



SOIL & CROP SCIENCES | RESEARCH ARTICLE

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Modulation in growth, photosynthesis and yield attributes of black mustard (*B. nigra* cv. IC247) by interactive effect of wastewater and fly ash under different NPK levels

Seema Sahay^{1*}, Akhtar Inam², Arif Inam¹ and Saba Iqbal¹

Abstract: Waste for fertilization of crop plants has received attention during recent years. The present experiment was conducted for a year on *Brassica nigra* cv. IC247 using two wastes, i.e. fly ash and wastewater with fertilizers and without fertilizers. The crop was irrigated with ground water and wastewater alone and along with the application of different levels of fly ash (FA₀, FA₁₀ and FA₂₀) and different levels of NPK (N₀P₀K₀, N₄₀P₁₅K₁₅, N₆₀P₃₀K₃₀ and N₈₀P₄₅K₄₅). Growth, photosynthesis and yield attributes of the test crop were evaluated at different plant age (A). Result showed that wastewater with fertilizers enhanced growth, photosynthesis and yield parameters as compared to that of ground water with fertilizers. The use of fly ash at 20 t ha⁻¹ performed better than 10 t ha⁻¹. Furthermore, the interactive use of wastewater and fly ash proved to be applicable in making N, P and K optimum at their lower doses, as maximum enhancement in growth, photosynthesis and yield was observed with N₆₀P₃₀K₃₀FA₂₀ + WW and further input of N₈₀P₄₅K₄₅FA₂₀ + WW was not beneficial. It was due to the application of fly ash and wastewater has shown the improvement in physiochemical properties of soil and also in the nutrient status.

ABOUT THE AUTHOR



Seema Sahay

Seema Sahay received her PhD in Advance Plant Physiology at the Aligarh Muslim University, India. During her research period, she has been awarded as JRF and SRF under RGNF by UGC, New Delhi, Govt of India. Her research interests include issues related to soil and plant mineral nutritional dynamics, wastes utilization and phytoremediation using different *Brassica* species and cultivars. Her research group has been engaged under major research projects on effect of various industrial wastes on soils and crop quality and achieved outstanding results and maintained clear sense of purpose. The major issues concern with the study of heavy metals accumulation, detoxification in soil and various edible crops to promote the sustainable and safe utilization of wastes for farmers' fields around and near the Aligarh city. Her research group has focused on the conservation of inorganic fertilizers, natural resources and improvement in environment quality.

PUBLIC INTEREST STATEMENT

Rapeseed-mustard crops are known for their rapid fall growth, great biomass production and nutrient-scavenging ability. Among various oilseed *Brassica* species, *Brassica nigra* has also been covered as an economically important crop, which is widely cultivated for leafy vegetables, edible oil and seeds. It has been reported for its ability to survive under various diverse agro-climatic conditions. However, it is attracting renewed interest primarily because of its defence mechanism property against herbivores and pathogens by synthesizing chemical compounds viz. sinigrin, etc. The seeds also have important medicinal uses such as treatment of rheumatism and joint pains, indurations of liver and spleen, tooth pain and throat tumours. Further, the application of fly ash and wastewater improves soil properties and also the nutrient status, which ultimately led to enhanced growth and yield. Thus, the general perception of "wastes as only pollutant" has changed into "wastes as nutrients".

Subjects: Agriculture & Environmental Sciences; Crop Science; Environment & Agriculture; Environmental Sciences; Soil Sciences

Keywords: physiochemical properties; wastewater; fly ash; black mustard; growth; photosynthesis; yield

1. Introduction

In India, *Brassica nigra* is commonly known by the name “black mustard” and belongs to the taxonomic family Brassicaceae. It is primarily (BB) a diploid species ($2n = 16$) and plays a role in developing the amphidiploid species viz. *Brassica juncea* (AABB) and *Brassica carinata* (BBCC) (Morinaga, 1934; Nagaharu, 1935). It is an economically important crop plant which is widely cultivated for leafy vegetables and edible oil and seeds sources (Vaughan & Hemingway, 1959). This crop is been considered because of its ability to survive and grow under diverse agro-ecological conditions, such as at relatively low temperatures, at highly disturbed soils or waste places (Angelova & Ivanova, 2009). This characteristic makes them well adapted to cultivate for both domestic and industrial uses. A typical plant image includes tap roots, lower leaves large and upper leaves reduced in size and stem covered with soft hairs. This species can be easily distinguished from other oilseed *Brassica* species in that it produced siliquae of very short size, a rosette of basal leaves and sharp pungency of its seeds. In view of current population growth with their advanced living style, consumption of oil per capita is increasing per annum. The increase in world demand for edible oils and more recently for biodiesel has led to a rapid growth in the production of most oilseeds, with total seed oil about 4% each year. In order to meet this demand, synthetic fertilizers have been supplied to soil due to their well-known application in increasing the crop yield in more quantity. The effect of fertilizers mainly focuses primarily on the use of nitrogen (N), phosphorus (P) and potassium (K) because these three macro-nutrients are the main nutrients in sustainable crop production (Food and Agriculture Organization [FAO], 2006, 2011). Compared to most other grain crops, *Brassica* oilseed crops require greater nutrient inputs, generally assume, about 25% more N, P and K and up to five times more S than a wheat crop to achieve high yields. Interestingly, synthetic fertilizers and other chemical nutrients now have been replaced with municipal wastes, if not fully, but partially, from few decades. The wastes were long considered as either a non-essential or toxic element. However, more is being learnt about the role of wastes as nutrients and their activity in plants; they have shown benefits in yield production (Chalkoo, Sahay, Inam, & Iqbal, 2014; Iqbal et al., 2015; Kiziloglu et al., 2007; Sahay, Inam, Inam, & Tak, 2013).

Among wastes, in general, one is wastewater, which has found to be used in raw (direct) and dilute (indirect) forms with planned (treated) and unplanned (untreated) ways. Exploding population growth, industrialization and urbanization (World Health Organization, 2009; Wyman, 2013) posed declination of fresh water, on one hand, and the production of huge volume of wastewater day by day, very fast, throughout the world, on the other hand. This is creating a pressure on farmers to adopt their farming with wastewater irrigation, as they are unaware of the pollution of air, water and land and that the cost of treatment of wastewater is too high in developing countries including India. However, in this context, reusing of such recycled wastewater for agriculture practices is a potential solution to reduce fresh water demand and a feasible option of various nutrients, which are believed to have a positive effect on soil properties and crop production in a sustainable way. But, it is the necessity of the present era to think about the existing urban wastewater disposal infrastructure, wastewater agriculture practices, quality of water used and awareness related to pollution issues. The important references in the aspect of use of wastewater come from fourteenth and fifteenth centuries in the Milanese Marcites and in the Valencian huertas, respectively (Soulie & Trema, 1991). It is still continued in practices and has gained interest worldwide due to it being rich in essential nutritive plant elements, especially in N, P and K in addition to Na, Ca, Mg, S, Cl, Cu, Fe, B, Zn, salts, pH, organic matter and microbial activity. According to a recent report of Australian Academy of Technological Sciences and Engineering (2004) and Jimenez and Asano (2008), wastewater irrigation is now quite common in many countries like Europe, the USA, Mexico, Australia, China, Chile, Peru, Egypt, Lebanon, Morocco, Vietnam, South Africa and India, where it has been used

as a source of crop nutrients and concluded that the use of wastewater in agriculture irrigation responds to multiple benefits: solving the problem of disposal, minimizing the risk of high demand for clean drinking water, reducing the direct input and need of high amounts of inorganic fertilizers and improving soil with its fertile quality. Further, it encountered the food demand, as it consequently permits higher yield of various range of field crops, including vegetables as compared to clean water irrigation (Drechsel et al., 2004; Ensink, Mahmood, van der Hoek, Raschid-Sally, & Amerasinghe, 2004; Lai, 2002; Singh, Deshbhratar, & Ramteke, 2012).

Another waste, fly ash (solid waste), is defined as an end by-product produced after coal combustion at high temperature in thermal power stations. Like water scarcity and increased food demand, the energy/power crisis is one of the considered factors in India. In order to meet the energy demand, use of coal as the prime energy source, calls for burning in large amount, leading to the generation of large amount of this solid waste product. It has been expected that the production of fly ash will likely exceed up to 225 million tons in the next two years, i.e. by 2017 (Singh, 2012). However, the utilization percentage of fly ash in India is still very low, especially in the agriculture sectors. But the use has increased substantially year wise (Ministry of Environment and Forests, 2007). Fly ash due to its efficacy in modification of soil's physical and chemical health has been reported with great potency to improve crop productivity in Indian agriculture. As a matter of fact, fly ash practically consists of all the essential elements present in the soil except very low amount of organic carbon and nitrogen, if any. Thus, its utilization would not only be a solution of disposal but might also reduce the inorganic fertilizers, especially non-nitrogenous.

The task of bringing together the three sources of nutrients by optimum fertilization is an attractive scope for getting a beneficial harvest per unit area, atleast where the two waste products are easily available, because application if given in excess or below the rate of requirement may have some negative effects on crop yield and environmental quality. Therefore, the present study deals with the application of wastewater and fly ash with different NPK levels and their effect of soil properties, crop yield and qualities.

2. Materials and methods

2.1. Study site

The experiment was conducted in a green net house of department of Botany, Aligarh Muslim University at Aligarh (an area of 3,747 sq kms, 27.88° N latitude, 78.08° E longitude, an elevation of 178.45 m above the sea level). The metrology of the study area indicated the temperature ranged from 32–35°C to 46–47.5°C in winter and summer, respectively, with annual rainfall of around 600–650 mm and humidity from 42–90%. India is a country of vast dimensions with varied conditions of geology, relief, climate and vegetation. Therefore, India has a large variety of soil groups, distinctly different from one another. Sandy, loamy, sandy loam and clayey loam under the alluvial soil group are characterized soil types at Aligarh. The soil at the experimental site is having sandy loam. The experimental soil was classified adopting the USDA (United States Department of Agriculture) soil taxonomy concepts.

2.2. Plant material and growth conditions

The authentic seeds of *B. nigra* cv. IC247 were collected from the National Research Centre on Plant Biotechnology (NRCPB) of the Indian Agriculture Research Institute (IARI), New Delhi, India. In the laboratory, seeds were disinfected with 0.01% aqueous solution of mercuric chloride (HgCl_2), followed by repeated washings with double-distilled water (DDW) and then dried in shade before sowing. Seeds were sown at the rate of 10 in each pot to avoid germination failure. The square, earthen pots (length \approx 20 inch and diameter \approx 23) filled with 10-kg soil were placed in a green net house with natural day-night conditions (photosynthetically active radiation $>$ 950 $\text{mmol m}^{-2} \text{s}^{-1}$, temperature $23 \pm 3^\circ\text{C}$, relative humidity $75 \pm 5\%$ and rainfall 30–40 mm).

Table 1. Scheme of treatments given in randomized complete block design

Treatments (Fertilizer)	Treatments/Waters		Remarks (per hectare)
	GW	WW	
$N_0P_0K_0 FA_{10}$	+	+	0 kg N, 0 kg P, 0 kg K, 0 t FA
$N_{40}P_{15}K_{15} FA_{10}$	+	+	40 kg N, 15 kg P, 15 kg K, 10 t FA
$N_{60}P_{30}K_{30} FA_{10}$	+	+	60 kg N, 30 kg P, 30 kg K, 10 t FA
$N_{80}P_{45}K_{45} FA_{10}$	+	+	80 kg N, 45 kg P, 45 kg K, 10 t FA
$N_{40}P_{15}K_{15} FA_{20}$	+	+	40 kg N, 15 kg P, 15 kg K, 20 t FA
$N_{60}P_{30}K_{30} FA_{20}$	+	+	60 kg N, 30 kg P, 30 kg K, 20 t FA
$N_{80}P_{45}K_{45} FA_{20}$	+	+	80 kg N, 45 kg P, 45 kg K, 20 t FA

N.B.: The NPK fertilizer doses and fly ash were calculated on the basis of their composition and that one hectare of land contained 2×10^6 kg effective soil. GW = Ground water and WW = Wastewater.

2.3. Treatment details

The present experiment was carried out with the treatment of WW and GW alone as well as with different FA and NPK levels. The schematic representation of the treatments of NPK and FA set-up along with GW and WW is shown in Table 1. Fly ash was applied at the rate of 10 t ha^{-1} (44.0 mg per pot) and 20 t ha^{-1} (88.0 mg per pot) into the soil and mixed well in each pot containing 10 kg soil. Before sowing, the NPK fertilizers were added into the soil at 40, 60 and 80 kg N ha^{-1} soil (189.9, 284.8 and 379.7 mg per pot); 15, 30 and 45 kg P ha^{-1} soil (139.0, 279.0 and 418.5 mg per pot); and 15, 30 and 45 kg K ha^{-1} soil (96.0, 192.0 and 225.0 mg per pot), respectively. NPK were given in the form of urea, single super phosphate (SSP) and muriate of potash (KCl), respectively.

2.4. Sampling and analyses of soil, fly ash and wastewater

The soil for pot experiment was collected from a farmer's field (from Aligarh). Before filling the pots, the soil was autoclaved for 20 min at 137.9 kPa and thoroughly mixed with suitable quantity of organic manure. Fly ash (FA) was collected from a pond from a thermal power station, Kasimpur, located 15 km away from the experimental area, producing 2.71 million tons of fly ash/year. It was dried in sun for few days before use. The samples of soil and fly ash were passed through a 2-mm sieve. The various physiochemical characteristics were determined for the soil before mixing and after mixing of fly ash, which are presented in Table 2.

The soil texture was determined using the soil texture triangle at www.usp.edu/geo/faculty/ritter/glossary/s_u/soil_texture_triangle.html (Chopra & Kanwar, 1991). The texture of fly ash was determined by the field method, by rubbing or feeling the fly ash between the thumb and fingers. The fly ash used was slightly silty in nature. An important chemical property of soil/fly ash as a medium for plant growth is its pH value since essential ions that enter into plant are highly dependent upon hydrogen concentration of soil solution. It was estimated with the help of Thermo Orion Model 290 pH meter, which was calibrated with a standard buffer of known pH (4.0, 7.0 and 9.2) as per the method given by Jackson (1973). CEC of the samples was determined using phenolphthalein titrating method of Ganguly (1951). Water-holding capacity of soil/fly ash was measured as per the method of Black et al. (1965). Soil and fly ash were analysed for total organic carbon by dichromate oxidation and titration with ferrous ammonium sulphate method (Walkley & Black, 1934). Nitrate-nitrogen (NO_3^- -N) estimation was carried out following Ghosh, Bajaj, Hasan, and Singh (1983)'s method. Analysis of phosphorus (P) in soil/fly ash extract was done calorimetrically after HClO_4 digestion according to the method of Dickman and Bray (1940). Potassium (K) was read with the help of flame photometer, after setting zero for the blank and 100 for 40 ppm of K. Calcium (Ca) and magnesium (Mg) were measured following Chopra and Kanwar (1982). Estimation of carbonates and bicarbonates was also done following the titration method of Richards (1954). For chloride, to 50 ml of extract, 2 ml of 5% potassium chromate indicator was added. It was titrated against 0.02 N silver nitrate solutions and calculated. The determination of sodium (Na) was carried out directly

Table 2. Physiochemical characteristics of experimental soil mixed with fly ash before sowing the plant

Characteristics	Soil	Flyash	Soil + FA (10 t ha ⁻¹)	Soil + FA (20 t ha ⁻¹)
Colour	Light brownish	Greyish	-	-
Texture	Sandy loam	Slightly silty	-	-
CEC (meq 100 g ⁻¹ soil)	2.92 ± 0.23	6.9 ± 0.62	3.1 ± 0.21	3.6 ± 0.32
pH	7.8 ± 0.62	8.7 ± 0.78	7.8 ± 0.54	8.2 ± 0.73
Porosity (%)	40.71	64.92	41.00	45.42
Water holding capacity (%)	35.84	55.61	37.11	44.23
Organic carbon (%)	0.758 ± 0.06	0.222 ± 0.02	0.953 ± 0.06	1.28 ± 0.11
NO ₃ -N (g kg ⁻¹ soil)	0.292 ± 0.02	0.060 ± 0.01	0.264 ± 0.01	0.289 ± 0.02
Phosphorus (g kg ⁻¹ soil)	0.136 ± 0.01	0.021 ± 0.001	0.136 ± 0.01	0.158 ± 0.01
Potassium	21.00 ± 1.68	15.00 ± 1.35	22.00 ± 1.54	35.00 ± 3.15
Magnesium	31.42 ± 2.51	21.95 ± 1.97	33.11 ± 2.31	38.23 ± 3.44
Calcium	19.31 ± 1.54	19.56 ± 1.76	20.16 ± 1.41	30.90 ± 2.78
Sodium	12.02 ± 0.96	16.13 ± 1.45	13.34 ± 0.93	18.19 ± 1.63
Bicarbonate	19.33 ± 1.54	13.42 ± 1.20	24.22 ± 1.69	32.86 ± 2.95
Carbonate	78.29 ± 6.26	70.14 ± 6.31	81.20 ± 5.68	96.82 ± 8.71
Sulphate	17.66 ± 1.41	26.25 ± 2.36	21.66 ± 1.51	35.9 ± 3.23
Chloride	28.22 ± 2.25	19.71 ± 1.77	33.34 ± 2.33	37.47 ± 3.37

Notes: All determinations in mg l⁻¹ in 1:5 ratio (Soil:Water extract), except pH or as specified.
 All the values are mean of three replicates ± SE.

from soil/fly ash extract with the help of flame photometer. For sulphate (SO₄²⁻), 50 ml soil/fly ash extract was mixed with 2.5 ml of conditioning reagents and then stirred on a shaker, and during shaking, small quantity of BaCl was added. The solution was read with the help of a digital nephelometer.

The wastewater (WW) used is a mixture of sewage, household and industrial wastewater. It was collected at weekly intervals from drains located 06 km away from the experimental area. The tap water of the University was used as a ground water (GW) source. Prior to application, wastewater and ground water analysis was done according to Standard methods of American Public Health Association (1985) and their physiochemical characteristics are presented in Table 3. The pots were watered with GW everyday, while on alternate days, plants were supplied with 300 mL of WW starting after 15 days of germination up to maturity of crops.

2.5. Measurements of growth, photosynthesis, physiological and yield characteristics

The experiment was set up in a complete randomized block design (CRBD) with three replicates of the plant. These plants were dug out carefully from each pot after they reached a particular age, 35, 70 and 105 days after sowing (DAS), for measuring the various parameters of growth and photosynthesis. The plants were washed for removing adhering foreign particles, and soaked on blotting sheets. Water adhering to the plants was soaked with blotting paper and fresh mass of shoot and root was measured separately using a digital weighing balance. Dry weight of the shoot and root was determined after drying the samples in an oven at 80°C. Length of the plant was recorded using a measuring scale. Leaf number was measured manually by counting the leaves per plant. Leaf area was measured by a LA211 leaf area meter (Systronics, Hyderabad, India).

Among physiological parameters, net photosynthetic rate (Pn) was recorded in fully expanded leaves, using an infrared gas analyzer (IRGA, LiCor, Lincoln, NE) on a sunny day between 10:30 am

Table 3. Physiochemical characteristics of ground water (GW) and wastewater (WW) before sowing the plant

Characteristics	GW	WW
pH	6.9–7.3	7.9–8.3
EC ($\mu\text{mhos cm}^{-1}$)	711–735	840–870
Total solids (T.S.)	902–912	1,209–1,279
Total dissolved solids (T.D.S.)	525–549	632–683
Total suspended solids (T.S.S.)	420–431	675–690
Dissolved oxygen (DO)	7.40–6.70	2.22–2.45
Biological oxygen demand (B.O.D.)	15.99–16.73	160.75–168.34
Chemical oxygen demand (C.O.D.)	35.20–41.28	119.19–128.73
Hardness	110.0–117.0	320.0–331.0
Magnesium (Mg^{++})	17.48–17.35	128.17–123.21
Calcium (Ca^{++})	23.91–22.68	41.48–42.66
Potassium (K^+)	6.08–7.52	16.67–19.14
Sodium (Na^+)	16.36–17.39	46.67–51.28
Bicarbonate (HCO_3^-)	61.00–60.00	86.00–94.00
Carbonate (CO_3^{--})	33.20–46.80	118.24–121.29
Chloride (Cl^-)	59.73–65.48	113.10–120.42
Phosphate (PO_4^-)	0.37–0.15	1.04–1.15
Sulphates (SO_4^{--})	35.28–79.87	46.52–83.13
Nitrate N (NO_3N)	0.74–0.76	1.20–1.23
Ammonium N (NH_3N)	1.13–1.29	5.21–5.41
Chromium (Cr)	BDL	0.011–0.021
Copper (Cu)	BDL	0.195–0.263
Nickel (Ni)	BDL	0.375–0.461
Lead (Pb)	BDL	0.038–0.051
Cadmium (Cd)	BDL	0.008–0.018

Notes: All determinations in mg l^{-1} or as specified except pH.

BDL: Below Detectable Limit.

and 11:30 am. The atmospheric conditions during the measurement were: photosynthetically active radiation (PAR), $1,000 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$, relative humidity $66 \pm 5\%$, atmospheric temperature $25 \pm 2^\circ\text{C}$ and atmospheric CO_2 , $355 \mu\text{mol mol}^{-1}$. Nitrate reductase (NR) activity (EC 1.7.99.4) was estimated by the intact tissue method given by Jaworski (1971). Carbonic anhydrase (CA) activity (EC 4.2.1.1) was measured in enzyme assay of fresh leaves by adopting the method of Dwivedi and Randhawa (1974). Total chlorophyll and carotenoid contents were estimated using dimethyl sulphoxide (DMSO), following the method as described by Hiscox and Israelstam (1979). Leaf nitrogen (N) and phosphorus (P) contents were estimated according to method of Lindner (1944) and Fiske and Subba Row (1925), respectively. Potassium was estimated by directly reading the samples on a flame photometer. Before estimating these three nutrients in leaf powder, the samples were digested according to Lindner's digestion method (1944).

The crop was harvested on 15 March to record the yield by measuring the number of siliquae, seed number per siliqua, 1,000 seed weight, siliqua length, seed yield, oil content and oil yield. After separating the seed samples from extraneous material, they were crushed to get a fine meal for extracting the oil. The oil was extracted in Soxhlet's extractor using petroleum ether method. The percentage oil content when multiplied with seed yield gave the oil yield. The oil content was calculated as:

Table 4. Model of analysis of variance (ANOVA) experimental design; randomized complete block

Source of variation	df (degree of freedom)	SS (Sum of square)	MSS (Mean sum of square)		F (variance value)	Significant ($p < 0.05$)
Waters	$(n-1) = 1$	SS_{water}	MS_{water}	$= \frac{SS_{\text{waters}}}{n-1}$	$F1 = \frac{MS_{\text{water}}}{MS_{\text{interaction}}}$	if $F1 > F_{0.05}$
Treatments	$(k-1) = 6$	$SS_{\text{treatment}}$	$MS_{\text{treatment}}$	$= \frac{SS_{\text{treatments}}}{k-1}$	$F2 = \frac{MS_{\text{treatment}}}{MS_{\text{interaction}}}$	if $F2 > F_{0.05}$
Interactions	$(n-1) \times (k-1) = 6$	$SS_{\text{interaction}}$	$MS_{\text{interaction}}$	$= \frac{SS_{\text{interactions}}}{(n-1)(k-1)}$		
Error	26					
Total	41	SS_{total}	$(nk-1) = (N-1)$			

Notes: F1 is variance of water with df at $(n-1)$ vs. $(n-1)(k-1)$.

F2 is variance of treatments with df at $(n-1)$ vs. $(n-1)(k-1)$.

$$\text{Oil content(\%)} = \frac{m_o}{m_s} \times 100$$

where, m_o = sum of 3 the mass of oil and m_s = seed sample mass.

2.6. Statistical analysis

The experimental data were statistically analysed using the analyses of variance techniques according to Gomez and Gomez (1984). In applying the “F” test, the error due to replicates was also determined. When “F” value was found to be significant at the level of probability, the least significant difference (LSD) was calculated. The model of two-way analyses of variance (ANOVA) for experiment is given in Table 4. Correlation coefficient (r) values and regression analysis of seed yield with some related physiological parameters were also worked out. Values are presented as mean \pm S.E. ($n = 3$). The graphs are plotted using scientific softwares ORIGIN 6.1 and SIGMA PLOT.

3. Result and discussion

3.1. Fly ash characteristics and its effect on soil and crop

Physiochemical analysis of fly ash (pure) showed that it was lower in organic carbon, N, P, K, Ca, Mg, Cl, carbonates and bicarbonates except pH, CEC, Na and sulphate as compared to field soil used in the present study. However, the addition of fly ash to the soil had the greatest effect on nutrient status (Table 2). Soil mixed with fly ash at 10 t ha^{-1} and 20 t ha^{-1} separately showed a higher value of all available elements as compared to control soil. In addition, FA amendment increases the porosity and water-holding capacity, 40–45% and 35–44%, over control soil, respectively, due to the fine-textured nature of fly ash, which helps in improving the physical health of the soil for supplying all essential nutrients in significant quantities for plant growth. Further, FA_{10} - and FA_{20} -amended soils were recorded with an increase of 25.72 and 68.86% in organic carbon content over non-fly ash-amended soil. Thus, the addition of fly ash into soil increased the organic carbon content which helps in binding soil particles in aggregates and improving the water-holding capacity of soil. Such improvement in agronomic properties of soil by constituents of fly ash has also been reported elsewhere as well at Aligarh (Jala & Goyal, 2006; Lee, Ha, Lee, Lee, & Kim, 2006; Sikka & Kansal, 1995; Singh & Siddiqui, 2003). FA at both levels was not found injurious during any age of the three sampling stages and harvest of the plant was as discussed earlier. It significantly enhanced growth, photosynthesis and yield as compared to non-fly ash-amended soil. FA at 20 t ha^{-1} was more enriched with utilizable plant nutrients as compared to 10 t ha^{-1} ; therefore, former dose of fly ash was found to be more effective for *Brassica* crop.

3.2. Wastewater characteristics and its nutritional effect on crop

The physiochemical analysis of the Aligarh city’s wastewater (Table 3) has revealed that it has an alkaline nature (pH-8.3) and the EC ($855 \mu \text{ mhos cm}^{-1}$), TDS (657), Cl^- (116.76), Ca^{++} (42.07), Mg^{++} (125.69), Na^+ (48.97), K^+ (17.90) and SO_4^{--} (64.82) mg L^{-1} were within the permissible limits of irrigation water quality except the potassium set by FAO (1985). The chloride contents were also low,

Table 5. Effect of ground water and wastewater alone and along with different fly ash (t ha⁻¹) and N, P and K levels (kg ha⁻¹) on shoot fresh and dry and root fresh and dry weight of black mustard (*B. nigra* cv. IC 247) at age 35, 70 and 105 days after sowing (DAS)

Age (DAS)	Treatments	Fresh weight (g)				Dry weight (g)							
		Shoot		Root		Shoot		Root					
		GW	WW	GW	WW	GW	WW	GW	WW				
35	N ₀ P ₀ K ₀ FA ₀	2.44 ^j	2.82 ⁱ	0.32 ^k	0.34 ^{jk}	0.47 ^f	0.49 ^{ef}	0.031 ^h	0.033 ^{gh}				
	N ₄₀ P ₁₅ K ₁₅ FA ₁₀	3.00 ^{hi}	3.72 ^g	0.35 ^{j-k}	0.40 ^{efg}	0.52 ^{de}	0.59 ^c	0.036 ^{fgh}	0.049 ^{a-h}				
	N ₆₀ P ₃₀ K ₃₀ FA ₁₀	3.98 ^f	4.16 ^{d-f}	0.42 ^{def}	0.46 ^{bcd}	0.59 ^c	0.61 ^c	0.044 ^{b-h}	0.053 ^{abcdefg}				
	N ₈₀ P ₄₅ K ₄₅ FA ₁₀	4.06 ^{ef}	4.36 ^{b-d}	0.44 ^{cde}	0.51 ^a	0.61 ^c	0.70 ^b	0.051 ^{a-h}	0.062 ^{ab}				
	N ₄₀ P ₁₅ K ₁₅ FA ₂₀	3.05 ^h	4.23 ^{c-e}	0.36 ^{h-k}	0.49 ^{ab}	0.54 ^d	0.69 ^b	0.036 ^{e-h}	0.058 ^{abcde}				
	N ₆₀ P ₃₀ K ₃₀ FA ₂₀	3.15 ^h	4.91 ^a	0.37 ^{g-j}	0.53 ^a	0.55 ^d	0.83 ^a	0.039 ^{d-h}	0.067 ^a				
	N ₈₀ P ₄₅ K ₄₅ FA ₂₀	3.66 ^g	4.54 ^b	0.39 ^{f-i}	0.52 ^a	0.58 ^c	0.72 ^b	0.040 ^{c-h}	0.066 ^a				
70	N ₀ P ₀ K ₀ FA ₀	17.57 ^h	18.78 ^h	1.38 ^k	1.47 ^{jk}	2.53 ^l	3.17 ^k	0.34 ^m	0.38 ^{lm}				
	N ₄₀ P ₁₅ K ₁₅ FA ₁₀	19.30 ^h	24.71 ^{ef}	1.53 ^{ijk}	1.75 ^{e-h}	3.49 ^j	3.85 ^{fgh}	0.40 ^{kl}	0.50 ^{ghi}				
	N ₆₀ P ₃₀ K ₃₀ FA ₁₀	24.83 ^{ef}	25.36 ^e	1.79 ^{d-g}	1.92 ^{bcd}	3.87 ^{efg}	4.07 ^d	0.53 ^{fg}	0.55 ^{def}				
	N ₈₀ P ₄₅ K ₄₅ FA ₁₀	24.88 ^{ef}	37.49 ^c	1.83 ^{c-g}	2.19 ^a	4.07 ^{de}	4.34 ^{bc}	0.53 ^{efg}	0.58 ^{bcd}				
	N ₄₀ P ₁₅ K ₁₅ FA ₂₀	21.61 ^g	30.56 ^d	1.60 ^{hij}	2.00 ^b	3.53 ^{ij}	4.30 ^c	0.45 ^{jk}	0.57 ^{cdef}				
	N ₆₀ P ₃₀ K ₃₀ FA ₂₀	23.44 ^{fg}	42.51 ^a	1.68 ^{ghi}	2.24 ^a	3.64 ^{hij}	4.64 ^a	0.47 ^{ij}	0.65 ^a				
	N ₈₀ P ₄₅ K ₄₅ FA ₂₀	24.01 ^{ef}	39.97 ^b	1.73 ^{fgh}	2.20 ^a	3.72 ^{ghi}	4.44 ^{abc}	0.48 ^{hij}	0.62 ^{ab}				
105	N ₀ P ₀ K ₀ FA ₀	43.28 ^j	46.36 ^{ij}	3.28 ^k	3.83 ^j	9.37 [*]	11.19	1.35 ^j	1.56 ⁱ				
	N ₄₀ P ₁₅ K ₁₅ FA ₁₀	49.15 ^{hi}	52.81 ^{d-g}	4.01 ^{ij}	5.08 ^{gh}	12.29 [*]	13.54 [*]	1.65 ^{hi}	1.91 ^{defg}				
	N ₆₀ P ₃₀ K ₃₀ FA ₁₀	53.23 ^{def}	60.06 ^c	5.10 ^{fgh}	5.30 ^{d-g}	13.68 [*]	14.58 [*]	2.05 ^d	2.43 ^b				
	N ₈₀ P ₄₅ K ₄₅ FA ₁₀	56.18 ^d	65.72 ^b	5.28 ^{efg}	5.68 ^{bcd}	14.36 [*]	15.38 [*]	2.24 ^c	2.46 ^b				
	N ₄₀ P ₁₅ K ₁₅ FA ₂₀	49.38 ^{ghi}	62.01 ^c	4.14 ^{ij}	5.46 ^{c-g}	12.48 [*]	15.19 [*]	1.77 ^{gh}	2.46 ^b				
	N ₆₀ P ₃₀ K ₃₀ FA ₂₀	50.26 ^{fgh}	70.50 ^a	4.34 ⁱ	6.53 ^a	13.36 [*]	16.53 [*]	1.87 ^{fg}	2.82 ^a				
	N ₈₀ P ₄₅ K ₄₅ FA ₂₀	51.20 ^{e-h}	68.08 ^{ob}	4.76 ^h	5.95 ^b	13.53 [*]	15.62 [*]	1.87 ^{efg}	2.75 ^a				
	LSD at 5%	35	70	105	35	70	105	35	70	105	35	70	105
	W =	0.07	0.72	1.30	0.01	0.06	0.14	0.01	0.08	0.68	0.008	0.146	0.06
	T =	0.14	1.35	2.44	0.03	0.11	0.27	0.02	0.15	1.27	0.01	1.352	0.12
	I =	0.20	1.91	3.46	0.04	0.15	0.38	0.03	0.21	*N.S	0.02	2.449	0.17

Notes: Values are presented as mean (n = 3). Significant difference at p < 0.05 was determined by least significant difference (LSD) test to compare the means. Superscript letters with different values denote DMR test of significance. W: water, T: Treatment and I: Interaction.

*N.S: non-significant at interaction.

although the wastewater contained sewage could not cause toxicity. Presence of phosphorus (PO₄⁻) and nitrogen (NO₃-N) in wastewater may be a cause of concern if it is diverted towards any water body causing eutrophication. But the values (1.09 and 1.21 mg L⁻¹) were also not in excess and their presence in wastewater made it an excellent source, supplementing them, and thereby lowering the fertilizer requirement as well as lessening the environmental degradation (Singh et al., 2012). The concentration of all nutritive elements was higher in WW as compared to GW. Of all these essential nutrients, nitrogen (N) is often the most limiting element and is invariably required in large quantities as pointed out by Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management, Council of Australia and New Zealand (2000) and Tucker (2004). In wastewater it was present in both ionic forms, i.e. NH₄⁺-N (cation) and NO₃⁻-N (anion), representing a positive-negative relationship and resulting in the highest growth rate and yield on combined supply (Kirkby, 1981) and the utility of this form of nitrogen also confirmed the enhanced growth and yield under WW with N₆₀ instead of N₈₀ containing treatment in the present study. The regular supply of P in good quantity (1.15 and 1.04 mg L⁻¹) in the form of phosphate with each

wastewater irrigation and its presence with P₁₅ instead of P₄₅ fertilizers containing treatment therefore made it suitable and effective, as observed in improved performance of the tested crop which recorded higher root fresh weight, dry weight (Table 5), seed yield, oil content and oil yield (Table 9). Potassium (K), the third major essential osmotically active cation nutrient, has an impact on various physiological processes, especially during the process of opening and closing of stomata. It is well established that only adequate quantity of K is needed for attaining full utilization of N for growth and yield (Mengel & Kirkby, 1982). Therefore, observed enhancement in growth of the tested crop was not only due to its own physiological role (Wolf, Kimbrough, & Blaser, 1976) but also by enhancing the effect of nitrogen. It is likely in the present study that K with WW gave higher N and K contents in leaves as compared to ground water. Sulphur (S) nutrition next to NPK is of importance, especially for mustard crop (Bhowmik, 2003) in oil production. Another important aspect is that it also increases phosphorus availability, thus enhancing the assimilation rate leading to higher seed production. Expectedly in the present study, yield parameters improved significantly in WW × NPK combination than the control and GW × NPK treatments. Calcium and magnesium are also considered important as Ca⁺⁺ is involved in cell division and Mg⁺⁺ is a central atom of chlorophyll, required for structural integrity of chloroplast on which photosynthesis is dependent. The enhancement in total chlorophyll content, carotenoid content and net photosynthetic rate in *B. nigra* grown under wastewater treatment may therefore be due to the involvement of Mg⁺² in pigment synthesis (Table 7; Figure 1). Cl⁻ has an important role in stomata regulation. Na⁺ although not essential, has been placed in the category of beneficial elements, but its presence in wastewater may also be responsible for growth stimulation due to cell expansion and water balance of plants. All factors have played a cumulative role in enhancing the growth, physiological and yield production and made wastewater an efficacious source of irrigation due to easy availability all year round.

3.3. Plant growth, physiological and yield characteristics

An increasing concentration of NPK and FA in the soil showed a positive effect on growth parameters of *B. nigra*; however, all the treatments of NPK together with FA used showed higher values with WW over GW at all three growth samplings. Wastewater gave an increase of 23.08, 40.96 and 20.65% shoot fresh weight, 23.57, 19.26 and 22.35% root fresh weight, 19.77, 15.93 and 14.56% shoot dry weight, 34.89, 20.21 and 27.96% root dry weight, 8.98, 18.67 and 23.44% leaf number and 17.01, 20.79 and 37.05% leaf area than GW at 35, 70 and 105 age of the plant (DAS), respectively. Effect of fly ash at 10 and 20 t ha⁻¹ with NPK-(kg ha⁻¹) amended soil showed significant improvement in all growth parameters as compared to non-fly ash-amended soil (NFFS) at different age of observations (Tables 5 and 6). Among all treatments of NPK + FA, maximum enhancement in growth was recorded by N₆₀P₃₀K₃₀FA₂₀ + WW. The treatments N₄₀P₁₅K₁₅FA₁₀ and N₄₀P₁₅K₁₅FA₂₀ with GW as well as WW

Figure 1. Effect of ground water and wastewater alone and along with different fly ash (t ha⁻¹) and N, P and K levels (kg ha⁻¹) on net photosynthetic rate (Pn) of black mustard (*B. nigra* cv. IC 247) at age 35 and 70 days after sowing (DAS).

Note: Values are presented as Mean ± SE (n = 3). Significant difference at p < 0.05 was determined by least significant difference (LSD) test to compare the means.

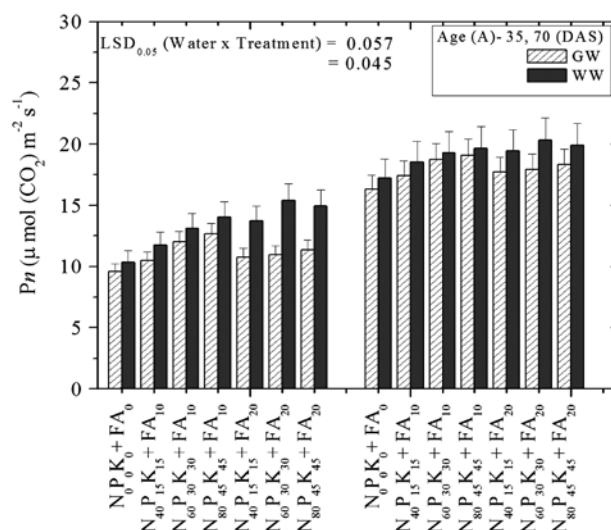


Table 6. Effect of ground water and wastewater alone and along with different fly ash (t ha⁻¹) and N, P and K levels (kg ha⁻¹) on leaf number, leaf area and shoot and root length of black mustard (*B. nigra* cv. IC 247) at age 35, 70 and 105 days after sowing (DAS)

Age (DAS)	Treatments	Leaf characteristics				Length (cm)							
		Leaf number		Leaf area (cm ²)		Shoot		Root					
		GW	WW	GW	WW	GW	WW	GW	WW				
35	N ₀ P ₀ K ₀ FA ₀	6.67*	7.33*	27.08*	28.66*	11.30 ^m	12.60 ^l	5.87 ^m	6.63 ^{lm}				
	N ₄₀ P ₁₅ K ₁₅ FA ₁₀	7.67*	8.33*	29.33*	33.50*	13.00 ^{kl}	14.23 ^{ghij}	6.90 ^{klm}	7.40 ^{hijkl}				
	N ₆₀ P ₃₀ K ₃₀ FA ₁₀	8.33*	8.67*	34.25*	37.32*	14.27 ^{ghij}	14.67 ^{defghi}	7.43 ^{ghijkl}	7.80 ^{defghijk}				
	N ₈₀ P ₄₅ K ₄₅ FA ₁₀	8.67*	9.00*	35.75*	40.17*	14.30 ^{efghij}	15.37 ^{bcdef}	7.53 ^{efghijkl}	7.93 ^{bcdefghi}				
	N ₄₀ P ₁₅ K ₁₅ FA ₂₀	8.00*	9.00*	31.40*	38.40*	13.20 ^{kl}	15.23 ^{cdefg}	7.07 ^{kl}	7.87 ^{cdefghijk}				
	N ₆₀ P ₃₀ K ₃₀ FA ₂₀	8.00*	9.33*	32.32*	42.28*	13.90 ^{jk}	17.23 ^a	7.27 ^{ijkl}	9.40 ^a				
	N ₈₀ P ₄₅ K ₄₅ FA ₂₀	8.33*	9.00*	32.73*	40.47*	14.20 ^{hij}	16.07 ^{abc}	7.40 ^{hijkl}	8.87 ^{abcd}				
70	N ₀ P ₀ K ₀ FA ₀	10.67 ^k	11.00 ^{jk}	50.40 ^l	53.73 ^{kl}	84.17 ^h	88.37 ^g	11.70 ^f	12.93 ^e				
	N ₄₀ P ₁₅ K ₁₅ FA ₁₀	11.67 ^{ijk}	13.33 ^{efghi}	55.30 ^{kl}	66.45 ^{efghi}	91.27 ^{fg}	99.97 ^e	15.07 ^d	17.13 ^c				
	N ₆₀ P ₃₀ K ₃₀ FA ₁₀	13.67 ^{defghi}	14.33 ^{bcdefgh}	67.12 ^{efgh}	71.68 ^{cdef}	104.27 ^d	107.40 ^{cd}	17.13 ^c	18.00 ^{bc}				
	N ₈₀ P ₄₅ K ₄₅ FA ₁₀	14.00 ^{cdefgh}	16.33 ^{ab}	68.43 ^{defgh}	77.63 ^{bc}	107.10 ^{cd}	114.83 ^b	17.40 ^{bc}	18.20 ^b				
	N ₄₀ P ₁₅ K ₁₅ FA ₂₀	12.67 ^{ghijk}	16.17 ^{ab}	60.82 ^{ij}	75.28 ^{bc}	93.90 ^f	108.87 ^c	15.27 ^d	18.20 ^b				
	N ₆₀ P ₃₀ K ₃₀ FA ₂₀	12.67 ^{ghijk}	17.00 ^a	63.63 ^{hi}	93.33 ^a	97.33 ^e	120.00 ^e	15.30 ^d	20.70 ^a				
	N ₈₀ P ₄₅ K ₄₅ FA ₂₀	13.00 ^{ghij}	16.67 ^a	64.38 ^{ghi}	81.40 ^b	98.03 ^e	115.27 ^b	15.43 ^d	20.60 ^a				
105	N ₀ P ₀ K ₀ FA ₀	41.33 ^j	45.33 ^{ij}	38.40 ^l	40.40 ^k	122.40 ^k	130.84 ⁱ	25.80 ^j	27.97 ⁱ				
	N ₄₀ P ₁₅ K ₁₅ FA ₁₀	46.67 ⁱ	59.00 ^{fg}	43.35 ⁱ	48.32 ^f	145.33 ⁱ	156.00 ^{ef}	28.30 ^h	31.10 ^g				
	N ₆₀ P ₃₀ K ₃₀ FA ₁₀	60.00 ^{efg}	68.00 ^{cd}	48.35 ^e	63.25 ^c	156.87 ^{def}	160.00 ^d	34.63 ^f	37.60 ^{de}				
	N ₈₀ P ₄₅ K ₄₅ FA ₁₀	64.33 ^{de}	70.33 ^{abc}	55.52 ^d	69.92 ^{ab}	159.33 ^{de}	168.73 ^c	36.23 ^{ef}	39.50 ^{bcd}				
	N ₄₀ P ₁₅ K ₁₅ FA ₂₀	46.67 ⁱ	68.33 ^{bcd}	45.25 ⁱ	67.20 ^b	148.87 ^{hi}	165.10 ^c	29.30 ^{ghi}	38.27 ^{cd}				
	N ₆₀ P ₃₀ K ₃₀ FA ₂₀	52.67 ^h	74.33 ^a	45.42 ^h	78.42 ^a	150.13 ^{gh}	204.43 ^a	29.58 ^{ghi}	42.53 ^a				
	N ₈₀ P ₄₅ K ₄₅ FA ₂₀	58.00 ^g	71.00 ^{abc}	46.68 ^g	75.15 ^a	153.40 ^{fg}	189.67 ^b	30.70 ^g	40.63 ^{ab}				
	LSD at 5%	35	70	105	35	70	105	35	70	105	35	70	105
	W =	0.57	0.80	1.92	1.98	2.33	1.57	0.44	1.29	1.42	0.40	0.35	0.77
	T =	1.07	1.50	3.60	3.71	4.38	2.94	0.83	2.42	2.65	0.76	0.66	1.44
	I =	*N.S	2.12	5.09	*N.S	6.20	4.17	1.18	3.92	3.37	1.08	0.94	2.04

Notes: Values are presented as mean (n = 3). Significant difference at p < 0.05 was determined by least significant difference (LSD) test to compare the means. Superscript letters with different values denote DMR test of significance. W: water, T: Treatment and I: Interaction.

*N.S: non-significant at interaction.

were found to be deficient, giving less growth and yield. It may be because of the short supply of mineral nutrients, i.e. the plant cannot produce the potential number of leaves and as such it cannot reach the potential area per leaf; thus, maintaining the elements' concentration in various plant parts for unrestricted growth may become restricted (Greenwood et al., 1990). It may also be pointed out that these deficient doses proved better than N₀P₀K₀FA₀ + GW and N₀P₀K₀FA₀ + WW, which indicated the importance of fertilizers that cannot be substituted fully with wastewater. Further, it was observed that N₈₀P₄₅K₄₅FA₁₀ + WW and N₈₀P₄₅K₄₅FA₂₀ + WW exerted no more changes on growth as the values were at par to N₆₀P₃₀K₃₀FA₁₀ + WW and N₆₀P₃₀K₃₀FA₂₀ + WW; hence, the former two treatments proved luxury doses. Thus, excess NPK affects growth through disturbance in optimum levels of nutrient elements for various morpho-physiological processes and nutrient uptake from soil (Donahue, Miller, & Shickluma, 1977). The inhibitory effect and wasteful use of high level of fertilizers in plant growth have also been reported previously in the work of Chalkoo et al. (2014) and Iqbal et al. (2015). It may be said that plants required N, P and K up to N₆₀P₃₀K₃₀ with FA₂₀ and WW, and further input as N₈₀P₄₅K₄₅ was of no additional benefit, and thus appeared as excess NPK (N₈₀P₄₅K₄₅)

Table 7. Effect of ground water and wastewater alone and along with different fly ash (t ha⁻¹) and N, P and K levels (kg ha⁻¹) on nitrate reductase (NR) activity, carbonic anhydrase (CA) activity, chlorophyll (Chl) content and carotenoid content of black mustard (*B. nigra* cv. IC 247) at age 35, 70 and 105 days after sowing (DAS)

Age (DAS)	Treatments	Enzymes characteristics				Pigments							
		Nitrate reductase (NR) activity		Carbonic anhydrase (CA) activity		Chlorophyll (Chl) content		Carotenoid content					
		GW	WW	GW	WW	GW	WW	GW	WW				
35	N ₀ P ₀ K ₀ FA ₀	218.21*	227.22*	2.35 ^k	2.52 ^j	0.860 ^m	0.920 ^l	0.303 ^j	0.345 ⁱ				
	N ₄₀ P ₁₅ K ₁₅ FA ₁₀	235.52*	259.18*	2.55 ^j	2.81 ^f	0.920 ^{kl}	0.960 ^{ghi}	0.366 ^h	0.404 ^{fg}				
	N ₆₀ P ₃₀ K ₃₀ FA ₁₀	265.26*	270.77*	2.96 ^e	3.19 ^c	0.980 ^{ef}	1.02 ^d	0.417 ^{ef}	0.428 ^{cde}				
	N ₈₀ P ₄₅ K ₄₅ FA ₁₀	268.39*	278.38*	3.10 ^d	3.23 ^c	1.000 ^{de}	1.11 ^{bc}	0.428 ^{de}	0.447 ^a				
	N ₄₀ P ₁₅ K ₁₅ FA ₂₀	243.32*	274.62*	2.62 ⁱ	3.20 ^c	0.940 ^{ijkl}	1.07 ^c	0.373 ^h	0.439 ^{bcd}				
	N ₆₀ P ₃₀ K ₃₀ FA ₂₀	250.20*	290.21*	2.68 ^h	3.46 ^a	0.940 ^{hijkl}	1.19 ^a	0.381 ^h	0.460 ^a				
	N ₈₀ P ₄₅ K ₄₅ FA ₂₀	253.52*	283.45*	2.74 ^g	3.30 ^b	0.950 ^{ghijkl}	1.12 ^b	0.398 ^g	0.450 ^{ab}				
70	N ₀ P ₀ K ₀ FA ₀	310.22*	331.15*	3.32 ^k	3.48 ^j	1.60 ^l	1.68 ^k	0.465 ^j	0.508 ⁱ				
	N ₄₀ P ₁₅ K ₁₅ FA ₁₀	338.35*	377.34*	3.56 ⁱ	3.81 ^g	1.69 ^{jk}	1.80 ^g	0.518	0.563 ^f				
	N ₆₀ P ₃₀ K ₃₀ FA ₁₀	388.47*	408.20*	3.90 ^f	4.13 ^{de}	1.86 ^f	1.90 ^e	0.582 ^e	0.601 ^d				
	N ₈₀ P ₄₅ K ₄₅ FA ₁₀	394.53*	424.11*	4.07 ^e	4.23 ^{bc}	1.88 ^{ef}	1.98 ^c	0.585 ^e	0.620 ^c				
	N ₄₀ P ₁₅ K ₁₅ FA ₂₀	346.63*	420.13*	3.60 ⁱ	4.19 ^{cd}	1.71 ^{ijk}	1.94 ^d	0.520 ^{hi}	0.616 ^c				
	N ₆₀ P ₃₀ K ₃₀ FA ₂₀	353.35*	447.91*	3.68 ^h	4.41 ^a	1.74 ^{hi}	2.23 ^a	0.530 ^h	0.658 ^a				
	N ₈₀ P ₄₅ K ₄₅ FA ₂₀	368.29*	434.42*	3.75 ^{gh}	4.29 ^b	1.77 ^h	2.11 ^b	0.544 ^g	0.638 ^b				
105	N ₀ P ₀ K ₀ FA ₀	279.78*	296.81*	2.86 ⁱ	2.95 ^k	1.14 ^k	1.19 ^j	0.377 ^j	0.408 ^j				
	N ₄₀ P ₁₅ K ₁₅ FA ₁₀	309.66*	339.49*	3.07 ^j	3.31 ^{fg}	1.21 ^{ij}	1.35 ^g	0.415 ^{hi}	0.439 ^{ef}				
	N ₆₀ P ₃₀ K ₃₀ FA ₁₀	344.63*	355.18*	3.33 ^{ef}	3.40 ^d	1.38 ^f	1.47 ^d	0.442 ^e	0.480 ^c				
	N ₈₀ P ₄₅ K ₄₅ FA ₁₀	350.28*	368.10*	3.37 ^{de}	3.58 ^b	1.42 ^e	1.59 ^b	0.463 ^d	0.492 ^c				
	N ₄₀ P ₁₅ K ₁₅ FA ₂₀	320.72*	361.09*	3.15 ^j	3.48 ^c	1.23 ^j	1.51 ^c	0.417 ^{ghi}	0.486 ^c				
	N ₆₀ P ₃₀ K ₃₀ FA ₂₀	327.53*	400.15*	3.22 ^h	3.71 ^a	1.24 ^j	1.71 ^a	0.429 ^{fgh}	0.535 ^a				
	N ₈₀ P ₄₅ K ₄₅ FA ₂₀	333.38*	379.21*	3.25 ^{gh}	3.63 ^b	1.27 ^h	1.61 ^b	0.434 ^{ef}	0.515 ^b				
	LSD at 5%	35	70	105	35	70	105	35	70	105	35	70	105
	W =	10.99	14.02	8.82	0.017	0.027	0.024	0.012	0.012	0.011	0.006	0.004	0.006
	T =	20.56	26.24	16.51	0.033	0.050	0.045	0.024	0.023	0.021	0.011	0.008	0.011
	I =	*N.S	*N.S	*N.S	0.047	0.071	0.064	0.034	0.033	0.030	0.016	0.012	0.016

Notes: Values are presented as mean (n = 3). Significant difference at p < 0.05 was determined by least significant difference (LSD) test to compare the means. Superscript letters with different values denote DMR test of significance. W: water, T: Treatment and I: Interaction.

*N.S: non-significant at interaction.

requirement for those plants which grown under GW condition only. Under GW treatments, plant utilized fertilizers increasingly up to N₈₀P₄₅K₄₅ more efficiently due to increased demand of nutrients. Therefore, the use of WW and FA proved applicable in making N, P and K fertilization optimum or sufficient at their lower levels, instead of higher doses. It may be because of the presence of essential macro- and micro-elements such as Na, K, Ca, P, N, Mg, Cl, S, Mg, Cu, Ni, etc. in their ionic forms in the two wastes tested and their management together with inorganic fertilizers.

ANOVA test also showed the significant effect of age with different treatment levels on the response of physiological parameters viz. nitrate reductase activity, carbonic anhydrase activity, total chlorophyll, carotenoid content, nitrogen content, phosphorus content, potassium content and net photosynthetic rate. Use of wastewater proved their application was beneficial over ground water in providing the suitable nutrients required for physiological processes of growth. WW improved 8.61, 16.96 and 10.32% NR activity, 14.26, 10.27 and 8.13% CA activity, 12.15, 11.37 and 17.13% total

Table 8. Effect of ground water and wastewater alone and along with different fly ash ($t\ ha^{-1}$) and N, P and K levels ($kg\ ha^{-1}$) on leaf nitrogen (N), leaf phosphorus (P) and leaf potassium (K) contents of black mustard (*B. nigra* cv. IC 247) at age 35, 70 and 105 days after sowing (DAS)

Age (DAS)	Treatments	Macro-nutrients								
		Nitrogen (N) content		Phosphorus (P) content		Potassium (K) content				
		GW	WW	GW	WW	GW	WW			
35	$N_0P_0K_0FA_0$	2.55 ^m	2.73 ^l	0.319 ^l	0.333 ^{kl}	2.02 ^m	2.16 ^{lm}			
	$N_{40}P_{15}K_{15}FA_{10}$	2.86 ^k	3.17 ^{fg}	0.342 ^{jk}	0.375 ^{fg}	2.23 ^{kl}	2.45 ^{ghij}			
	$N_{60}P_{30}K_{30}FA_{10}$	3.17 ^{gh}	3.28 ^{de}	0.378 ^{efg}	0.393 ^{cd}	2.50 ^{ghij}	2.58 ^{defgh}			
	$N_{80}P_{45}K_{45}FA_{10}$	3.24 ^{efg}	3.33 ^{bcd}	0.385 ^{def}	0.409 ^{ab}	2.53 ^{efgh}	2.63 ^{bcdefg}			
	$N_{40}P_{15}K_{15}FA_{20}$	2.98 ^j	3.31 ^{cde}	0.350 ^{ij}	0.401 ^{bc}	2.28 ^{kl}	2.61 ^{cdefg}			
	$N_{60}P_{30}K_{30}FA_{20}$	3.07 ⁱ	3.46 ^a	0.359 ^{hi}	0.422 ^a	2.30 ^{ijkl}	2.84 ^a			
	$N_{80}P_{45}K_{45}FA_{20}$	3.15 ^{hi}	3.38 ^{abc}	0.367 ^{gh}	0.415 ^{ab}	2.38 ^{hijk}	2.69 ^{abcdef}			
70	$N_0P_0K_0FA_0$	3.48 ^l	3.65 ^k	0.429 ^l	0.444 ^{kl}	3.02 ^l	3.17 ^k			
	$N_{40}P_{15}K_{15}FA_{10}$	3.71 ^{jk}	3.97 ^g	0.453 ^{jk}	0.504 ^{fg}	3.25 ^{jk}	3.48 ^{fg}			
	$N_{60}P_{30}K_{30}FA_{10}$	4.08 ^f	4.20 ^{de}	0.512 ^{ef}	0.545 ^{cd}	3.55 ^{ef}	3.76 ^{cd}			
	$N_{80}P_{45}K_{45}FA_{10}$	4.13 ^{ef}	4.49 ^c	0.528 ^{de}	0.555 ^{bc}	3.66 ^{de}	3.86 ^{bc}			
	$N_{40}P_{15}K_{15}FA_{20}$	3.77 ^{ij}	4.30 ^d	0.466 ^{ij}	0.551 ^{bc}	3.31 ^{ijk}	3.80 ^{bc}			
	$N_{60}P_{30}K_{30}FA_{20}$	3.82 ^{hi}	4.81 ^a	0.475 ^{hi}	0.586 ^a	3.33 ^{hij}	4.08 ^a			
	$N_{80}P_{45}K_{45}FA_{20}$	3.89 ^{gh}	4.63 ^b	0.490 ^{gh}	0.568 ^b	3.40 ^{ghi}	3.93 ^b			
105	$N_0P_0K_0FA_0$	2.95 ^j	3.10 ^k	0.388 ^k	0.408 ^j	2.28 ^k	2.45 ^j			
	$N_{40}P_{15}K_{15}FA_{10}$	3.18 ⁱ	3.51 ^g	0.415 ^{ij}	0.464 ^{ef}	2.57 ^{ij}	2.84 ^{fg}			
	$N_{60}P_{30}K_{30}FA_{10}$	3.53 ^{fg}	3.62 ^e	0.470 ^{def}	0.484 ^{bcd}	2.90 ^{efg}	3.03 ^{cde}			
	$N_{80}P_{45}K_{45}FA_{10}$	3.60 ^{ef}	3.88 ^c	0.475 ^{cdef}	0.490 ^b	2.95 ^{defg}	3.14 ^{abc}			
	$N_{40}P_{15}K_{15}FA_{20}$	3.26 ^j	3.75 ^d	0.428 ^{hi}	0.486 ^{bcd}	2.63 ^{hi}	3.10 ^{bcd}			
	$N_{60}P_{30}K_{30}FA_{20}$	3.30 ⁱ	4.13 ^a	0.444 ^{gh}	0.597 ^a	2.79 ^{gh}	3.30 ^a			
	$N_{80}P_{45}K_{45}FA_{20}$	3.38 ^h	3.97 ^b	0.457 ^{fg}	0.496 ^b	2.79 ^{gh}	3.20 ^{ab}			
LSD at 5%		35	70	105	35	70	105	35	70	105
W =		0.033	0.038	0.027	0.005	0.006	0.007	0.079	0.053	0.618
T =		0.062	0.071	0.052	0.010	0.012	0.013	0.148	0.099	0.115
I =		0.088	0.101	0.073	0.014	0.017	0.018	0.209	0.140	0.163

Notes: Values are presented as mean ($n = 3$). Significant difference at $p < 0.05$ was determined by least significant difference (LSD) test to compare the means. Superscript letters with different values denote DMR test of significance. W: water, T: Treatment and I: Interaction.

chlorophyll, 11.51, 12.28 and 12.69% carotenoid content, 7.80, 11.79 and 11.89% nitrogen content, 9.92, 10.63 and 10.02% phosphorus content and 10.59, 10.88 and 11.36% potassium content at age 35, 70 and 105 days of the plant, respectively, over GW (Tables 7 and 8). The net photosynthetic rate was recorded at age 35 and 70 days after sowing, where WW gave 19.86 and 7.04% increase over GW (Figure 1). Like growth parameters, consumption of $20\ t\ ha^{-1}$ fly ash was recorded as harmless and was found to be effective more than $10\ t\ ha^{-1}$ and without fly ash soil. Among the interaction effects of fertilizers, fly ash treatments with both waters was recorded and found that under GW set of treatments, all physiological parameters enhance linearly up to N_{80} with both FA_{10} - and FA_{20} -containing treatments. However, under WW, the pattern of linear increase in all parameters was found to be optimum at the $N_{60}P_{30}K_{30}FA_{20}$ + WW as it was at par to $N_{60}P_{30}K_{30}FA_{10}$ and $N_{80}P_{45}K_{45}FA_{20}$, indicating the efficacy of WW in reducing the fertilizers' needs. The treatments containing N_{40} with FA_{10} and FA_{20} were recorded as deficient with GW and WW as well.

Yield is the final manifestation of growth. Yield parameters viz. siliquae number, seed number, 1,000 seed weight, seed yield and oil yield are presented in Table 9. The observations of yield

Table 9. Effect of different NPK + FA concentrations along with ground water (GW) and wastewater (WW) on yield characteristics like number of seeds siliqua⁻¹, number of siliqua plant⁻¹, 1,000 seed weight and seed and oil yields of *B. nigra* cv. IC 247 at 150 days after sowing

Treatments	Yield characteristics (Age—105 DAS)							
	Siliquae number		Seed number		Siliqua length		1,000 seed weight	
	GW	WW	GW	WW	GW	WW	GW	WW
N ₀ P ₀ K ₀ FA ₀	234.33*	271.00*	5.67*	6.00*	1.47 ^l	1.60 ^{kl}	1.44 ⁱ	1.58 ^{ij}
N ₄₀ P ₁₅ K ₁₅ FA ₁₀	296.33*	349.33*	6.00*	7.00*	1.60 ^{kl}	1.93 ^{ghij}	1.64 ^{hi}	1.74 ^{efghi}
N ₆₀ P ₃₀ K ₃₀ FA ₁₀	353.67*	370.00*	7.33*	8.67*	2.00 ^{efgh}	2.40 ^{cde}	1.76 ^{defghi}	1.85 ^{bcdef}
N ₈₀ P ₄₅ K ₄₅ FA ₁₀	366.33*	386.67*	7.67*	8.67*	2.23 ^{de}	2.63 ^{abc}	1.80 ^{cdefgh}	1.87 ^{abcdef}
N ₄₀ P ₁₅ K ₁₅ FA ₂₀	325.33*	375.33*	6.33*	8.67*	1.70 ^{ijkl}	2.47 ^{bcde}	1.65 ^{ghi}	1.87 ^{abcdef}
N ₆₀ P ₃₀ K ₃₀ FA ₂₀	331.00*	400.67*	6.67*	9.00*	1.77 ^{hijk}	2.83 ^a	1.70 ^{fghi}	2.04 ^a
N ₈₀ P ₄₅ K ₄₅ FA ₂₀	347.00*	394.00*	6.67*	9.00*	1.83 ^{ghijk}	2.77 ^a	1.74 ^{efghi}	1.94 ^{abcd}
LSD at 5% W =	7.546		0.627		0.095		0.070	
T =	14.11		1.174		0.179		0.132	
I =	N.S*		N.S*		0.253		0.183	
	Biological yield		Seed yield		Oil content		Oil yield	
	GW	WW	GW	WW	GW	WW	GW	WW
N ₀ P ₀ K ₀ FA ₀	11.63*	13.57*	3.83 ^l	4.25 ^k	35.29 ^k	35.87 ^j	135.03*	152.53*
N ₄₀ P ₁₅ K ₁₅ FA ₁₀	12.33*	13.93*	4.40 ^k	5.16 ^{gh}	36.17 ^j	37.44 ^g	159.12*	193.33*
N ₆₀ P ₃₀ K ₃₀ FA ₁₀	12.64*	14.86*	5.27 ^{fgh}	5.47 ^{def}	37.76 ^f	40.14 ^d	198.85*	219.75*
N ₈₀ P ₄₅ K ₄₅ FA ₁₀	14.30*	15.27*	5.34 ^{efg}	5.75 ^{bcd}	39.42 ^e	40.60 ^{bcd}	210.66*	233.44*
N ₄₀ P ₁₅ K ₁₅ FA ₂₀	14.53*	14.99*	4.72 ^j	5.60 ^{cde}	36.71 ⁱ	40.34 ^{cd}	173.25*	225.90*
N ₆₀ P ₃₀ K ₃₀ FA ₂₀	13.02*	15.94*	4.83 ^{ij}	6.10 ^a	36.83 ^{hi}	41.12 ^a	178.02*	250.99*
N ₈₀ P ₄₅ K ₄₅ FA ₂₀	13.28*	15.54*	5.05 ^{hi}	5.87 ^{abc}	37.18 ^{ghi}	40.84 ^{ab}	187.76	239.64
LSD at 5% W =	0.590		0.116		0.187		7.747	
T =	1.140		0.217		0.349		14.49	
I =	N.S*		0.307		0.494		N.S*	

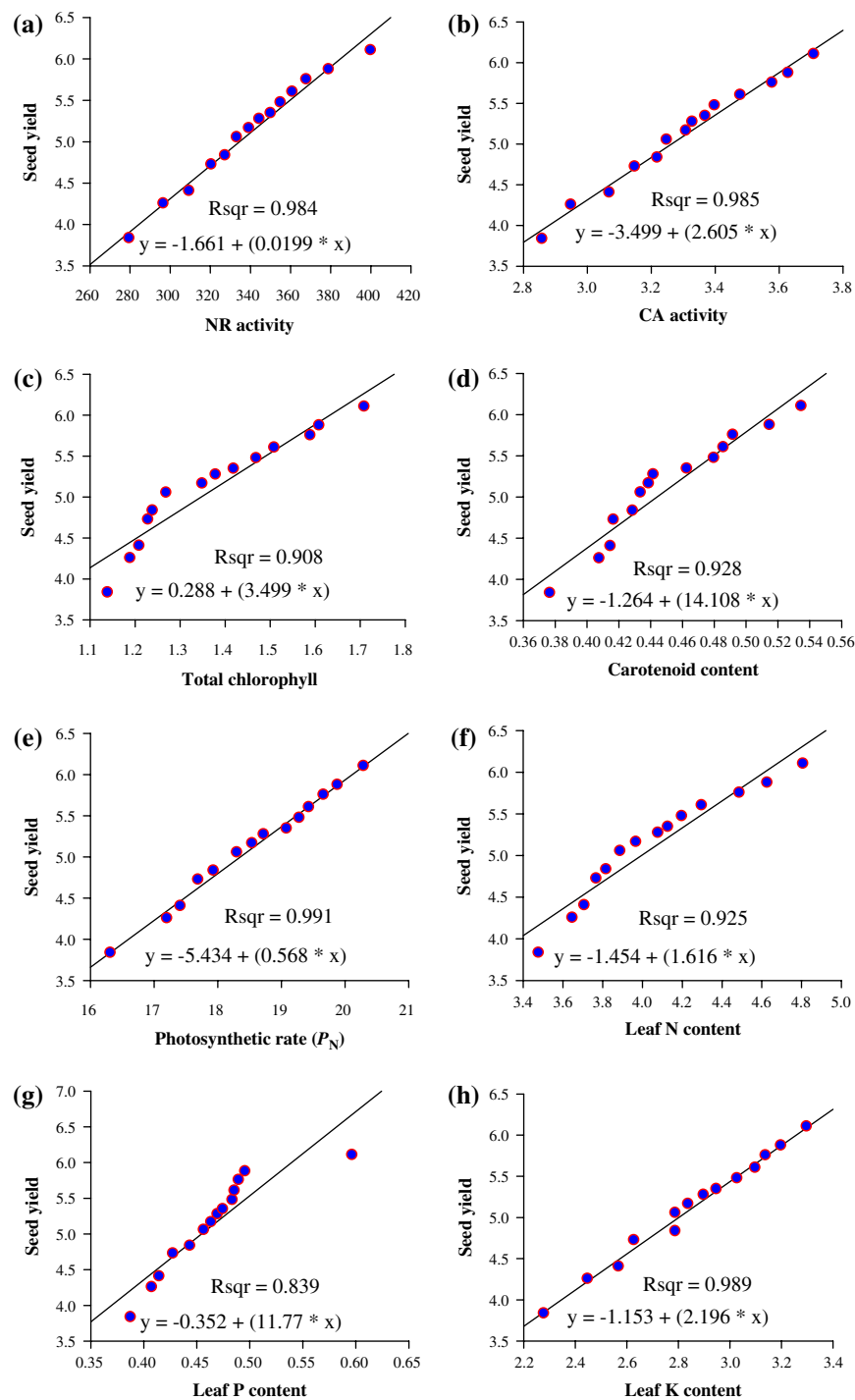
Notes: Values are presented as mean (n = 3). Significant difference at p < 0.05 was determined by least significant difference (LSD) test to compare the means.

Superscript letters with different values denote DMR test of significance. W: water, T: Treatment and I: Interaction.

*N.S: non-significant at interaction.

characteristics at all treatment levels were found to be on a similar pattern as observed in growth and physiological parameters. Hence, with increase in level of NPK + FA, the values of yield parameters also increased and the maximum production of yield was recorded with WW at medium fertilizers' level (N₆₀P₃₀K₃₀FA₂₀), while higher fertilizer levels (N₈₀P₄₅K₄₅FA₂₀ × WW) proved luxury as the values were at par with N₆₀P₃₀K₃₀FA₂₀ × WW. And N₄₀-containing treatments such as N₄₀P₁₅K₁₅FA₁₀ × WW, N₄₀P₁₅K₁₅FA₂₀ × WW, N₄₀P₁₅K₁₅FA₁₀ × WW and N₄₀P₁₅K₁₅FA₁₀ × WW had deficient doses, but recorded better than control and N₀P₀K₀FA₀ × WW (WW only). Result of two-way ANOVA test N₆₀P₃₀K₃₀ + FA₂₀ + WW treatment increased the seed yield by 59.26% in *B. nigra* in comparison with the control. The other yield parameters such as number of siliqua per plant, seeds per siliqua, 1,000 seed weight and oil yield increased by 70.98, 58.73, 41.66 and 85.87% in *B. nigra*, respectively, compared with the control under this optimum treatment. However, all these parameters decreased under N₈₀P₄₅K₄₅ + FA₂₀ + WW when compared with N₆₀P₃₀K₃₀ + FA₂₀ + WW by 3.77, 1.66, 0.00, 4.90 and 4.52%,

Figure 2. Regression relationship between seed yield and (a) nitrate reductase (NR) activity (b) carbonic anhydrase (CA) activity (c) chlorophyll content (d) carotenoid content (e) net photosynthetic rate (f) leaf nitrogen (N) (g) leaf phosphorus (P) and (h) leaf potassium (K) content of *B. nigra* cv. IC247 under different FA and NPK levels.



respectively. Chalkoo et al. (2014), Sahay, Inam, and Iqbal (2014) and Iqbal et al. (2015) reported significant reduction or not much enhancement in yield characteristics of *Capsicum annuum* L. and *B. juncea* cv. Pusa Bold as a result of higher dose of NPK and/or under NPK stress. Hence, nutrient quantities are growth- and yield-limiting factors in all plants; thus, quite but useful correlation from the above discussion can be explained as;

NPK Stress/Deficient NPK—less production in yield
Excess/High/luxury NPK—reduction in yield
Optimum/sufficient NPK—significant production in yield

3.4. Logarithmic relationship model: physiological parameters vs. seed yield

A relationship between positive and negative correlation coefficient values was also calculated when regression linear curves were drawn between various physiological parameters (independent variable) and seed yield (dependent variable). Further, an equation of linear regression (y) was also calculated to determine the distance between the straight line and all of the data points. Therefore, increased activity of all physiological parameters led to enhanced yield and yield attributes which were also evident from Figure 2, showing the linear regression correlations obtained between seed yield and physiological parameters (Figure 2(a–f)). The R^2 values showed a strong positive relationship between both dependent and independent factors at different levels of NPK + FA with GW or/ and WW and further confirmed that the synergistic interaction effect of nutrients of the two wastes tested with NPK fertilizers caused the higher yield as compared to control.

4. Conclusion

The results concluded that farmers can use comparatively less inorganic fertilizers ($N_{60}P_{30}K_{30}$ in present case) with up to 20 t ha⁻¹ FA and application of WW for cultivation of *B. nigra* cv. IC247 at Aligarh's soil for better growth and yield, which was definitely due to the presence of utilizable essential nutrients such as N, P, K, Ca, Mg, S, Cl, etc. in FA and WW because field soil used in the experiment was low in plant nutrients. Also, the soil qualities, like porosity and water-holding capacity, and nutrient status can be enhanced under waste (fly ash) application. Therefore, the importance of the present comprehensive investigation has been considered for the purpose of conservation of inorganic nutrients and fresh water for the disposal of the two wastes generated in large quantities at Aligarh.

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Competing interests

The authors declare no competing interest.

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