS AND DISCUSSION

Soil Properties and Climate Conditions at Experimental Location

The result of soil analysis showed pH value of 5.95 (slightly acid), content of C-organic was 1.48% (low), and N-total was 0.16% (low). Meanwhile, the P₂O₅ content was 73.43 mg 100 g⁻¹ (very high) and K₂O was 19.43 mg 100 g⁻¹ (moderate). The soil texture at the study location was silty clay loam (Table 1). Generally, nitrogen is a macro essential nutrient that is needed by plant for

vegetative growth. *M. pruriens* that belongs to Leguminosae has the ability to fix nitrogen from the air into a form available to plant (Magaddelela et al., 2021). Phosphorus and potassium are also essential macronutrients needed by plant. Specifically, phosphorus is one of the components of nucleic acid and phospholipids (Lambers, 2022). Potassium plays a role in several biochemical processes such as stomatal regulation, photosynthesis, and increasing plant tolerance to abiotic stress (Johnson et al., 2022).

In the early of plant growth, rainfall conditions were low because it was in the dry season with an average rainfall of 131.85 mm month⁻¹. Therefore, watering was carried out every day to prevent drought stress. When plant entered harvest time, rainfall was approximately 488 mm month⁻¹ or in the rainy season. There were no significant pests and diseases attacking plant, thereby no control activity was not carried out.

Yield Component of *M. pruriens*

The yield component of *M. pruriens* seed production was pod number, pod weight, weight of 100 seeds, and seed weight per plant. These components affected the yield of *M. pruriens*,

Table 1
Soil properties at experimental location

Parameters	Value	Status
pH H ₂ O	5.95	Slightly acid
C-organic (%)	1.48	Low
N-total (%)	0.16	Low
P ₂ O ₅ (mg 100 g ⁻¹)	73.43	Very high
K ₂ O (mg 100 g ⁻¹)	19.43	Medium
Soil Texture		
Clay (%)	65	Silty Clay Loam
Silt (%)	28	
Sand (%)	7	

including the treatment combinations of NPK fertilizer dose and plant density, as shown in Table 2. The combinations of 1.5 recommended dose and 1 seed per plant hole produced the highest value. Yield component of *M. pruriens* tended to increase with high fertilizer doses. In each dose of NPK fertilizer, there was a decrease of yield component with increasing plant density. Weight of 100 seeds was not affected by treatment.

High plant density caused competition for nutrients, water, and sunlight for plant. The high competition caused decreasing of plant growth and yield. Nutrient competition also occurs when plant population increases. The previous studies on white lupine showed that lower plant density increased the number of seed per pod and seed weight per plant (Tobiasz-Salach et al., 2023). High number of seed per plant hole also increases intraspecific competition. At low plant density, the rate of photosynthesis per plant increases, thereby the supply of C to the root nodules has an impact on increasing nodulation and the rate of nitrogen fixation (Luca & Hungria, 2014).

Table 2
Effect of NPK fertilizer and plant density on yield component of *M. pruriens*

Treatment	Pod number per plant	Pod weight per plant (g)	100 seeds weight (g)	Seed weight per plant (g)
0 RD, 1 seed	35.6 ± 9.57 bc	156.28 ± 38.59 bc	78.17 ± 3.47 a	85.16 ± 21.38 bc
0 RD, 2 seed	19.9 ± 10.12 ab	90.52 ± 36.47 ab	74.36 ± 4.92 a	47.57 ± 23.25 ab
0 RD, 3 seed	16.0 ± 3.90 a	67.01 ± 17.30 a	72.77 ± 4.31 a	42.67 ± 15.77 ab
0.5 RD, 1 seed	50.4 ± 20.97 cd	233.37 ± 117.14 cd	75.95 ± 5.59 a	125.79 ± 63.86 cd
0.5 RD, 2 seed	18.7 ± 1.83 ab	79.88 ± 9.00 ab	75.29 ± 2.82 a	43.72 ± 5.46 ab
0.5 RD, 3 seed	13.9 ± 4.55 a	54.25 ± 17.75 a	69.37 ± 3.87 a	29.92 ± 10.08 a
1 RD, 1 seed	52.5 ± 15.09 cd	217.77 ± 58.65 cd	75.06 ± 2.78 a	119.95 ± 31.18 cd
1 RD, 2 seed	21.9 ± 3.77 ab	96.27 ± 14.53 ab	76.60 ± 3.76 a	52.78 ± 8.40 ab
1 RD, 3 seed	12.8 ± 10.69 a	54.75 ± 46.02 a	74.99 ± 8.27 a	30.89 ± 26.52 a
1.5 RD, 1 seed	61.1 ± 21.11 d	260.23 ± 96.88 d	74.74 ± 4.40 a	141.13 ± 53.37 d
1.5 RD, 2 seed	23.9 ± 3.57 ab	103.91 ± 22.22 ab	69.16 ± 1.65 a	57.12 ± 11.37 ab
1.5 RD, 3 seed	16.7 ± 5.80 a	71.90 ± 22.54 ab	73.38 ± 6.99 a	39.65 ± 12.60 ab

Note. The number followed by the same letter in a column is not significantly different by the DMRT test at the level of $\alpha=5\%$. Numbers were followed by $\pm$ standard deviation. RD = recommended dose of NPK fertilizer

Nutritional dan Bioactive Content of *M. pruriens* Seed

The nitrogen and phosphorus content in *M. pruriens* seed were not affected by dose of fertilization and plant density. More importantly, the potassium content was found to be higher in the control treatment (without fertilization) in all treatments of the number of seed per planting hole (Table 3). The potassium accumulation in seed function plant resistance to oxidative stress (Johnson et al., 2022). When plant is not fertilized, there is a tendency to face nutrient stress, leading to the accumulation of potassium in the seed.

Table 3
Effect of NPK fertilizer and plant density on nutrient content of *M. pruriens* seed

Treatment	N (%)	P (%)	K (%)
0 RD, 1 seed	4.57 ± 0.04 a	0.27 ± 0.03 a	1.66 ± 0.26 b
0 RD, 2 seed	4.47 ± 0.08 a	0.27 ± 0.01 a	1.72 ± 0.11 b
0 RD, 3 seed	4.42 ± 0.26 a	0.29 ± 0.01 a	1.86 ± 0.13 b
0.5 RD, 1 seed	4.48 ± 0.11 a	0.28 ± 0.01 a	1.35 ± 0.07 a
0.5 RD, 2 seed	4.38 ± 0.19 a	0.28 ± 0.01 a	1.15 ± 0.08 a
0.5 RD, 3 seed	4.40 ± 0.17 a	0.25 ± 0.02 a	1.20 ± 0.10 a
1 RD, 1 seed	4.34 ± 0.17 a	0.26 ± 0.02 a	1.22 ± 0.10 a
1 RD, 2 seed	4.76 ± 0.30 a	0.26 ± 0.03 a	1.24 ± 0.03 a
1 RD, 3 seed	4.58 ± 0.34 a	0.27 ± 0.03 a	1.29 ± 0.20 a
1.5 RD, 1 seed	4.60 ± 0.10 a	0.28 ± 0.04 a	1.27 ± 0.14 a
1.5 RD, 2 seed	4.50 ± 0.16 a	0.25 ± 0.00 a	1.35 ± 0.10 a
1.5 RD, 3 seed	4.61 ± 0.14 a	0.27 ± 0.03 a	1.21 ± 0.08 a

Note. The number followed by the same letter in a column is not significantly different by the DMRT test at the level of $\alpha=5\%$. Numbers were followed by $\pm$ standard deviation. RD = recommended dose of NPK fertilizer

The nitrogen content in seed is related to protein content (Lu et al., 2020). Based on Pearson correlation analysis, there was a positive correlation between protein and nitrogen ($P < 0.01$, $r = 0.650$) (Figure 1). The nitrogen and phosphorus content in seed was also positively correlated with the weight of 100 seeds with correlation coefficients of $r = 0.404$ and $r = 0.503$, respectively. In this study, protein content was not significantly different between treatments (Table 4). This was because the nitrogen content in seed did not differ between treatments. Previous studies showed that the enhanced protein levels were affected by nitrogen delivery to seed (Lu et al., 2020).

Legume plants are able to fix free nitrogen from the air, thereby meeting their needs. The ability to fix atmospheric nitrogen shows the potential of plant to be included in low-input agricultural systems (Kebede, 2021). In this study, protein content of *M. pruriens* ranged from 22-25%. Previous studies have shown that protein content of white *M. pruriens* seed was 28.82 % and black was 26.26% (Baby et al., 2023). However, protein content of *M. pruriens* was lower than soybeans, which ranged 33-37% (Kudelka et al., 2021).

Crude fat content of *M. pruriens* seed was affected by combination of fertilizer dose and plant density, although there was no clear trend regarding those treatment. The highest fat content was found in the 1 recommended dose and 2 seeds per plant hole. At the same dose, the treatment of 1 and 3 seeds per plant hole gave the lowest crude fat content. In this study, crude fat content was 2.21-6.92% in line with previous report, where content of lipids in *M. pruriens* seed without processing was 4.69% (Ezegbe et al., 2023).

The analysis of fatty acid profile in *M. pruriens* showed that linoleic acid was found in higher concentrations (Figure 2), followed by palmitic acid. The composition of linoleic acid

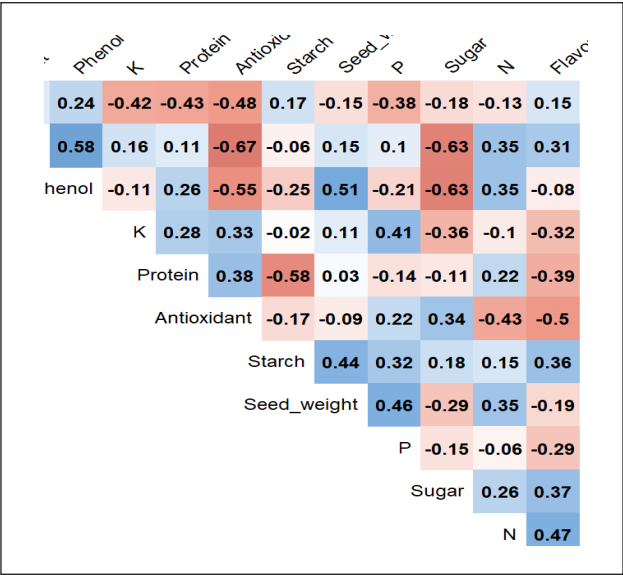


Figure 1. Pearson correlation analysis of seed weight and nutritional content of *M. pruriens* seed

Table 4
Effect of NPK fertilizer and plant density on protein, crude fat, and starch content of *M. pruriens* seed

Treatment	Protein (%)	Crude fat (%)	Starch (%)
0 RD, 1 seed	24.23 ± 0.16 a	5.03 ± 0.20 cd	8.97 ± 0.20 bcd
0 RD, 2 seed	23.71 ± 0.43 a	4.97 ± 0.24 cd	7.23 ± 1.15 ab
0 RD, 3 seed	23.46 ± 1.38 a	5.00 ± 1.21 cd	7.04 ± 1.02 ab
0.5 RD, 1 seed	23.73 ± 0.59 a	5.78 ± 0.43 de	7.92 ± 1.41 abc
0.5 RD, 2 seed	23.21 ± 0.99 a	5.07 ± 0.47 cd	7.43 ± 2.10 ab
0.5 RD, 3 seed	23.34 ± 0.89 a	5.24 ± 0.47 cd	7.63 ± 1.41 ab
1 RD, 1 seed	23.06 ± 0.97 a	2.82 ± 0.67 a	7.54 ± 0.58 ab
1 RD, 2 seed	23.54 ± 0.44 a	6.57 ± 0.39 e	6.98 ± 0.24 ab
1 RD, 3 seed	23.16 ± 1.12 a	3.67 ± 0.34 ab	9.72 ± 0.09 cd
1.5 RD, 1 seed	22.54 ± 0.06 a	4.69 ± 1.06 bcd	10.55 ± 0.59 d
1.5 RD, 2 seed	23.82 ± 0.84 a	4.54 ± 0.73 bc	5.87 ± 1.50 a
1.5 RD, 3 seed	24.45 ± 0.77 a	3.75 ± 0.79 ab	5.90 ± 1.25 a

Note. The number followed by the same letter in a column is not significantly different by the DMRT test at the level of $\alpha=5\%$. Numbers were followed by $\pm$ standard deviation. RD = recommended dose of NPK fertilizer

was relatively the same in each treatment, which was approximately 34.5%. Meanwhile, palmitic acid had a higher concentration in the 0.5 of the recommended doses with 1 seed planted per hole (Figure 2). The presence of linoleic acid, palmitic acid, and oleic acid in *M. pruriens* seed has the potential to be used as a source of vegetable oil (Baby et al., 2023). Linoleic acid is included in the n-6 polyunsaturated fatty acid (PUFA) with 18

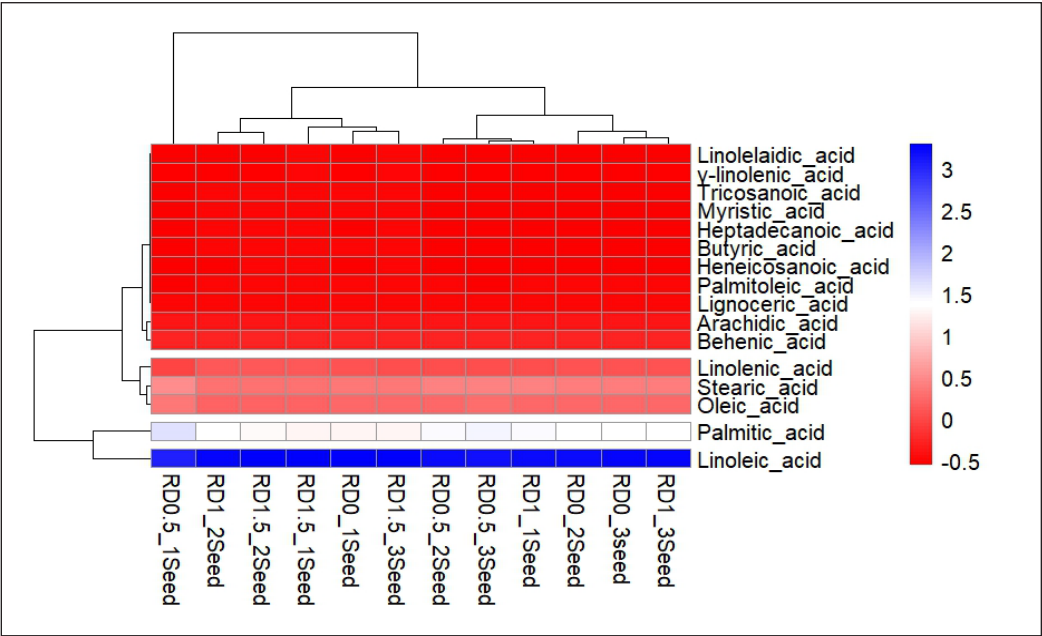


Figure 2. Heatmap profile of fatty acid in *M. pruriens* seed
Note. RD = Recommended dose

carbon atoms. It is also found in high concentrations in nuts and lower concentrations in some cereals including legumes (Marangoni et al., 2020).

Starch is a form of carbohydrate storage in seed (MacNeill et al., 2017). The highest starch content is found in the treatment of 1.5 recommended doses and 1 seed per hole. Increasing the number of seed per hole decreases the starch content in seed. The decrease in starch levels with increasing plant density is attributed to competition between plant which has an impact on reducing the rate of photosynthesis and its derivative product such as starch. Like protein, starch is positively correlated with the weight of 100 seed ($P < 0.05$, $r = 0.330$). Starch plays a role during seed development (Liu, Luo et al., 2021).

Sugar (sucrose) is the product of photosynthesis in plant (Aluko et al., 2021) and is converted into stored starch during seed development (Li et al., 2022). Based on the ANOVA results, the sugar content in *M. pruriens* seed was affected by fertilizer dose treatment. Compared to potassium content in seed, the highest sugar content was found in the treatment of 1.5 recommended doses (Table 5). In this study, there was a negative correlation between sugar and fat content ($P < 0.01$, $r = -0.509$). Fat content in seed is mainly synthesized from sugar (Kaur et al., 2021). Therefore, high sugar accumulation reduces the fat content in seed.

The crude fiber content in *M. pruriens* seed was affected by the treatment. Based on the results, the highest crude fiber content was found in the treatment of 0.5 recommended doses

Table 5
Effect of NPK fertilizer and plant density on sugar and crude fiber content of *M. pruriens* seed

Treatment	Sugar (%)	Crude fiber (%)
0 RD, 1 seed	5.11 ± 0.26 ab	5.25 ± 0.14 ab
0 RD, 2 seed	4.90 ± 0.27 ab	5.49 ± 1.62 ab
0 RD, 3 seed	4.94 ± 0.42 ab	4.75 ± 0.32 a
0.5 RD, 1 seed	4.87 ± 0.33 a	4.36 ± 0.77 a
0.5 RD, 2 seed	4.97 ± 0.05 ab	6.86 ± 0.50 c
0.5 RD, 3 seed	5.13 ± 0.61 ab	6.93 ± 0.59 c
1 RD, 1 seed	5.73 ± 0.10 b	5.42 ± 0.47 ab
1 RD, 2 seed	4.76 ± 0.19 a	6.07 ± 0.41 bc
1 RD, 3 seed	6.72 ± 0.47 c	6.28 ± 0.44 bc
1.5 RD, 1 seed	6.99 ± 0.55 c	5.32 ± 0.50 ab
1.5 RD, 2 seed	6.71 ± 0.59 c	5.32 ± 0.54 ab
1.5 RD, 3 seed	6.93 ± 0.70 c	4.51 ± 0.16 a

Note. The number followed by the same letter in a column is not significantly different by the DMRT test at the level of $\alpha=5\%$. Numbers were followed by $\pm$ standard deviation. RD = recommended dose of NPK fertilizer

with 2 and 3 seeds per planting hole, but not significantly different from 1 recommended dose. Increasing fertilizer dose to 1.5 recommended doses reduced the crude fiber content in seed. The components of crude fiber include cellulose and lignin (Liu et al., 2022; Yang et al., 2021). Previous studies on rice showed that the addition of nitrogen fertilizer reduced the cellulose level in the endosperm (Midorikawa et al., 2014). Lignin, cellulose, and hemicellulose also decreased with the increase of nitrogen fertilizer in maize (Liu, Gu et al., 2021).

The development of *M. pruriens* seed into a functional food needs to consider bioactive content that has the potential to act as an antioxidant. Previous studies have shown that *M. pruriens* has a function as an antioxidant, antidiabetic, and anti-inflammatory agent (Kumbhare et al., 2023; Yadav et al., 2024). The main compounds that function as antioxidant includes phenolics and flavonoids. Total flavonoids and total phenolics in *M. pruriens* seed were not affected by treatment (Table 6). However, antioxidant activity was affected by the treatment of fertilizer doses and the number of seed per plant hole. The highest antioxidant activity was found in the treatment of 1.5 recommended doses and 3 seeds per hole. Furthermore, the average percentage of inhibition of DPPH free radical compounds produced by antioxidant compounds in *M. pruriens* seed was 90.97%. The high antioxidant activity in *M. pruriens* seed allows for incorporation into human diet to promote healthy living (Jimoh et al., 2020). In this study, the antioxidant activity of *M. pruriens* seed did not correlate with the phenolic and flavonoid content (Figure 1), which varied significantly from several previous reports.

Table 6.
Effect of NPK fertilizer and plant density on total phenolic content, total flavonoid content, and antioxidant activity of M. pruriens seed

Treatment	TPC (mg GAE 100 g ⁻¹ dw)	TFC (mg QE 100 g ⁻¹ dw)	Antioxidant activity (% inhibition)
0 RD, 1 seed	4221.20 ± 503.82 a	62.87 ± 11.11. a	91.51 ± 1.08 bc
0 RD, 2 seed	4078.00 ± 455.38 a	64.75 ± 5.09 a	91.48 ± 0.68 bc
0 RD, 3 seed	3849.14 ± 385.40 a	59.15 ± 1.79 a	91.29 ± 0.28 bc
0.5 RD, 1 seed	4089.88 ± 477.87 a	65.47 ± 6.08 a	90.89 ± 0.52 bc
0.5 RD, 2 seed	4194.04 ± 356.85 a	61.82 ± 6.12 a	91.03 ± 0.69 bc
0.5 RD, 3 seed	3978.34 ± 272.83 a	67.61 ± 5.95 a	90.04 ± 1.42 ab
1 RD, 1 seed	4061.95 ± 140.60 a	60.77 ± 1.22 a	91.48 ± 0.28 bc
1 RD, 2 seed	4623.95 ± 968.47 a	69.26 ± 20.26 a	88.90 ± 0.76 a
1 RD, 3 seed	3849.14 ± 514.03 a	64.78 ± 13.10 a	91.02 ± 0.12 bc
1.5 RD, 1 seed	3763.21 ± 100.93 a	75.95 ± 10.95 a	90.55 ± 1.27 bc
1.5 RD, 2 seed	3906.86 ± 282.87 a	70.10 ± 9.39 a	91.56 ± 0.38 bc
1.5 RD, 3 seed	3946.23 ± 171.05 a	61.90 ± 5.82 a	91.93 ± 0.32 c

Note. The number followed by the same letter in a column is not significantly different by the DMRT test at the level of $\alpha=5\%$. Numbers were followed by $\pm$ standard deviation. RD = recommended dose of NPK fertilizer; TPC = Total phenolic contents; TFC = Total flavonoid contents; GAE = Gallic acid equivalent; QE = Quercetin equivalent; dw = dry weight

CONCLUSION

In conclusion, this study showed that an increase in plant density decreased seed yield of *M. pruriens*. The combination of 1.5 recommended doses (168.75 kg N/ha, 135 kg P₂O₅/ha, and 162 kg K₂O/ha) and 1 seed per plant hole gave a higher number of pods per plant, pod weight per plant, and seed weight per plant. These treatments combination also produced higher starch and sugar levels in the seed. Protein content was not affected by the treatment, including total phenol and flavonoid levels. The highest antioxidant activity was obtained in the combination of 1.5 recommended doses and 3 seeds per hole. Moreover, further studies were recommended to analyze the effect of organic fertilizer on the yield and quality of *M. pruriens* seed.

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