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IMPACT OF TOBACCO INDUSTRY WASTEWATER ON GERMINATION AND SEEDLING GROWTH OF MUNGBEAN

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Abstract

An experiment was conducted in the month of November, 2021 at Crop Physiology and Ecology Laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh to study the impact of tobacco industry wastewater on germination and early seedling traits of five mung bean accessions (BD-10022, BD-10023, BD-10024, BD-10026 and BD-10027) following factorial completely randomized design (CRD) with three replications. Mung bean accession, tobacco industry wastewater and their interaction significantly interacted on germination and seedling traits of mung bean. Tobacco industry wastewater reduced the germination percentage, germination rate and co-efficient of germination but insisted longer shoot and root as well as escalated the seedling dry weight of mung bean as compared to tap water. Among the five mung bean accessions, BD-10024 and BD-10027 performed comparatively better performance regarding germination and seedling traits, respectively under wastewater conditions.

Introduction

The scarcity of freshwater is increasing day by day due to the increasing demand of water in domestic, municipal, agricultural and industrial sectors, especially in the developing countries of the world (UNDP, 2016). Due to the shortage of freshwater resources the use of industrial wastewater for growing agricultural crops is emerging (Niroula, 2003; Dash, 2012), particularly in arid and semi-arid regions (Pramanik, 2021). Industrial wastewater may contain organic matter, N, P, K, Ca, $CaCO_3$ and other essential plant nutrients which are beneficial for crop growth but heavy metal present in excess quantity affects germination of seed (Mehta and Bharadwaj, 2012). Industrial wastewater significantly affects the seed germination and seedling growth of various crop species (Huma *et al.*, 2012; Cabral *et al.*, 2010; Acharya, 2001). Industrial wastewater at higher concentration significantly affects germination and growth of wheat (Patil *et al.*, 2020), rice (Islam *et al.*, 2017) and onion (Pokharel *et al.*, 2000). But there can be both beneficial and damaging effects of wastewater irrigation on crops (Ramana *et al.*, 2002; Saravanamoorthy and Kumari, 2007). Therefore, it is necessary to study the impact of wastewater on crop production before recommending for irrigation (Thamizhiniyan *et al.*, 2009). The tobacco industry is one of the biggest industries in the world that generates and disposes large quantities of wastewater containing many chemical compounds like nicotine, glycogen, alcohol, absorbable organic halogens and exudates of tobacco leaves (Sponza, 2002), which may be toxic to the flora (Pramanik and Sikder, 2020; Pramanik, 2021), fauna, public health as well as environment (Adenike, 2014; Alabi *et al.*, 2014).

Bangladesh is one of the most tobacco producing and consuming countries. Tobacco industry is one of the largest industries in Bangladesh and there are so many active firms of tobacco. Tobacco is manufactured or processed by different steps where huge amount of wastewater are originated. For manufacturing of tobacco, first step is the tobacco production that does not contaminate the water. But in second step, nicotine, synthetic flavoring agents and residues of pesticides contribute to produce wastewater (Chidambara and Han, 2004). Untreated or allegedly treated tobacco wastewater may contaminate seed germination and crop growth under irrigation as it contains significant amounts of heavy metals like arsenic, cadmium, chromium, copper, lead, mercury, nickel and zinc (Sharma *et al.*, 2004). In spite of the large quantities of wastewater generated by the tobacco industry which has been affecting the environmental and public health, there is little information on toxicity of that wastewater on different flora as well as on economic crop growth.

Mung bean (*Vigna radiata* L.), self-pollinating grain legume belonging to the family Fabaceae also known as the moong bean, mugdal or green gram is one of the most important pulse crops in Bangladesh. Being a short duration (<60 days) crop, it is an ideal legume for catch cropping, intercropping, and relay cropping. The crop is supporting food security by ensuring excellent and easily digestible protein source for humans especially for poor people where meat is lacking (Bangar *et al.*, 2019). The raw and mature seeds are rich in carbohydrates, protein, fibers, minerals, antioxidants and phenolics (Guo *et al.*, 2012) and has become one of the popular legumes in the tropic and sub-tropics for its rich nutritional components (Thomas *et al.*, 2004). Mung bean grows mainly in rain-fed conditions at high temperatures (27-30 C) with low humidity and moderate rainfall ranging from 60 to 80 cm (Bangar *et al.*, 2019). In Bangladesh, the Barind Tract region is the major mung bean cultivating area where warm and humid climate generally exist with comparatively little rainfall. Despite being an economically important crop, the annual overall production (41000 MT) as well as average yield (0.93 MT ha^{-1}) of mung bean (BBS, 2022) is low in Bangladesh. The crop faces scarcity of water at different growth and developmental stages which ultimately loss of yield. Therefore, one or two supplementary irrigation(s) is/are essential for successful production of mung bean in that region of Bangladesh. In the northern part of Bangladesh, most of the farmers face severe shortage of irrigation water during dry season. The lower socio-economic group of people living near the tobacco industry use tobacco industry effluent wastewater for cultivating different crops including mung bean without giving any concern on suitability of the water for irrigation. It is hypothesized that different toxic chemicals and heavy metals present in tobacco industry wastewater could interfere in germination, growth and development of mung bean. For the sake of that, the experiment was conducted to evaluate the impact of tobacco industry wastewater on germination and early seedling traits of some mung bean accessions.

Materials and Methods

The experiment was carried out at Crop Physiology and Ecology laboratory, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh in the month of November, 2021 with two factors viz. Factor A: Five mung bean accessions (BD-10022, BD-10023, BD-10024, BD-10026 and BD-10027) and factor B: Two irrigation water (i. tap water ii tobacco industry wastewater). The completely randomized design (CRD) with three replications was followed to conduct the experiment. Healthy seeds of five mung bean accessions were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur. The wastewater samples were collected from the drainage system of three tobacco industries (Akij Tobacco Industry Ltd., Abul Khayer Tobacco Industry Ltd. and British American Tobacco Bangladesh Ltd.) situated in

Rangpur City. Samples were collected in plastic bottles that had been cleaned with hydrochloric acid (1:1) and then rinsed with tap water followed by rinsing with distilled water. The water samples collected from three industries were mixed thoroughly and considered as 100% wastewater. Clean, air dried and sterilized petridishes (11cm diameter) were taken for germination test. Twenty seeds of each accession were placed on filter paper sprinkled with respective treatment solution. Before sowing, seeds were surface sterilized in 0.1% mercuric chloride solution for 30 seconds, and then were washed thoroughly with tap water followed by distilled water. Each petridish containing the seeds was irrigated with respective treatment water throughout the experimental period.

Germination was counted at 24-hours interval and continued up to 6th day. A seed was considered germinated when plumule and radicle came out and larger than 2 mm long. The percentage of germination was counted at 6th day of placement of seeds. The rate of germination was calculated according to Krishnasamy and Seshu (1990) and co-efficient of germination was calculated using the formulae of Copeland (1976). The length of shoot and root of seedling was recorded by centimeter scale at 8 days after placement. Five seedlings from each petridish were sampled for shoot and root length. Then the seedlings were dried separately at 70°C for 72 hours in an electric oven and weight was recorded with an electrical balance. The mean of length and dry weight were calculated for each treatment combination. Data were analyzed by partitioning the total variance using Statistix 10 program and the means were compared by Tukey's Test at 5% level of probability.

Results and discussion

Germination characteristics

Percent germination, rate of germination and co-efficient of germination of mung bean seeds were significantly varied due to different accessions and wastewater (Table 1 and 2). Among the accessions, maximum germination percentage (95.61%) and highest rate of germination (94.17%) were noted in BD-10026 which was statistically similar with that of BD-10022 (93.77% and 93.12%, respectively). Comparatively lower percentage of germination and rate of germination were observed in other accessions compared to BD-10022 and BD-10026. Maximum co-efficient of germination (40.49) was calculated in BD-10022. Lower but statistically similar co-efficient of germination of mung bean seeds were recorded in BD-10023 (40.13) and BD-10026 (39.95) whereas minimum value of the trait (34.41) was found in BD-10024 followed by BD-10027 (36.77). Lower percent germination (81.91%), germination rate (79.33%) and co-efficient of germination (35.44) compared to that of control (97.28%, 97.42%, and 41.26, respectively) indicates that the germination of mung bean seeds was inhibited by tobacco industry wastewater.

The interaction effect of mung bean accessions and irrigation water indicated that significant reduction was observed in the germination percent of all mung bean accessions in different extends under tobacco industry wastewater. The maximum and statistically similar germination percentage and rate of germination of mung bean seeds were recorded under control condition except BD-10024 which was reduced at tobacco industry wastewater treatment. Tobacco industry wastewater reduced percent germination by 9.03, 27.67, 3.66, 8.79 and 28.92%, while germination rate by 12.09, 31.67, 3.53, 11.67 and 32.23% in BD-10022, BD-10023, BD-10024, BD-10026 and BD-10027, respectively (Table 2). The highest co-efficient of germination (44.85) was found in BD-10023 at control condition, whereas the lowest value of the trait (33.14) was recorded in BD-10027 under wastewater condition which was statistically at par with that of BD-10024 under same growing condition. Tobacco industry wastewater

reduced the trait by 12.44, 21.05, 6.66, 11.02 and 17.95 % in BD-10022, BD-10023, BD-10024, BD-10026 and BD-10027, respectively (Table 2). Results reveal that BD-10024 performed better compared to other accessions in terms of germination capacity.

Table 1. Effect of tobacco industry wastewater on germination of seeds of mung bean accessions

Treatments	Germination characteristics (mean ± SE)		
	Germination (%)	Germination rate (%)	Co-efficient of germination
Mung bean accessions			
BD-10022	93.77 2.442 a	93.12 3.388 a	40.49 1.350 a
BD-10023	86.17 6.511 bc	84.17 7.290 bc	40.13 2.335 a
BD-10024	89.09 3.003 b	87.10 1.847 b	34.41 0.818 c
BD-10026	95.61 2.609 a	94.17 3.581 a	39.95 1.423 a
BD-10027	83.36 6.585 c	83.33 7.475 c	36.77 1.786 b
Level of significance	**	**	**
Critical value for comparison	3.4522	2.9430	0.9787
Standard error for comparison	1.142	0.973	0.324
Irrigation water			
Control (Tap water)	97.28 1.668 a	97.42 1.838 a	41.26 0.971 a
Wastewater	81.91 2.671 b	79.33 2.709 b	35.44 0.734 b
Level of significance	**	**	**
Critical value for comparison	1.5178	1.2939	0.4303
Standard error for comparison	0.722	0.616	0.205
Coefficient of variation (%)	2.21	1.91	1.46

In a column, values having same letter(s) did not differ significantly by Tukey at p≤ 5% level.
** indicates significantly different at 1% level of probability. SE indicates standard error.

Table 2. Interaction effect of tobacco industry wastewater and mung bean accessions on germination of mung bean

Mung bean accessions	Irrigation water	Germination percentage		Rate of germination		Co-efficient of germination	
		%	% Change over control	%	% Change over control	-	% Change over control
BD-10022	Control (Tap water)	98.20 a	-9.03	99.11 a	-12.09	43.17 b	-12.44
	Wastewater	89.33 b		87.12 b		37.80 d	
BD-10023	Control (Tap water)	100.00 a	-27.67	100.00 a	-31.67	44.85 a	-21.05
	Wastewater	72.33 c		68.33 c		35.41 e	
BD-10024	Control (Tap water)	90.75 b	-3.66	88.66 b	-3.53	35.59 e	-6.66
	Wastewater	87.43 b		85.53 b		33.22 f	
BD-10026	Control (Tap water)	100.00 a	-8.79	100.00 a	-11.67	42.28 b	-11.02
	Wastewater	91.21 b		88.33 b		37.62 d	
BD-10027	Control (Tap water)	97.45 a	-28.92	99.34 a	-32.23	40.39 c	-17.95
	Wastewater	69.27 c		67.32 c		33.14 f	
Level of significance		**	-	**	-	**	-
Critical value for comparison		5.7866	-	4.9331	-	1.6404	-
Standard error for comparison		1.615	-	1.376	-	0.458	-
Coefficient of variation (%)		2.21	-	1.91	-	1.46	-

In a column, values having same letter(s) did not differ significantly by Tukey at p≤ 5% level.
** indicates significantly different at 1% level of probability.

However, the accession BD-10027 was found the most susceptible in terms of germination percentage and rate of germination, whereas BD-10023 was found the most susceptible accession in respect to co-efficient of germination at tobacco industry wastewater application.

Different degrees of germination capacity of mung bean seeds under wastewater application might be due to genetic variability of the crop (Pramanik, 2021). Tobacco industry wastewater knowingly declined the studied germination characteristics of mung bean accessions which might be due to the chemical fraction that leads to the inhibition of germination (Bhalla *et al.*, 1973). Suseelamma and Venkataraju (1994) reported that the inhibition of germination is dependent on the concentration of extract from cigarette tobacco leaf and also its entry to growing points of seedlings. Another finding reported that allelochemicals acid and phthalate released from the smoke and tobacco leaf as well as from wastewater probably interfered the plant growth regulators during germination and inhibited germination as well as early seedling growth of plant (Jianhua *et al.*, 2012). Our findings are very much consistent to findings of other researchers who reported that tobacco industry wastewater significantly hampered the germination characteristics of wheat (Pramanik and Sikder, 2020) and mung bean (Pramanik, 2021).

Early seedling traits

Different mung bean accessions showed significant variation in their shoot length, root length, shoot to root ratio and dry weight of seedling (Table 3). Among the accessions, BD-10024 produced the longest shoot (9.91 cm), maximum seedling dry weight (14.95 mg plant^{-1}) and showed maximum shoot to root ratio (2.66), whereas BD-10026 produced the highest root length (5.74 cm). The shortest shoot (7.42 cm) and shortest root (3.20 cm) were recorded in BD-10027 but the minimum shoot to root ratio (1.44) and minimum dry matter accumulation in seedling (11.97 mg plant^{-1}) were recorded in BD-10026. Achieved results on early seedling traits of mung bean accessions were significantly influenced by irrigation water except shoot to root ratio (Table 3). Mung bean accessions increased their shoot and root length, shoot to root ratio and seedling dry weight by 9.27 cm, 4.69 cm, 1.98 and 13.69 mg plant^{-1}, respectively when germination of seed allowed in tobacco industry wastewater application as compared to that produced at control condition by 8.14 cm, 4.15 cm, 1.96 and 12.24 mg plant^{-1}, respectively.

Table 3. Impact of tobacco industry wastewater on early seedling traits of mung bean accessions

Treatments	Early seedling traits (mean ± SE)			
	Shoot length (cm)	Root length (cm)	Shoot to root ratio	Seedling dry weight plant^{-1} (mg)
Mung bean accession				
BD-10022	8.85 0.345b	5.02 0.138b	1.76 0.135 c	11.99 0.365 c
BD-10023	9.08 0.233b	4.40 0.157c	2.06 0.112bc	13.05 0.563 b
BD-10024	9.91 0.428a	3.73 0.129d	2.66 0.152 a	14.95 0.415 a
BD-10026	8.27 0.214c	5.74 0.250a	1.44 0.097 d	11.97 0.360 c
BD-10027	7.42 0.374d	3.20 0.158e	2.32 0.107 b	12.88 0.433 b
Level of significance	**	**	**	**
Critical value for comparison	0.4557	0.4429	0.3251	0.4769
Standard error for comparison	0.1507	0.1465	0.1405	0.1577
Growing media				
Control	8.14 0.251 b	4.15 0.241 b	1.96 0.083	12.24 0.334 b
Waste water	9.27 0.260 a	4.69 0.262 a	1.98 0.104	13.69 0.341 a
Level of significance	**	**	NS	**
Critical value for comparison	0.2003	0.1947	0.1142	0.2097
Standard error for comparison	0.095	0.093	0.094	0.099
Coefficient of variation (%)	3.00	5.75	1.46	2.11

In a column, values having same letter(s) did not differ significantly by Tukey at p≤ 5% level. ** indicates significantly different at 1% level of probability. NS indicates not significant. SE indicates standard error.

The shoot length, root length, shoot to root ratio and seedling dry weight plant^{-1} were also varied significantly by the interaction effect of mung bean accessions and irrigation water where tobacco industry wastewater considerably increased the seedling traits of mung bean in different extends compared to control condition except shoot to root ratio in BD-10023 and BD-10026.

The interaction effect on early seedling traits of mung bean expressed that the longest shoot (10.69 cm), maximum shoot to root ratio (2.72) and the maximum seedling dry weight (15.66 mg plant^{-1}) were recorded in BD-10024, while BD-10026 produced the longest root (6.20 cm) under wastewater condition which was statistically similar to root length (5.27 cm) produced by the same accession under control condition (Table 4). The shortest shoot and root length 6.67 and 2.93 cm, respectively were observed in BD-10027 under control. Minimum shoot to root ratio (1.32) were recorded in accession BD-10026 germinated at wastewater and minimum seedling dry weight (11.33 mg plant^{-1}) were recorded in BD-10022 at control.

Table 4. Interaction effect of tobacco industry wastewater and mung bean accessions on early seedling traits of mung bean

Mung bean accession	Growing media	Shoot length		Root length		Shoot to root ratio		Seedling dry weight	
		cm	% Change over control	cm	% Change over control	-	% Change over control	mg	% Change over control
BD-10022	Control (Tap water)	8.17 ef	+16.65	4.83 bc	+7.66	1.69 f	+8.28	11.33 d	+11.65
	Wastewater	9.53 b		5.20 b		1.83 e		12.65 c	
BD-10023	Control (Tap water)	8.75 cde	+7.54	4.17 cde	+10.79	2.09 d	-2.39	12.17 c	+14.46
	Wastewater	9.41 bc		4.62 bcd		2.04 d		13.93 b	
BD-10024	Control (Tap water)	9.12 bcd	+17.21	3.53 ef	+11.33	2.58 b	+5.43	14.24 b	+9.97
	Wastewater	10.69 a		3.93 de		2.72 a		15.66 a	
BD-10026	Control (Tap water)	7.98 f	+7.14	5.27 a	+17.65	1.51 g	-12.58	11.36 d	+10.74
	Wastewater	8.55 def		6.20 a		1.32 h		12.58 c	
BD-10027	Control (Tap water)	6.67 g	+22.34	2.93 f	+18.09	2.28 c	+3.50	12.11 c	+12.63
	Wastewater	8.16 ef		3.46 ef		2.36 c		13.64 b	
Level of significance		**	-	**	-	**	-	*	-
Critical value for comparison		0.7638	-	0.7424	-	0.1352	-	0.7994	-
Standard error for comparison		0.213	-	0.207	-	0.1639	-	0.223	-
Coefficient of variation (%)		3.00	-	5.75	-	1.46	-	2.11	-

In a column, values having same letter(s) did not differ significantly by Tukey at p≤ 5% level. **and * indicate significantly different at 1% and 5% level of probability, respectively.

Tobacco industry wastewater increased the seedling growth by 16.65, 7.54, 17.21, 7.14 and 22.34% for shoot length; 7.66, 10.79, 11.33, 17.65 and 18.09% for root length and 11.65, 14.46, 9.97, 10.74 and 12.63% for seedling dry weight in BD-10022, BD-10023, BD-10024, BD-10026 and BD-10027, respectively. Considerable increment (8.28, 5.43 and 3.50%) in shoot to root ratio were found in BD-10022, BD-10024 and BD-10027, correspondingly but accession BD-10023 decreased the trait by 2.39% and BD-10026 by 12.58%. The meaningful

increment in the shoot and root length of mung bean seedlings under wastewater might be due to the organic matter content in industrial wastewater which accelerated more accumulation of dry matter in seedling of mung bean (Messrouk *et al.*, 2014). Wastewater may trigger some growth stimulating enzymes during germination, which in turn increase the amount of hydrolyzates like glucose and amino acids required for growth of embryo axes (Pramanik and Sikder, 2020) that might be another possible reason for enhancement of early seedling growth. Hossain *et al.* (2018) observed that irrigation with tobacco industry wastewater increased the shoot and root length in cucumber, radish and long yard bean seedlings under laboratory condition, similar facts might be happened in the present findings. Tobacco industry wastewater significantly increased the shoot and root length as well as shoot and root dry weight in wheat seedlings (Pramanik and Sikder, 2020) and in mung bean seedlings (Pramanik, 2021). These findings are very much congruent with the results of our present investigation. Mojiri *et al.* (2013) also found increase in shoot and root length of *Lepidium sativum* when irrigated with wastewater that consistent with our findings.

Conclusion

The achieved findings from the present investigation support our hypothesis that the tobacco industry wastewater hinders the germination traits though leads to beneficial changes in early seedling traits to some extent. Therefore, tobacco industry wastewater needs a thorough assessment for its suitability as irrigation water. Among the studied accessions, BD-10024 showed comparatively better performance in relation to germination and BD-10027 performed better regarding seedling traits under wastewater treated condition.

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