

Original Article

## Role of beneficial microbes with nitrogen and phosphorous levels on canola productivity

Papel de microbes benéficos em associação com níveis de nitrogênio e fosforos na produtividade de canola

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### Abstract

A research was conducted to evaluate the impact of various nitrogen and phosphorus levels along with beneficial microbes to enhance canola productivity. The research was carried out at Agronomy Research Farm, The University of Agriculture Peshawar in winter 2016-2017. The experiment was conducted in randomized complete block factorial design. The study was comprised of three factors including nitrogen (60, 120 and 180 kg ha<sup>-1</sup>), phosphorous (70, 100 and 130 kg ha<sup>-1</sup>) and beneficial microbes (with and without BM). A control treatment with no N, P and BM was also kept for comparison. Application of beneficial microbes significantly increased pods plant, seed pod, seed filling duration, 1000 seed weight, biological yield and seed yield as compared to control plots. Nitrogen applied at the rate of 180 kg ha<sup>-1</sup> increased pods plant<sup>-1</sup>, seed pod, seed filling duration, seed weight, biological yield and seed yield. Maximum pods plant<sup>-1</sup>, seed pod, early seed filling, heavier seed weight, biological yield, seed yield, and harvest index were observed in plots treated with 130 kg.ha<sup>-1</sup> phosphorous. As comparison, the combine treated plots have more pods plant<sup>-1</sup>, seeds pod<sup>-1</sup>, seed filling duration, heaviest seeds, biological yield, seed yield and harvest index as compared to control plots. It is concluded that application of beneficial microbes with N and P at the rate of 180 kg ha<sup>-1</sup> and 130 kg ha<sup>-1</sup>, respectively, increased yield and its attributes for canola.

**Keywords:** nutrients, micro-organisms, production, *Brassica napus*.

### Resumo

Uma pesquisa foi realizada para avaliar o impacto de vários níveis de nitrogênio e fósforo, juntamente com micróbios benéficos, para aumentar a produtividade da canola. A pesquisa foi realizada no inverno de 2016-17 no Agronomy Research Farm, Universidade de Agricultura do Peshawar. O experimento foi conduzido por planejamento fatorial aleatorizado em blocos. O estudo focou-se em três fatores, incluindo o teor de nitrogênio, N, (60, 120 e 180 kg.ha<sup>-1</sup>), o teor de fósforo, P, (70, 100 e 130 kg ha<sup>-1</sup>) e a presença de micróbios benéficos (com BM e sem BM). Para fins de comparação, um tratamento controle sem N, P e BM também foi incluído no estudo. A aplicação de micróbios benéficos aumentou significativamente as vagens das plantas e de sementes, a duração do enchimento das sementes, o peso de 1000 sementes, o rendimento biológico e o rendimento de sementes em comparação com os resultados do controle. O nitrogênio aplicado na taxa de 180 kg ha<sup>-1</sup> aumentou as vagens por planta, vagem, duração do enchimento, peso da semente, rendimento biológico e rendimento de sementes. Vagens máximas por planta, vagem, enchimento precoce de sementes, peso maior de semente, rendimento biológico, rendimento de sementes e índice de colheita foram observados em parcelas tratadas com 130 kg.ha<sup>-1</sup> de fósforo. Em comparação aos blocos cultivados de controle, os blocos cultivados tratados combinados têm mais vagens por planta e sementes por vagem, maior duração do enchimento das sementes, maior número de sementes mais pesadas e maior rendimento biológico, rendimento de sementes e índice de colheita. Conclui-se que a aplicação de micróbios benéficos junto com N e P nas doses de 180 kg ha<sup>-1</sup> e 130 kg ha<sup>-1</sup>, respectivamente, aumentou a produtividade e atributos de produtividade para a canola.

**Palavras-chave:** nutrientes, microrganismos, produção, *Brassica napus*.

### 1. Introduction

World wide food demands are increasing day by day, especially in developing countries, where crop lands and resources hardly contribute to an efficient crop production (Bargaz et al., 2018). Majority of Farmers only rely on

chemical fertilizer which not only deteriorates quality of soil but also increases cost of production. Using of beneficial microbes with fertilizers are integrated approaches to reduce dependency on chemical fertilizers with the

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advantage of low cost of production (Imran, 2019). In Pakistan rapeseed is a vital palatable oilseed crop though its average yield is very low i.e. less than 812 kg ha<sup>-1</sup> compared to Canada where average yield is 3200 kg ha<sup>-1</sup> and Australia (2000 kg ha<sup>-1</sup>). The yield is low in Pakistan because it is cultivated on marginal land with poor fertility status, the lack of proper crop nutrition and unavailability of beneficial microbes. Nitrogen is an important element in the chemical arrangement of chlorophyll, the molecule accountable for changing light into chemical energy that drives photosynthesis (Havlin, 2005). Moreover, most of the applied N is lost in the environment through volatilization or leaching down. Therefore for efficient utilization of applied N fertilizer, proper strategy should be followed in timely and balance application of nitrogenous fertilizer to crop. The soils of Pakistan are mostly calcareous in nature. P availability in calcareous soil is a major problem because in calcareous soil P is adsorbed in to insoluble form and become unavailable to the plants (Barber, 1995). The application of nitrogen and phosphorous are essential for getting maximum crop production. But their inadequate and imbalance reduced the yield. Beneficial microbes not only enhance nutrient uptake, but also regulate plant growth, improved photosynthetic efficiency nutrient use efficiency, tolerance to stress condition i.e (heat tolerance, resistance to insects and resistance to plant diseases) (Calabi-Floody et al., 2018).

Beneficial microbes composed of different mixed strains of bacteria, naturally-occurring such as photosynthetic bacteria (e.g., *Rhodospseudomonas palustris*, *Rhodobacter sphaeroides*), lactobacilli (e.g., *Lactobacillus plantarum* L. casei, and *Streptococcus lactis*), yeasts (e.g., *Saccharomyces* spp.), and Actinomycetes (*Streptomyces* spp.) (Javaid, 2010). Providing photosynthetic bacteria to the field enhances other beneficial microbes. For example, vesicular-arbuscular mycorrhiza level were increased in the rhizosphere due to the availability of nitrogenous compounds by photosynthetic bacteria (Kleiber et al., 2014). Vesicular-arbuscular mycorrhiza increase the availability and solubility of phosphorous in the soils for crops. As lactic acid acts to sterilise soils and suppress harmful microorganisms, as well as increasing the decomposition of organic matter (Cóndor et al., 2007). Lactic acid bacteria also enhance the breakdown of organic matter. Similarly yeasts synthesis, anti-microbial compounds that also promote growth of crop from sugars and amino acids secreted by photosynthetic bacteria. Yeasts, can promote active cell and root division. Actinomycetes, produce anti-microbial substances can suppress harmful fungi and bacteria. These microorganisms are possible tools for sustainable agriculture and in combination of these different microbial strains could be a good multifunctional biofertilizer for sustainable agriculture (Kumar and Gopal, 2015) and when applied to seed, plant surfaces or soil colonize the plant and encourage its growth by increasing the nutrient availability (Thakur et al., 2018). It was confirmed that application of beneficial microbes to the soil can improve soil fertility and soil quality (Maheswari and Elakkiya,

2014). Moreover, beneficial Microbes have an effect on the availability of nutrients (Górski and Kleiber, 2010).

The application of beneficial microbes along with NPK enhanced yields significantly (Joshi et al., 2019). Beneficial microbes appeared to promote early growth and yield in different crops (Backer et al., 2018). The application of beneficial microbes enhance the fertilizer use efficiency (Bargaz et al., 2018). It was reported that beneficial microbes have the beneficial effects on crop yield and yield components of cereal crops (rice, wheat) and in legume i.e. mung-bean and vegetables (Seran and Shahardeen, 2013). Therefore, the present research was carried out to evaluate the effect of different nitrogen, phosphorus levels with beneficial microbes on yield and yield components of canola. To sustain fertility status of the soil, it is important to provide nutrients and beneficial soil organisms for intensive sustainable agriculture (Pankhurst and Lynch, 1995).

Application of adequate and balanced major nutrients are must to securing higher productivity in a sustainable manner, therefore nutrient management with relevant beneficial micro-organisms are highly required.

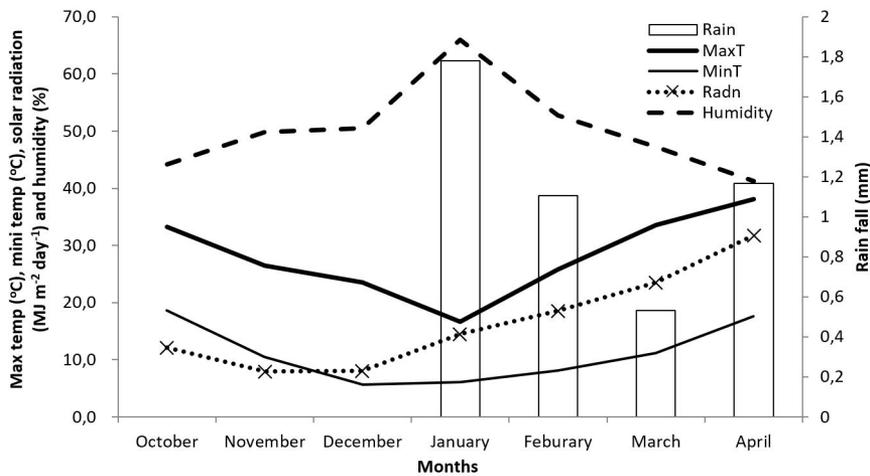
## 2. Materials and Methods

### 2.1. Experimental design and treatments

The experiment was carried out in randomized complete block design having three replications. The experiment consisted of three nitrogen levels (60, 120, 180 Kg ha<sup>-1</sup>), three phosphorous levels (70, 100 and 130 Kg ha<sup>-1</sup>) and application of beneficial microbes (with and without). Urea and single super phosphate were used for nitrogen and phosphorous as sources, respectively. The cultivar of Brassica 'Zahoor' was sown at the seed rate of 10 kg ha<sup>-1</sup> in the 3<sup>rd</sup> week of October 2016. The size of the plot was 3.5 m × 2.8 m, having 8 rows, 40 cm apart. The field was irrigated four times when required. The canola field was weeded three times after every 30 days before flowering.

### 2.2. Soil and environmental characteristics

The field experiment was located in Peshawar 34°01'06"N, 71°27'59"E, at an altitude of 350 m above sea level. Peshawar is located about 1600 km north of the Indian Ocean and has semiarid climate. The soil sample of the experimental site have pH (7.5), EC (605 µS/cm), soil texture (Silt loam), organic matter (0.80%), 0.065% N, 13 mg kg<sup>-1</sup> available P, 230 mg kg<sup>-1</sup> available K and there were no said beneficial microbes according to the soil microbiology laboratory, Department of Soil and Environmental Sciences. Data on different meteorological parameters (Maximum and minimum temperature, relative humidity, rainfall and solar radiation) of experimental site was measured during growing season of the crop (2016-17). Mean monthly maximum and minimum temperature (°C), solar radiation (MJ m<sup>-2</sup> day<sup>-1</sup>), relative humidity (%) and rainfall (mm) of the growing season of canola crop (2,016-2,017) (Figure 1).



**Figure 1.** Mean monthly maximum & minimum temperature (°C), solar radiation ( $\text{MJ m}^{-2} \text{day}^{-1}$ ) and humidity (%) and rainfall (mm) of the growing season of canola crop (2,016-2,017).

### 2.3. Beneficial microbes application

Beneficial microbes stock solution was obtained from National Agriculture Research Center, Islamabad, Pakistan. Beneficial microbe's solution consisted of predominant population of photosynthetic bacteria i.e. *Rhodo pseudomonas plastris* and *Rhodobacter sphaeroides*; lactobacilli (*Lactobacillus plantarum* L. casei and *Streptococcus lactis*), yeasts (*Saccharomyces cerevisiae*) and actinomycetes viz. *Strptomycetes griseoviridis*. Before its application to the field a solution was made containing one part of beneficial microbes with one part of 1% sugar solution plus 20 parts water by volume kept in dark for several hours. The activated beneficial microbes solution was diluted to 1:1000 by adding sterilized water. And the applied to the plots at the rate of  $2 \text{ L m}^{-2}$

### 2.4. Procedure for recording data

Data concerning the days to emergence was recorded by counting the number of days from sowing to 50% of the seedlings' emergence in each subplot. Days to seed filling were measured by counting number of days when seed become fully filled at the growth stage "80". About thirty five (35) to forty five (45) days after the flower opens seed filling is complete. when seed filling complete, it contains nearly 40 to 45% moisture (Thurling, 1974). Pods  $\text{plant}^{-1}$  and seeds  $\text{pod}^{-1}$  were measured by counting pods and seed  $\text{pod}^{-1}$  in five randomly selected plants in each subplot and averaged it respectively (Manaf et al., 2017). Thousand grains weight (g) was measured by taking seed lot of thousands grains from each subplot and weighed with digital balance repeated three times. Data on biological yield ( $\text{kg ha}^{-1}$ ) was recorded by harvesting three central rows in each sub plot, sun dried, weighed and after that data was converted to  $\text{kg ha}^{-1}$  using following Formula 1 (Ahmad et al., 2005):

$$\text{Biological yield} \left( \frac{\text{kg}}{\text{ha}} \right) = \frac{\text{Biological yield (kg / ha)}}{r - r \text{ distance} \times \text{no. of rows} \times \text{row length}} \times 10,000\text{m} \quad (1)$$

Seed yield ( $\text{Kg ha}^{-1}$ ) was measured by threshing, cleaning and weighing the seeds from harvested rows and data was converted in  $\text{kg ha}^{-1}$  using Formula 2 (Younis et al., 2020):

$$\text{Grain yield} \left( \frac{\text{kg}}{\text{ha}} \right) = \frac{\text{Grain yield (kg / ha)}}{r - r \text{ distance} \times \text{no. of rows} \times \text{row length}} \times 10,000\text{m} \quad (2)$$

### 2.5. Data analysis

The data collected were analysed using the procedure appropriate for randomized complete block (RCB) design. Means was compared using Least Significant Difference (LSD) test at the 5% level of probability when F values were significant (Jan et al., 2009).

## 3. Results and Discussion

### 3.1. Days to emergence

Analysis of variance exhibited that days to emergence of canola was not significantly affected by nitrogen, phosphorus, beneficial microbes (Table 1). The planned mean comparison of control vs rest and all the interactions were also found non-significant for days to emergence (Table 1). However maximum days to emergence (11) was recorded in control plots as compared with beneficial microbes treated plots (10).

### 3.2. Days to seed fill duration

Days to seed fill duration of canola were significantly affected by different nitrogen levels, beneficial microbes and mean comparison of control vs rest (Table 1). The effect of phosphorus levels and all the interactions were found non-significant. The planned mean comparison of control with fertilized plots exhibited that more days (122) were taken to seed fill duration by the fertilized treatment as compared to control (110). Regarding nitrogen levels, seed filling stage was delayed with increasing nitrogen level from 60 to 180  $\text{kg ha}^{-1}$  and more days (126) to seed filling

stage were taken with application of 180 kg N ha<sup>-1</sup>, followed by 120 kg N ha<sup>-1</sup> (120). Nitrogen influenced vegetative growth and crop canopy, which may delay phenology of maize. Earlier seed filling stage (119) was observed with addition of 60 kg N ha<sup>-1</sup>. Application of beneficial microbes took more days (120) to seed filling stage as compared with no BM application (123). Phosphorus at the rate of 122 kg ha<sup>-1</sup> increased days to seed filling (122) as compared to higher doses (130 kg ha<sup>-1</sup>) which decreased days to seed filling (121).

### 3.3. Thousand seed weight (g)

Different nitrogen and phosphorus levels, beneficial microbes and mean comparison of control vs rest

significantly affected thousand seeds weight of canola (Table 2). All the interactions except BM x N were found non-significant. The mean comparison of control vs rest revealed that heavier grains (4.5 g) were produced by fertilized treatment as compared to control (3.8 g). Mean values of nitrogen revealed that application of 180 kg N ha<sup>-1</sup> produced heavier seeds (4.7 g) which was statistically equal to thousand seeds weight (4.5 g) noticed with 120 kg N ha<sup>-1</sup>. Lowest thousand grains weight was recorded with 60 kg N ha<sup>-1</sup> (4.2 g). The phosphorous applied at the rate of 130 kg ha<sup>-1</sup> produced heavier grains (4.7 g), which was statistically at par with 100 kg P ha<sup>-1</sup> (4.5 g). Addition of 70 kg P ha<sup>-1</sup> produced lighter seeds (4.2 g). Application of BM produced heavier seeds (4.8 g) in comparison with no treatment of plots with BM (4.2 g).

**Table 1.** Days to emergence, pods plant<sup>-1</sup>, seeds pod<sup>-1</sup> and days to seed filling duration as affected beneficial microbes with nitrogen and phosphorus levels.

Treatments	Emergence	Pods plant <sup>-1</sup>	Seeds pod <sup>-1</sup>	Seed Filling
<b>Beneficial microbes</b>				
Without	10	224 b	20.2 b	120 b
With	10	241 a	23.3 a	123 a
<b>Nitrogen (kg ha<sup>-1</sup>)</b>				
60	10	218 c	20.2 b	119 c
120	10	235 b	22.5 b	120 b
180	10	246 a	22.7 a	126 a
<b>Phosphorus (kg ha<sup>-1</sup>)</b>				
70	10	222 c	19.9 c	122
100	10	230 b	21.6 b	122
130	10	240 a	23.8 a	121
<b>Control</b>	11	182 b	17.7 b	110 b
<b>Rest</b>	10	233 a	21.8 a	122 a

**Table 2.** Thousand grain weight (g), biological yield (kg ha<sup>-1</sup>), seed yield (kg ha<sup>-1</sup>) and Harvest index (%) of canola as affected beneficial microbes with nitrogen and phosphorus levels.

Treatments	1000 seed wt	Biological yield	Seed yield	Harvest index
<b>Beneficial microbes</b>				
Without	4.2 b	9583 b	1394 b	14.6 b
With	4.8 a	10357 a	1484 a	14.4 a
<b>Nitrogen (kg ha<sup>-1</sup>)</b>				
60	4.2 b	9479 c	1380 b	14.6 ab
120	4.5 a	9950 b	1487 a	15 a
180	4.7 a	10481 a	1450 a	13.9 b
<b>Phosphorus (kg ha<sup>-1</sup>)</b>				
70	4.2 b	9581 b	1311 c	13.8 b
100	4.5 a	10002 a	1437 b	14.4 b
130	4.7 a	10327 a	1569 a	15.3 a
<b>Control</b>	3.8 b	8009 b	1002 b	12.5 b
<b>Rest</b>	4.5 a	9970 a	1439 a	14.5 a

Means of the same category followed by different letters are significantly different at 5% level of probability using LSD test.

The interaction between BM and nitrogen indicated that increasing nitrogen level from 60 to 180 kg ha<sup>-1</sup> increased thousand seeds weight with BM application. While plots with no BM application, seeds weight were decreased with increasing nitrogen (180 kg ha<sup>-1</sup>) (Figure 2c). Application of beneficial microbes in sesame significantly increased 1000 seed weight and seed yield (Yasari et al., 2008). These beneficial microbes produced some useful substances i.e. amino acids, polysaccharides, nucleic acids, bioactive substances, and sugars, all of which promote plant growth and development. The metabolites which are produced by these microbes are readily absorbed by the crops (Higa, 2000). The crops required high NPK during the initial growth, therefore the crops that gain high supply of nitrogen would increase the vegetative growth and yield (Rorie et al., 2011).

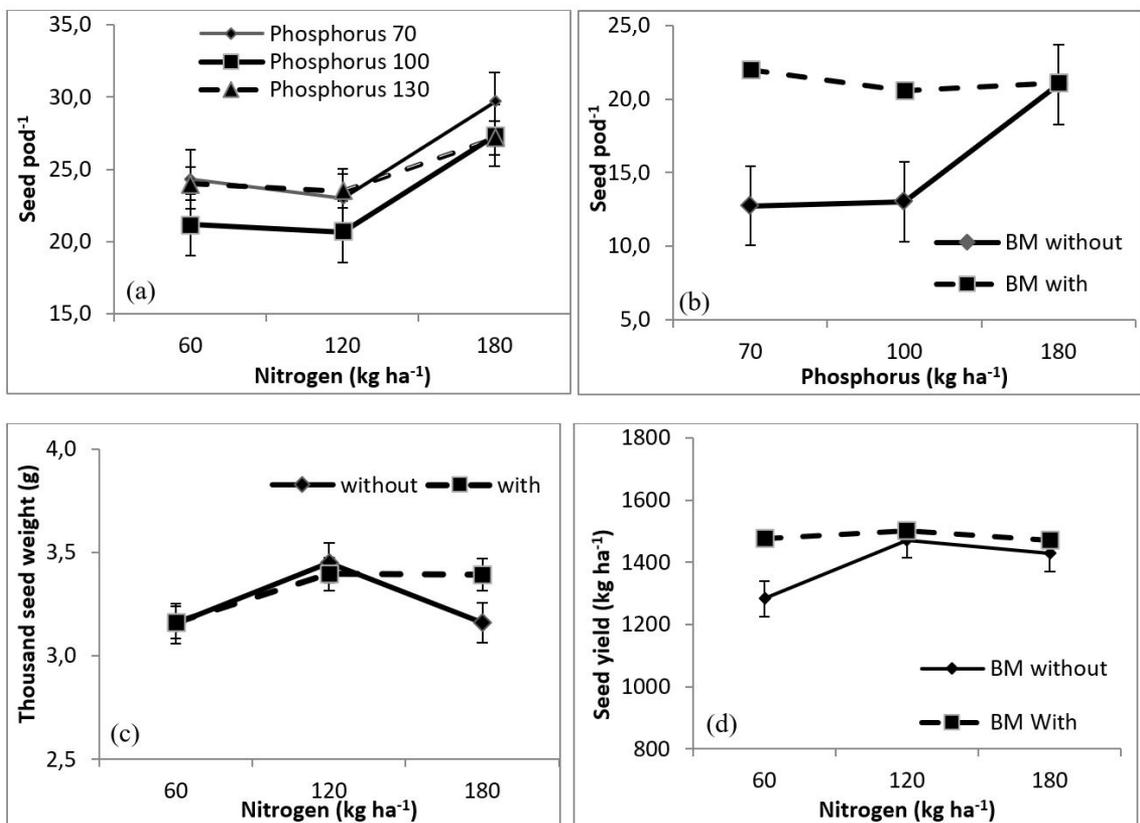
#### 3.4. Number of pods plant<sup>-1</sup>

Different nitrogen and phosphorus levels, beneficial microbes and mean comparison of control vs rest significantly affected number of pods plant<sup>-1</sup> of canola (Table 1). All the possible interactions were found non-significant. Mean comparison of control vs rest indicated that more pods were produced in fertilized plots (233) as compared to unfertilized plots (182). Mean values of nitrogen revealed that increasing nitrogen level consistently increased pods plant<sup>-1</sup> and more pods (246) were produced

with addition of 180 kg N ha<sup>-1</sup>, followed by 120 kg N ha<sup>-1</sup> (235). Addition of 60 kg N ha<sup>-1</sup> produced lower pods (218). Likewise, increasing phosphorus application consistently increased pods plant<sup>-1</sup> and application of 130 kg P ha<sup>-1</sup> produced higher pods (246), followed by 100 kg P ha<sup>-1</sup> (230). Pods was lowest (222) with addition of 70 kg P ha<sup>-1</sup>. Regarding beneficial microbes, BM application increased pods (241) as compared to no BM application (224).

#### 3.5. Number of seeds pods<sup>-1</sup>

Number of seeds pod<sup>-1</sup> of canola was significantly affected by different nitrogen and phosphorus levels, beneficial microbes and mean comparison of control vs rest significantly affected number of pods plant<sup>-1</sup> of canola (Tables 1 and 3). All the interactions between N x P and BM x P were found non-significant (Tables 4). Mean comparison of control vs rest indicated that more number of seeds pod<sup>-1</sup> were produced in fertilized plots (21.8) as compared to unfertilized plots (17.7). Mean values for nitrogen revealed that increasing nitrogen level from 60 to 180 kg ha<sup>-1</sup>, seeds pod<sup>-1</sup> were increased from 20.2 to 22.7. Which was statistically similar with number of seeds (22.5) produced with 120 kg N ha<sup>-1</sup>. Addition of 60 kg N ha<sup>-1</sup> produced lower number of pods (20.2). The increase in seeds pod<sup>-1</sup> might be due to the fact that nitrogen enhanced the growth of the crop and produced more dry matter that resulted in more seeds pod<sup>-1</sup>. Likewise, increasing



**Figure 2.** Interaction between nitrogen x phosphorus for seeds pod<sup>-1</sup> (a), phosphorus x beneficial microbes for seeds pod<sup>-1</sup> (b), nitrogen x beneficial microbes for grains weight (c), and nitrogen x beneficial microbes for seed yield (kg ha<sup>-1</sup>) of canola (d).

**Table 3.** LSD at (0.05) for days to emergence, pods plant<sup>-1</sup>, seeds pod<sup>-1</sup>, days to seed filling duration, thousand seed weight (g), biological yield (kg ha<sup>-1</sup>), seed yield (kg ha<sup>-1</sup>) and harvest index (%) of canola.

Parameters	Nitrogen (N)	Phosphorus (P)	Beneficial microbes (BM)
Days to emergence	Ns	Ns	Ns
Pods plant <sup>-1</sup>	8	8	6
Seeds pod <sup>-1</sup>	1.7	1.7	1.4
Seed filling duration	5	NS	4
Thousand grain weight	0.3	0.3	0.2
Biological yield	459	459	375
Seed yield	41	41	33
Harvest index	0.8	0.8	NS

**Table 4.** Analysis of variance for days to emergence, pods plant<sup>-1</sup>, seeds pod<sup>-1</sup> and days to seed filling duration of canola as affected by beneficial microbes with nitrogen and phosphorus levels.

SOV	Df	Emergence	Pods plant <sup>-1</sup>	Seeds pod <sup>-1</sup>	Seed fill duration
Replication	2	15.368	133.737	2.333	1.333
Nitrogen (N)	2	2.074 Ns	3514.889**	35.167**	226.741**
Phosphorus (P)	2	0.963 Ns	2641.556**	66.500**	0.352 Ns
Beneficial microbes (BM)	1	2.667 Ns	3986.963**	130.667**	192.667**
Control vs Rest	1	1.887 Ns	7328.035**	48.035**	377.080**
N x P	4	1.519 Ns	158.194 Ns	21.250**	5.491 Ns
N x BM	2	3.556 Ns	234.963 Ns	9.056 Ns	96.222 Ns
P x BM	2	1.556 Ns	58.963 Ns	26.722**	6.056 Ns
N x P x S	4	2.111 Ns	138.546 Ns	2.194 Ns	75.361 Ns
Error	36	2.035	125.700	6.333	45.833
Total	56				
CV (%)		14.42	4.87	11.67	5.60

SOV=Source of variation, DF=Degree of freedom, \*, \*\*, \*\*\*: significant at P≤ 0.05, 0.01, and 0.001, respectively.

phosphorus application consistently increased number of seeds pod<sup>-1</sup>. Application of 130 kg P ha<sup>-1</sup> produced higher number of seeds pod<sup>-1</sup> (23.8), followed by 100 kg P ha<sup>-1</sup> (21.6). Number of seeds pod<sup>-1</sup> was lowest (19.9) with addition of 70 kg P ha<sup>-1</sup>. Regarding beneficial microbes, BM application produced more number of seeds pod<sup>-1</sup> (23.3) as compared to no BM application (20.2). The interaction N x P indicated that number of seeds pod<sup>-1</sup> increased with the increasing N from 60 to 180 kg ha<sup>-1</sup> and phosphorus from 70 and 100 kg P ha<sup>-1</sup>, however slight decrease was observed with increasing N at 130 kg P ha<sup>-1</sup> (Figure 2a). The interaction between BM and P revealed that increasing P level from 70 to 130 kg ha<sup>-1</sup> increased number of seeds pod<sup>-1</sup> without BM application however the increase was more prominent with BM application plots (Figure 2b).

### 3.6. Biological yield (kg ha<sup>-1</sup>)

Statistical analysis of the data showed that biological yield of canola was significantly affected by nitrogen and phosphorus levels, beneficial microbes and mean comparison of control vs. rest. While all the possible interactions were found non-significant (Table 2). Mean

values of the data indicated that control plots produced less biological yield (8009 kg ha<sup>-1</sup>) as compared to rest of the fertilized plots (9970 kg ha<sup>-1</sup>). Nitrogen applied at 180 kg ha<sup>-1</sup> produced more biological yield (10480 kg ha<sup>-1</sup>), which was followed by 120 kg N ha<sup>-1</sup> (9950 kg ha<sup>-1</sup>). Lower biological yield (9479 kg ha<sup>-1</sup>) was obtained with nitrogen application at the rate of 60 kg ha<sup>-1</sup>. Among different phosphorus levels, 130 kg P ha<sup>-1</sup> produced higher biological yield (10327 kg ha<sup>-1</sup>), which was statistically similar with yield (10002 kg ha<sup>-1</sup>) obtained from 100 kg P ha<sup>-1</sup>. The application of phosphorus at the rate of 70 kg ha<sup>-1</sup> produced lowest biological yield (9581 kg ha<sup>-1</sup>).

Beneficial microbes produced more biological yield (10357 kg ha<sup>-1</sup>) as compared to no beneficial microbes applied plots (9583 kg ha<sup>-1</sup>).

### 3.7. Seed yield (kg ha<sup>-1</sup>)

Statistical analysis of the data showed that seed yield of canola was significantly affected by nitrogen, phosphorus levels, beneficial microbes and control vs. rest (Table 2). All the possible interactions except BM x N were found non-significant. Mean comparison of control vs. rest

indicated that the fertilized plots produced more seed yield ( $1439 \text{ kg ha}^{-1}$ ) as compared to control plots ( $1002 \text{ kg ha}^{-1}$ ). Mean values for nitrogen ( $120 \text{ kg N ha}^{-1}$ ) observed maximum seed yield ( $1487 \text{ kg ha}^{-1}$ ), which was statistically at par with  $180 \text{ kg N ha}^{-1}$  ( $1450 \text{ kg ha}^{-1}$ ). The nitrogen application at the rate of  $60 \text{ kg ha}^{-1}$  produced lower seed yield ( $1380 \text{ kg ha}^{-1}$ ). Application of phosphorous at the rate of  $130 \text{ kg ha}^{-1}$  produced higher seed yield ( $1569 \text{ kg ha}^{-1}$ ), followed by  $100 \text{ kg P ha}^{-1}$  ( $1437 \text{ kg ha}^{-1}$ ). Lowest seed yield was recorded ( $1311 \text{ kg ha}^{-1}$ ) at  $70 \text{ kg P ha}^{-1}$ . Regarding BM, addition of BM increased seed yield ( $1484 \text{ kg ha}^{-1}$ ) as compared to without BM applied ( $1394 \text{ kg ha}^{-1}$ ). The interaction between BM and N revealed that seed yield increased with increasing N from 60 to  $120 \text{ kg ha}^{-1}$ . However the increase was more prominent in without BM application. Further, increasing N beyond  $120 \text{ kg ha}^{-1}$  decrease seed yield (Figure 2d).

### 3.8. Harvest index (%)

Harvest index of canola was significantly affected by nitrogen and phosphorus levels, as well as control vs rest (Table 2). The effect of BM and all the possible interactions were found non-significant (Table 5). Mean comparison of control vs rest showed that higher harvest index (14.5%) was observed in fertilized plots as compared to control plots (12.5%). The nitrogen applied at the rate of  $120 \text{ kg N ha}^{-1}$  had higher harvest index (15.0%), followed by  $60 \text{ kg N ha}^{-1}$  (14.6%). The nitrogen application at the rate of  $180 \text{ kg ha}^{-1}$  had lowest harvest index (13.9%). In case of phosphorus levels, application of  $130 \text{ kg P ha}^{-1}$  had higher harvest index (15.3%), followed by  $100 \text{ kg P ha}^{-1}$  (14.4%).

## 4. Discussion

Days to emergence was non-significantly affected by nitrogen, phosphorus, beneficial microbes and its interactions. It might be due that the young plants take

nutrients from the store food in the endosperm of seed and there is no response of fertilizer at early germination stage. It was reported that seedling emergence is mostly related to the reserved food present in seed (Le Gouis et al., 1999). Seeds used their own endosperm for germination and did not utilize food from any other sources (Hadi et al., 2012).

The increase in days to seed fill duration with application of nitrogen might be due to the fact that increasing nitrogen increased the vegetative growth of the plants therefore it delayed phenology of the crop (Banziger et al., 1999). Similar results were previously reported that increasing nitrogen application delayed the maturity of the crop (Kutcher et al., 2005). Delayed phenology is associated with the increase in leaf area duration, vegetative growth and light use efficiency with increased nitrogen levels (Rehman et al. 2010).

Pods  $\text{plant}^{-1}$  was increased through using nitrogen at the rate of  $200 \text{ kg ha}^{-1}$  and  $150 \text{ kg ha}^{-1}$  with beneficial microbes as compared to lowest pods  $\text{plant}^{-1}$  was observed in control plots (Asl, 2017). Regarding beneficial microbes, BM application increased pods (241) as compared to no BM application (224). Application of beneficial microorganisms increased pods  $\text{plant}^{-1}$ , pods cluster $^{-1}$ , seeds pod $^{-1}$ , hundred seed weight (g), pod yield  $\text{plant}^{-1}$  and pod yield hectare $^{-1}$  in common bean (Ramana et al., 2011). Highest cabbage seed yields and yield attributes were recorded with the application of  $150 \text{ kg Phosphorous ha}^{-1}$  combined with BM (Olle and Williams, 2015). Beneficial microbes significantly increased all the seed yield and their attributes i.e., seed  $\text{plant}^{-1}$ , seed dry weigh  $\text{plant}^{-1}$  and seed pod $^{-1}$  (Iriti et al., 2019). Beneficial microbe's application increased the pod production in Chili (Khaing and Kyu, 2016). A much greater effect of BM on the yield of chilli and bell pepper (Kodippili and Nimalan, 2018). According to Moraditochae et al., (2011), nitrogen application could fulfill the requirement for N and increase number of fruits and total yield. Chickpea pods  $\text{plant}^{-1}$  were significantly increased with application

**Table 5.** Analysis of variance for thousand grain weight (g), biological yield ( $\text{kg ha}^{-1}$ ), seed yield ( $\text{kg ha}^{-1}$ ) and harvest index (%) of canola as affected by beneficial microbes with nitrogen and phosphorus levels.

SOV	Df	1000 grains weight	Biological yield	Seed yield	Harvest index
Replication	2	0.286	1266744	6647	6.094
Nitrogen (N)	2	1.260**	4515306**	52843**	5.293**
Phosphorus (P)	2	1.031**	2516587**	299834**	9.954**
Beneficial microbes (BM)	1	5.019**	8098366**	108631**	0.527Ns
Control vs Rest	1	1.460**	10929788**	542478**	10.770**
N x P	4	0.026 Ns	135796 Ns	2047 Ns	0.129 Ns
N x BM	2	0.832**	20146 Ns	36981**	3.183 Ns
P x BM	2	0.081 Ns	429324 Ns	975 Ns	1.211 Ns
N x P x S	4	0.015 Ns	382790 Ns	2631 Ns	1.026 Ns
Error	36	0.159	460463	3660	1.300
Total	56				
CV (%)		8.98	6.88	4.27	

SOV=Source of variation, DF=Degree of freedom, \*, \*\*, \*\*\*: significant at  $P \leq 0.05$ , 0.01, and 0.001, respectively.

of beneficial microbes as compared with control plots (Rabieyan et al., 2011). Seeds pod and pods plant increased significantly with the application of beneficial microbes with inorganic fertilizers (Singh Kothiyari et al., 2017).

The increase in seeds pod<sup>-1</sup> might be due to the fact that nitrogen enhanced the growth of the crop and produced more dry matter that resulted in more seeds pod<sup>-1</sup>. The plants took more nitrogen and enhanced the rate of photosynthesis which resulted in more vigorous growth (Tusar-Patra et al., 2006). Integrated application of beneficial microbes with chemical nitrogen fertilizer increased yield and yield components of canola compared to control (treated plants with beneficial microbes without using chemical nitrogen fertilizer (Keivanrad and Zandi, 2012).

Number of seeds pod<sup>-1</sup> was lowest (19.9) with addition of 70 kg P ha<sup>-1</sup>. Application of chemical and beneficial microbes increased physiological and metabolic activities in sesame accumulating more dried materials in plants and seeds pod<sup>-1</sup> (Yasari et al., 2008). Regarding beneficial microbes, BM application produced more number of seeds pod<sup>-1</sup> (23.3) as compared to no BM application (20.2). It was concluded that use of beneficial microbes significantly increased yield and yield attributes of canola (Jashankar and Wahab, 2005).

The increase in days to seed fill duration with application of nitrogen might be due to the fact that increasing nitrogen increased the vegetative growth of the plants therefore it delayed phenology of the crop (Banziger et al., 1999). Similar results were previously reported that increasing nitrogen application delayed the maturity of the crop (Kutcher et al., 2005). Delayed phenology is associated with the increase in leaf area duration, vegetative growth and light use efficiency with increased N levels (Rehman et al., 2010). Effective microbes had positive impact on growth and play a vital role in crop phenology (Vaid et al., 2017).

Lowest thousand grains weight was recorded with 60 kg N ha<sup>-1</sup> (4.2 g). Increasing nitrogen levels increased grain weight for which the probable reason is that higher availability of nitrogen increases the nutrients uptake and enhanced the dry matter accumulation in grains which resulted in heavier grain (Hamidi et al., 2007). The increase in yield is strongly correlated with increased in yield components such as siliques plant<sup>-1</sup>, grains silique<sup>-1</sup>, and grains weight (Malidarreh, 2010; Ogradowczyk and Wawrzyniak, 2004). Application of beneficial microbes in sesame significantly increased 1000 seed weight and seed yield (Yasari et al., 2008). These beneficial microbes produced some useful substances i.e. amino acids, polysaccharides, nucleic acids, bioactive substances, and sugars, all of which promote plant growth and development. The metabolites which are produced by these microbes are readily absorbed by the crops (Higa, 2000). The crops required high NPK during the initial growth, therefore the crops that gain high supply of nitrogen would increase the vegetative growth and yield (Rorie et al., 2011).

The increase in biological yield with increasing nitrogen levels might be due to vigorous plant growth with addition of nitrogen. Moreover, nitrogen is a key nutrient and is constituent of plant tissues and play vital role in photosynthesis, thus increasing crop growth and dry matter

production. It was observed that lower fertilizers rate had little effect on plant growth and biomass as compared to higher rates (Assainar et al., 2018). Similarly, application of nitrogen with beneficial microbes increased synthesis of assimilates and more dry matter were produced with higher level of nitrogen (Asl, 2017). Likewise, nitrogen is also known for increasing vegetative growth hence produced taller plants with greater canopy which leads to increased biological yield (Banziger et al., 1999). The increase in biological yield might be because of nitrogen enhanced the growth of the crop and produced more dry matter that resulted in increasing net biological yield. Increasing nitrogen levels increased biomass for which the probable reason is that higher availability of nitrogen increased the nutrients uptake and enhanced the dry matter accumulation in seeds (Hamidi et al., 2007). The increase in biological yield caused by the nitrogen levels might be due to increase in production of biomass and seed yield (Diepenbrock, 2000; Ahmadi and Bahrani, 2009). Application of phosphorous increased biological yield and dry matter production which might be due to its role in plant growth and developments. Phosphorous plays important role in early root development, which leads to more nutrient uptake and increased plant growth. These results are in agreement with the findings of several researchers who revealed that Phosphorous application increased the vegetative growth and biomass production effectively (Dinesh et al., 2010; Meena et al., 2018; Minorsky, 2008). Moreover, it was found that application of Phosphorous increased the growth, dry matter accumulation, yield and quality of plant (Mishra and Jain, 2013). Application of mineral Phosphorous with beneficial microbe significantly enhanced the availability of photo assimilates which in turn increased biological yield. Beneficial bacteria were used in combination they perform better in comparison to their individual use, and significantly increase the mean grain and straw yield by 34% and 52.4% over none inoculated control (Vaid et al., 2017).

The increase in biological yield with addition of BM might be due to the ability of BM which increased nutrients availability through mineralization which leads to increased crop growth. BM increased nutrients availability which increased plant growth and thus more dry matter was accumulated in plant's tissues. Beneficial microbes improved crop growth and has significant effect on crop yield (Ghetiya et al., 2018).

The probable reason for increasing seed yield with application of nitrogen might be that nitrogen play an important role in plant growth and development. It helps in increasing photosynthetic rate and in results more dry matter is produced. Moreover seed yield is associated with yield components such as pods plant<sup>-1</sup>, seeds pod<sup>-1</sup> and grain weight. Increasing nitrogen rate increased siliques plant<sup>-1</sup> therefore resulted in more siliques m<sup>-2</sup> (Ali et al., 2002). The increase in seeds pod<sup>-1</sup> might be due to the fact that nitrogen enhanced the growth of the crop and produced more dry matter that resulted in more seeds pod<sup>-1</sup>. Increasing nitrogen levels increased seeds weight for which the probable reason is that higher availability of nitrogen increases the nutrients uptake and enhanced the dry matter accumulation in grains which resulted in heavier

grain (Hamidi et al., 2007). The crop yield increased due to the strongly correlated with increased in yield components such as siliques plant<sup>-1</sup>, grains silique<sup>-1</sup>, and grains weight (Malidarreh, 2010; Ogrodowczyk; Wawrzyniak, 2004). The probable reason for increasing seed yield with application of phosphorus might be that phosphorous played important role in root development during early growth stages and provided better crop stand which lead to vigorous crop growth and yield. Similarly increase in seed yield and yield components with addition of phosphorous as compared to no phosphorous application (Meena et al., 2018; Minorsky, 2008). Moreover, application of phosphorous increased the growth, dry matter accumulation (Manhas et al., 2010), yield and quality of plant (Ramesh et al., 2011; Mishra and Jain, 2013). Regarding BM, addition of BM increased seed yield (1484 kg ha<sup>-1</sup>) as compared to without BM applied (1394 kg ha<sup>-1</sup>). The probable reason for increase in yield and yield components of canola with the addition of BM might be that BM improved soil fertility and increase nutrients availability which lead to higher nutrients uptake and increased crop production. Application of BM increased soil fertility and enhanced nutrient use efficiency which lead to higher crop yield (Javaid, 2006; Khaliq et al., 2006). Similarly beneficial microorganism increase crop yield by enhancing nutrient availability in the soil (Priyadi et al., 2005). BM improve crop health and yield by increasing photosynthesis, producing bioactive substances such as hormones and enzymes (Higa, 2000; Hussain et al., 2002). The application of BM increased yield and yield components of maize (Dehghani et al., 2013). This might be due to high photosynthetic activity with higher nitrogen availability and the plants consumed nitrogen efficiently due to its higher availability and produced more dry matter resulting in higher number of siliques plant<sup>-1</sup>. The nitrogen rate affected the crop physiology, in terms of a slight delay in the first day of flowering and time until maturity (Karamzadeh et al., 2010).

Harvest index was not affected by the microbial inoculants but was decreased with all fertilizer treatments as compared to control (Assainar et al., 2018). Grain yield and harvest index increased with beneficial microbes application and NPK fertilizers (Javaid and Bajwa, 2011). The beneficial microbes increased the grain yield and harvest index of the crop (Xu, 2001). The beneficial microbes application either in combination with organic matter or mineral NPK significantly enhanced cotton yield as compared to the treatments, where these soil amendments were used without BM application (Khaliq et al., 2006).

## 5. Conclusions

Nitrogen applied at the rate of 180 Kg ha<sup>-1</sup> increased pods plant<sup>-1</sup>, seeds pod<sup>-1</sup>, seed filling duration, seed weight, and biological yield. While phosphorous applied at the rate of 130 Kg ha<sup>-1</sup> increased pods plant<sup>-1</sup>, seeds pod<sup>-1</sup>, seed wt, biological yield, seed yield and harvest index. The crops treated with beneficial microbes had maximum pods plant<sup>-1</sup>, seeds pod<sup>-1</sup>, seed filling duration, seed weight, biological yield and seed yield.

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