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Effect of *Azotobacter chroococcum* Application on Quantity and Quality Forage of Rapeseed Cultivars

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Abstract: The study was conducted to determine the effect of *Azotobacter chroococcum* (Azotobacter) application on quality and quantity forage of the rapeseed cultivars (RGS 003, Hyolla 401 and Hyolla 330) and the possibility of cultivation the rapeseed forage in summer. The experimental design was spilt plot laid out in randomized complete block with three replications. Different levels of Azotobacter (zero and one kg ha^{-1}) and cultivars were randomized to main plot and sub plot units, respectively. Results showed that Azotobacter significantly affected on all quantity traits except plant height. There were significant differences among cultivars for dry matter, biomass, sub branch number, plant height and forage glucosinolate concentration. Therefore Azotobacter can be considered as growth promoting for rapeseed in the future studies. Also, RGS003 cultivar was better than other cultivars when Azotobacter was applied.

Key words: Forage rape, Azotobacter, quality and quantity yield

INTRODUCTION

Forage Shortage in Iran has imposed the intense pressure on the pastures and followed by the lateral problems such as soil erosion. Increasing cultivation area is not also possible due to water and land limitation. At present the most ready lands are cultivated, so that increasing yield per area and advantages of the second cultivation that fill the time interval between the production of a winter crop (seed) and its further cultivation, is very important.

Most researchers about forage production such as Goihl and Mcelliney (1994), Neilsen *et al.* (2002) and Amin *et al.* (2002) have emphasized on compatibility of rapeseed for forage production. They explain the advantages of rapeseed forage production, when it is placed in the cropping pattern and crop rotation of the region.

Clay Pool *et al.* (1985) also found that 45 day old Holstein calves weight increased 900 kg daily, when they were feed by rapeseed meal during 7 weeks before weaning and 8 weeks after weaning.

Schwab *et al.* (2001) reported that damage of rodent on potato and carrot fields was reduced by mixed cropping with forage rape.

Application of rapeseed and barley together can increase digestion of forage in rumen of sheep (Jochmann *et al.*, 2002).

Result of research on field without weed showed that forage rape has the capacity of producing up to 12 ton ha^{-1} (Anonymous, 2004).

Banuelos *et al.* (2002) also showed that forage rape is appropriate replacement for other forage plants at dry regions.

Therefore, forage production comparison among different rapeseed cultivars as the second cultivation is very important, because the wasting usage of the chemical fertilizer especially potash, nitrogen and phosphorus fertilizers to increase yield in different crops has caused very problems from the economical and environmental perspectives. It seems that the best way is substitution of biological fertilizers instead of chemical fertilizers.

The biological fertilizers attribute not only to the materials from dung and plant remainders, but also including all products that are made from the activity of the microorganisms and its microorganisms. The soil bacteria such as Azotobacter are one of these microorganisms (Kennedy *et al.*, 2004). The different species of Azobacter can be categorized in the group of

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the plant growth promoting *rhizobacteria* (PGPR) that in the medium of the root (rhizosphere), has necessary potential to occupy the root system of the plants (Vessey, 2003).

At present, in some of the countries, this bacteria is used as biological fertilizer to produce very agricultural products including cereals, vegetables (Kadder *et al.* 2002). Ravikumar *et al.* (2004) studied the effect of Azotobacter on the plant species in the coastal regions and observed that the plant inoculation with Azotobacter increased plant growth and quantity of chlorophyll in the leaf.

Rodelas *et al.* (1999) reported that Azotobacter increased the yield of the products like sugar beet, carrot and cabbage as much as 10%. These bacteria also help to preserve the health of the plant by controlling the pathogenic agent indirectly as that growth improvement and the crop yield increment is the ultimate aim (Mrkovacki and Milic, 2001).

Kwon *et al.* (2003) have reported that rape is a useful forage fodder crop with high content of crude protein and low contents of Neutral Detergent Fibre (NDF).

Prasad and Prasad (2004) showed positive effect of azotobacter on the yield and height of Brassica plant. Gupta and Samnotra (2004) conducted several researches in Kashmir, India and concluded that the simultaneous application of azotobacter and manure had a significant effect on plant height and yield of *Brassica*.

In another study the effect of azotobacter and other biofertilizers were examined on the vegetative growth (shoot length, fresh and dry weights and number of leaves per plant) of mustard. Azotobacter, applied alone, resulted in the highest values for the parameters measured (Abdul *et al*., 2003).

With respect to above explanation, determining the effects of growth stimulating bacteria on the crop yield in the soils of every region are completely necessary for extension the usage of the biological fertilizers and sustainable agriculture. Therefore the purpose of the present study was to evaluate Azotobacter effects on growth, development and yield of tested varieties.

MATERIALS AND METHODS

This research was conducted in Mahdasht of Karaj at a field with 3000 m^2 area during growing season 2005. The soil texture was clay loam with pH 7.4. The experimental design was split plot laid out in randomized complete block with three replications. Two levels of the Azotobacter (supplied by Mehr Asia Biotechnology Company, Tehran, Iran, P.O. Box 15875-9498) (0 as control and 1 kg ha^{-1}) and three cultivars (Hyolla 401, RGS 003 and Hyolla 330) were randomized to the main and sub plot units respectively. In this experiment, every sub plot had 6 lines with 3 m long and the interval between the plots was 1 m. At the end of June, land preparation was done with disk to break the clods, leveling the land by leveler and creating the stream and the mound by furrower. Weed was controlled by the terflan herbicide (2-2.5 L ha^{-1}) before cultivation and it was blended with the soil.

Seeds were inoculated with Azotobacter in 2005/June/20. Then inoculation and control seeds were planted directly to soil. The first irrigation was done immediately after seed planting. All treatments were irrigated every 7 days once until harvesting time. The harvesting area was 2.4 m^2 per plot. When 25% of the plants had flower they were harvested.

Before final harvesting, 10 plants were selected randomly from each plot and the number of sub branch and plant height was measured. Forage dry and wet weights of all four rows per plot were determined after omitting the effect of the margin (half meter from the beginning and the end of each line).

The raw protein percent (with Kjeldahl method) ash percentage (by the method of burning in the electrical jug with the temperature 500°C for 5 h), organic material percent (the ash-the dry material = the organic material) were determined and glucosinolate concentration (quantity of glucose in the sample by spectrophotometer). The data were subjected to statistical analysis using SAS statistical software (SAS Institute Inc., 1997). When analysis of variance showed significant treatments effects, Duncan's multiple range test was used to analyze the mean at $p \leq 0.05$.

RESULTS AND DISCUSSION

Analysis of variance showed that the variety and Azotobacter had significant effects on the biomass, so that the inoculated plants with Azotobacter produced more wet forage (39.701 ton ha^{-1}) than control (Table 1). Kennedy (2004), Ravikumar *et al.* (2004) and Rodelas *et al.* (1999) reported that the yield in different crops could be increased when crops were inoculated with Azotobacter. Mean comparison among varieties showed that RGS 003 had higher forage (46.957 ton ha^{-1}) than other varieties. This can be attributed to the high genetic potential of RGS 003 cultivar. Some researchers such as McGregor (1987) and Morrison *et al.* (1997) also attributed difference between varieties for forage to the difference of their genotypes.

The effect of Azotobacter and cultivar on dry weight was significant, but their interaction effect was non

Table 1: Analysis of variance on traits of inoculated canola cultivars with Azobacter

SOV	df	Biomass	Forage yield	Plant height	Sub branch No.	Protein content	Organic matter	Ash	Glucosinolate
Replication	2	31421361.5ns	6159.5ns	22.70ns	1.00ns	1.12ns	3.00ns	0.1ns	0.03ns
Azotobacter (A)	1	420169445**	5896178**	266.80ns	2.12ns	6.00ns	8.60ns	5.0ns	0.06ns
E(a)	2	8633860	31986.5	44.60	0.12	5.60	2.10	1.1	0.37
Variety (V)	2	371071875**	2041884.5**	522.60**	12.70**	0.28ns	0.12ns	1.3ns	0.20*
A*V	2	2692966ns	21861.5ns	35.60ns	0.05ns	0.32ns	6.90ns	1.9ns	0.00ns
E(b)	8	1838840	28976.2	27.68	0.03	2.19	2.63	1.3	0.03
CV (%)		4.17	2.8	9.30	4.10	10.50	20.70	6.6	7.00

* and ** significant at 5% and 1% probability levels respectively; ns: Non-significant; V: Variety (V1 = Hyola 401, V2 = Hyola 330, V3 = RGS 003, A: Azotobacter (A1 non application, A2: application)

Table 2: Means comparison for yield and quality in, variety and use of Azotobacter

Treatments	Biomass (kg ha^{-1})	Forage yield (kg ha^{-1})	Protein content (%)	Organic matter (%)	Ash (%)	Plant height (cm)	Sub branch No.	Glucosinolate (μmol g^{-1})
Variety								
V1	36176b	6732b	15.6a	82.4a	17.6a	62.0a	4.3b	2.8a
V2	30141c	6336c	15.1a	83.0a	17.0a	60.0a	6.1a	2.5ab
V3	46957a	7645a	14.9a	82.5a	17.6a	54.0b	3.2c	2.3b
Azotobacter								
A1	35617b	6083b	14.9a	83.4a	16.6a	52.5a	4.3a	2.4a
A2	39707a	7365a	15.8a	82.4a	17.6a	60.5a	5.4a	2.9a

Mean fallowed by the same letter(s) in each column are not significantly different (Duncan multiple rang test 5%); V: Variety (V1 = Hyola 401, V2 = Hyola 330, V3 = RGS 003); A: Azotobacter; (A1: non application, A2: application)

significant for dry weight (Table 1). Inoculated plants with Azobacter had more dry weight (7.365 ton ha^{-1}) than control (Table 2). The researchers like Narula *et al.* (2000), Ahmad *et al.* (2005) and Ravikumar *et al.* (2004) reported that plant inoculation with Azotobacter had positive effect on the dry weight and it was attributed to Azotobacter that stimulated growth hormones production. Dry matter was difference among cultivars. RGS 003 cultivar produced more dry matter than other cultivars.

There were significant differences among cultivars for plant height. Azotobacter did not affect on this trait. The height difference of cultivars is related to their genetic background. Hyolla 401 cultivar was taller than others, but it was ranked with Hyolla 330 in a statistical group (Table 2).

Varieties had different sub branch number ($p \leq 0.01$). Inoculated plants with Azotobacter had more sub branch (5.4) number than control, but it was not statistical significant. Barea and Azcon (1975) also reported that sub branch number enhancement could be attributed to production of growth stimulation hormone by Azotobacter. Hyolla 330 cultivar had the highest sub branch number and it was ranked with Hyolla 401 cultivar in two separately statistical groups. RGS 003 had the lowest sub branch number. It was located at third rank. Probably, this difference has the genetic backgrounds (Table 2).

Protein percent was not different among cultivars. This trait was not also affected by Azotobacter (Table 1). Zamber *et al.* (1984) reported that plant inoculation with Azotobacter increased forage protein percent, but Zaied *et al.* (2003) observed that seed protein percent in inoculated wheat plants with Azotobacter did not increased.

Cultivars had different glucosinolate. This trait was the lowest in RGS003 cultivar (Table 2). Scientific reports show that herbaceous species of *Brassica* as naturally and originally have the poison composition that glucosinolate is one of them. The difference in the quantity of glucosinolate rises from genetic difference among the cultivars (Gustine and Jung, 1985). Brassica forage had the lowest glucosinolate concentration (Tookey *et al.* 1980). The researchers have proved that the most quantity of glucosinolyate was in the non-herbaceous species of Brassica, primary seeds and brassica meal (Vanetten and Daxenbichler, 1977).

In this experiment, the treatments of Azotobacter, cultivar and the interaction effect of the cultivar* Azotobacter did not influence on ash percent. In the standard table (NRC, 2001) researchers has reported that the quantity of ash in the forage of rapeseed is 11.4% of the dry matter, whereas our record showed that ash quantity in rapeseed forage was 17%. Plant population density, urea fertilizer application, type and quantity of the irrigation, sowing season and time of harvest influence effectively on the quality traits (Chao *et al.*, 1998). In the standard table, the harvesting time of product was in the beginning of vegetative growth period, but in this experiment forages were harvested in the beginning of flowering period that perhaps, this case has caused to increase the ash in the forages of varieties.

None of the used treatments did not significantly influence on the organic material percent (Table 1). In the standard table organic material and hay of the rapeseed forage (NRC, 2001; MAFF, 1990) have been reported 88.6 and 91.2, respectively. The little difference in the organic material of our rapeseeds with standard table is due to harvesting time: harvesting time of rapeseed plants in the standard table was in the beginning of the germination, whereas the harvesting time of our rapeseeds was at flowering.

CONCLUSIONS

Generally, plant growth promoting rhizobacteria including Azotobacter has advantages in compared to the chemical fertilizers that justify its usage. Environmental pollution reduction, less cost, simple availability, power of reproduction and propagation are the major important factors of growth stimulating bacteria and biological fertilizers in compared to chemical fertilizers.

RGS 003 cultivar had the highest forage yield and the best forage quality. Therefore mentioned cultivar can be used to cultivate in Summer.

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