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Influence of nitrogen to phosphorus supply ratio on growth, yield and biochemical parameters of kalonji (*Nigella sativa* L.)

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Abstract

Kalonji (*Nigella sativa* L.) is an important medicinal plant, cultivated as a rabi crop in Pakistan. A hydroponic sand culture experiment was conducted to know the effect of N:P supply ratio at two N and P supply levels on kalonji. N:P ratios maintained in nutrient solution ranged from 2 to 18 at two levels of N and P designated as high and low. The plants produced more biomass and seeds with higher antioxidant activity, total phenolics and flavonoids at high N and P supply levels. Biological weight, seed weight, antioxidant activity and total phenolics were not affected by N:P supply ratio. However, high N:P supply ratios *i.e.,* 15 and 18 reduced total flavonoids in kalonji seeds. N uptake by the plant was not influenced by N:P supply ratio however, P uptake was found maximum at ratios 6 to 9. It is concluded from the results that kalonji plants with better quality seeds can be more successfully grown if N:P supply ratio is maintained at or near 7 in the growth medium.

Key words: Kalonji, N:P supply ratio, antioxidant activity, phenolics, flavonoids

Introduction

Fennel (*Nigella sativa*) or Kalonji belongs to the family Ranunculaceae and is a famous medicinal plant. Seeds of this plant contain many healing components that help to make a strong immune system and provide strength to human body parts (Ahmad *et al*., 2018). It is extensively used in traditional medicine as antidiarrheal, antihypertensive, liver tonic, appetite stimulant, antidiabetic, anti-inflammatory and antimicrobial (Gilani *et al*., 2004).

Nitrogen (N) and phosphorus (P) are macronutrients that significantly affect the growth, yield and biochemical parameters of kalonji plants (Yimam *et al*., 2015). The interaction among these nutrients promotes their uptake and increases crop yields (Rietra *et al*., 2017). Wilkinson *et al*. (2000) described that N can enhance P absorption in plants by increasing root growth. However, excess amounts of P fertilizer can negatively affect plant growth by inducing zinc deficiency (Mandal and Haldar, 1980). Tomassen *et al.* (2003) suggested that plant species respond differently to N:P supply ratios. Plants with high and low N:P supply ratios differ in various traits irrespective of their fast growth (Güsewell, 2004).

If P is limiting, increased N deposition does not stimulate plant growth but rather reduces it (Carroll *et al*., 2003). In short-term experiments, P deficiency reduces the growth of young plants less than N deficiency, probably because these plants can adjust to P deficiency through a reduction in internal P concentrations (Keddy *et al*., 2001; Güsewell, 2004). Conversely, P deficiency seems to have a more negative impact than N deficiency on long-term plant performance (Brouwer *et al*., 2001). Field studies also suggested that P deficiency induced by N enrichment increased root turnover and decreased nutrient retention by the vegetation (Güsewell *et al*., 2002; El-Kahloun *et al.,* 2003). In a growth experiment with 15 wetland species, plants receiving high N supply retained a smaller fraction of the supplied nutrients in their biomass than those receiving high P supply (Güsewell and Bollens*,* 2003). Given the medicinal importance of Kalonji, the interaction of N and P in controlling its various growth parameters has not been well assessed. In this study, growth, yield, and biochemical parameters of kalonji were investigated under different N:P supply ratios at two N and P supply levels in the nutrient solution.

Material and methods

This sand culture hydroponic experiment was conducted at the Department of Soil Science, University of Punjab, Lahore in plastic pots. Sand (particle size 1 to 2 mm diameter) was washed, air-dried, and filled to plastic pots of height 15 cm and diameter 13 cm at 2.5 kg per pot. Before sand filling the bottom of each pot was drilled with three equally spaced holes for nutrient solution absorption and drainage. Each sand-filled pot was placed in a plastic plate having a diameter more than that of the base of the pot by 3 cm.

Experimental plan: The experimental plan was comprised of sixteen nutrient treatments, eight N:P supply ratios at two supply levels. N and P concentration in the nutrient solutions was calculated by using the following formulae as reported by Gusewell (2005) and are presented in Table 1.

$N = L.\sqrt{N:P}$ and $P = \frac{1}{\sqrt{N:P}}$

Where, L is the overall supply level (geometric mean of N and P supply), being either 80 mg for high supply or 26 mg for low supply.

All the pots with their bottom plates were placed according to two-factor factorials completely randomized design (CRD) with three replications. Before seed sowing, each treatment solution at 200 mL per pot was applied in underlying plates. At absorption of the treatment solution by pot sand, 8-10 seeds of Kalonji (*Nigella*

Table 1. Treatment plan: concentrations of nitrogen (N) and phosphorus (P) in treatment solutions to maintain various N:P ratios at high and low N and P level

Treatment	N:P Ratio	N and P level	N content	P content
T1	2	High	114.1	57.0
T2	3	High	139.7	46.6
T3	6	High	197.6	32.9
T4	7	High	213.5	30.5
T5	9	High	242.0	26.9
T6	12	High	279.5	23.3
T7	15	High	312.5	20.8
T8	18	High	342.3	19.0
T9	2	Low	38.03	19.01
T10	3	Low	46.58	15.52
T11	6	Low	65.87	10.97
T12	7	Low	71.15	10.16
T13	9	Low	80.68	8.96
T14	12	Low	93.16	7.76
T15	15	Low	104.15	6.94
T16	18	Low	114.09	6.34

sativa) were sown in each pot and after seedling emergence, two plants per pot were maintained. The pots were supplied with treatment solutions once a week. The water requirement of the plants was fulfilled by applying 100 mL distilled water per pot as and when needed. The weeds were controlled manually and at the bud formation stage, an aphid attack was controlled by spraying the plants with Imidacloprid at 1 cm^{-3} L^{-1}.

Parameters studied: It took 135 days for the plants to attain physiological maturity. Shoots and roots were separated, weighed, measured, and the statistical analysis used the average of two plants in a pot. The seed pods were counted, weighed, and threshed. The seeds per plant were recorded. 100 seeds per treatment were counted and weighed. Weighed down to 100 seeds when total seed count fell short.

For total phenolic and flavonoid contents, weighed amount of crushed seed mass was extracted with 80% methanol. In case of total phenolics, the supernatant extract was mixed with Folin Ciocalteu Reagent (FCR) and Na_2CO_3 followed by incubation in darkness for 2 hours at room temperature. The optical density was measured at 765 nm. Total phenolics were determined against a gallic acid standard curve and expressed as gallic acid equivalent (Velioglu *et al*., 1998). For flavonoid determination, the supernatant extract was mixed with $NaNO_2$ solution and $AlCl_3$ solution one after the other with 6 min interval. After a further 5 min incubation, 1.0 N NaOH was added and optical density was measured instantly at 510 nm. Total flavonoids were then determined against a quercetin standard curve and expressed as quercetin equivalent (Chang *et al*., 2002).

FRAP (Ferric Reducing Antioxidant Power) method was used for determining the antioxidant activity of Kalonji seeds. In this method, 50 µL of sample extract and 1.50 mL of FRAP reagent were added in pre-labeled falcon tubes and mixed gently. After 15 minutes of incubation in dark at room temperature, absorption was measured with a Spectrophotometer at 593 nm. Ascorbic acid standards curve was used to determine antioxidant activity as ascorbic acid equivalent (Thaipong *et al.,* 2006).

For P determination the plant samples were extracted with a mixture of nitric acid and perchloric acid. The P in the digests was determined by using the ammonium vanadate ammonium molybdate method (Jones *et al*., 1991). For N determination plant samples were extracted with concentrated sulfuric acid followed by Kjeldahl distillation. N as ammonia from distillation was absorbed in boric acid solution and determined by titration against 0.01 N H_2SO_4 (Hu and Barker, 1999). Total uptake of both N and P was estimated by multiplying dry plant weight with N and P concentrations in the plant, respectively.

Data were subjected to analysis of variance and means were compared through the Tuckey HSD test by using Statistics 8.1 computer software.

Results

Growth and yield parameters: It is evidentif from the analysis of variance that N:P supply ratio and N and P supply level in nutrient solution significantly affected the shoot length of kalonji (Table 2). The length increased with increasing N:P supply ratios and the highest was noted in the treatments where the ratio was maintained as 6 or 7. At ratios 2 to 7, high N and P level gave more shoot length than that of low level. However, at ratios from 9 to 18 the shoot lengths at high and low nutrient levels were statistically non-significant (Fig. 1).

The root length of kalonji plant was also significantly affected by both N:P supply ratios and N and P supply levels. However, their interaction remained non-significant (Table 2). On an average basis, maximum root length was observed where a high N and P level was maintained, compared to that of low level (Table 4). All N:P supply ratios, except 12, 15 and 18, produced roots with statistically similar lengths. At ratios from 12 to 18, the length was significantly less than that of other treatments (Table 3).

On an average basis at high N and P level, root, shoot, seed and biological weights were 29, 28, 20 and 28% more than that at low level, respectively (Table 4). Other factors *i.e*., N:P supply ratio and interaction of the ratio and the level did not affect root, shoot and seed weights (Table 2).

Table 2. F-ratios and significance levels for the effects of nitrogen (N) and phosphorus (P) supply ratio and N and P supply level on N and P status, various growth, yield and biochemical parameters of kalonji

Parameters	F-value		
	N:P supply ratio	Nutrient level	Ratio × level
Shoot length (cm)	30.06**	180.31**	3.35*
Root length (cm)	5.58**	30.75**	0.53$^{N.S}$
Root weight (g plant^{-1})	3.51$^{N.S}$	17.77**	0.30$^{N.S}$
Shoot weight (g plant^{-1})	0.96$^{N.S}$	24.72**	0.33$^{N.S}$
Seed weight (g plant^{-1})	0.41$^{N.S}$	5.91*	0.11$^{N.S}$
Biological weight (g plant^{-1})	1.00$^{N.S}$	33.10**	0.32$^{N.S}$
No. of capsules per plant	10.60**	17.42**	0.51$^{N.S}$
100 Seed weight (g)	1.74$^{N.S}$	0.00$^{N.S}$	0.50$^{N.S}$
Antioxidant activity (AA eq.)	2.07$^{N.S}$	21.6**	0.53$^{N.S}$
Total phenolics (GA eq.)	1.66$^{N.S}$	15.4**	0.05$^{N.S}$
Total flavonoids (Quercetin eq.)	5.68**	11.37*	0.76$^{N.S}$
N conc. in plant (%)	5.60**	9.63*	0.34$^{N.S}$
P conc. in plant (%)	10.03**	12.10**	0.22$^{N.S}$
N uptake (mg plant^{-1})	1.65$^{N.S}$	30.54**	0.32$^{N.S}$
P uptake (mg plant^{-1})	6.25**	25.27**	0.32$^{N.S}$
N:P ratio in plant (uptake)	7.36**	3.51$^{N.S}$	0.51$^{N.S}$

**=Highly significant at $P \leq 0.001$, *=significant at $P \leq 0.05$, NS=Non-significant. AA=Ascorbic acid, GA=Gallic acid

Table 3. Variations in variables of kalonji (*N. sativa* L.) seeds scored in response to N:P supply ratio

Parameters	N:P Supply Ratio							
	2	3	6	7	9	12	15	18
Root length (cm)	10.4a-c	10.4a-c	11.6a	10.8ab	11.1ab	9.2c	9.7bc	9.0c
No. of capsules	3.2bc	3.3bc	3.8ab	4.6a	3.2bc	2.6c	2.7c	3.2bc
Total flavonoids	5.9ab	6.4a	6.0ab	5.7ab	4.9bc	5.1a-c	4.2c	4.0c
N in plant (%)	0.6ab	0.8a	0.6a-c	0.6bc	0.6bc	0.5c	0.7ab	0.6bc
P in plant (%)	0.04c	0.05c	0.08ab	0.10a	0.06bc	0.05c	0.05c	0.05c
P uptake (mg)	6.0c	7.2bc	12.5ab	13.4a	9.2a-c	7.6bc	5.9c	6.2c
N:P ratio (uptake)	15.3a	12.9ab	7.6bc	5.7c	9.8a-c	8.8bc	14.6a	11.8ab

*Means sharing common letters in a row do not differ significantly at $P < 0.05$.

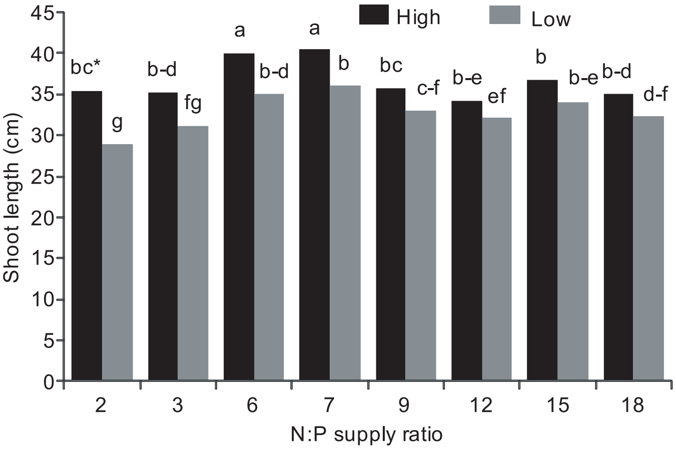

Fig.1. Response of shoot length of kalonji (*N. sativa* L.) plant to high and low N and P supply level maintained at various N:P supply ratios. *Bars sharing common letters do not differ significantly at $P < 0.05$.

Table 4. Variations in variables scored in response to N and P supply level in nutrient solution.

Parameters	N and P supply level	
	High	Low
Shoot length (cm)	36.6a*	32.3b
Root Length (cm)	11.17a	9.88b
Root Weight (g)	0.50a	0.38b
Shoot Weight (g)	11.98a	9.39b
Seed Weight per plant (g)	1.89a	1.46b
Biological Weight (g plant^{-1})	14.38a	11.24b
No. of capsules per Plant	3.60a	3.02b
Antioxidant activity (ascorbic acid eq.)	0.55a	0.47b
Total phenolics (GA eq.)	0.55a	0.47b
Total flavonoids (quercetin eq.)	5.81a	5.10b
N conc. in plant (%)	0.65a	0.57b
P conc. in plant (%)	0.07a	0.05b
N uptake of plant (mg plant^{-1})	93.9a	65.1b
P uptake of plant (mg plant^{-1})	10.6a	6.4b

*Means sharing common letters in a row do not differ significantly at $P < 0.05$.

The number of capsules per plant of kalonji was significantly affected with N:P supply ratios and N and P supply levels however, the interaction effect of both factors was non-significant (Table 2). The number of capsules was higher at high N and P supply level and found maximum at N:P supply ratio of 6 and 7, whereas, capsules on plants cultivated on other ratios were significantly less than that at 6 and 7 (Table 3). The analysis of variance indicated that N:P supply ratios, N and P supply levels and the interaction of the ratios and levels did not significantly affect 100 seeds weight of kalonji (Table 2).

Biochemical parameters and nutrient status: N:P supply ratio and interaction effect of ratio and level did not affect both the antioxidant activity and total phenolic contents in kalonji seeds; however, the N and P supply level significantly affected both these parameters (Table 2). Antioxidant activity and total phenolic contents increased with increasing N and P supply level in the solution. Table 4 indicates that at high N and P level, antioxidant activity and total phenolic contents were 16 and 17% more than that of low N and P level, respectively.

It is obvious from the analysis of variance that N:P supply ratio and N and P supply level significantly affected the total flavonoid contents of kalonji seeds (Table 2). Plant-produced seeds with more flavonoid contents at a high N and P level compared to that at the low level (Table 4). Statistically similar flavonoid content were noted in the treatments with N:P supply ratios from 2 to 12. However, compared to other treatment ratios, total flavonoids significantly decreased at extreme N:P ratios of 15 and 18 (Table 3).

N:P supply ratio and N and P supply level affected N and P concentration in kalonji plants (Table 2). The concentration of both the nutrients was found maximum at a high N and P levels as compared to that at low level (Table 4). When studied at different N:P supply ratios, the N concentration in the plant was found maximum at ratio 3 which was statistically similar to that at ratios 2 and 6. The response of N concentration to other N:P supply ratios did not differ significantly from that where the ratio was maintained at 6 (Table 3). At N:P supply ratio 6 and 7, P concentration in plants was found maximum. The response of plant P to other N:P supply ratios was statistically similar, and less than that at ratios 6 and 7 (Table 3).

At a high N and P level, kalonji plants had more N and P uptake compared to that at a low N and P supply level (Table 4). At N:P supply ratios 6 and 7, P uptake of the plant was found maximum followed by that at ratio 9. The response of plant P to other N:P supply ratios was similar to that at ratio 9 (Table 3). No significant effect of N:P supply ratio was noted on N uptake of kalonji plant.

The minimum N:P ratio in the plant was noted at N:P supply ratio 7. An increase or decrease in supply ratio from 7 resulted in a gradual increase in the N:P ratio of the plant (Table 3).

Discussion

Weights of kalonji plant parts, antioxidant activity and total phenolics of the seeds were affected only with N and P supply level. The values of these parameters were more at a high N and P supply than that at a low level where N and P concentrations were maintained three times less. It is evidentif from the results that at low level of N and P concentrations in nutrient solution was not enough to fulfill plant requirements.

Shoot and root length, number of capsules per plant, N and P in the plant, and flavonoid contents in seeds were significantly affected with both N and P supply levels and N:P supply ratios.

However, the effect of the ratios was generally weaker than that of two supply levels as is evidentif from much lower F-ratios (Table 2). This seems to be related to plant type as it is already known that plant species respond differently to N:P supply ratios (Tomassen *et al*., 2003).

The N:P ratio in plant biomass was the only unaffected parameter by N and P supply levels. The plant's N:P ratio was found to be lowest (around 5) in the treatment where it was kept at 7. This supply ratio increased growth, P demand, and P uptake (Terman *et al*. 1977). The low root and shoot length in these high N:P ratio treatments indicates that P was too limiting to negatively affect plant N use efficiency. It is well known that in a P limiting environment, N deposition does not promote growth but inhibits it (Gotelli and Ellison, 2002; Carroll *et al*., 2003).

Generally, N:P supply ratios of 2 to 3 promoted N:P supply. This may have been mediated by cytokinin. Shoot cytokinin levels boost growth and delay leaf ageing (Smart, 1994; Gan and Amasino, 1997). The reduction in N supply reduces cytokinin production and transport from roots to shoots, suppressing growth before N becomes directly limiting (Lambers *et al*., 1998; Forde, 2002). For plants that already take up N sufficiently, increased N concentration in the rooting medium stimulates cytokinin production (De-Groot *et al*., 2003). When P is not limiting, the effect of P supply on cytokinin levels is less rapid and less pronounced (De-Groot *et al*., 2003).

Less number of capsules per plant produced at a high N:P supply ratio could be a response of low P concentration. This assumption is supported by previous findings where P deficiency impaired reproduction resulting decreased fruit and seed setting (Brouwer *et al*., 2001).

The results show that the kalonji plants can be grown best with a N:P ratio of 7 or near 7. The study should be repeated in a soil medium with multiple factors affecting nutrient availability to the plants for validation.

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