

Biological Forum – An International Journal

14(2a): 499-504(2022)

ISSN No. (Print): 0975-1130 ISSN No. (Online): 2249-3239

Effect of Nano Urea vs Conventional Urea on the Nutrient Content, Uptake and Economics of Black Wheat (*Triticum aestivum* L.) along with Biofertilizers

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ABSTRACT: To study the effect of nitrogen sources and biofertilizers on the nutrient content, uptake and economics of black wheat, a field research experiment in factorial randomized block design was performed at Instructional Farm, Department of Agronomy, Rajasthan College of Agriculture, Udaipur (Rajasthan). The experiment comprised of five levels of nitrogen sources *i.e.*, N₁ (100 % Conventional urea fertilizer), N₂ (75 % Conventional urea + 25 % of Nano urea fertilizer), N₃ (50 % Conventional urea + 50 % of Nano urea fertilizer), N₄ (25 % Conventional urea + 75 % of Nano urea fertilizer) and N₅ (100 % of Nano urea fertilizer) and four levels of biofertilizers i.e., (B₁: No biofertilizer, B₂: Azotobacter, B₃: PSB and B₄: Azotobacter + PSB) which were replicated thrice. The maximum N, P, K, Fe and Zn content and uptake in grain and straw were recorded under N3 (50 % Conventional urea + 50 % of Nano urea fertilizer) as compared to the remaining treatments. The utmost net return (109637 ₹ ha⁻¹) and benefit-cost ratio (2.37) were also obtained under N_3 (50 % Conventional urea + 50 % of Nano urea fertilizer). Inoculation of seed with conglomerated mixture of Azotobacter + PSB (B₄) appreciably increases the N, P, K, Fe and Zn content and uptake in grain and straw. It also leads to significant enhancement in net return (105367 ₹ ha ¹) and benefit-cost ratio (2.27) as compared to all other treatments of biofertilizer. But under the diverse treatment combination of both these factors, there was no any significant variation reported for anthocyanin content in black wheat grain. So nanofertilizer application proved more practical and efficient in improving nutrient content, uptake and economics of black wheat as compared to conventional fertilizer.

Keywords: Nano urea, Black wheat, Biofertilizer, Azotobacter, PSB.

INTRODUCTION

Wheat (Triticum aestivum L.) is the most important and widely grown cereal crop of the globe which is grown since pre-historic times and according to De Candole, it had originated in the Valley of Euphrates and Tigris. It is a self-pollinated crop having chromosome number (2n = 42) belongs to the family *Poaceae*. Wheat plays a significant role in increasing the economic growth of the nation and ensuring food as well as nutrition security. It is grown on an area of 215.9 million hectares, producing 765.8 million metric tonnes of wheat in the world (FAO, 2020). China is ranked first which is followed by India and Russia and together they contribute 41 per cent of the global production. In India, wheat is the second most important cereal crop next to rice. Among winter cereals, it contributes about 49 per cent of total food grain production. India had 31.45 million of hectares area, production 107.86 million tonnes with productivity of 3.42 tonnes hectare⁻¹ (Pocket Book of Agricultural Statistics, 2020). Rajasthan state stands on fifth position in terms of wheat production after Uttar Pradesh, Punjab, Haryana and Madhya Pradesh. Rajasthan produced 10.57 million tonnes from 29.32 lakh hectares area with the average productivity of 3.46 tonnes hectare⁻¹ (Government of Rajasthan, 2021).

It has lots of nutritive value in the form of carbohydrates (70 %), protein (10-12 %), fat (2.0 %), minerals (1.8 %) crude fibers (2.2 %) vitamins *viz.*, thiamin, riboflavin, niacin and small amounts of vitamin A, but during the milling process most of the nutrients get eliminated with the bran and germ (Britannica, 2021). It is also a good source of fiber, manganese and magnesium in an unrefined state (Yadav *et al.*, 2013). Now a day's consumers changed his demands and they prefer a balanced nutrient profile instead of energy providing diets, which provides metabolic, physiological and functional health benefits. At present, a large population of the world is suffering

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from various diseases and health issues because of inadequate quantities of protein, vitamins, essential macro and micro nutrients including Fe and Zn in daily dietary (Balyan *et al.*, 2013), to overcome such problems scientists worked on biofortification of wheat resulted, black wheat came in existence. Black wheat contains all the nutrients and minerals which are important for human dietary needs, it's coloured pigment *i.e.*, anthocyanins and other phytochemicals are getting popular around the world owing to the associated health benefits. Coloured wheat has proven to be helpful in preventing and fighting against various chronic diseases like cancer, cardio vascular disease (CVD), diabetes, inflammation, obesity and aging (Garg *et al.*, 2016).

Urea contributes about 82 per cent of the total fertilizer consumption in India and about 55 per cent of the total fertilizer nitrogen consumed in the world. Around 30-40 per cent of nitrogen from urea is utilized by plants and the rest gets wasted due to quick chemical transformation as a result of leaching, volatilization, denitrification and run off, thereby low use efficiency. Whereas, nano urea has high nitrogen use efficiency and also it is environment friendly. This fertilizer is popularly known as "smart fertilizer" because it reduces the emission of nitrous oxide which is primarily responsible for contaminating soil, air and water bodies and also helps in reduction of global warming. These properties make it a promising alternative over conventional urea. Micro-organism plays a vital role in fixing, solubilizing, mobilizing, recycling of macro and micro nutrients in an agricultural eco-system. Although, they are occurring naturally in soil but their population is generally insufficient to bring about the desired level of nutrient mobilization (Welbaun et al., 2004). Azotobacter and Azospirillum biofertilizer inoculant are used in non-leguminous crops like wheat, rice, maize and barley etc. They fix atmospheric nitrogen in soil and helps in saving 15-20 kg N ha⁻¹.

MATERIALS AND METHODS

This field experiment was conducted at Instructional Farm, Department of Agronomy, Rajasthan College of Agriculture, Udaipur (Rajasthan) during the *rabi* season of 2021-22. The region of the experimental site falls

under the agro-climatic zone IVa (Sub-Humid Southern Plains and Aravalli Hills) of Rajasthan and soil of the experimental field was clay loam in texture, slightly alkaline (pH 7.75) in reaction, low in organic carbon (0.66 %), nitrogen (286.50 kg ha⁻¹) and medium in available phosphorus (21.60 kg ha⁻¹) but high in potassium (369.70 kg ha⁻¹). The experiment consisted of 20 treatment combination which comprises of five levels of nitrogen sources *i.e.*, N₁ (100 % Conventional urea fertilizer), N_2 (75 % Conventional urea + 25 % of Nano urea fertilizer), N₃ (50 % Conventional urea + 50 % of Nano urea fertilizer), N₄ (25 % Conventional urea + 75 % of Nano urea fertilizer) and N₅ (100 % of Nano urea fertilizer) and four levels of biofertilizers *i.e.*, (B₁: No biofertilizer, B₂: Azotobacter, B₃: PSB and B₄: Azotobacter + PSB) which were laid out in a factorial randomized block design (FRBD) and replicated thrice. Black wheat crop was sown on 28th November, 2021, for optimizing plant stands 100 kg seed rate was used and seed was sown at 4-5 cm depth with 20 cm row spacing. After pre-sowing irrigation, total 5 irrigations were applied during the whole growing period. Recommended dose of phosphorous and potassium *i.e.*, $60 \text{ and } 40 \text{ kg ha}^{-1}$ were applied through SSP and MOP. But the total recommended dose of nitrogen *i.e.*, 120 kg ha⁻¹ was provided through the combination of two sources *i.e.*, nano urea and conventional urea according to the treatment. Nano urea was applied in the form of spray solution. As one bottle of nano urea (500 ml) is equal to one bag of conventional urea so according to the total urea dose required in wheat crop in one hectare, a total of 2604 ml nano urea is required ha⁻¹. First spray of nano urea was given at tillering stage and second at jointing stage. The microbiological fertilizers i.e., Azotobacter and PSB were used in the form of liquid equally for seed treatment of black wheat. A recommended dose of liquid biofertilizers were used for seed inoculation of black wheat.

Nutrient content and uptake estimation. For estimation of N, P, K, Fe, Zn and anthocyanin contents, the plant samples were collected at the time of harvest and oven dried at 70° C for 72 hours to obtain constant weight. Fully dried samples were grinded to fine powder and nutrient content in grain and straw were estimated as per the following method.

Nutrients	Method of analysis	Reference
Nitrogen	Nesseler's reagent colorimetric method	Linder (1944)
Phosphorus	Ammonium vanadomolybdo phosphoric acid method	Richards (1968)
Potassium	Flame photometer method	Jackson (1973)
Iron	Atomic absorption spectrophotometrically	Lindsay and Norvell (1978)
Zinc	Atomic absorption spectrophotometrically	Lindsay and Norvell (1978)
Anthocyanin	Spectrophotometric method	Abdel-Aal and Hucl (1999)

Total N, P, K, Fe and Zn uptake in grain and straw samples were calculated by multiplying per cent nutrient content with their respective dry matter accumulation as per the formula given below: Nutrient uptake ((kg ha⁻¹) =

$$\frac{\text{Nutrient content (\%)} \times \text{dry matter accumulation (kg ha^{-1})}}{100}$$

Net return and B-C ratio. Gross return was calculated by multiplying the total grain and straw yield with prevalent market prices of the items and then presented on per hectare basis as per treatments. Net return was computed by deducting the total cost of cultivation from the gross return as per treatments.

Net return $(\mathbf{F} \text{ ha}^{-1}) = \text{Gross return } (\mathbf{F} \text{ ha}^{-1}) - \text{Cost of cultivation } (\mathbf{F} \text{ ha}^{-1}).$

Treatment-wise benefit-cost ratio was calculated to analyze and determine the economic viability of the treatments by using the formula:

$$B - C ratio = \frac{\text{Net return (ha}^{-1})}{\text{Total cost (ha}^{-1})}$$

RESULTS AND DISCUSSION

A. Effect of Nitrogen Sources

Nutrient content and uptake. Application of nano urea along with conventional urea leads to elevated amount of nitrogen, phosphorous, potassium, iron and zinc content in gain and straw as well as their uptake by grain, straw and total under the treatment N_3 (50 % Conventional urea + 50 % of Nano urea fertilizer) as compared to other treatments of nitrogen sources (Table 1). Data elucidated in Table 1 clearly demonstrates that there was significant improvement in the nitrogen (2.02 and 0.48 %), phosphorous (0.47 and 0.17 %), potassium (0.51 and 2.31 %), iron (66.11 and 128.41 ppm) and zinc (58.74 and 54.22 ppm) content in gain and straw, respectively under the treatment N₃ (50 % Conventional urea + 50 % of Nano urea fertilizer) was recorded and this was found statistically analogous to effect of treatment N₂(75 % Conventional urea + 25 % of Nano urea fertilizer) however, found significantly superior over rest all of the treatments of nitrogen sources. But there wasn't any significant variation noticed in anthocyanin content of grain among various treatments of nitrogen sources. Furthermore, results showed that amount of N, P, K, Fe and Zn contentin grain under the treatment N₃ (50 % Conventional urea + 50 % of Nano urea fertilizer) increased with the tune of 4.45, 2.12, 5.88, 14.83 and 17.79 per cent over the treatment N_1 (100 % Conventional urea fertilizer).

Table 1: Effect of nitrogen sources and biofertilizers on nutrient content in grain and straw of black wheat.

	Nutrient content											
Treatments				Grain			Straw					
	N	Р	K	Fe	Zn	Anthocyanin	N	Р	K	Fe	Zn	
	(%)	(%)	(%)	(ppm)	(ppm)	(ppm)	(%)	(%)	(%)	(ppm)	(ppm)	
Nitrogen Sources												
N_1	1.93	0.46	0.48	56.30	48.29	120.42	0.46	0.16	2.21	110.60	44.29	
N_2	1.99	0.47	0.50	64.86	57.71	120.69	0.47	0.16	2.27	127.34	53.06	
N_3	2.02	0.47	0.51	66.11	58.74	121.21	0.48	0.17	2.31	128.41	54.22	
N_4	1.88	0.45	0.47	50.27	42.27	119.49	0.45	0.16	2.16	98.76	36.93	
N_5	1.82	0.44	0.47	45.76	36.92	118.99	0.43	0.15	2.13	91.48	32.92	
SEm ±	0.01	0.00	0.00	0.45	0.48	0.79	0.00	0.00	0.02	0.76	0.50	
CD (P=0.05)	0.04	0.01	0.01	1.28	1.39	NS	0.01	0.00	0.04	2.17	1.42	
Biofertilizers												
B_1	1.91	0.44	0.48	54.39	46.48	119.54	0.45	0.16	2.19	106.28	41.98	
B ₂	1.93	0.46	0.49	57.01	49.11	120.32	0.46	0.16	2.22	112.15	44.61	
B ₃	1.92	0.46	0.49	56.28	48.50	119.72	0.45	0.16	2.21	110.41	44.00	
B_4	1.96	0.47	0.49	58.95	51.05	121.06	0.47	0.16	2.25	116.43	46.55	
SEm ±	0.01	0.00	0.00	0.40	0.43	0.71	0.00	0.00	0.01	0.68	0.44	
CD (P=0.05)	0.03	0.01	0.01	1.14	1.24	NS	0.01	0.00	0.04	1.94	1.27	

Similarly, the uptake of N, P, K, Fe and Zn by grain, straw and total also showed the similar kind of trend as shown by nutrient content (Table 2). There was appreciably utmost amount of nutrient uptake of these nutrients *i.e.*, N, P, K, Fe and Zn by grain (85.08, 19.96, 21.36, 0.28 and 0.25 kg ha⁻¹), straw (40.72, 14.07, 195.39, 1.09 and 0.46 kg ha⁻¹) and total (125.80, 34.03, 216.75, 1.36 and 0.71 kg ha⁻¹) noticed under the application N₃ (50 % Conventional urea + 50 % of Nano urea fertilizer). Uptake of N, P, K and Fe by straw under N₃ was found statistically equivalent to the uptake under N2 (75 % Conventional urea + 25 % Nano urea fertilizer) but superior over rest of all. Again, the total uptake of potassium under N₃ was found at par with the treatment N₂ (75 % Conventional urea + 25 % of Nano urea fertilizer) but superior over others. Total nutrient uptake of N, P, K, Fe and Zn increased by means of 11.69, 10.40, 10.62, 19.11 and 23.94 per cent over the treatment N1 (100 % Conventional urea fertilizer).

Nutrient content and uptake were significantly improved under the treatment N_3 (50 % Conventional urea + 50 % of Nano urea fertilizer). Nano fertilizers have large surface area and particle size smaller than the pore size of plant leaves, allowing for greater

penetration into plant tissues from the applied surface and improved absorption and nutrient use efficiency. The pore diameter of plant cell wall ranging from 5 to 50 nm. Hence, only nanoparticles or nanoparticle aggregates with diameter less than the pore diameter of the plant cell wall could easily pass through and reach the plasma membrane. Nanoparticles with having the size less than 5 nm go through the cuticular pathway, whereas those with larger sizes travel through the stomatal pathway before arriving to the conducting system, where they aid in the rapid and simple absorption of nutrients by leaves (Dimkpa et al., 2015; Qureshi et al., 2018). Moreover, coating of nano and sub nano-composites are capable of regulating the release of nutrients from the fertilizer capsule and nano particles have both positive and negative charged binding site that adsorbed available nitrogen in soil and curtail different type of losses resulted in increased uptake of nitrogen by crop Nanoparticles triggered metabolic activity in plants which results in increased exudation and acidity. Subsequently, release of PO₄ may occur as a result of a ligand exchange reaction triggered by plant root exudation, potentially disrupting the adsorption-desorption equilibrium and releasing P into the soil solution where it is easily available for

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uptake. Application of nano particles improves carbon balance in crops, accelerates plant growth, leads to increase in the efficiency of micro and macronutrients of plants and reducing the use of chemical fertilizers per unit area which causes environmental problems. These results are in correlation with results of Junrungrean *et al.* (2002); Aljabri (2010); Junejo *et al.* (2012); Soliman *et al.* (2016); Shrivastava *et al.* (2017); Togas *et al.* (2017); Mahil and Kumar (2019); Hasan and Saad (2020).

Table 2: Effect of nitrogen sources and biofertilizers on nutrient uptake of black wheat.

	Nutrient uptake (kg ha ⁻¹)														
Treatments			Grain			Straw					Total				
	Ν	Р	K	Fe	Zn	N	Р	K	Fe	Zn	Ν	Р	K	Fe	Zn
Nitrogen															
Sources															
N ₁	74.69	17.80	18.77	0.22	0.19	36.40	12.68	174.96	0.88	0.35	111.09	30.49	193.73	1.10	0.54
N_2	79.07	18.56	19.85	0.26	0.23	38.53	13.32	184.84	1.04	0.43	117.61	31.88	204.69	1.30	0.66
N ₃	85.08	19.96	21.36	0.28	0.25	40.72	14.07	195.39	1.09	0.46	125.80	34.03	216.75	1.36	0.71
N_4	69.13	16.44	17.42	0.18	0.16	34.88	12.14	168.31	0.77	0.29	104.01	28.58	185.73	0.96	0.44
N ₅	62.15	14.99	15.97	0.16	0.13	32.77	11.30	161.40	0.69	0.25	94.92	26.29	177.37	0.85	0.38
SEm ±	1.72	0.41	0.40	0.01	0.01	0.78	0.30	4.20	0.02	0.01	2.33	0.65	4.49	0.02	0.01
CD (P=0.05)	4.93	1.18	1.139	0.01	0.01	2.241	0.85	12.012	0.05	0.03	6.66	1.86	12.846	0.06	0.04
Biofertilizers															
B_1	70.04	16.01	17.75	0.20	0.17	34.37	11.99	168.00	0.82	0.32	104.41	28.00	185.75	1.02	0.50
B_2	73.95	17.77	18.65	0.22	0.19	36.72	12.78	176.77	0.90	0.36	110.67	30.54	195.42	1.12	0.55
B ₃	72.64	17.26	18.41	0.22	0.19	35.88	12.52	174.03	0.87	0.35	108.51	29.78	192.43	1.09	0.53
B_4	79.46	19.15	19.87	0.24	0.21	39.68	13.54	189.13	0.98	0.39	119.14	32.69	209.00	1.22	0.60
SEm ±	1.54	0.37	0.36	0.00	0.00	0.70	0.27	3.75	0.02	0.01	2.08	0.58	4.01	0.02	0.01
CD (P=0.05)	4.41	1.05	1.019	0.01	0.01	2.005	0.76	10.744	0.05	0.02	5.95	1.66	11.490	0.06	0.04

Economics. A careful evaluation of the information decoded from Table 3 uncovers that the maximum net return (₹ 109637) and benefit-cost ratio (2.37) was obtained under the application of N_3 (50 % Conventional urea + 50 % of Nano urea fertilizer) which was statistically analogous to N_2 (75 % Conventional urea + 25 % of Nano urea fertilizer) with net return of ₹ 102044 and benefit-cost ratio of 2.20 howbeit, net return and benefit-cost ratio under N_3 were found significant over the other treatments and net return was greater than by 10.54, 15.79 and 22.90 per

cent over N₁ (100 % Conventional urea fertilizer), N₄ (25 % Conventional urea + 75 % of Nano urea fertilizer) and N₅ (100 % of Nano urea fertilizer), respectively. Greater net return was fetched as a consequence of lower cultivation costs due to reduced urea application and effective use of foliar nano fertilizers, which resulted in higher grain and straw yield and as a result, higher net return. These findings were in accordance with Mehta and Bharat (2019); Manikandan *et al.* (2016); Kumar *et al.* (2020).

Transformer	Economics						
Treatments	NR (2 ha ⁻¹)	B-C ratio					
Nitrogen Sources							
N_1	98076	2.12					
N_2	102044	2.20					
N ₃	109637	2.37					
N_4	92318	1.99					
N ₅	84529	1.82					
SEm ±	2922	0.06					
CD (P=0.05)	8367	0.18					
Biofertilizers							
B ₁	91421	1.98					
B ₂	97004	2.09					
B ₃	95492	2.06					
B_4	105367	2.27					
SEm ±	2614	0.06					
CD (P=0.05)	7483	0.16					

B. Effect of Biofertilizers

Nutrient content and uptake. In the present study data explicated that nitrogen, phosphorus, potassium, iron and zinc content and uptake by grain and straw of wheat appreciably improved by inoculation of seed with different biofertilizers. The highest same nutrient was reported with cumulative inoculation of seed with *Azotobacter* + PSB over control and single inoculation. The N, P, K, Fe and Zn content increased by 2.55, 6.38, 2.04, 7.73 and 8.95 per cent in grain and 4.25, 2.98,

2.66, 8.71 and 9.81 per cent in straw, respectively with combine inoculation of seed with *Azotobacter* + PSB over control *i.e.*, B_1 (No biofertilizer), respectively. Whereas, the uptake was found to increase more significantly with combine inoculation of *Azotobacter* + PSB. Considerably the loftiest amount of nutrient uptake of N, P, K, Fe and Zn by grain (79.46, 19.15, 19.87, 0.24 and 0.21 kg ha⁻¹), straw (39.68, 13.54, 189.13, 0.98 and 0.39 kg ha⁻¹) and total (119.14, 32.69, 209.00, 1.22 and 0.60 kg ha⁻¹) was recorded when seed

of the black wheat was inoculated with the conglomerated mixture of *Azotobacter* + PSB and statistically it was found superior over rest all of the biofertilizers treatment. But only in case of P and K uptake by straw under B_2 (*Azotobacter*) was found at par with uptake under B_4 (*Azotobacter* + PSB).

Through biological processes, biofertilizers are capable of transforming essential nutritional components in the soil from non-usable to usable form for crop plants. Azotobacter inoculated seeds facilitated the efficient uptake of N, P, and micronutrients like Fe and Zn in wheat. Mineralization of organic nitrogen and phosphorus enhances nitrogen and phosphorus availability in soil, resulting in increased nutrient absorption by plants via inoculation of nitrogen and phosphorus fixing bacteria. Azotobacter promoted the activity of nitrogenase and nitrate reductase enzymes in soil for higher nitrogen fixation. The nutrient content and uptake by plants appreciably improved when seeds were inoculated with Azotobacter and PSB prior to sowing because Azotobacter can be attributed to enhanced specific activities of iso citric and malic dehydrogenase enzyme, the source of electrons during nitrogen fixation, resulting in a more favourable nutritional environment (Kurtz and Larue, 1975) and PSB solubilize both natural and added phosphorus (Singh et al., 2012). Hameeda et al. (2008) reported that PSB solubilizes insoluble inorganic phosphate compounds in soil, such as tricalcium phosphate and dicalcium phosphate, by the excretion of various organic acids from root exudates. Potassium absorption from soil also rises when N and P availability increased. Thus, synergistic effect of biofertilizers enhanced the content and uptake of nitrogen, phosphorus and potassium in grain and straw. These results are in accordance with the Abbasi and Yousra (2012); Singh et al. (2018); Moradgholi et al. (2021); Radwan et al. (2021).

Economics. The results revealed that the utmost net return *i.e.* ₹105367 was recorded under seed treatment with B_4 (*Azotobacter* + PSB) which was statistically significant over the all-remaining treatments and it was higher with the tune of 13.23, 7.93 and 9.37 per cent over B_1 (No biofertilzer), B_2 (*Azotobacter*) and B_3 (PSB), respectively. Similarly, when seeds were inoculated with amalgamated mixture of *Azotobacter* and PSB (B_4) then highest B-C ratio (2.27) was fetched and this was statistically superior over all other treatments of biofertilizer.

So, the results showed that bio-fertilizers inoculation significantly affected the net return and benefit-cost ratio. The highest amount of net return and benefit- cost ratio was fetched with dual inoculation of *Azotobacter* + PSB (Table 3). The use of effective strains of bio-fertilizers is an environmentally benign, low-cost agricultural input that plays an essential role in enhancing nutrient availability to crops while also lowering production costs (Kumar, 2013). These non-traditional fertilizer sources not only save money, but they also boost soil and crop output dramatically. These observations complement the findings of Ram and Mir (2006); Davari *et al.* (2012).

CONCLUSION

Based on the above cited results of the experiment it could be concluded that nano urea spray in combination with application of conventional urea fertilizer *i.e.*, treatment N₃ (50 % Conventional urea + 50 % of Nano urea fertilizer) significantly increased the nutrient content and uptake of the black wheat crop and also improve the economic return. Similarly, seed inoculation of black wheat with conglomerated mixture of *Azotobacter* and PSB (B₄) considerably improved the nutrient content, uptake, net return and benefit-cost of the crop. So, both of these factors can be used in combination to improve the nutrient content, uptake and economics of the black wheat.

Acknowledgement. The author(s) gratefully acknowledge the Department of Agronomy, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur, India for providing the research facilities for this research.

Conflicts of Interest. None.

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How to cite this article: Kannoj, J. Choudhary, Devendera Jain, Manish Tomar, Ritesh Patidar and Ruchika Choudhary (2022). Effect of Nano Urea vs Conventional Urea on the Nutrient Content, Uptake and Economics of Black Wheat (*Triticum aestivum* L.) along with Biofertilizers. *Biological Forum – An International Journal*, 14(2a): 499-504.