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ORIGINAL RESEARCH

Potential of *Azolla pinnata* R. Br. green manure for boosting soil fertility and yield of terrestrial crops in Uganda: a case study of *Eleusine coracana* (L.) Gaertn

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Abstract

Azolla fern has traditionally been used over centuries as a source of nutrients for rice grown in paddies with minimal attention on crops grown terrestrially. In this study, we aimed at examining the feasibility of using *Azolla pinnata* green manure to improve soil fertility and yield of terrestrial crops using *Eleusine coracana* as a test plant. Infertile soil samples were subjected to five *A. pinnata* green manure treatment levels that were laid out in a completely randomized design and replicated five times in a screenhouse-based experiment. Seeds of *E. coracana* were germinated in the treated soils and their growth performance and yield assessed. Data were analyzed with the R statistical package and Graph Pad Prism 8. *A. pinnata* green manure treatment levels of 150 to 400 g/kg significantly improved the pH, Total N, Available P and cation exchange capacity of the soil. All the treatment levels significantly enhanced the growth performance, leaf width and length, number of ears per panicle, length of panicle ears, number of grains per panicle, and shoot dry matter production of *E. coracana*. Weight of 1000 grains, tiller formation and root dry matter production were only significantly enhanced by treatment levels of 150 to 400 g/kg. Correlation results demonstrated *A. pinnata*'s potential to improve soil fertility under terrestrial conditions and ultimately the yield of crops. The findings of this study are suggestive of a high potential of *A. pinnata* green manure for restoration of soil fertility and enhancement of terrestrial crop production.

Keywords Green manure · *Azolla pinnata* · *Anabaena azollae* · Soil fertility · Food security · Yield · *Eleusine coracana*

Introduction

Food security and livelihood improvement are threatened by declining soil fertility and consequently low crop yields, especially in communities that rely on small-scale farming. Although increasing inorganic fertilizer use is recognized as a key strategy for increasing crop yield (Selim 2020), fertilizer application is still low in Sub-Saharan Africa (Bonilla et al. 2020) and consequently the yields have remained significantly lower than in other regions (Bjornlund et al. 2020). Fertilizer use in Uganda is one of the least globally (Mbowa et al. 2015), with only 8% of the smallholder farmers applying them at an estimated rate of 1 kg of nutrient per hectare per year (Okoboi and Barungi 2012). This rate of application is far below the internationally recommended rate of 200 kg per hectare per year (MAAIF 2010) and is mainly associated with the prohibitive costs of the fertilizers in the region.

Traditionally, small-scale farmers have always relied on the application of cattle manure to maintain soil fertility and crop yield as a substitute for inorganic fertilizers. Nevertheless, cattle manure application is increasingly becoming unfeasible due to its inadequate supply and thus escalating costs especially in regions with less livestock. Furthermore, lack of fodder and non-provision of supplementary feeds due to lack of capital has resulted in less cow dung with low nutrient contents leading to low level of crop yield (Muhereza et al. 2014). It has therefore

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become necessary to look for more sustainable, affordable and renewable alternative sources of nutrients for crop production to ensure food security (Irungbam et al. 2019) and household income improvement most especially among the resource-poor communities. Nitrogen-fixing crops such as *Azolla* are one of the least-cost alternative sources of nitrogen and other nutrients which have been adopted for several past decades by farmers especially in rice growing.

Azolla is a free-floating water fern, which occurs in symbiotic association with the cyanobacteria *Anabaena azollae* in its cavities that fixes atmospheric N_2 (Ali et al. 2012; Bhuvaneshwari and Singh 2015). The cyanobacterium symbiotically fixes about 30–60 kg N ha^{-1}, making *Azolla* an important biological source of N for agriculture production (Brouwer et al. 2014; Kollah et al. 2016; Lakshmanan et al. 2017). The fern is endowed with numerous attributes that make it agronomically more outstanding. Its tissue contains 5% N and other nutrients such P, K and Ca, which are slowly released into the soil upon decomposition (Oyange 2020). The fern has the ability to rapidly multiply with a doubling time of 2 to 5 days (Sadeghi et al. 2013), ensuring its continuous supply to end-users as opposed to other alternative sources of soil nutrients. Through decomposition, the fern is known to slowly release nutrients, thus promoting efficient absorption of nutrients by crops. The fern also has a low carbon to nitrogen ratio of about 10:1 which ensures that *Azolla* nitrogen will not be tied up by bacteria that are involved in the decomposition of an overabundance of carbonaceous plant residues (Raut et al. 2017). Thus, the fern has been recommended for use in crop production as a fertilizer, either as green manure or biofertilizer (Syamsiyah et al. 2017; Maswada et al. 2020).

Azolla fern has traditionally been used over centuries for fertilization in paddies as a source of nitrogen (Oyange 2019). Application of *Azolla* in rice paddy fields has been reported to improve nutrient use efficiency (NUE) (Lin et al. 2018), reduce the application of urea fertilizer by 25% without affecting the rice yields (Malyan et al. 2019) and to reduce N_2O emissions (Kimani et al. 2020). Despite its valuable agronomic and ecological attributes in paddy rice growing, minimal attention has been directed to its utility for other crops most especially those grown terrestrially. Hence, this study was undertaken to examine the feasibility of using *A. pinnata* as a green manure to improve soil fertility and yield of terrestrial crops using *E. coracana* as a test plant. We selected wild finger millet as a test crop because of its declining productivity in Uganda (UBOS 2017), attributed to declining soil fertility, mainly arising from excessive land use and poor farming methods (Owere et al. 2014). Furthermore, under low nutrient input conditions, the crop expresses poor yields (Maitra 2020), yet it is majorly grown by resource-poor farmers in low input agricultural systems of Asia and Africa who cannot afford to buy expensive inorganic fertilizers (Thilakarathna and Raizada 2015).

Materials and methods

Study site

This experiment was conducted from the screenhouse at the Botanical Gardens of Makerere University, Kampala, Uganda. This is located in the equatorial region along coordinates 00° 20′ 18.0″ N latitude and 32° 37′00″ E longitude. The area experiences tropical climate with an average annual temperature of 21.3 °C and about 1293 mm of precipitation.

Azolla pinnata inoculum collection and cultivation

Azolla pinnata inoculum was collected from streams with slow moving water in Namulonge and cultivated in constructed ponds following procedures described by Reddy (2016). The ponds were shaded using dry elephant grass (*Pennisetum purpureum*) to limit light penetration. During the period of cultivation, the temperature and pH of the water in the ponds was respectively in the range of 28.4–31.2 °C and 6.2–6.8. After three weeks of cultivation, *A. pinnata* was harvested, spread out on a wire mesh supported by poles to drain out all the water held on its phytomass.

Experimental soil sample collection and preparation

The infertile soil samples were collected from Wakiso at a site with plants of very low vigor and clear symptoms of nutrient deficiency. At the site, ten spots were randomly selected. Soil sub-samples were collected from each spot surface within the 4–10 cm depth using a handheld soil auger and thoroughly mixed to constitute a composite sample that was packed in polythene bags and transported to Makerere University Botanical Gardens for further processing. The composite sample was spread on a polythene sheet and air dried for 3 weeks in well-secured place to eliminate any contaminants. The dry soil was crushed and then sieved through a plastic sieve with a mesh of 5 mm openings to remove rocks, clods and large pieces of organic matter to obtain soil with uniform particle size.

Experimental design

A screen house experiment was conducted during August-November 2019 laid out in a completely randomized design (CRD), with five replications to evaluate the effect of *A. pinnata* green manure treatment on soil nutrient composition and agro-morphological characters of millet (stem height, leaf size-length and width, dry matter production, yield and its components). Five treatment levels were adopted: 0 g, 50 g, 150 g, 300 g and 400 g of *A. pinnata* tissues per kg of dry infertile soil. They were prepared by mixing 16 kg of the soil with 0 g, 800 g, 2400 g, 4800 g and 6400 g of *A. pinnata* green manure, respectively, in large thick polythene bags. Each *A. pinnata*-soil homogeneous mixture was moistened, thoroughly tied at the top opening to avoid loss of nitrogen in form of ammonia. Due to the high lignin content of the *Azolla* tissues, the respective mixtures were kept in a moist place for a period of 6 weeks as reported by Raut et al. (2017) to be ideal for maximum release of the nutrients from its tissues into the experimental soils.

Planting of *E. coracana* seeds

After 6 weeks of decomposition, each *A. pinnata*-soil treatment mixture was sub-sampled into five 3 kg portions and 1 kg portion. The 3 kg portions of each treatment, were respectively, transferred into well labelled pots (19 cm top diameter, 14 cm bottom diameter and 17 cm height) and watered to field capacity with a sprinkler. In each pot, 5 seeds of *E. coracana* were sowed and later transferred to the screenhouse. The remaining 1 kg portion of each treatment was packed in zip-lock plastic bags and taken to the Soil Science Laboratory of the College of Agriculture and Environmental Science of Makerere University for physico-chemical characterization.

Physico-chemical characterization of soil samples

The soil samples were oven dried at 40 °C for 24 h (Okalebo et al. 2002). The dried samples were pulverized to pass through a 2 mm sieve to remove any coarse particles. The samples were then ground to a very fine powder in a mortar. Soil pH (soil:deionised water = 1:2.5 w/v) was determined using a calibrated pH meter, soil organic matter (SOM) content by Walkley–Black potassium dichromate wet oxidation (Nelson and Sommers 1982) and Total N by the semi-micro Kjeldahl method (Bremner and Mulvaney 1982). Available P and exchangeable cations (Ex-Ca and Ex-K) were determined using the M-3 solution [0.2 M CH_3COOH, 0.25 M NH_4NO_3, 0015 M NH_4F, 0.013 M HNO_3, and 0.001 M ethylene diamine tetra-acetic acid (EDTA)] according to Mehlich (2008). The Available P in the extract was determined following ammonium molybdate-ascorbic acid method (Knudsen and Beegle 1988) using a UV/Visible spectrophotometer at 860 nm. The concentrations of the two exchangeable cations were determined with an atomic absorption spectrophotometer (SHIMADZU AA-6800).

Agro-morphological characterization of millet

Morphological characteristics of stem height, length and width of fully expanded mid leaf for 6 randomly selected plants for each treatment were measured at regular time intervals of 2 weeks until the week for panicle emergence. The height of the stem from the foot of the millet plant to the emerging leaf, length of leaf from the sheath to the tip and the maximum leaf width (taken at the widest point perpendicular to the length measurement) were measured with a 60 cm fully flexible steel rule calibrated in mm. At 96 days after planting, the number of ears per panicle, number of grains per panicle and number of tillers per plant were physically counted. At the same time, shoot and root dry weights and weight of 1000 grains were determined with analytical balance (Mettler Toledo, New Classic MF, ML204/01, Switzerland) after drying them to a constant mass in an electric oven (Panasonic Healthcare Co. Ltd, MOV-112F-PE, Sakata, Japan) maintained at 60 °C.

Data analysis

Statistical analyses were performed using R statistical package 4.0.2 (R-Development Core Team 2020) and with GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA). Prior to any statistical analysis, data distributions were checked for normality and homogeneity of variances. In case of deviations from normality or homoscedasticity, the statistical assumptions of the analysis were fulfilled by log-transformation but actual values were used in data presentation. Variability in means among parameters was analyzed with analysis of variance (ANOVA) followed by a post hoc test (Tukey's Honest Significant Multiple Comparison) in case of significant variations, with means considered to be significantly different at $p < 0.05$.

Quality assurance

Prior to the determination of the physico-chemical characteristics of soil samples, glassware was thoroughly cleaned and oven dried to avoid external nutrient sources. Weighing of soil sample and preparation of reagents were done with authentic and tested instruments and all the reagents were of analytical grade. Double distilled water was used in all preparations, and all analyses were done in triplicates with blank solutions included for each. All experimental setups were subjected to similar environmental conditions of temperature, light intensity and water supply and an over-head transparent screen cover was used to prevent direct precipitation from interfering with the potted plants and to minimize excess irrigation. Measurements were often taken on the same day to avoid temporal variations. To ensure the validity of seed counts, researcher triangulation was done by involving three researchers on each count.

Results

Effect of *A. pinnata* green manure treatment on the physico-chemical characteristics of soils

All the physico-chemical characteristics under study increased with an increase in *A. pinnata* green manure treatment levels (Table 1). Furthermore, A. *pinnata* green manure treatment levels led to significant variations in pH ($F = 93.31$, $p < 0.001$), SOM ($F = 55.5$, $p < 0.001$), Total *N* ($F = 62.09$, $p < 0.001$), Available P ($F = 10{,}005.00$, $p < 0.001$), K ($F = 90.22$, $p < 0.001$) and Ca ($F = 27.88$, $p < 0.001$). Soil pH, Total N, Available P and exchangeable cations were significantly enhanced by treatment levels of 150 g/kg to 400 g/kg (Tukey's test, $p < 0.05$). For SOM, the enhancement was only significant for treatment levels of 300 g/kg and 400 g/kg while for K the enhancement was significant even at the treatment level of 50 g/kg. The increment in organic matter was only significant for 300 g/kg and 400 g/kg as compared to the control.

Effect of *A. pinnata* green manure treatment on growth performance of *E. coracana*

A. pinnata green manure treatment significantly enhanced the growth performance of *E. coracana* with respect to height. The rate of growth progressively increased with the increase in the application rate as evidenced by the slopes of each treatment level (Fig. 1). Overall, the slopes of the different treatments were extremely different ($F = 49.48$, d*fd* = 140, $p < 0.0001$). The slopes of 50 g/kg, 150 g/kg, 300 g/kg and 400 g/kg were 2.7-, 2.9-, 3.1- and 3.5-folds higher than that of the control, respectively. The treated plants grew faster, reaching reproductive maturity after 8 weeks before the control plants. Correspondingly, the grains of the treated plants matured after 12 weeks before those of the control.

Table 1 Effect of *A. pinnata* green manure treatment on the physico-chemical characteristics ($\bar{x} \pm$ SD, $n = 3$) of agronomic soil

Treatment (g/kg)	pH	SOM (%)	Total N (%)	Available P (mg/kg)	K (cmol/kg)	Ca (cmol/kg)
0	5.18 ± 0.12a	1.43 ± 0.04a	0.08 ± 0.01a	1.72 ± 0.17a	0.15 ± 0.01a	1.73 ± 0.29a
50	5.44 ± 0.03a	1.47 ± 0.21a	0.09 ± 0.01a	3.19 ± 0.34a	0.25 ± 0.01b	2.23 ± 0.15a
150	6.30 ± 0.22b	2.20 ± 0.23a	0.19 ± 0.01b	13.73 ± 0.90b	0.41 ± 0.05c	3.73 ± 0.42b
300	6.34 ± 0.03b	3.17 ± 0.06b	0.24 ± 0.01b	18.58 ± 1.40c	0.47 ± 0.04c	4.07 ± 0.29b
400	6.66 ± 0.04c	4.56 ± 0.26c	0.31 ± 0.02c	37.40 ± 0.45d	0.61 ± 0.19d	5.13 ± 0.38c

[a]Means in each column followed by different letters of the alphabet are significantly different (Tukey's test $p < 0.05$)

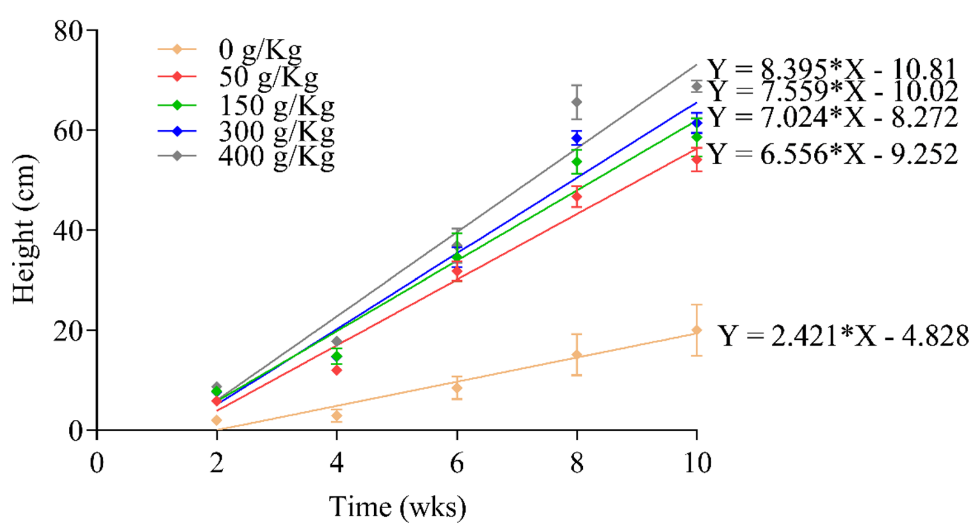

Fig. 1 Linear regression plot comparing the growth performance of *E. coracana* treated with different levels of *A. pinnata* green manure over the growth period

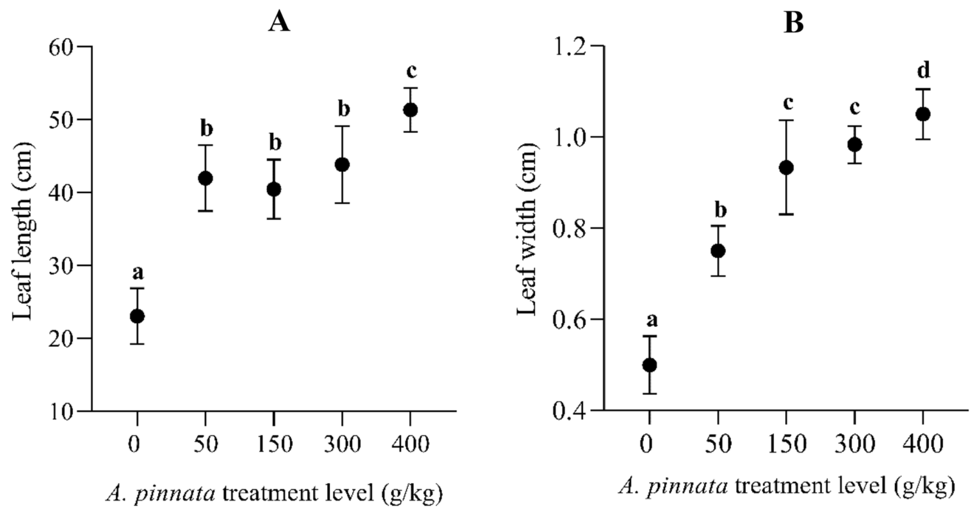

Fig. 2 Effect of *A. pinnata* green manure treatment on the mean leaf length (**A**) and width (**B**) ($\bar{x} \pm$ SD, $n = 6$) of *E. coracana* at 10 weeks after planting. Means with different letters of the alphabet are significantly different (Tukey's test, $p < 0.05$)

Effect of *A. pinnata* treatments on the leaf length and width of *E. coracana*

Leaf length and width progressively increased with an increase in the level of *A. pinnata* green manure applied (Fig. 2A, B). All levels of *A. pinnata* green manure treatments significantly increased both leaf length and width. Leaf length and width of *E. coracana* treated with 400 g/kg of the green manure were significantly higher than that of *E. coracana* treated with lower levels of the green manure (Tukey's test, $p < 0.05$). Despite similar leaf lengths for *E. coracana* plants treated with 50 g/kg, 150 g/kg and 300 g/kg of *Azolla* green manure (Tukey's test, $p > 0.05$), their leaf width varied significantly, with 50 g/kg plants having significantly lower width (Tukey's test, $p < 0.05$) as compared to 150 k/kg and 300 g/kg that had similar width.

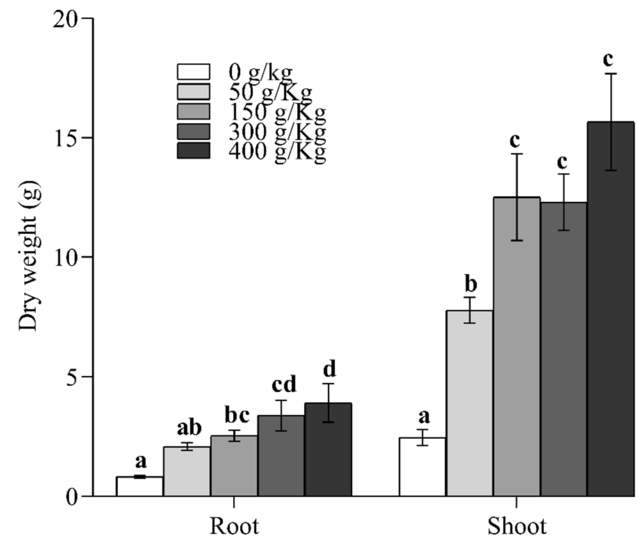

Fig. 3 Effect of *A. pinnata* green manure treatment on *E. coracana* dry matter production at harvest-96 days after planting, ($\bar{x} \pm$ SD, $n = 6$). Bars with different letters of the alphabet are significantly different (Tukey's test, $p < 0.05$)

Effect of *A. pinnata* green manure treatments on dry matter accumulation

Dry matter production at harvest generally increased with increase in the quantities of the *A. pinnata* green manure applied to the soil (Fig. 3). With the exceptional case of 50 g/kg for the root dry matter, all *A. pinnata* green manure treatment levels significantly enhanced root and shoot dry matter accumulation (Tukey's test, $p < 0.05$). For higher treatment levels ranging between 150 and 400 g/kg, the differences in shoot dry matter production were not statistically significant.

Effect of *A. pinnata* manure treatments on the agronomic characteristics of *E. coracana*

The study revealed a progressive increase in the number of panicle ears, length of panicle ears and number of tillers with increase in *A. pinnata* green manure treatment levels (Table 2). With the exceptional case of number of tillers and weight of 1000 grains (g), all the green manure treatment levels significantly enhanced the agronomic characteristics of *E. coracana* that were under study (Tukey's test, $p < 0.05$). Significantly higher tiller formation and weight of 1000 grains (g) were only observed for treatment levels between 150 and 400 g/kg of dry soil. *A. pinnata* green manure treatment increased grain formation within 3.9–6.2 folds higher than in the untreated *E. coracana* plants. We recorded the highest number of grains per panicle in *E. coracana* plants treated with 400 g/kg of *A. pinnata* green manure. A correlation analysis demonstrated positive correlations between yield attributes, yield and the physico-chemical characteristics of the experimental soils (Table 3) were all significant for the number of ears

Table 2 Effect of *A. pinnata* green manure treatment on the agronomic characteristics ($\bar{x} \pm SD$, $n=6$) of *E. coracana*

Treatment (g/kg of soil)	Yield attributes			Grain yield	
	No. of ears/panicle	Panicle ear length (cm)	No. of tillers	No. of grains/panicle	Weight of 1000 grains (g)
0	2.00 ± 0.71a	3.00 ± 0.35a	0.00 ± 0.00a	153.92 ± 69.12a	1.89 ± 0.47a
50	3.83 ± 0.75b	4.42 ± 0.34b	0.33 ± 0.52ab	442.75 ± 91.13b	2.13 ± 0.15ab
150	4.33 ± 0.52bc	5.02 ± 0.29c	1.33 ± 1.03bc	498.33 ± 135.65b	2.64 ± 0.24bc
300	5.00 ± 0.00c	5.07 ± 0.27c	2.00 ± 0.89c	531.58 ± 75.70b	3.14 ± 0.25c
400	6.83 ± 0.98d	6.12 ± 0.12d	2.33 ± 0.52c	787.42 ± 249.03c	3.12 ± 0.15c

Means in each column followed by the same letter of the alphabet are not significantly different (Tukey's test, $p < 0.05$)

Table 3 Pearson correlation coefficients (r) between soil physico-chemical parameters and agro-morphological characteristics of *E. coracana*

Parameter	Yield attributes			Grain yield	
	Number of ears/panicle	Length of panicle ears	Number of tillers	Number of grains/panicle	Weight of 1000 grains
pH	0.6305^{*}	0.6907^{*}	0.1270^{NS}	0.5804^{*}	0.5447^{*}
OM	0.8791^{***}	0.8295^{**}	0.4873^{NS}	0.8633^{***}	0.1734^{NS}
N	0.8334^{**}	0.8715^{***}	0.4871^{*}	0.8500^{***}	0.3882^{NS}
P	0.9043^{***}	0.8759^{***}	0.5611^{*}	0.9190^{***}	0.2265^{NS}
K	0.9277^{***}	0.9522^{***}	0.4634^{NS}	0.9109^{***}	0.4773^{NS}
Ca	0.9325^{***}	0.9004^{***}	0.5888^{*}	0.9016^{***}	0.3474^{NS}

NS not significant

*Level of significance < 0.05

**Level of significance $p < 0.01$

***Level of significance: pairwise two-sided p values

per panicle, length of panicle ears and number of grains per panicle. Overall, these results are in accordance with findings reported by Krishna et al. (2019) who reported a corresponding increase in yield and yield attributes of *E. coracana* with increased N and P levels.

The correlations between leaf size (leaf length and width) and agronomic characteristics that were under study were all positive (Fig. 4). Leaf length was significantly correlated with, number of panicles ears ($F = 29.25$, $p = 0.001$), length of panicle ears ($F = 95.55$, $p < 0.001$), number of grains formed per panicle ($F = 32.56$, $p < 0.001$) and weights of 1000 grains ($F = 6.369$, $p = 0.0254$). The same relationship was also evident between leaf width and number of panicles ($F = 42.70$, $p = 0.001$), length of panicle ($F = 129.9$, $p < 0.001$), number of grains formed per panicle ($F = 31.09$, $p < 0.001$) and weight of 1000 grains ($F = 11.96$, $p = 0.0042$). In light of the above correlation results, *E. coracana* yield and its attributes were significantly enhanced by *A. pinnata* green manure treatment as a result of enhanced leaf size. The enhancement of leaf size could have correspondingly led to a rise in the photosynthetic efficiency and production of photo-assimilates that supported yield and its attributes.

Discussions

Enhancement of soil fertility

The study examined the effect of *A. pinnata* green manure application on the physico-chemical characteristics of infertile soil. Originally, the soil had pH of 5.14 that was within the range 5 to 7 to which the test plant *E. coracana* is adapted to grow (Oyange et al. 2019). We observed a progressive increase in the pH of the soil with *A. pinnata* green manure application rate. The observed increase in soil pH is in agreement with earlier findings of Kotpal and Bali (2003) and Bhubaneshwari and Kumar (2013) who reported *Azollas*' ability to raise pH value. *A. pinnata* green manure treatment levels in the range of 150 g/kg to 400 g/kg increased the pH within the range of 6.0 to 6.8, known to be optimal for growth of grain crops (Heggenstaller 2012). Soil organic matter contents are usually positively related to specific soil properties or process fostering crop growth, such as cation exchange capacity, rainfall infiltration or soil structure (Vaulaurve et al. 2004). Thus, the rise in the organic matter content was reminiscent *A.*

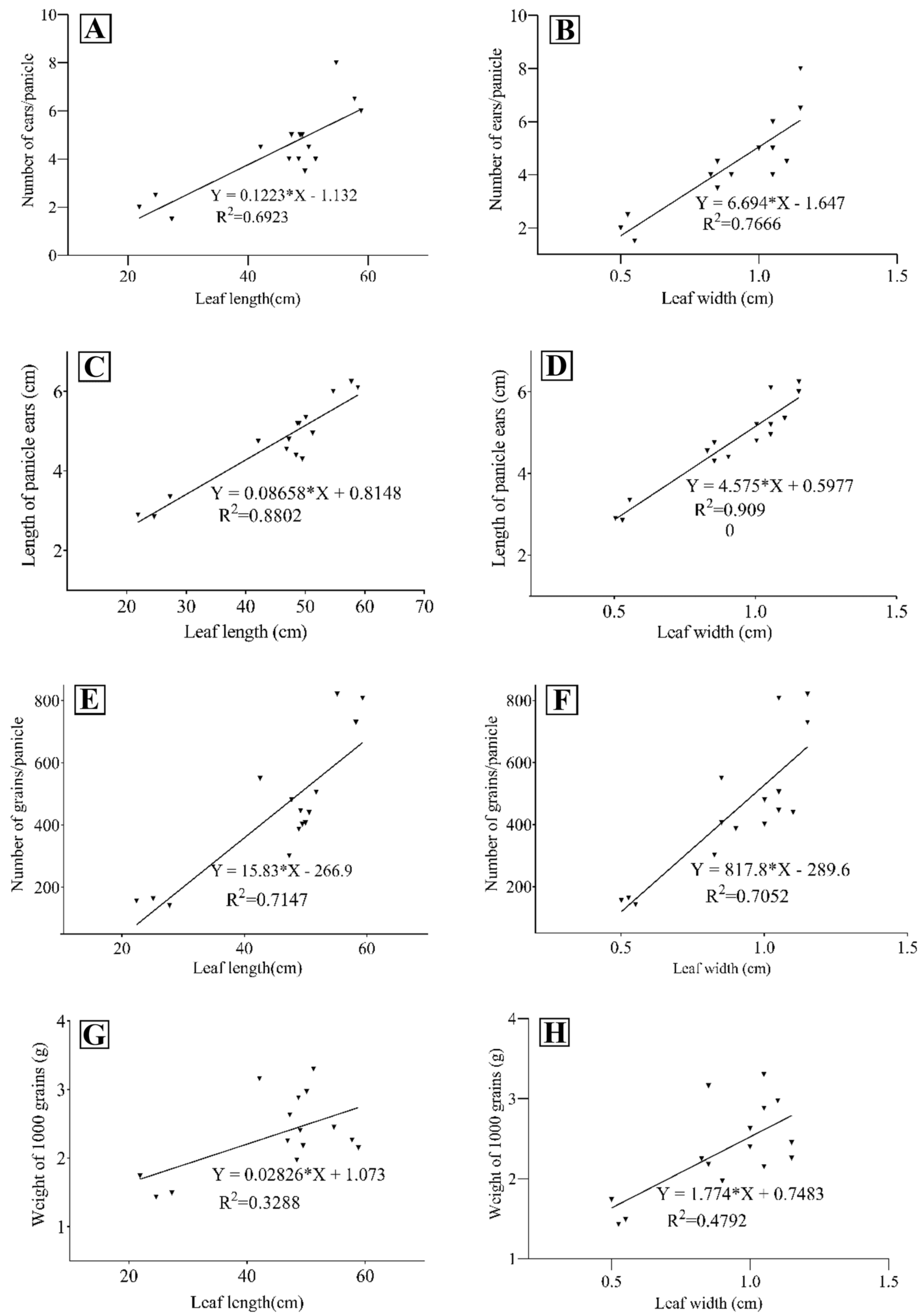

Fig. 4 Linear regression plots for leaf length 10 weeks after planting and **A** number of ears per panicle, **C** length of panicle ears, **E** number of grains per panicle and **G** weight of 1000 grains and leaf width 10 weeks after planting with **B** number of ears per panicle, **D** length of panicle ears, **F** number of grains per panicle and **H** weight of 1000 grains

pinnata green manure potential for enhancement of processes that foster the growth of crops. In line with previous studies by Bhubaneshwari and Kumar (2013) and Setiawati et al. (2018), *A. pinnata* green manure treatment also improved total nitrogen, Available phosphorous and the cation exchange capacity (K and Ca) of the soils, which are known to be the key inputs in the production of cereals (Ricker-Gilbert 2020). The rise in the nutrient status after green manure application is usually associated with the increase in the active role that soil bacterial communities play (Zhang et al. 2017), of enhancing nutrient cycling and utilization efficiency by the crop (García-Fraile et al. 2016; Xiong et al. 2016). Integration of *A. piñata* green manure into the soil could have also led to increased cellulolytic and urea hydrolyzing activities, increase in the population of heterotrophic bacteria (Thanikachalam et al. 1984; Kannaiyan and Subramani 1992; Kannaiyan and Kalidurai 1995) and phosphatase activity (Thanikachalam et al. 1984; Thangaraju and Kannaiyan 1989). These could have enhanced the mineralization of the *A. pinnata* mineral nutrient-laden tissues to release the mineral nutrients that were positively correlated with yield and its attributes.

Effect on growth performance

We observed a direct proportionality between *A. pinnata* green manure treatment levels and the growth performance of *E. coracana*. This observation is suggestive of the latter's dependency on the nutrients released into the soil that were also directly proportional to green manure application level. This argument is consistent with earlier findings of Charate et al. (2018) who reported an increase in height due to higher nitrogen and potassium levels in little millet (*Panicum sumatrense*), through enhancement of meristematic activities of cell enlargement and elongation. Similar results in rice attributed to increased availability of nutrients and nitrogen as a macronutrient through mineralization of *A. pinnata* green manure have been reported by Frageria (2004) and Sainju (2013) and in pearl millet (*Pennisetum glaucum* (L.) *R.Br.*) by Ayub et al. (2009) due to increased nitrogen through the application of inorganic nitrogen fertilizer. In this respect, the effects of *A. pinnata* green manure were comparable with those of standard inorganic fertilizer and thus suggestive of its potential for enhancement of crop yield.

Leaf size

We used leaf width and length as proxies of leaf size. Thus, *A. pinnata* green manure treatment enhanced leaf size of *E. coracana*. Like any other plant, the higher leaf size could have resulted in higher photosynthetic activity and production of larger amounts of phototoassimilates by finger millet at all stages of growth (Patil et al. 2015). We believe that the enhancement in leaf size could have been due to higher quantity of macro- and micronutrients added to soil through the decomposition of the green manure tissues. This might have resulted in increased availability of nutrients in the root zones and their subsequent uptake by *E. coracana*.

Dry matter production

At lower green manure application levels, a significant and proportionate increase in dry matter production with increase in the green manure application levels was evident. Comparable results were reported by Shah and Kumar (2014) and Jeyajothi et al. (2016) in rice (*Oryza sativa* L.) treated with organic sources of nitrogen including *Azolla* attributed to increased nitrogen supply through mineralization of organic nitrogen forms (Devi et al. 2020). At higher application levels (150–400 g/kg), shoot dry matter production was comparable most probably due to the attainment of optimal nutrient supply from *A. pinnata* tissues. These findings are consistent with research findings by Krishna et al. (2019) who reported a comparable trend in dry matter production by *E. coracana* at higher N application levels.

Grain yield and yield attributes

In line with previous studies by Ullasa et al. (2017), *A. pinnata* green manure application significantly enhanced grain yield and its attributes relative to the untreated *E. coracana*. Grain yield and yield attributes were largely a function of yield attributes which were significantly enhanced by the application of *A. pinnata* green manure. These results are directly in line with previous findings of Charate et al. (2018), who worked on Little Millet (*Panicum sumatrense*). Even though within treatment levels of 50 to 300 g/kg grain formation increased with *A. pinnata* green manure treatment levels, it was not significantly different for each level (Tukey's test, $p > 0.05$). Furthermore, weights of the 1000 grains were not significantly different for *E. coracana* treated with 150 g/kg, 300 g/kg and 400 g/kg and were not correlated with all physico-chemical characteristics apart from pH (Table 3). This could possibly be due to the role of other factors that influenced photo-assimilate partitioning to the grains. Additionally, the photo-assimilates formed could not suffice for grain filling of the numerous seeds formed especially for 400 g/kg. Taking into account the variation in yield attributes and tiller formation, overall yield in terms of weight was in the order of 400 g/kg > 300 g/kg > 150 g/kg > 50 g/kg > 0 g/kg. The high millet yield may be ascribed to the enhancement of total soil nitrogen levels and partly due to increased availability of K and P that are known to improve the agronomic efficiency of N (Brar et al. 2011). Furthermore, plants treated with higher doses of *A. pinnata* green manure were significantly taller. This enabled them to acquire more leaf area and green stem tissues that intercept more solar radiation (Ausiku et al. 2020), to synthesize more photo-assimilates for grain filling.

Conclusions and recommendations

A. pinnata green manure treatment significantly improved soil fertility to levels that substantially enhanced the growth and yield of *E. coracana*. Its application accelerated the rate of growth and dry matter production as compared to the untreated *E. coracana*. The *A. pinnata* green manure treatment substantially improved leaf size, yield attributes and ultimately the yield of *E. coracana*. The findings of this study are suggestive of a high potential of *Azolla pinnata* green manure for restoration of soil fertility and enhancement of terrestrial crop production. However, the applicability of the findings under field conditions needs to be verified. There is an urgent need to innovate protocols for application on specific terrestrial crops under field conditions, to realize the widely acclaimed agronomic potential of *Azolla* to revolutionize agriculture.

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Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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