

1 Potential of *Tithonia diversifolia* Biomass as Alternative for Inorganic Fertilizer
2 to Improve Production of African Nightshade (*Solanum nigrum* L.)

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4 Christopher Ngosong^{1*}, Clifford M. Njobe¹, Clovis B. Tanyi¹, Lawrence T.
5 Nanganoa², Aaron S. Tening¹

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7 ¹Department of Agronomic and Applied Molecular Sciences, Faculty of
8 Agriculture and Veterinary Medicine, University of Buea, P.O.Box 63 Buea,
9 South West Region, Cameroon.

10 ²Institute of Agricultural Research for Development (IRAD), Ekona, P.M.B 25,
11 Buea, South West Region, Cameroon.

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13 *Corresponding author: ngosongk@yahoo.com;

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19 **Authors' contributions**

20 This work was carried out in collaboration between all authors. CMJ, CN and
21 AST designed the experiment. CMJ established the experiment and collected
22 data. LTN and AST conducted soil analysis. CMJ, CBT and CN processed
23 data performed statistics. CN wrote the first manuscript draft. All authors read
24 and approved the final manuscript.

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36 **ABSTRACT**

37 Poor soil fertility is a major constraint for crop production that is commonly
38 corrected with inorganic fertilizers, but their high cost or environmental and

39 health effects have necessitated alternative management strategies. Hence,
40 the biomass of Mexican sunflower (*Tithonia diversifolia*) was evaluated as a
41 sustainable alternative resource to improve soil fertility and production of
42 African nightshade (*Solanum nigrum* L.) A field experiment was laid out as
43 randomized complete block design with four treatments (Control – no fertilizer,
44 urea, NPK and *Tithonia*) and four replications. Soil available phosphorus
45 ranged from 10.3–16.3 mg kg⁻¹ and differed significantly ($P < 0.05$) across
46 treatments, with the highest in *Tithonia* and NPK as compared to urea and
47 control. In comparison to the baseline soil, residual soil phosphorus increased
48 by 7.7% for *Tithonia*, but decreased by 1.7% for NPK, 27.7% for control, and
49 46.3% for urea. The yield of African nightshade correlated significantly ($P <$
50 0.05) with the content of soil available phosphorus, and ranged from 8.6–14.9
51 t ha⁻¹ fresh and 1.5–2.5 t ha⁻¹ dry biomass that differed significantly ($P < 0.05$)
52 across treatments, with the highest in NPK as compared to control. Plant
53 height ranged from 24.6–33.2 cm and differed significantly ($P < 0.05$) across
54 treatments, with the highest in NPK. The number of leaves ranged from 80–
55 117 per plant and differed significantly ($P < 0.05$) across treatments, with the
56 highest in NPK as compared to urea. *Tithonia* and fertilizers increased yield
57 comparatively, which demonstrates the potential of *Tithonia* biomass as a
58 sustainable alternative resource for soil fertility management.

59 **Keywords:** Fertility, leafy vegetables, NPK, sunflower, urea.

60 1. INTRODUCTION

61 Sub-Saharan Africa (SSA) accounts for about 9% of the global population with
62 40% under malnutrition [1]. Vegetables are considered as the most affordable

63 and accessible source of micronutrients, vitamins and health-promoting
64 secondary metabolites [2,3]. Indigenous African leafy vegetables such as
65 African nightshade (*Solanum spp.*) are important for food and nutrition
66 security, but the method of cultivation and processing influences their
67 nutritional value and health-promoting potential [4]. However, poor and soil
68 fertility resulting from continuous crop cultivation is one of the main constraints
69 of crop production in SSA, with nitrogen and phosphorus as the most limiting
70 elements [5,6]. This is exacerbated by low fertilizer inputs that result in low
71 productivity with huge gaps of over 30% between actual production and the
72 attainable potential [7,8]. SSA accounts for only 0.1% of global mineral
73 fertilizer production and 1.8% of global mineral fertilizer use, which is less
74 than 10 kg ha⁻¹ fertilizer use compared to 87 kg ha⁻¹ for some developed
75 nations [9]. Inorganic fertilizers are commonly used to improve soil fertility and
76 plant nutrition, but their enduring use eventually damages the soil [10,11].
77 According to The Economist [12], simply using more fertilizers to produce
78 more food is not sustainable for the feeding of about nine billion people on
79 earth by 2050. This requires gains from narrowing the yield gaps through
80 sustainable cost-effective techniques [12]. Meanwhile, high cost of fertilizers
81 and the need for resource conservation has led to renewed interest in
82 alternative nutrient resources for soil fertility management.

83 Alternatively, organic inputs enhance soil organic matter and nutrients
84 with improved soil structure, aeration, water holding capacity and infiltration
85 [10,11,13]. Plant biomasses have demonstrated efficacy as mulch to improve
86 soil health and foster sustainable development [14,15]. However, reliance on
87 plant biomass for soil fertility management largely depends on the quality and

availability. *Tithonia diversifolia* (Hemsley) is abundant in Buea Cameroon and rich in essential elements, with demonstrated potential to improve soil fertility and tomato production [16]. *Tithonia* biomass possesses both fertilizer attributes and allelopathic or phytotoxic growth inhibiting qualities [17,18]. *Tithonia* has high biomass with nutrient contents of about 3.5% nitrogen, 0.4% phosphorus and 4.1% potassium [19,20]. *Tithonia* also contains low recalcitrant compounds with 6.5% lignin and 1.6% polyphenol [21]. *Tithonia* demonstrated strong potential for soil rejuvenation and crop protection due to sesquiterpene lactones (tagitinins-terpene) and other antimicrobial substances against pests and diseases [20,22]. Hence, this study was conducted to evaluate the potential of *Tithonia* as a sustainable alternative for inorganic fertilizer to improve soil fertility and performance of African nightshade *Solanum nigrum* L. (Solanaceae). It was hypothesized that *Tithonia* biomass will enhance soil fertility and yield of African nightshade as compared to inorganic fertilizers.

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104 2. MATERIALS AND METHODS

105 2.1 Experimental site and setup

106 This experiment was conducted from April–August 2018 at the research and
107 teaching farm of the Faculty of Agriculture and Veterinary Medicine, University
108 of Buea. The site is located at Bulu Mile 16–Buea between latitudes 4°3'N and
109 4°12'N and longitudes 9°12'E and 9°20'E. The soil is derived from weathered
110 volcanic rocks dominated by silt, clay and sand [23,24]. Buea has mono-
111 modal rainfall regime with less pronounced dry season and 86% relative
112 humidity. The dry season starts from November to March, with mean annual

113 rainfall of 2800 mm and monthly air temperature ranging from 19–30 °C. Soil
114 temperature at 10 cm depth decreases from 25–15 °C with increasing
115 elevation from 200–2200 m, respectively, above sea level [23,25,26].

116 The experiment was laid out as a randomized complete block design
117 with four treatments each replicated four times, giving a total of 16
118 experimental plots measuring 4×4 m each. The experimental plots and
119 surrounding areas were separated from each other by 1 m buffer zone. This
120 experiment comprised four treatments including a control – no fertilizer, two
121 inorganic (urea and NPK) and organic (*Tithonia diversifolia* – Mexican
122 sunflower) fertilizers. Both urea and NPK fertilizers are commonly used in
123 vegetable production systems, while *Tithonia* biomass was used as a cost-
124 effective and environmentally friendly alternative. The field site was manually
125 cleared using a cutlass and tilled at about 30 cm depth using a hoe.

126

127 2.2 Crop cultivation

128 Seeds of the African nightshade leafy vegetable (*Solanum nigrum* L.) were
129 purchased from an agro-shop and pre-germinated on nursery beds before
130 they were transplanted on the experimental plots. The nursery comprised
131 three (1×10 m each) raised-beds produced by tilling at about 30 cm depth
132 using a hoe. The African nightshade seeds were planted at about 1 cm soil
133 depth and covered lightly with soil and dry grass mulch. Depending on rainfall
134 regime, the nursery was manually irrigated twice a day (morning and
135 evening), starting on the day of sowing. After three weeks, irrigation was
136 reduced to once a day or after two days depending on the frequency of
137 rainfall. Vigorous African nightshade seedlings of approximately same sizes

138 were transplanted to experimental plots after four weeks in the nursery. The
139 seedlings were planted at 40×40 cm spacing between and within rows, giving
140 a total of 81 plants per plot. Seedlings that did not survive after transplanting
141 were immediately replaced with supplies from the nursery in order to maintain
142 uniform field capacity.

143

144 2.3 Fertilizer resources and crop protection

145 A total of 1.5 kg inorganic fertilizer NPK 20:10:10 + CaO (ADER® Cameroon)
146 was applied on the 30 m² nursery beds. *Tithonia* biomass (leaves and stems)
147 were harvested from roadsides within the university of Buea and sun-dried for
148 two weeks, and spread on respective plots two weeks before African
149 nightshade seedlings were transplanted. The inorganic urea and NPK
150 20:10:10 + CaO (ADER® Cameroon) fertilizers were purchased from a local
151 agro-shop and broadcasted on respective plots after African nightshade
152 seedlings were transplanted. Both inorganic (urea and NPK) and organic
153 (*Tithonia*) amendments were applied at the rate of 312.5 kg ha⁻¹.

154 A mixture of 2 kg wood ash and 0.2 kg MOCAB (ingredient as
155 ethoprop: o-ethylms, s-dipropyl phosphorodithioate; Amvac®, USA) was
156 spread in the nursery to protect African nightshade seedlings against insect
157 pests. MOCID (active ingredient as metaldehyde; SAVANA-Horizon Phyto
158 Plus®, Cameroon) was broadcast in the nursery (total of 0.3 kg at the rate of
159 0.1 kg every two weeks) and experimental site (total of 1 kg at the rate of 0.25
160 kg every two weeks) for protection against snails. For control of insect pests
161 and diseases, 40 ml K-Optimal (active ingredient lambda-cyhalothrine 15g/l
162 plus acetamipride 20g/l; SCPA SIVEX International®, France) was mixed in

163 16L water and a knapsack sprayer was used to spray plants one week after
164 seedlings were transplanted. Experimental plots and alleys were regularly
165 monitored for weed emergence and weeded manually using a hoe.

166

167 **2.4 Sampling and data collection**

168 One day after the experimental site was cleared, five soil sub-samples were
169 collected at 0–15 cm depth in randomized distribution (Z-form) using 3.5 cm
170 diameter auger and bulked to form a composite baseline sample. Sampling at
171 crop maturity was conducted two days before the first crop harvest (64 and 50
172 days after application of *Tithonia* and inorganic fertilizers, respectively). Five
173 sub-soil samples were randomly collected at 0–15 cm depth (using 3.5 cm
174 diameter auger) and bulked together to form a composite sample for each
175 experimental plot, giving a total of 16 soil samples. The soil samples were air-
176 dried and sieved using a 2-mm sieve, and the soil particle size was
177 determined using the pipette method with sodium hexametaphosphate as the
178 dispersing agent [27]. Soil pH was determined potentiometrically in both water
179 (H₂O) and one molar potassium chloride (1 M KCl) solutions after 24 hours in
180 soil suspension (soil/liquid = 1/2.5 w/v). Exchangeable bases were extracted
181 with neutral ammonium acetate solution. Calcium (Ca) and magnesium (Mg)
182 were determined by atomic absorption spectrophotometry while potassium (K)
183 and Sodium (Na) were determined by flame photometry [28]. Exchangeable
184 acidity was determined by KCl extraction method [28]. Total nitrogen was
185 determined by macrokjeldahl digestion method [29], while available
186 phosphorus (P) was determined by Bray II method [28]. Soil organic carbon
187 was determined by Walkley-Black method [30].

188 During the experimentation, five plants were randomly selected from
189 each treatment replicate and tagged for assessment of growth parameters at
190 three to five weeks after transplanting. Plant height (cm) was measured from
191 the soil surface to the apical tip of the plant using a measuring tape. The
192 number of fully developed leaves on the nodes of the main stem and the
193 number of branches were visually observed and counted per plant. Stem girth
194 was measured with a vernier calliper at 5 cm from soil surface to the nearest
195 cm. Three manual harvests were conducted using a sharp knife every two
196 weeks over four weeks to determine the cumulative yield. Plants were
197 harvested by cutting the main stem at 6 cm from soil surface, and primary
198 branches cut at 3 cm from the main stem and considered as marketable yield.
199 The three harvests of African nightshade were weighed using a top-loading
200 balance and the data reported in tons per hectare fresh and dry (after oven-
201 drying at 60 °C for 72 hours) weights.

202

203 2.5 Statistical analysis

204 Data sets were subjected to statistical analyses using STATISTICA 9.1 for
205 Windows [31]. Dependent variables as performance of African nightshade
206 (yield, plant height, stem girth, number of leaves or branches) were subjected
207 to univariate analysis of variance (ANOVA, $P < 0.05$) to test effects of
208 treatments ($n=4$) as categorical predictors. Significant data means were
209 compared by Tukey's HSD ($P < 0.05$). Where applicable, Spearman Rank
210 Correlation ($P < 0.05$) was performed to determine the degree of association
211 between dependent variables and categorical predictors.

212

213 **3. RESULTS**

214 **3.1 Influence of treatments on soil properties**

215 Soil texture at the experimental site before application of treatments indicated
216 that the site is dominated by clay (47.19%), silt (44.44%) and sand (8.37%).
217 The soil was highly acidic and ranged from 4.1–5.4, but soil pH was not
218 affected by the different treatments (Table 1A,B). The content of soil available
219 phosphorus differed significantly (ANOVA: $F_{3,15} = 21.17$, $P < 0.001$; Fig. 1)
220 across treatments, ranging from 10.3–16.3 mg kg⁻¹. The highest soil available
221 phosphorus occurred in the *Tithonia* and NPK treatments, which differed
222 significantly ($P < 0.05$; Fig. 1) from urea and control, but urea and control did
223 not differ significantly ($P > 0.05$; Fig. 1). Compared to the baseline soil
224 phosphorus (Table 1A), the residual soil phosphorus (Fig. 1) increased by
225 7.7% for *Tithonia* plots, but decreased by 1.7% for NPK, 27.7% for control and
226 46.3% for urea after treatments were applied (Fig. 2).

227

228 **3.2 Impact of treatments on yield and vegetative parameters**

229 The yield of African nightshade ranged from 8.6–14.9 t ha⁻¹ fresh biomass
230 (Fig. 3a) and 1.5–2.5 t ha⁻¹ dry biomass (Fig. 3b) across treatments. Fresh
231 biomass yield differed significantly (ANOVA: $F_{3,14} = 4.53$, $P < 0.05$; Fig. 3a)
232 across treatments, with the highest in NPK as compared to the control.
233 Similarly, dry biomass yield differed significantly (ANOVA: $F_{3,14} = 4.34$, $P <$
234 0.05 ; Fig. 3b) across treatments, with the highest in NPK as compared to the

235 **control.** The content of soil available phosphorus influenced the yield of
236 African nightshade as demonstrated by the significant ($P < 0.05$) positive
237 correlation of fresh ($r = 0.67$) and dry ($r = 0.75$) biomass yield with soil
238 phosphorus. The plant height ranged from 24.6–33.2 cm and differed
239 significantly (ANOVA: $F_{3,15} = 7.77$, $P < 0.01$; Table 2) across treatments, **with**
240 **the highest in NPK as compared to the other treatments.** The number of
241 leaves ranged from 80–117 per plant and differed significantly (ANOVA: $F_{3,15}$
242 $= 4.09$, $P < 0.05$; Table 2) across treatments, **with the highest in NPK as**
243 **compared to urea.** The stem girth ranged from 1.4–2.1 cm per plant while the
244 number of branches ranged from 11–15 per plant across treatments, but did
245 not differ ($P > 0.05$; Table 2).

246

247 **4. DISCUSSION**

248 **4.1 Effect of treatments on soil properties**

249 According to the guidelines for tropical soils elaborated by Landon [32], high,
250 medium and low soil contents correspond to >10 , 4–10 and $<4\%$ for organic
251 carbon; >0.5 , 0.2–0.5 and $<0.2\%$ for total nitrogen; 10–15, 5–10 and 0–5
252 cmol/kg for phosphorus; >0.4 –0.8, 0.2–0.4 and <0.03 –0.2 cmol/kg for
253 potassium. Therefore, organic carbon and total nitrogen contents were low in
254 this study, while phosphorus was high and potassium was medium [32].
255 Meanwhile, the observed highly acidic soil pH corresponds to the standard
256 tropical acid soil pH range [33]. Liasu et al. [34] reported high nutrient
257 contents in *Tithonia* biomass, but the ability to exert effects on soil fertility
258 depends on the quantity of material used and the rate of decomposing and

259 mineralization. Therefore, the low amount of *Tithonia* used did not adequately
260 supply the essential nutrients as compared to Ngosong et al. [16] who
261 reported an increase in soil fertility and tomato yield for *Tithonia* than NPK. In
262 addition, Odhiambo [35] reported reduced nitrogen mineralization in high clay
263 soils, which might have influenced the mineralization of *Tithonia* biomass in
264 the present study with high clay content of 47.19%. Ngosong et al. [16]
265 reported 0.55% phosphorus content in *Tithonia* biomass from Buea, which
266 means that by applying 312.5 kg ha⁻¹ *Tithonia*, the total phosphorus input was
267 only 1.72 kg ha⁻¹ as compared to the 78.13 kg ha⁻¹ phosphorus supplied by
268 the 312.5 kg ha⁻¹ NPK fertilizer applied. This suggests that much of the
269 phosphorus from NPK fertilizer was either leached or adsorbed by the highly
270 acidic tropical soil [6]. The two highest peaks of phosphorus fixation occur in
271 the acid range of pH 4 and 5.5, where phosphorus precipitates with iron and
272 aluminium. Also the clay fraction of the soil (47.19%) is likely to be the main
273 site of phosphorus fixation. Low phosphorus content observed in control and
274 urea treatments is likely due to high P-sorption capacity of the highly acidic
275 tropical soil in this study area with 47.19% clay composition, since clay
276 particles correlate strongly with P-sorption [36]. The high soil available
277 phosphorus recorded for *Tithonia* treatment might be due to increase in labile
278 pools of soil phosphorus [21]. Net phosphorus mineralization likely increased
279 because of the concentration of phosphorus in the biomass, in relation to the
280 critical P-levels required for mineralization of soil organic-P to inorganic-P
281 [37–39]. Accordingly, the interaction of phosphorus sources and different soil
282 types reportedly influenced the availability of soil phosphorus [40]. However,

283 this result does not support the hypothesis and *Tithonia* and NPK recorded
284 similar contents of soil available phosphorus.

285

286 **4.2 Impact of treatments on crop yield**

287 The low yield recorded in control demonstrates lack of essential soil nutrients,
288 while the high yield recorded for *Tithonia* and inorganic fertilizers reflects high
289 nutrient supply that favoured plant growth. However, the similarity in yield for
290 *Tithonia* and inorganic fertilizers does not support the hypothesis of this study
291 that advocated greater yield for *Tithonia*. Nonetheless, the similarity in yield
292 for *Tithonia* and inorganic fertilizers indicates strong potential of *Tithonia* as a
293 sustainable alternative amendment for soil fertility management. The high
294 yield recorded for *Tithonia* biomass that is comparable to NPK treatment can
295 be attributed to high nutrient status of *Tithonia*, fast decomposition and
296 nutrient release [22,37]. In addition, the *Tithonia* biomass might have created
297 a favourable soil environment that probably enhanced root growth and
298 nutrient acquisition [41]. Moreover, the application of higher amounts of
299 *Tithonia* biomass might have increased crop growth more than the inorganic
300 fertilizers [16,42]. Hence, the similarity in yield for *Tithonia* and inorganic
301 fertilizers compared to higher performance of *Tithonia* reported by other
302 studies is likely because of the low amount of *Tithonia* biomass used in this
303 study [16,42]. Meanwhile, the release of readily available and balanced
304 macronutrients in the NPK fertilizer probably stimulated the crop growth and
305 yield [43]. Overall, the observed yield is consistent with the report of Yengoh
306 [44] that nutrient inputs and farm management are important determinants of
307 yield differences in small-scale food crop farming systems in Cameroon.

308

309 **5. CONCLUSION**

310 *Tithonia* and inorganic fertilizers increased the growth and yield of African
311 nightshade as compared to the control. However, the comparable crop yield
312 for *Tithonia* and inorganic fertilizers demonstrate the potential of *Tithonia* as a
313 sustainable alternative for inorganic fertilizers to improve soil fertility and crop
314 production. The results highlight the importance of fertilizers to improve soil
315 fertility and vegetable production, which emphasizes the need for more cost-
316 effective and sustainable alternative resources for soil fertility management.

317

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324

325 **COMPETING INTERESTS**

326 Authors have declared that no competing interests exist.

327

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468 Table 1. **A – baseline** soil physicochemical properties of the experimental site
 469 before treatments; **B – effect** of treatments (control – no fertilizer, urea, NPK
 470 and *Tithonia*) on soil physicochemical properties (Mean \pm SD).

A													
	Moisture [%]	Organic carbon [%]	Total nitrogen [%]	C/N	pH [H ₂ O]	pH [KCl]	Sodium [cmol/kg]	Potassium [cmol/kg]	Magnesium [cmol/kg]	Calcium [cmol/kg]	Aluminium [cmol/kg]	ECEC [cmol/kg]	Available phosphorus [mg/kg]
Baseline soil	9.5	2.0	0.14	14.0	5.4	4.5	0.33	0.22	1.5	4.0	0.32	6.4	15.0

B													
Treatments	Moisture [%]	Organic carbon [%]	Total nitrogen [%]	C/N	pH [H ₂ O]	pH [KCl]	Sodium [cmol/kg]	Potassium [cmol/kg]	Magnesium [cmol/kg]	Calcium [cmol/kg]	Aluminium [cmol/kg]	ECEC [cmol/kg]	
Control	17.2 \pm 1.8a	2.5 \pm 0.3a	0.14 \pm 0.02a	18.3 \pm 3.8a	4.9 \pm 0.5a	4.4 \pm 0.2a	0.19 \pm 0.11a	0.52 \pm 0.22a	1.6 \pm 0.5a	4.5 \pm 1.0a	0.56 \pm 0.37a	7.3 \pm 1.2a	
Urea	16.3 \pm 1.8a	2.8 \pm 0.4a	0.13 \pm 0.02a	22.3 \pm 4.8a	4.5 \pm 0.2a	4.1 \pm 0.1a	0.11 \pm 0.11a	0.30 \pm 0.13a	1.4 \pm 0.3a	3.4 \pm 1.4a	1.13 \pm 0.47a	6.3 \pm 1.5a	
NPK	16.4 \pm 1.3a	2.4 \pm 0.2a	0.14 \pm 0.01a	17.3 \pm 4.1a	4.6 \pm 0.3a	4.2 \pm 0.1a	0.21 \pm 0.12a	0.49 \pm 0.13a	1.9 \pm 0.9a	4.7 \pm 1.3a	0.92 \pm 0.51a	8.3 \pm 1.6a	
<i>Tithonia</i>	15.5 \pm 0.5a	2.4 \pm 0.3a	0.16 \pm 0.01a	15.0 \pm 2.7a	4.8 \pm 0.4a	4.3 \pm 0.1a	0.23 \pm 0.14a	0.36 \pm 0.10a	1.7 \pm 0.6a	4.7 \pm 1.1a	0.71 \pm 0.39a	7.8 \pm 1.2a	

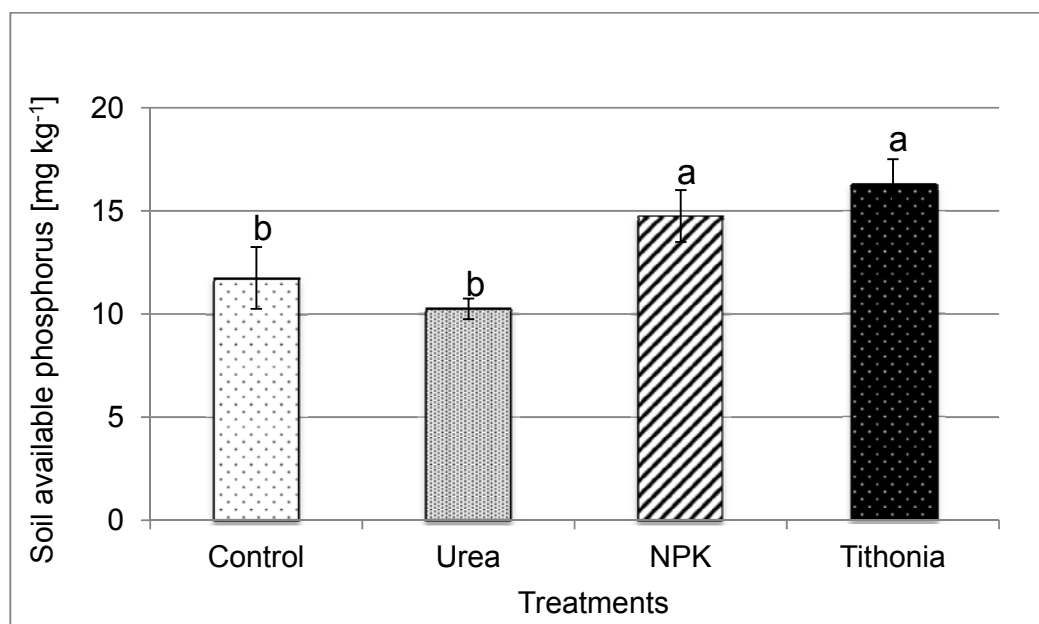
471
 472 Values within columns with the same letters are not significantly different ($P <$
 473 **0.05**).

474

475 Table 2. Effect of treatments (control – no fertilizer, urea, NPK and *Tithonia*)
 476 on the growth parameters of **African nightshade** (Mean \pm SD).

Treatments	Plant height [cm]	Number of leaves	Stem girth [cm]	Number of branches
Control	27.6 \pm 0.7b	90 \pm 12ab	1.8 \pm 0.3a	12 \pm 1a
Urea	24.6 \pm 2.4b	80 \pm 12b	1.4 \pm 0.3a	11 \pm 3a
NPK	33.2 \pm 2.2a	117 \pm 21a	2.1 \pm 0.5a	15 \pm 4a
<i>Tithonia</i>	26.6 \pm 4.1b	83 \pm 20ab	1.9 \pm 0.2a	13 \pm 4a

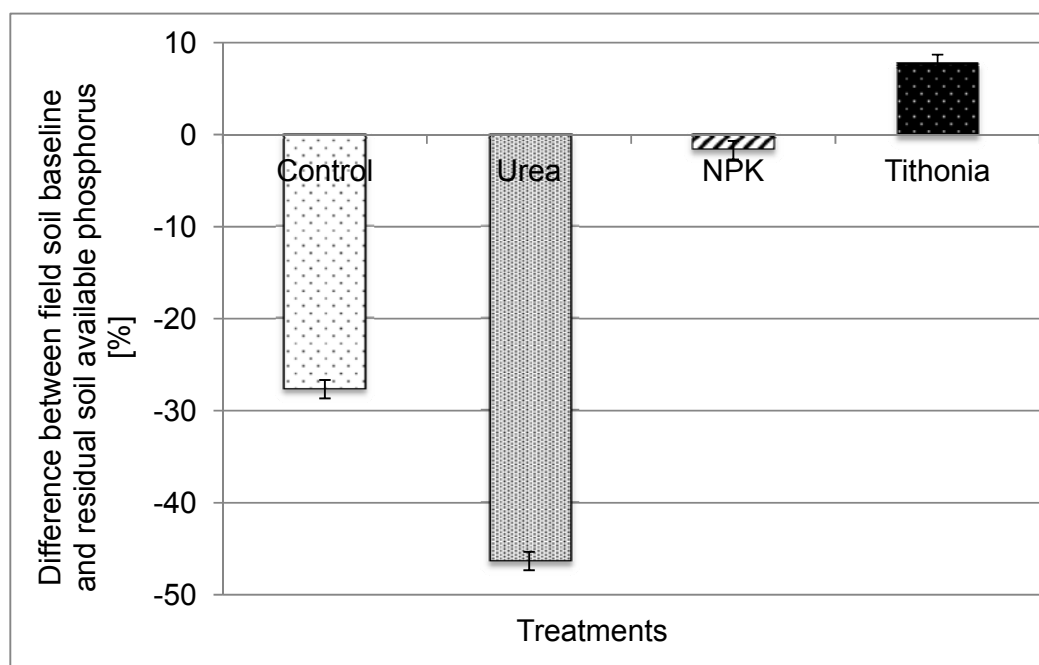
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 478 Values within columns with different letters are significantly different ($P <$
 479 **0.05**).



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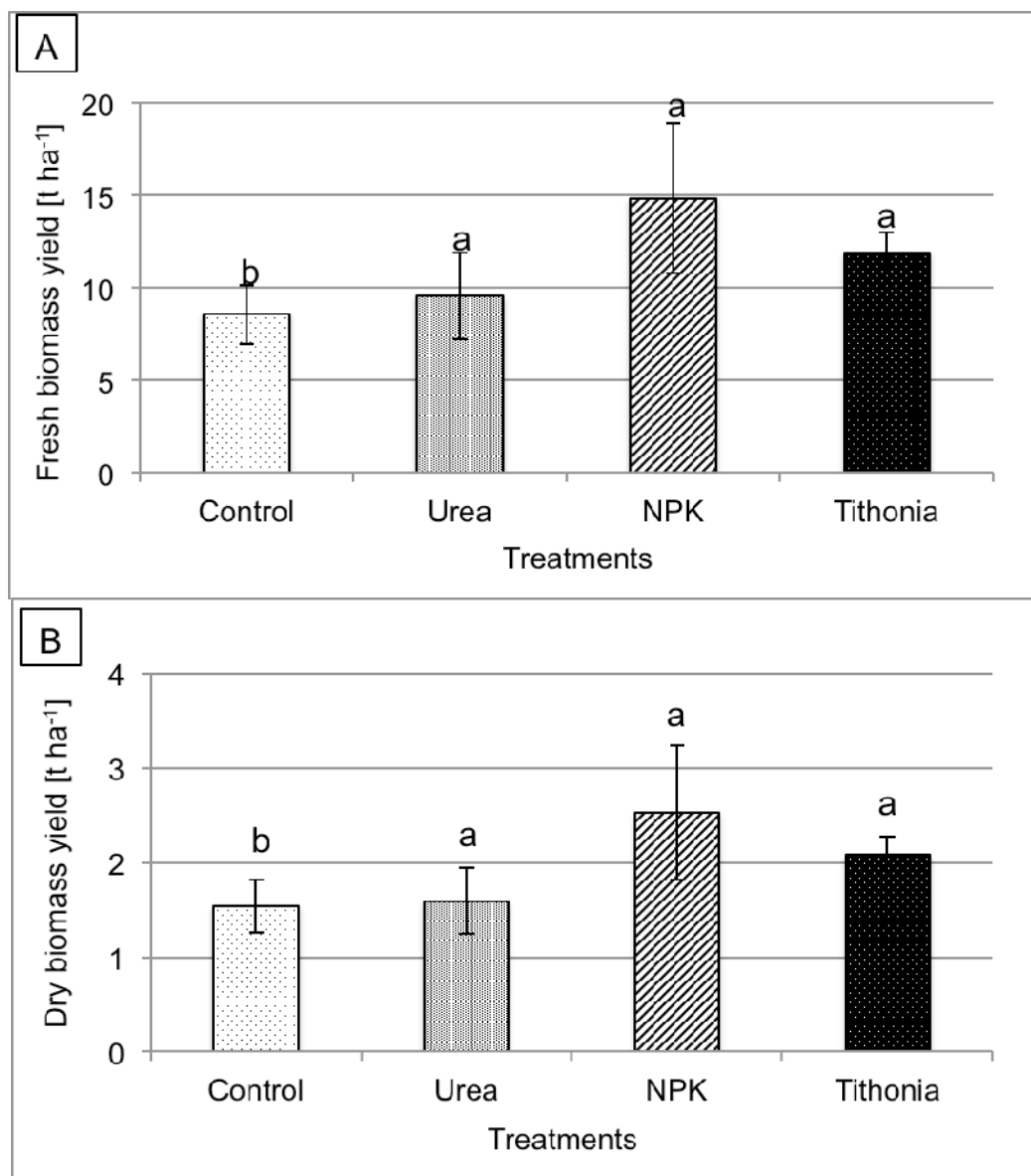
481 **Fig. 1** Effect of treatments (control – no fertilizer, urea, NPK and *Tithonia*) on
 482 soil available phosphorus (mg kg⁻¹, Mean ± SD). Values with different letters
 483 are significantly different ($P < 0.05$).

484



485

486 **Fig. 2** Percentage difference (% increase or decrease) between field soil
 487 baseline and residual soil available phosphorus after treatments.



488

489 **Fig. 3** Impact of treatments (control – no fertilizer, urea, NPK and *Tithonia*) on
 490 yield of African nightshade (t ha⁻¹, Mean ± SD); A – fresh biomass, B – dry
 491 biomass. Values with different letters are significantly different ($P < 0.05$).