Effects of (Azotobacter and Azospirillum) Inoculants and Chemical Fertilizers on Growth and Productivity of Canola (Brassica napus L.)

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Abstract: To investigate the effects of inoculants (biofertilizer) and chemical fertilizer on the yield, yield components and seed oil content of canola (Brassica napus L.), a split-plot experimental design with 20 treatments in 4 replications was carried out during 2004-2005 growing season at the Bagelara Agricultural Research Station in the Mazandaran province, Iran. Canola (cv. Hyola 401 hybrid), a high yielding early maturity variety, was grown in rotation after wheat. In the main plots, the biofertilizer treatments were at two different levels: 1) control (no seed inoculation) and 2) seed-inoculation with a combination of three different strains of bacteria Azotobacter chroococcum and Azospirillum brasilense and Azospirillum lipoferum. In the two sets of 10 sub-plots chemical fertilizers comprising N, P, K and their combinations, NPKS and NPK Zn were applied. The seed yield touched a high of 3741.5 kg ha⁻¹ at treatment T₁₆ (Bio + NPK Zn), that corresponded to 257.7 pods per plant and maximum CGR (18.3 g m⁻² day⁻¹). The highest weight of 1000 seeds (4.45 g) was obtained at treatment no. T₁₆ (Bio + NP S) which coincided with the maximum TDM (11.55 g⁻²) and maximum LAI (5.06). The maximum branching (4.43 branches per plant) was obtained at treatment T₁₆ (Bio + NPK Zn) showing a 46.2% increase over the control. The maximum oil content 47.73% was obtained at T₁₆ (Bio + NK). The application of inoculation with Azotobacter and Azospirillum helps to increase the yield by 21.17% over the control, raised the number of pods per plant (16.05%), number of branches (11.78%), weight of 1000 grain (2.92%) and the oil content of seeds (1.73%) but decreased (-0.24%) the number of seeds per pod.

Key words: Azospirillum, Azotobacter, biofertilizer, chemical fertilizers, canola

INTRODUCTION

Application of fertilizers has become a necessity in the crop production of oilseeds, especially canola, because of the ever increasing demand of the health conscious population of the world. In Iran also efforts are being made to increase the areas under cover for oil seed crops as much of the edible oil is imported. It is well known that N-P-K fertilizers help in the healthy growth of crops like canola, soybean and also in increasing the yield of food grains like wheat and rice. But recently there have been some reports that excessive and repeated use of chemical fertilizers may spoil the soil, ground water and pollute even the atmosphere (Kennedy and Teahan, 1992; Myton, 1993). These problems have renewed public interest in exploring alternative or supplementary non-polluting sources of N- fertilizers (Ladha et al., 1998), i.e., the biofertilizers. Besides the cost of importing chemical fertilizers is equally prohibitive and necessitates the finding of suitable alternative. Positive reports exist on the use of biofertilizers Azotobacter or Azospirillum for sorghum (Sorghum bicolor), (Singh et al., 2005), onion (Allium cepa), (Navala et al., 2004), wheat and mustard (Gupta and Gupta, 2006; Sharma et al., 1997). Rai and Caur (1988) reported that combined application of Azotobacter chroococcum and Azospirillum lipoferum resulted in higher increase in seed and stover yields of wheat compared to the application of each bacterium alone. Not much experimental work has been conducted on the use of such N₅ fixing bacteria on the growth and yield of canola. The only attempts made on canola refer to the application of inoculation with Penicillium bilai, Bacillus thuringiensis and phosphate solubilizing rhizobacteria for the P-uptake, vegetative growth and grain yield of canola (Gleddie et al., 1993, Fretas et al., 1997). Therefore the present study on effects of (Azotobacter and Azospirillum) inoculants and chemical fertilizers together on growth and productivity of canola (Brassica napus L.) was planned.

MATERIALS AND METHODS

A split-plot experimental design with 20 treatments and 4 replications was carried out in the period October-May, 2004-2005. The bacterial strains...
Azospirillum and Azotobacter inoculants were applied in the main plots and a combination of chemical fertilizers, both macro and micronutrients were applied in the sub plots. The experiment was carried out at the Baieola Agricultural Research Station in Mazandaran province (Iran).

The Azotobacter and Azospirillum strains were isolated from the different samples of the soils of local area. To facilitate the identification of Azotobacter sp., modified mannitol agar medium with 10 g of glucose, mannitol per liter as a carbon source (Thompson and Skerman, 1979) was used. For Azospirillum NFb a potato extract media (Baldani and Dobereiner, 1980) was used. The isolates were compared with the reference strains. Combined inoculants of Azotobacter chroococcum, Azospirillum lipovorum and Azospirillum brasilense strains were applied for the biofertilizer treatments.

The Canola (cv. Hyola 401 hybrid) crop, a high yielding early maturity variety, was taken as a second crop in rotation after wheat. It was grown under rain fed conditions. Soil samples were collected and analyzed to know the composition or the nutrients availability and the crop requirements for the nutrients. The chemical fertilizers were chosen and applied accordingly. The experimental soil was texturally silt-clay, with pH 7.6, 1.3% O.C, 180 ppm of available K, 7 ppm of available P, 18 ppm Mn, 10 ppm Fe, 1.1 ppm B and 0.96 ppm of Zn. The chemical fertilizers consisting N, P, K, S and Zn were applied prior to cultivation, except the nitrogen fertilizer which was applied in split stages, once basal and twice top-dressed.


Five plants were sampled randomly in each plot and averaged for recording the change in dry weight in shoots (above ground), at intervals of 34, 67, 82, 111, 133, 153 and 182 DAS (Days after sowing), relating to different stages of canola growth. The samples were first sun dried and thereafter in oven at 70°C till a constant weight was recorded. The leaf area (only one side) was determined by estimating the leaf weight and comparing it with the weight and surface of a standard paper. The dry matter accumulation rate per unit of land area (CGR), expressed as g m⁻² day⁻¹ was calculated using the formula \( CGR = \frac{W_f - W_i}{SA \times t} \), \( W_1 \) and \( W_2 \) are crop dry weights at the beginning and end of the interval \( t \), and \( SA \) is the soil area occupied by the plants at each sampling (Acuñaah, 2002; Gupta and Gupta, 2005). Data were analyzed following the analysis of variance technique (ANOVA) and then the mean differences were adjudged by Duncan’s multiple range tests (DMRT, Gomez and Gomez, 1984).

**RESULTS**

Dry-matter accumulation: The dry-matter accumulation was quite significantly improved by the treatments (Table 1). The treatments promoted a much healthier growth before the commencement of winter. A significantly higher TDM at rosette (68 to 82 DAS) was obtained at combined treatments of NPK, NPK S or NPK Zn along with biofertilizer (T13, T14 and T19) when compared to the corresponding non-biofertilizer treatments. A threshold value of about 100 g m⁻² was obtained in these treatments. This increase in TDM continued till (134-153 DAS), with a threshold value of 1080 g m⁻². The exuberant growth rate was observed during the 134-153 DAS, irrespective of the treatments. At maturity (154-182 DAS), the TDM gets lowered in all the treatments. Wysocki et al. (2005) have also reported such a decline in TDM after reaching a climax in full bloom.

Leaf Area Index (LAI): The rate of increase in the leaf area determines the photosynthetic capacity of plant. The application of different treatments did influence the LAI (Table 2) significantly. The canola plant growth is critical at the rosette stage and if it is able to produce enough leaves before rosette, the plant may spend a healthy winter. The LAI at rosette stage was higher when NP, NPK NPKS and NPK Zn with or without biofertilizer were applied, a threshold value of 3.4 observed in these treatments. The results showed 3.5% increase in LAI at rosette stage in the presence of biofertilizer. LAI increased further till 111 DAS then declines somewhat at the flowering stage. Correlating LAI with the yield, it observed that the canola yield suffered at treatments whenever the LAI is <4 at the flowering stage. Mendham et al. (1990) have also reported that a LAI <4 may results in decrease in the growth and yield of canola.

Crop Growth Rate (CGR): The different treatments resulted also in increasing the CGR (Table 3). During rosette the CGR was low at each treatment except in treatments with NPK, Bio + NPK, Bio + NPKS and Bio + NPKZn. In all the treatments the plants resumed growth rapidly after rosette. During flowering period (112 to 134 DAS) the CGR was the highest, especially in these treatments showing 10-12% increase over the non-biofertilizer treatments.
Unlike the TDM and LAI, the CGR was highest during the flowering period (112 to 134 DAS). It is more likely that the new and actively photosynthesizing tissues of pods might be responsible for the increase in CGR during this phase and the increase in dry weight during 135 to 153 DAS. Similar observation was made by Clarke and Simpson (1978). The CGR then declined eventually between the period 134-153 DAS and even to a negative value at final maturity (after 153 DAS). The steep decline in CGR in the last four weeks is on account of senescence of older leaves. Shukla et al. (2002) reported that using sulphur and zinc as supplementary nutrients resulted in 23 and 20.5% increase in CGR value of Indian mustard.

**Yield and Yield-components:** The yield, yield-components and the seed oil content of canola were all influenced significantly by the treatments (Table 4). The effect on yield was statistically significant at p<0.01, the best results again obtained at T<sub>21</sub> (Bio + NPK Zn), as the yield (374.5 kg h<sup>-1</sup>) i.e., five times increase over the control. The yield shot above 3280 kg h<sup>-1</sup> (almost two times more than average of canola yield in the country) by application of additional S and Zn in the presence of biofertilizer.

The effect on the number of pods per plant was also statistically significant p<0.01 (Table 4), showing a definite improvement. The best result was obtained at T<sub>21</sub> with 257.5 pods per plant, applying biofertilizer along with
NPK Zn, showing a 14.20% increase over the control. Results also showed that the Bio + NPK Zn (T _{30} ) treatment gave a 51% increase, compared to the non-biofertilizer treatment NPK Zn (T _{20} ).

The increase in the number of seeds per pod (of main stem) was also significant mainly at T _{10} , T _{15} and T _{20} . Otherwise did not change much from the control (LSD = 1.571). With NPK Zn the best result was obtained with 25.88 seeds per pod showing a distinct increase of 11.21% over the control. However seeds inoculated with the biofertilizer did result in further increasing the number of seeds/pod compared. The results indicated that the increase in the yield was consequent mostly to the proliferation of pods per plant. A similar observation was made by Hocking et al. (2003) in experiments using N-fertilizer in canola growing.

The number of branches appears to be statistically significant at p < 0.05 levels (Table 4), the best result was obtained at T _{20} = Bio + NPK Zn i.e., 46.20% increase over the control.

The effects on 1000 grain weight also was positive but did not vary with different treatments; the best result was at T _{10} = Bio + NPKS showed a 19.30% increase over the control. But the change was not statistically different from the other treatments. Despite the differences in the number of pods and the yield with every treatment,
the 1000 grain weight remained between 4 and 4.45 g. This appears to be the range or limit up to which only the improvement could be achieved using these treatments. The narrow range makes it obvious that the 1000 grain weight did not determine the improvement in the yield ($T_{19}$).

The effects on the seed oil content of seeds was statistically significant at p<0.01 level (Table 4), the maximum oil content (46.73%) obtained at $T_{19}$ (Bio + NK) showing a 11.68% increase over the control. The treatments $T_{19}$ = Bio + NPKS and $T_{20}$ = Bio + NPK Zn also resulted in improving the oil content to more than 46%.

**DISCUSSION**

In managing the crop production, it is important to understand how the yield and the yield components respond to the treatments of only chemical fertilizers and a combination of chemical and biofertilizer. Seed yield of canola was found to be related to certain plant characters which can be called as the yield-attributing characters. In the present experiment, it was found closely related to the total dry matter, LAI and CGR. The higher yield corresponded to an increased number of pods per plant and higher CGR and LAI. The additional application of strains of bacteria *Azo bacter chroococcum* and *Azospirillum brasilense* and *Azospirillum lipoferm* shot up the yield by 21.17% over the control (chemical fertilizers). This appeared mainly related to the proliferation of pod/plant (16.09%) though simultaneously the number of branches (11.78%) also increased. The weight of 1000 grain (2.92%) and the oil content of seeds (1.73%) also increased with this additional biofertilizer support. The present results are better than those obtained by Sharma et al. (1997) and Shukla et al. (2002) on Indian mustard, using only *Azotobacter* as biofertilizer. *Azotobacter* and *Azospirillum* are free living N$_2$ fixing bacteria which in the rhizospheric zone have the ability to synthesize and secrete some biologically active substances like B vitamins, nicotinic acid, pantothenic acid, biotin, heteroauxins, gibberellins etc. which enhances the root growth (Kader, 2002). The *Azotobacter* and *Azospirillum* association helps the crop improvement also by excretion of ammonia in the presence of root exudates that enhances and regulates the nutrient uptake by plants (Narula et al., 1993; Narula and Yadav, 1989). The higher dry-matter production by the inoculated plant might be because of the augmented uptake of nitrogen, phosphorous and potassium, which in turn was consequent to the root proliferation (Table 5).

**Table 5:** Gain of biofertilizers on yield, yield contributing characters and oil content of seeds comparing to control

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Inoculation (Biofertilizer)</th>
<th>Increase over the control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield (kg ha$^{-1}$)</td>
<td>2086.22</td>
<td>2577.99</td>
<td>21.17</td>
</tr>
<tr>
<td>Pods/plant</td>
<td>149.26</td>
<td>173.22</td>
<td>16.65</td>
</tr>
<tr>
<td>Seeds/pods (of main stem)</td>
<td>24.58</td>
<td>24.47</td>
<td>-0.24</td>
</tr>
<tr>
<td>Number of branches</td>
<td>3.48</td>
<td>3.89</td>
<td>11.18</td>
</tr>
<tr>
<td>1000 grain weight (g)</td>
<td>4.143</td>
<td>4.264</td>
<td>2.92</td>
</tr>
<tr>
<td>Seed oil content (%)</td>
<td>44.44</td>
<td>45.21</td>
<td>1.75</td>
</tr>
</tbody>
</table>

**REFERENCES**


