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Comparison of growth and yield performances of horsegram (*Macrotyloma uniflorum* L.) supplemented with organic and inorganic fertilizers

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***Abstract*—**Horsegram is an underutilized legume with greater potential to utilize as food and feed. In the Sri Lankan context, no fertilizer recommendations have been developed, as such impede commercial cultivation of horsegram. Hence, a field experiment was carried out to investigate the crop performance of two varieties viz; ANKK -black and ANKK-brown under different regimes of fertilizer applications. The treatment comprised of the full dose of recommended inorganic fertilizer of mung bean (T1), a half dose of recommended inorganic fertilizers (T2), compost mix (T3), and control treatment (T4) were laid down in a split-plot design replicated three times. The results revealed that variety ANKK-brown responded superior to the ANKK-black. The highest chlorophyll content (44.3), dry weight of shoot (29g Plant^{-1}), root (1.97g plant^{-1}), pods (160 plant^{-1}), and mean grain yield (1877.8 kg ha^{-1}) resulted in T1. However, a significantly increased number of root nodules (42 plant^{-1}) were produced with T3. Moreover, there was a positive association between chlorophyll content versus the number of pods per plant (0.45), shoot (0.52) and root (0.53) dry matter production. Therefore, the findings suggest that the horsegram can either be successfully grown with NPK inorganic fertilizers of 35 kg/ha urea, 100 kg/ha muriate of potash (MOP) and 75 kg/ha triple super phosphate (TSP)] or be with the application of organic manures at the rates of 10,000kg/ha (T3) without significant compromise in crop yields.

***Keywords*—**Chlorophyll content, Compost, Horsegram, Inorganic fertilizer, Root nodules.

I. Introduction

Horsegram (*Mycrotyloma uniflorum* L.) belonging to the family Leguminosae is an underutilized legume crop that thrives well under harsh soil conditions and it has diversely dispersed across South Asian region (Pavithra *et al*., 2020) and African continent. It is consumed as a food supplement rich in protein and carbohydrates (Raut *et al*., 2016) and also consist of increased level of lysine, an essential amino acid that makes the crop ideal feed for ruminants. In addition, horsegram exerts greater medicinal properties to minimize the risk of cardiovascular and heart-related disorders, treatment of kidney and urinary diseases (Ravishankar Vishnupriya, 2012). In Sri Lanka, this crop is widely cultivated in the districts of Monaragala, Hambantota and Badulla predominantly adopted with the newly released local varieties *viz*; ANKK Black and ANKK Brown.

Agronomically, horsegram can be excelled being as an intercrop with cereals, vegetables cultivations, and with grasses (Kumar, 2015) as it fixes the atmospheric nitrogen through the root nodules, as such improves soil nutrient contents. However, the crop suffers from substantial nutrient deficiency and plays significant losses in crop growth and yield potential. The deficiency of nitrogen (N), phosphorus (P), and potassium (K) in the soil that can lead to retarded growth and yield performances (Pal *et al*., 2006). Moreover, nutrient deficiencies that lead to cause premature flower drop and fruit setting and phytohormonal imbalances (Kumar, 2015). Hence, low soil nitrogen levels can be overcomed through the application of nitrogen fertilizer at a low dosage that prompts the root nodulation and induces the growth of vigorous horse gram canopies (Xia *et al*., 2017). To compensate for such negative effects, the basal application of fertilizer seems to promote vegetative growth and yield characteristics (Raut *et al*., 2016). However, in the local context, this crop is being cultivated in farmlands as a neglected crop with no fertilizers resulting in lower crop productivity. Thereby, investigating the optimal application rates of organic and/or inorganic fertilizers ensure crop growth and sustainable grain production.

The application of inorganic fertilizers certainly promotes crop productivity however, indiscriminate use of inorganic fertilizers that cause widespread damages to the agricultural lands, watercourses, wildlife and human health (Sha *et al*., 2019). Thereby, conserving the environment is utmost

requirement while maintaining crop productivity with the use of green technologies is an important. Alternatively, recent studies have revealed that the application of organic fertilizers has improved the crop growth in soybean (Rahayu *et al*., 2021), pigeon pea (Behera *et al*., 2012), velvet beans (Kavitha *et al*., 2008), cowpea and horsegram (Kumar 2015), and in rice-horsegram cropping systems (Pal *et al*., 2006). Considering these facts, judicial assessment of fertilizers is required prior to recommending fertilizers to the farmers and crop growers. However, with regards to horsegram, there is no fertilizer recommendation given by the Department of Agriculture (DOA) in Sri Lanka as crops are cultivated to a small extent in the country. Thereby, it is paramount to explore application rates of fertilizer dosage and to promote the cultivation of horsegram that can be promotes as one of the potential crops for global food security. Hence, the objectives of the study were to evaluate the effects of organic and inorganic fertilizers among two varieties of horsegram and to assess the growth and yield performances under field conditions. Further, this research will pave the way to take the initial step for a fertilizer recommendation for such underutilized crops.

II. Matherials and method

A. Experimental site

The research was carried out from November, 2020 to February, 2021 at Grain Legume and Oil Crops Research and Development Center (GLOCRDC), Agunakolapelessa. The experimental site belonging to Dry zone low country (DL1b) agro-ecological zone (6.16° N, 80.90° E) in Sri Lanka with reddish brown earth soil. It is located at 98 m mean sea level. The average temperature of the study area is 27.3 °C with annual rainfall of 1289 mm.

Agronomic practices Horsegram seeds of two varieties (ANKK Black and ANKK Brown) were used in this experiment as planting materials. The field experiment was laid out in a split plot design with two- factors, viz; the varieties and different levels of fertilizer applications, thereby making 4 treatment combinations replicated thrice in open field conditions. The main plot treatments were comprised of ANKK Black and ANKK brown varieties. The sub plot treatments were comprised of four fertilizer applications: T1; Full dosage of NPK inorganic fertilizers [35 kg/ha urea, 100 kg/ha Muriate of potash (MOP) and 75 kg/ha triple super phosphate (TSP)], T2; Half dosage of NPK inorganic fertilizers, [17.5 kg/ha urea, 50 kg/ha Muriate of potash (MOP) and 37.5 kg/ha triple super phosphate (TSP)] T3; compost mixture (cow dung: straw: Gliricidia sepium at 1:1:1) applied at the rates of 10,000kg/ha and T4; no any fertilizer as absolute control . The size of main plot was 6 × 8 m (48 m²) and the sub plot was 3 ×2 m (6 m²) respectively. The seeds were sown at 20 cm × 40 cm spacing per experimental plot. Subsequently, the fertilizer basal dose for each plot was calculated and applied through soil as per respective treatments. Then the other cultural practices such as irrigation, regular weeding, manual pest control were followed as recommended by DOA to provide the vigorous crop growth.

B. Data Collection

The data collection was commenced from the onset of 50% flowering using of horsegram varieties by five randomly selected plants per subplot. The plant height, number of leaves per plant, number of branches per plant were recorded, while the chlorophyll content of trifoliate leaf was taken by using chlorophyll meter (SPAD 502, Spectrum technologies). At 60 days after sowing, the number of root nodules were counted by obtaining 3 plants per plot by carefully uprooting the plants, cleaned delicately and counted the root nodules. At physiological maturity stage (100 days after seed sowing), horsegram pods were harvested manually from all plants within a 6 m2 of each plot. The pod length, number of pods per plant, seeds per pod, and the 1000-seed weight were measured by taking ten plants per plot. Then obtained ten randomly selected mature pods to record the average number of grains produced per pod. For obtaining 1000-seed weight, the harvested seeds were sun dried untill reach 10% moisture content, then 1000 seeds were weighed three times and the average weight was calculated. Finally, shoot and root dry matter content was recorded by oven drying samples at 70^{o}C until constant dry weight was obtained (Nagoor, 2013).

C. Data Analysis

The data were analyzed using SPSS package version 25. Prior to the data analysis, the test were performed on constant variance to ensure the data followed the normal distribution. Thereafter, data were subjected to statistical analysis to perform Two-way ANOVA in multiple comparisons of means. Post hoc (Tukey's pair-wise) were performed to compare the means at 5% significant level. Pearson's correlation analysis was carried out to evaluate strength of the linear relationship between tested variables.

III. Results

A. Effect of fertilizers on growth parameters of horsegram

According to the data, there were no significant differences ($p>0.05$) denoted among the tested varieties for their plant height, number of leaves per plant (Table 1). Conversely, the number of branches produced at harvesting stage had showed a significant ($p <0.05$) increase in ANKK black (8/plant, $p =0.029$) than the tested counterpart. Moreover, the interesting feature of this study was the significant variation in the number of root nodules produced among the tested varieties ($p = 0.001$). Overall, the variety ANKK-brown produced the highest number of root nodules (43.5/plant), particularly the field plots treated with DOA recommended rate of compost mixture (42.3 nodules/plant, T3). While the counterpart ANKK-black exhibited the lowest nodules (18.4/plant).

Table I: Number of days taken to 50% flowering and harvesting stage among the horsegram varieties

Treatments	Days to 50% flowering Means±(SD)	Days to harvest Means±(SD)
Factor 1 (Variety)		
V1 - ANKK Black	40.92±1.49	101.00±1.28
V2 - ANKK Brown	44.75±1.57	104.00±1.28
P <0.05	0.09	0.111
Factor 2 (Treatments)		
T1	42.50±2.63	102.50±2.01
T2	42.67±2.40	102.50±2.01
T3	42.67±2.28	102.50±2.01
T4	43.50±2.34	102.50±2.01
p<0.05	0.991	1

*: The mean difference is significant at 5% confidence level

B. Days taken to 50% flowering and harvesting

The study indicated that, no significant (p >0.05) difference was seen between the main plot effects (varieties) and the effect of fertilizers treatments on the days taken to 50 % flowering (40-44 d) and the days taken to harvest of horse gram varieties (100-104 d) (Table 1).

C. Chlorophyll content and dry matter accumulation

The fertilizer treatments had triggered significant variation in chlorophyll content in horsegram crop when treated with different fertilizers (p<0.05). According to our analysis, T1 and T3 displayed significantly higher chlorophyll content than the counterpart treatments (p=0.01). This increased chlorophyll content had displayed positive effects on the yield attributes.

According to the results, the application of fertilizers had caused significant (p <0.01) difference in dry matter accumulation. Superior shoot dry matter was produced in plots treated T1 (29 g/plant) followed by T3 (22.1 g/plant) whereas the least amount of dry matter (15.4 g/plant) was produced in T2. Conversely, significant different root dry matter production was seen among tested horse gram varieties. (P=0.007). Treatment-1 recorded significantly higher dry matter content of root per plant (1.97 g/plant, p=0.03) followed by T3 and T4 (control) and least in T2 (Table 3).

D. Yield attributes of horsegram

The experimental results revealed that the application of T1 with the inorganic fertilizers statistically at par with T3 and significantly (p <0.05) improved the yield attributing characters over the remaining treatments (Table 4). T1produced the maximum number of pods per plant (160.08 g) (P=0.037) and grain yield (1877.83 kg/ha) (p= 0.017) were recorded followed by T3 which was found significantly superior over T2 and T4. In contrary, a significantly lowest grain yield (1403.17 kg/ha) was recorded in T2. There were no significant differences for 1000 seed weight were seen among the tested treatments (p =0.90).

Moreover, the Pearson's correlation matrix analysis revealed that plant growth and yield parameters were significantly correlated (p <0.05) to each other (Table 5). In particularly, root nodulation was positively associated with yield and its component characters. (p >0.05), while leaf chlorophyll content was positively associated with root dry matter (r=0.53), shoot dry matter (r=0.52) and pods per plants (r=045) suggesting that the root nodulation and subsequent fixation of nitrogen in plants exerts greater control in determining the crop growth and dry matter production.

IV. Discussion

Overall, the variety ANKK-brown produced the highest number of root nodules particularly the field plots treated with DOA recommended rate of compost mixture (42.3 nodules/plant). In contrary, ANKK-black exhibited the lowest nodules (18.4 nodules/plant). This observation may indicate that the compost-based media promotes root nodulation as a result of providing idealized level of soil physiological and chemical properties as such that assist to maintain optimum temperatures, porosity and the exchange of oxygen and carbon dioxide gas within the soil and idealized soil pH (Goss *et al*., 2013). However, the disparity in the nodulation between two varieties may indicate as the intrinsic plant root factors such as the level of phytohormones particularly the auxin and cytokinin play an instrumental role in determining root symbiosis and nodulation through the exchange of molecular signals between the legume root and the microsymbiont (Hungriaa, Vargas, 2000; Desbrosses Stougaard, 2011). Our experimental results also indicated that the variety ANK-brown might have expressed the idealized amount of PGR that promote root nodulation pathways in horsegram. The level of nitrogen supplied by the rooting media is paramount in determining the growth and development of root nodules. Zaharan (1999) reported that nodulation and growth of soybean is significantly increased when N was applied at low levels, while Xia *et al*., (2017) found in soybean that the threshold level of nitrogen was 50 mg/l, while, at higher level of nitrogen tent to decline the root nodulation in pigeon pea (Behera *et al*., 2012). With regards to our findings, T3 produced the highest nodulation, indicating that the level of nitrogen in compost mixture may be optimum for horsegram root nodulation. While in contrary, crop treated with inorganic fertilizer (T1), the nitrogen level may slightly higher for root nodulation and exhibited retard in nature (Table 2). Hungriaa, and Vargas, (2000) reported that the compatible bacterial strain selection has been an integral part of nodule formation in both common bean and soybean cultivation. This may also be true for the horsegram trials as the inoculation of native Rhizobium bacterial communities were used in the present experiment which seems compatible for ANKK-brown, though less compatible for the variety ANK-black, hence in response caused lower number nodule were formed.

Chlorophyll content is one of the important leaf level parameters which is directly influenced by the level of nutrient (nitrogen) application and SPAD meter measurements serve as an indicator of crop nutrient balance. In this experiment, the highest chlorophyll content (44.3) was obtained in the field plots treated with DOA recommended inorganic fer-

Table II: Effects of different fertilizers on the growth and development of horsegram varieties

Treatments	Plant height Mean± (SD) cm		Number of branches Mean± (SD)		No of Leaves per plant Mean± (SD)	No. of nodules per plant Mean± (SD)	Pod length Mean± (SD) cm
	At 50% flowering	At harvest	At 50% flowering	At harvest	At 50% flowering	At 60 days	At harvest
Factor 1 (Varity)							
ANKK Black	56.09±5.21	122.60±3.69	4.32±0.20	8.19±0.20	28.78±2.72	18.42±2.75	5.69±0.06
ANKK Brown	68.44±6.62	128.67±3.42	4.48±0.26	7.33±0.31	34.20±3.03	43.166±4.99	5.82±0.07
p<0.05	0.157	0.241	0.637	0.029*	0.197	0.001**	0.183
Factor 2 (Fertilizers)							
T1	61.68±8.17	124.65±5.27	4.75±0.21	7.52±0.53	32.93±3.17	32.50±10.54	5.69±0.09
T2	66.77±11.82	120.30±5.86	4.18±0.40	8.42±0.36	31.02±5.14	17.50±3.57	5.75±0.01
T3	59.95±6.83	132.60±3.58	4.33±0.43	7.72±0.33	30.48±4.47	42.33±7.19	5.76±0.10
T4	60.67±9.14	124.98±5.33	4.32±0.24	7.40±0.32	31.52±4.67	30.83±5.27	5.82±.09
p<0.05	0.952	0.408	0.663	0.290	0.982	0.130	0.824

.*The mean difference is significant at 0.05 confidence level, ** significant at 0.01% confidence level respective

Table III: Effects of different fertilizers on chlorophyll content and biomass production of horsegram varieties

Treatments	Leaf chlorophyll content (SPAD units) Means ± SD	Shoot dry matter (g/Plant) Means ± SD	Root dry matter (g/Plant) Means ± SD
Factor 1 (Variety)			
ANKK Black	40.66±0.89	19.57±2.31	1.27±0.17
ANKK Brown	40.82±0.79	22.94±1.97	1.64± 0.13
p<0.05	0.097	0.280	0.007**
Factor 2 (Fertilizers)			
T1	44.37±0.55	29.01±1.73	1.97±0.19
T2	38.52±1.08	15.47±2.48	1.19±0.26
T3	40.63±0.49	22.18±1.92	1.49±0.15
T4	39.45±0.77	18.36±3.08	1.18±0.14
p<0.05	0.01**	0.004**	0.030*

* The mean difference is significant at the 0.05 level, ** significant at the 0.01 level, respectively.

Table IV: Effects of different fertilizers on the pods, seed weight and grain yield production of horsegram varieties

Treatments	Pods /Plant Means±(SD)	1000 Seeds Weight (g) Means±(SD)	Yield (kg/ha) Means±(SD)
Factor 1 (Variety)			
ANKK Black	126.68±8.26	32.90±0.53	1533.83±108.10
ANKK Brown	134.84±10.53	34.27±0.47	1585.67±66.45
p value	0.548	0.065	0.687
Factor 2 (Fertilizers)			
T1	160.08±8.65	33.47±0.80	1877.83±109.78
T2	124.43±5.74	33.58±0.79	1403.17±100.48
T3	129.12±18.55	33.83±0.85	1501.17±137.82
T4	109.42±8.79	33.45±0.74	1456.83±47.95
p<0.05	0.037	0.906	0.017

* The mean difference is significant at the 0.05 level, ** significant at the 0.01 level, respectively.

tilizer dosage (T1). The reason behind this scenario may indicate that the inorganic nitrogen fertilizers (from urea) is readily available in the form of NH4+ to the plant root zone that may favor absorption through roots and subsequent utilization the biosynthesis of chlorophyll in green tissues. In contrary, plants treated with compost alone (T3) require some times for the release nitrogen in mild dosages in the form of NH4+/nitrate ions into soil, which may delay to synthesis chlorophyll than in T1. In a previous study with soybean, the total chlorophyll content has increased with the inorganic N-fertilizer doses that supplemented with additional organic fertilizer (Pal *et al*., 2006).

A. Effect of different fertilizers on dry matter yield attributes

Overall, our results indicated that the performance of variety ANKK-brown in vegetative and yield characteristics when provided with full dose of inorganic fertilizer treatment (T1). This observation is generally true for many crops as the commercially available inorganic fertilizers have the ability to provide instantaneously larger amount of plant nutrient in the required ion forms (anions/cations) by the root zones, which can readily promote the plant physiology and the crop yield. However, our results confirmed that the application of compost to soil promotes the microbial activities (rhizobia), as a result, an increase in root nodulation was resulted in horsegram variety ANKK-brown than the counterpart variety

Table V: Pearson correlations between growth and dry matter traits of horsegram varieties

Plant trait	Number of nodules per plant	Chlorophyll content when?	Pods / plant	Seeds/ pod	Pod length	Dry matte content - shoot	Root dry matte content	plot yield(g)	1000 seeds weight (g)	Yield (kg/ha)
No.of nodules per plant	1									
Chlorophyll content at 50% flowering	0.065	1								
Pods /plant	0.035	.453*	1							
Seeds/pod	0.241	-0.251	-0.315	1						
Pod length	-0.209	-0.238	-0.195	0.324	1					
Shoot dry matte content	0.377	0.521**	.437*	-0.009	-0.245	1				
Root dry matte content	0.088	0.528**	.415*	-0.022	-0.311	.770**	1			
Net plot yield(g)	-0.254	0.333	.678**	-0.159	0.065	0.303	.457*	1		
1000 seeds weight (g)	0.182	0.005	0.323	0.193	-0.051	-0.178	0.04	0.283	1	
Yield (kg/ha)	-0.254	0.333	.678**	-0.16	0.065	0.303	.457*	1.000**	0.283	1

*. Correlation is significant at the 0.05 level, **. Correlation is significant at the 0.01 level respectively

ANKK-black. The compost mixtures have the ability to provide plant nutrients in a slower manner in mild doses. Hence, this may attribute horse gram varieties with mild, vigorous plant growth characteristics and eventually produced the 2nd highest crop yield among the tested fertilizers. Hence, our findings are in line with the previous leguminous crop study, reveal that in velvet beans (Mucuna pruriens L) that has the greater degree of positive influence on dry matter production when treated with correct dosage of combined organic and inorganic fertilizers (Kavitha Vadivel, 2008) and in pigeon pea (Behera *et al*., 2012). Similar combine effects of organic fertilizers proved to be economically advantages in tomato cultivation in Bangladesh (Sha *et al*., 2019). Moreover, an increase number of root nodulation trigger the nitrogenase activities in root nodules in field beans and soybeans which is reported to be associated with the carbohydrate metabolism (Hungriaa Vargas, 2000).

Furthermore, our study revealed that leaf chlorophyll content was positively associated with root dry matter (0.53), shoot dry matter (0.52) and pods per plants (045) suggesting that the root nodulation and subsequent fixation of nitrogen in plants exerts greater control in determining the crop growth and dry matter production. This may assist further in horsegram plants for the enhancement rates of photosynthetic and the charbohydrate accumulations in the crops through improving plant nitrogen content.

V. Conclusion

Based on the findings, it can conclude that horsegram varieties fertilized with inorganic fertilizers with Urea (35kg/ha), MOP (100kg/ha) and TSP (75 kg/ha) resulted in the highest crop growth and yield. Hence, this fertilizer mixture can be used as the first recommended package for cultivation. However, similar yield trends can be obtained by using inorganic fertilizers supplemented with compost mixture (10,000kg/ha) without compromising horsegram yield. As such, future experiments are needed to explore the variety ANKK-brown upon carrying out large-scale multiple location trials to confirm the above observations.

VI. Author contribution

BNS developed the concept and design the experiment. SWWY Performed field trails, maintained research data, and data collection. ANMM evaluated the results analyzed data statistically and contributed to writing manuscript. ADNTK contributed to revise manuscript critically and statistical analysis. All authors read the article and approved the final version to be published.

VII. Competing Interest

The authors have declared that no competing interest exist.

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