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Sub lethal mutagenic effects of glyphosate herbicides on juveniles of *Clarias gariepinus* using randomly amplified DNA primers

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Authors' Contribution Das, U. K has been setup experiment, manage experiment, collected data and complied, statistical analyzed and collected references, **M. M. A. Mondal** has been developed the concepts and also designed the experiments, supervise the experimenter and write up the article.

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ABSTRACT

An experiment was conducted at the pot yard of Bangladesh Institute of Nuclear Agriculture, Mymensingh during the period from February to May 2017 to investigate the effect of foliar application of nitrogen and micronutrients on crop characters, yield attributes and yield of two mungbean genotypes. The experiment comprised four levels of nutrients foliar application like, i) T_1 = Control; ii) T_2 = Foliar application of urea at the rate of 1.5% four times from flowering start to pod development stage with an interval of 4 days; iii) $T_3 = T_2$ + 0.1% micronutrients (B, Mo, Zn, Mn, Ca, Fe, Cu) four times from flowering start to pod development stage with an interval of 4 days and iv); $T_4 = T_3$ + side dressing (soil application) of 1.5% N and 0.1% micronutrients solution four times from flowering start to pod development stage with an interval of 4 days. The genotypes were Binamoog-6 and Binamoog-7. Results revealed that morphological (plant height, branch and leaf number, leaf area plant^{-1}), physiological (total dry mass plant^{-1}, specific leaf weight, chlorophyll), yield attributes (number of pods plant^{-1}, pod length, single pod weight, number of seeds pod^{-1} and 100-seed weight) and yield increased in foliar nutrients applied plants over control but the increment was greater in T_2 and T_3 than the T_4 treatment. The highest plant height, branch and leaf number, leaf area, total dry mass, pod number, pod length, 100-seed weight and seed yield were recorded in T_2 followed by T_3 with same statistical rank (in most cases). The lowest morpho-physiological, yield attributes and yield were recorded in T_1 (control) plants. Binamoog-7 was superior in most of plant parameters and yield compared to Binamoog-6.

Keywords: Foliar fertilization, nitrogen, micronutrients, yield, mungbean.

INTRODUCTION: Mungbean [*Vigna radiata* (L) Wilczek] is a widely grown, short duration grain legume crop in Southeast Asia. Mungbean contains 51% carbohydrate, 26% protein, 10% moisture, 4% mineral and 3% vitamins (Afzal and Abe, 1998). Besides providing valuable protein in the diet, mungbean has the remarkable quality of helping the symbiotic root rhizobia to fix atmospheric nitrogen and hence to enrich soil (Malek *et al.*, 2012). However, the mean yields in the farmers' fields are low, 903 kg ha^{-1}. Even in the experimental farms, grain yield rarely exceeds 2000 kg ha^{-1} (BINA, 2018). The slow rate of dry matter accumulation during the preflowering phase, onset of leaf senescence during the period of pod development and low partitioning efficiency of assimilates to grain were identified (Mondal *et al.*, 2011) as the main physiological constraints for increasing yield. Other region of low yield in mungbean is that like other grain legumes is characterized by prolific flower production with an extremely low proportion of pod set (Mondal *et al.*, 2013). However, many pods did not fill their seeds and grain yield did not increase. Oliveira *et al.* (2019) concluded that despite the greatly increased sink potential, the plants lacked the capacity for increasing assimilates supply during pod growth and development. Researchers have suggested the foliar fertilization minimizes nutrient depletion from the leaves in legumes (Boote *et al.*, 1978; Oliveira *et al.*, 2019). Concentration of N, P, K in leaves, stem, petioles and pod walls decline considerably during the seed-filling period and exogenous supply of elements may overcome these problems. Protein concentration in mungbean seed ranges from 24 to 26% on a dry weight basis and hence the N requirement for seed development is high. It was estimated to be 26 mg N g^{-1} of photosynthate (Mitra *et al.*, 1988). It is indicated that, at best, only 20 mg N g^{-1} photosynthate can available to the developing seed from the soil (Mitra *et al.*, 1988). Thus, from data it is apparent that mungbean is not able to meet the N demand of its seeds by uptake from the soil or by fixation. Researchers reported that foliar application of N during reproductive stages increased grain yield in legumes (Hamayun *et al.*, 2011).

The fertilizer management practices may have tremendous role to play on the growth, yield and quality of the crop. In many cases micronutrients may also play vital role in the improvement in the growth, yield, and quality of legume crops. Foliar application of micronutrient was better than direct soil application for increasing yield. In legume crops, requires not only adequate macronutrients but also micro-nutrients for increasing the bacterial activity of nodule. So, an optimum supply of micronutrients under balanced condition is very important for achieving higher productivity. Extensive research performed during 1970-2020s on foliar fertilization at reproductive stages in soybean showed increased grain yield. Research report on foliar nutrition effect in mungbean is still scarce. The experiment was therefore to investigate whether exogenous supply of nitrogen, in the form of urea and urea plus micronutrients, during flowering and pod development stages would increase seed yield in mungbean.

MATERIALS AND METHODS: A pot experiment was carried out at the pot yard of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, during the period from February to May 2017. The experiment comprised of four levels of foliar nutrients application from flowering to pod development stages, were: i) Control (no foliar application of nutrient, T_1); ii) Urea four times at the rate of 1.5% at an interval of 4 days (T_2); iii) 1.5% urea plus 0.1% micronutrients of B, Mo, Zn, Mn, Cu, Ca and Fe four times at an interval of 4 days (T_3) and iv) T_3 + side dressing (soil application) of 1.5% N and 0.1% micronutrients solution four times from flowers start to pod development stage with an interval of 4 days. Two mungbean genotypes, bold seeded (Binamoog-6) and small seeded (Binamoog-7) were selected. The pots were filled with 12 kg sandy loam soil. The urea, triple superphosphate, muriate of potash and gypsum were applied as basal doses at the rate of 40, 80, 60 and 40 kg ha^{-1}, respectively. The experiment was laid out in a two factor completely randomized design with four replications. Each pot contained two plants and denotes a replication. Seeds were sown on 28th February 2017. Intercultural operations like weeding, mulching and pest control were done as and when necessary for healthy plant growth and development.

At harvest, plant height, number of branches and leaves, leaf area, total dry matter and yield and yield contributing characters were recorded. Leaf area per plant was measured by automatic leaf area meter (LICOR 3000, USA). The total dry matter was recorded by drying (80 $^0C \pm 2$) for 72 hours and calculated from summation of leaves, stem, roots and pods was taken in an electronic balance. Specific leaf weight was calculated by dividing leaf weight to leaf area. Chlorophyll was determined during pod growth stages following the method of Yoshida *et al.* (1971). Harvest index (HI) was calculated by dividing economic yield to biological yield of plant by multiplying with 100 and expressed in percentage. The collected data were analyzed by CRD factorial design using the statistical computer package programme, MSTAT.

RESULTS AND DISCUSSION: The effect of foliar application of N, N with micronutrients and soil application of N and micronutrients had significant effects on morphological parameters like plant height, number of branches and leaves $plant^{-1}$ and leaf area $plant^{-1}$ (table 1). Result showed that morphological parameters increased over control in nutrient applied plant. Plant height, number of branches and leaves $plant^{-1}$ and leaf area $plant^{-1}$ were significantly increased in both N and N plus micronutrients treated plants over control with N treated plant being the highest of all treatments. Bold seeded genotype, Binamoog-6 showed inferior performance in respect of plant height, number of branches and leaves $plant^{-1}$ and leaf area $plant^{-1}$ compared to small seeded genotype, Binamoog-7. These results are in conformity with that of Malek *et al.* (2012) who evaluated 45 mungbean genotypes on the basis of morpho-physiological criteria and reported that in general, bold seeded mungbean genotypes produced lower plant height and LA than small seeded ones. Further, Mitra *et al.* (1988) observed that foliar application of N had increased LA compared to control in mungbean and soybean that supported the present results. On the other hand, application of N and micronutrients both foliar and soil during reproductive stages had no significant influence plant morphological parameters (table 1).

Treatment		Plant height (cm)	Branches $plant^{-1}$ (no)	Leaves $plant^{-1}$ (no)	Leaf area $plant^{-1}$ (cm^2)
Foliar application of nutrients					
T_1 = Control		20.5 b	1.64 c	8.85 b	245 c
T_2 = Urea		25.1 a	2.06 a	9.94 a	446 a
T_3 = Urea + micro-nutrients		24.2 a	1.80 b	8.24 c	305 b
T_4 = Urea + micronutrient +side soil dressing of T_3		21.8 b	1.43 d	7.50 d	262 c
F-test		**	**	**	**
Genotype					
Binamoog-6		21.7 b	0.65 b	5.75 b	266 b
Binamoog-7		24.1 a	2.82 a	11.5 a	372 a
F-test		**	**	**	**
Interaction of genotype and treatment					
Binamoog-6	T_1	19.3 e	0.57 e	5.86 e	202 e
	T_2	22.8bcd	1.00 d	5.87 e	309 bc
	T_3	23.6 bc	0.60 e	5.86 e	288 cd
	T_4	20.9 de	0.43 e	5.43 e	248 e
Binamoog-7	T_1	21.7 cde	2.71 b	11.9 b	301 bcd
	T_2	27.4 a	3.12 a	14.0 a	383 a
	T_3	24.8 b	3.00 a	10.6 c	323 b
	T_4	22.6bcd	2.43 c	9.57 d	283 d
F-test		*	*	**	**
CV (%)		7.02	8.07	6.20	5.17

Table 1. Effect of foliar application of nitrogen and micronutrients on morphological haracters in mungbean.
T1 = Control; T2 = Foliar application of at the rate of 1.5% four times from flowers start to pod development stage with an interval of 4 days; T3 = Foliar application of Urea + 0.1% micronutrients (B, Mo, Zn, Mn, Ca, Fe, Cu) four times from flowers start to pod development stage with an interval of 4 days and T4 = Foliar application of Urea + 0.1% micronutrients (B, Mo, Zn, Mn, Ca, Fe, Cu) + ground application of 1.5% N and 0.1% micronutrients solution four times from flowers start to pod development stage with an interval of 4 days.

The interaction effect of foliar application of nitrogen and micronutrients in relation to plant height, number of branches and leaves $plant^{-1}$ and leaf area $plant^{-1}$ (table 1). The tallest plant, higher number of branches and leaves $plant^{-1}$ and leaf area $plant^{-1}$ was recorded in Binamoog-7 with foliar application of N. The shorted plant, lower number of branches and leaves $plant^{-1}$ as well as leaf area $plant^{-1}$ was recorded in Binamoog-6 with no N and micronutrient applied plant.

The effect of foliar application of N, N with micronutrients and soil application of N and micronutrients had significant effects on physiological parameters (table 2). Total dry mass (TDM) $plant^{-1}$, specific leaf weight (SLW), chlorophyll content in leaves and harvest index were significantly increased in both N and N plus micronutrients treated plants over control. TDM $plant^{-1}$ and chlorophyll content in leaves were the highest in only N foliar applied plants whereas the highest SLW and HI was recorded in foliar application of N plus micronutrient applied plants. The treatment of T_4 (foliar application of N + micronutrients and soil application of N + micronutrients) showed inferiority in physiological parameters as compared to T_2 (foliar application of N) and T_3 (foliar application of N +

micronutrients) treatment indicating both foliar and soil application of N and micronutrient had no significant effect on physiological parameters in mungbean plants. Bold seeded genotype, Binamoog-6 showed inferior performance in respect of TDM, SLW and chlorophyll content in leaves compared to small seeded genotype, Binamoog-7 although HI was greater in the former than latter.

Treatment		Total dry mass plant^{-1} (g)	Specific leaf weight (mg cm^{-2})	Chlorophyll (mg g^{-1} fw)	Harvest index (%)
Foliar application of nutrients					
T_1 = Control		4.75 d	8.71 d	1.69 c	32.2 ab
T_2 = Urea		7.60 a	10.4 b	2.17 a	31.1 b
T_3 = Urea + micro-nutrients		6.22 b	10.9 a	2.13 ab	33.0 a
T_4 = Urea + micronutrient + side soil dressing of T_3		5.38 c	9.77 c	2.02 b	32.8 ab
F-test		**	**	**	*
Genotype					
Binamoog-6 Binamoog-7		4.38 b 7.60 a	8.78 b 11.1 a	1.98 2.02	35.3 a 29.2 b
F-test		**	**	NS	**
Interaction of genotype and treatment					
Binamoog-6	T_1	3.13 f	7.20 e	1.66 c	36.7 a
	T_2	5.35 d	9.59 c	2.11 ab	35.9 a
	T_3	5.33 d	9.87 c	2.03 ab	33.6 abc
	T_4	3.69 e	8.47 d	2.10 ab	35.2 ab
Binamoog-7	T_1	6.37 c	10.2 c	1.72 c	27.8 de
	T_2	9.85 a	11.2 b	2.23 a	26.3 e
	T_3	7.12 b	12.0 a	2.20 a	32.4 bc
	T_4	7.07 b	11.1 b	1.95 b	30.4 cd
F-test		**	*	*	*
CV (%)		3.19	4.66	5.73	7.95

Table 2. Effect of foliar application of nitrogen and micronutrients on physiological parameters of mungbean.

In a column, figures bearing same letter (s) do not differ significantly at $P \leq 0.05$ by DMRT; NS = Not significant; * and ** indicate significance at 5% and 1% level of probability, respectively. T1 = Control; T2 = Foliar application of at the rate of 1.5% four times from flowers start to pod development stage with an interval of 4 days; T3 = Foliar application of Urea + 0.1% micronutrients (B, Mo, Zn, Mn, Ca, Fe, Cu) four times from flowers start to pod development stage with an interval of 4 days and T4 = Foliar application of Urea + 0.1% micronutrients (B, Mo, Zn, Mn, Ca, Fe, Cu) + ground application of 1.5% N and 0.1% micronutrients solution four times from flowers start to pod development stage with an interval of 4 days.

These results are in conformity with that of Malek *et al.* (2012) who evaluated 45 mungbean genotypes on the basis of morpho-physiological criteria and reported that in general, bold seeded mungbean genotypes produced lower LA, TDM and chlorophyll content in leaves with higher HI than small seeded ones. Further, several researchers observed that foliar application of N had increased TDM, chlorophyll content in leaves to control in mungbean and soybean Mitra *et al.* (1988) that supported the present results. The effect of foliar and soil application of N, N with micronutrients and soil application of N and micronutrients on yield contributing characters and yield such as number of pods plant^{-1}, pod length, single pod and 100-seed weight and number of seeds pod^{-1} was statistically significant (table 3). Results revealed that all the yield attributes were greater in both foliar nutrients and soil nutrients applied plants (T_2, T_3 and T_4) than the control (T_1). The highest seed yield was recorded in foliar application of N though it was not significantly different from N + micronutrients application. The yield was the highest in foliar application of N alone might be due to increased number of pods, number of seeds pod^{-1} and seed size. In contrast, the lowest yield contributing characters and yield were recorded in T_1 (control). Several studies (Hamayun *et al.*, 2011; El-Azab, 2016; Oliveira *et al.*, 2019; Bahadri *et al.*, 2020) confirmed that foliar application of N and/or micronutrients increased grain yield in legumes that supported the present experimental results. The effect of variety on yield attributes and yield was significant (table 3).

Treatment		Pods plant^{-1} (no)	Pod length (cm)	Single pod weight (mg)	Seeds pod^{-1} (no)	100-seed weight (g)	Seed yield plant^{-1} (g)
Foliar application of nutrients							
T_1 = Control		9.22c	6.89 b	445 b	4.23c	7.53 c	2.79c
T_2 = Urea		12.5b	7.34 a	469 a	5.02a	9.09 a	4.13a
T_3 = Urea + micronutrients		14.2a	7.02 b	478 a	5.08a	8.84 a	4.00a
T_4 = Urea + micronutrient + side soil dressing of T_3		12.1b	6.87 b	421 c	4.79b	8.08 b	3.32b
F-test		**	**	**	**	**	**
Genotypes							
Binamoog-6 Binamoog-7		9.07 b 15.0 a	7.89 a 6.17 b	546 a 361 b	5.59 a 3.98 b	8.25 b 8.52 a	3.37 b 3.76 a
F-test		**	**	**	**	*	**
Interaction of genotype and treatment							
Binamoog-6	T_1	6.30 f	8 ab	544 b	5.09c	7.06 d	2.64e
	T_2	10.1d	8.30 a	559 b	5.7ab	9.30 a	4.29a
	T_3	11.5c	7.75b	594 a	5.91a	9. ab	3.7c
	T_4	8.4e	7.50 c	488 c	5.5b	7.63c	2.8de
Binamoog-7	T_1	12.1c	5.78 e	346 e	3.38 f	8.00 c	2.9 d
	T_2	15b	6.38 d	379 d	4.28d	8.88b	3.98b
	T_3	17.0a	6.28 d	362 de	4.25d	8.68 b	4.31a
	T_4	15.7b	6.23 d	355 de	4.0e	8.53 b	3.80bc
F-test		*	**	**	*	**	**
CV (%)		6.60	3.84	4.06	3.07	3.55	4.90

Table 3. Effect of foliar application of nitrogen and micronutrients on yield contributing characters and yield in mungbean.

In a column, figures bearing same letter (s) do not differ significantly at $P \leq 0.05$ by DMRT; * and ** indicate significance at 5% and 1% level of probability, respectively. T1 = Control; T2 = Foliar application of at the rate of 1.5% four times from flowers start to pod development stage with an interval of 4 days; T3 = Foliar application of Urea + 0.1% micronutrients (B, Mo, Zn, Mn, Ca, Fe, Cu) four times from flowers start to pod development stage with an interval of 4 days and T4 = Foliar application of Urea + 0.1% micronutrients (B, Mo, Zn, Mn, Ca, Fe, Cu) + ground application of 1.5% N and 0.1% micronutrients solution four times from flowers start to pod development stage with an interval of 4 days.

Results revealed that the genotype Binamoog-7 showed superiority in number of pods plant^{-1} and seeds pod^{-1} and seed yield over Binamoog-6 whereas Binamoog-6 was superior in single pod and 100-seed weight and harvest index over Binamoog-7. The interaction effect of foliar application of nutrients and variety in relation to yield contributing characters and yield was significant. The highest number of pods and the highest seed yield was recorded in Binamoog-7 with foliar application of N and micronutrients and the lowest was recorded in Binamoog-6 with no foliar application of N and micronutrients.

CONCLUSION: Based on the experimental results, it may be concluded that foliar application of nitrogen along or in combination with micronutrients had significant positive influence on yield attributes and seed yield of mungbean over control. Though T_2 (only urea) and T_3 (urea + micronutrients) treatments performed similarity in vegetative and yield performance of mungbean, we should practice in T_2 only because the farmers of the Indian sub-continent are not habituated to use micronutrients as foliar spray on field crops. Therefore, the farmers may use only N as foliar spray for increased mungbean yield. This result needs conformation with further experimentation under field conditions.

CONFLICT OF INTEREST: Authors have no conflict of interest

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