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Research Article



Effect of inoculants & herbicide on nitrate reductase activity and nitrogen uptake of vegetative parts and yield of wheat crop

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Abstract

Modern agriculture faces challenges such as loss of soil fertility fluctuating climatic factors and an increase in pathogen and pest attacks. The sustainability and environmental safety in agriculture production needs eco-friendly approaches like the use of biofertilizers, biopesticide, green manuring etc. The multiple beneficial effects of microbial inoculants apart from nitrogen fixation need further research and their use in modern agriculture. The effect of inoculants and herbicide (Arelon) on nitrate reductase activity and nitrogen uptake of vegetative parts and yield of wheat crop clearly show that in presence of different inoculants, the NR activity was significantly more than control at all stages of growth.

Keywords: *Azospirillum*, *Azotobacter*, Nitrate reductase, Herbicide.

Introduction

In recent years attention has been focused on the fact that nitrogen is one of the most plant limiting nutrient in Indian soils. Our soils are poor in organic matter and nitrogen. If biological nitrogen fixation can replace the chemical nitrogen for crop productivity even in part, food production targets could be obtained without the application of high level of chemical fertilizer. Of the two groups of micro-organisms involved in the fixation of nitrogen the symbiotic nitrogen fixation in legume rhizobia is well documented.

However, the role of nonsymbiotic nitrogen fixing microorganisms in cereals remain to be established (Henis et al. 1980; Nausova et al., 2009). *Azospirillum* has gained importance recently as energy needed for its metabolism is much less as compared to other nonsymbiotic bacterial inoculants. The occurrence of *Azospirillum* in rhizosphere and its establishment in plant roots, tolerance of high temperature and high efficiency of nitrogen fixation make it ideally suited for cereal crops under the subtropical and tropical conditions. The insecticide/pesticide has come into extensive use in

agriculture and public health and the benefits are reflected in enhanced farm productivity and control of insect vector-borne disease. Nitrate reductase (NR) is the key enzyme of nitrate assimilation subjected to inhibition by excessive use of pesticides. nowadays pesticides are used commonly at a large scale in field. The effect of pesticides on nitrate reductase activity in *Trigonella foenum* i.e. Fenugreek was seen by Pankaj (2015). In the present study, the effect of pesticides on nitrate reductase activity has been studied on wheat crops and decrease in activity of nitrate reductase enzyme was found, there by affecting nitrogen metabolism.

Materials and methods

A pot culture experiment was conducted. In all, there were 24 treatments and each treatment was replicated six times. The three bacterial strains of *Azospirillum lipoferum*, *Azotobacter chroococcum*, *Bacillus polymyxa* were used as an inoculant with or without. Arelon at field rate and half of it.

The treatments are:

Control	(T1)
Azospirillum lipoferum A1.	(T2)
Azotobacter chroococcum A2	(T3)
Bacillus polymyxa. B	(T4)
A1+A2	(T5)
A1+B	(T6)
A2+B	(T7)
A1+A2+B	(T8)
1/2x Arelon (1/2 a)	(T9)
1/2 a +A1	(T10)
1/2 a + A2	(T11)
1/2 a +B	(T12)
1/2a +A1 +A2	(T13)
1/2a +A1 + B	(T14)
1/2 a +A2 + B	(T15)
1/2 a + A1 +A2 + B	(T16)
X Arelon (a)	(T17)
a+A1	(T18)
a + A2	(T19)
a+B	(T20)
a +A1 +A2	(T21)
a + A1 + B	(T22)
a +A2 +B	(T23)
a+A1+A2 +B	(T24)

The effect of inoculants and herbicide Arelon on Nitrate reductase activity was seen at all the stages of growth. Seeds were inoculated with different bacterial cultures as per treatment and dried in shade. Treated and untreated seeds as per schedule were sown. Arelon application at the rate of 1.0 kg /ha having 75% active ingredient as 0.66 ppm indicated as x and half of field rate of application (1/2x) was done after 35 days of sowing by spraying on soil. Samples were drawn at vegetative and flowering stages analysed for total nitrogen content by autoanalyzer. Samples were also drawn at three intervals for enumeration of Azotobacter in Jensen’s medium, Azospirillum in malate medium, and phosphorus solubilizers in pilkovskaya agar medium (Pikovskaya, 1948).

Estimation of nitrate reductase

The nitrate reductase activity was estimated at vegetative (45 days after sowing) flowering and just after anthesis during growth period.

The method followed for in vivo NR assay was essentially that of Hageman Huckerby (1971) as modified by Nair and Abrol (1973).

Extraction procedure

Leaf blades were taken and washed with tap water. These were kept in between moistened filter paper. Leaves were cut with fine pair of scissors into pieces of about 2-3 mm length. Cut leaf tissue (300 mg) was put in tubes containing 6 ml of infiltration medium (3 ml of 0.2 M KNO₃ and 3 ml of 0.1 M K₂(PO₄)₃(Ph7.5). These tubes were kept in cool ice. There

replicates were taken for each treatment, and one was blank. Air was evacuated from tubes with the help of vacuum pump for about 30 seconds and then vacuum was released. This process was repeated until leaf pieces were wetted and they sank below the surface of the medium. 0.5 ml of medium was pipetted out in tube which served as zero-time control. Rest of the tubes were wiped and incubated at 30°C in shaking water bath in dark. The tubes were removed after 1 hr and were put in hot boiling water for 5 minutes to stop the reaction and for effective extraction of nitrate accumulated in leaf tissues.

Measurement of nitrite

Nitrite was estimated by diazotization following the method of Snell and Snell (1949). 0.2 ml aliquots from test solutions were pipette out into test tubes and 1 ml of 1% sulphanilamide in 1 N-HCl was added and shaken thoroughly. To the colourless diazo-compounds o formed, was added 1ml of 0.01% N- (1-Naphthyl)- ethylene diammonium dichloride (GR) in water. The colour was allowed to develop for 25 minutes after which the volume was made up to 6 ml with distilled water. The absorbance of the pink-coloured solution was read at 540 nm using a Bausch and Lomb (Spectronic 20) colorimeter. The amount of nitrate was calculated with the help of a calibration curve prepared with a standard solution of known nitrite concentration.

Results and Discussion

Nitrate reductase activity

The effect of inoculants and herbicide (Arelon) on nitrate reductase (NR) activity presented in Table... show clearly that in presence of different inoculants the NR activity was significantly more than control at all the stages of growth. On 30th day, the maximum activity was found in A1 +A2 +B (63.41 n moles NO₃⁻) followed by A2 (61.30 n moles NO₃⁻) and A1 (58.88 n moles NO₃⁻).

When Arelon was applied at normal field rate and half of the field rate, these brought a drastic reduction in NR activity. At half field rate, in some treatments no NR activity was observed. All treatments had significantly low activity than control except in case of mixed inoculums (A1 +A2+ B).

On 60th day in comparison to 30th day, there was significantly increase in NR activity in all the cultural treatments. In herbicidal treatments also, there was significant increase compared to 30th day. At half field rate of Arelon the NR activity (n moles NO₃⁻) was significantly more in different treatments (soil alone 99.9, 1/2 a + A1 91.94, 1/2a + A2 88.78 and 1/2a +A1+A2+B 101.28) than control (86.55) while other treatments having 62.89 – 84.78 n moles NO₃⁻) and B (103.75 n moles NO₃⁻) all had significantly lower values than control.

On 75th day, stagewise there was reduction in activity in comparison to 60th day but increase compared to 30th day significantly. At half field rate, the activity was significantly

more in all treatments except Arelon alone (55.5 n moles NO₃⁻) and A₁+A₂ (49.1 n moles NO₃⁻).

At normal field rate, the activity was more in soil alone, B and A₁+A₂ while in others it was significantly less.

Table 1. Effect of inoculants and herbicide on nitrate reductase activity and nitrogen uptake of vegetative parts and yield of wheat crop

Treatments	n Moles NO ₃	Reduced/hr/	g dry wt.
	Stages	Of	Growth(days)
	30	60	75
Control	41.83 (1749.74)	86.55 (7490.9)	57.50 (3306.25)
Azospirillum lipoferum (A ₁)	58.88 (3466.85)	97.22 (9549.19)	64.61 (4174 0.45)
Azotobacter chroococum (A ₂)	61.30 (3757.69)	140.90 (19852.81)	63.51 (4033.52)
Bacillus polymyxa (B)	51.79 (2682.20)	105.41 (11111.26)	59.42 (3530.73)
A ₁ +A ₂	58.50 (3422.25)	87.15 (7595.12)	73.10 (5343.61)
A ₁ +B	52.95 (2803.70)	85.34 (7282.91)	58.50 (3422.25)
A ₂ +B	54.79 (3001.94)	94.34 (8900.03)	60.45 (3654.20)
A ₁ +A ₂ +B	63.41 (4020.82)	91.53 (8377.74)	73.13 (5347.99)
½ x Arelon (1/2a)	7.94 (63.04)	99.92 (9984.00)	55.50 (3080.25)
1/2a+A ₁	14.68 (215.50)	91.94 (8452.96)	61.71 (3802.12)
1/2a+A ₂	0.71 (0.50)	88.78 (7881. 88)	59.85 (3534.30)
1/2a+B	0.71 (0.50)	62.89 (3955.15)	82.66 (6832.67)
1/2a+A ₁ +A ₂	0.71 0.50)	84.50 (7140.25)	49.10 (2410.81)
1/2a+A ₁ +B	11.06 (122.32)	73.88 (5384.62)	57.70 (3329.29)
1/2a+A ₂ +B	0.71 (0.50)	84.77 (7185.95)	59.41 (3529.54)
1/2a+A ₁ +A ₂ +B	53.00 (2809.00)	101.28 (10257.63)	89.33 (7979.84)
x Arelon (a)	41.32 (1707.34)	83.97 (7050.96)	85.25 (7267.56)
a+A ₁	37.73 (1423.55)	79.41 (6305.94)	55.82 (3115.87)
a+ A ₂	33.05 (1092.30)	87.81 (7710.59)	44.54 (1983.81)
a +B	38.64 (1493.04)	103.75 (10764.06)	70.23 (4932.25)
a+A ₁ +A ₂	30.70 (942.49)	83.40 (6955.56)	81.29 (6608.06)
a+A ₁ +B	31.09 (966.08)	76.78 (5895.16)	43.54 (1895.73)
a+A ₂ +B	29.28 (857.31)	58.40 (3410.56)	42.92 (1842.12)
a +A ₁ +A ₂ +B	23.05 (531.30)	80.82 (6531.87)	33.69 (1135.01)

Stages bye(S) **treatment(T)** **SXT**
 .0245 0.0707 0.122
 .0685 0.1975 .3423
 C.D. 5%

Actual values are inside the parenthesis. values obtained after transformation are outside the parenthesis.

Nitrogen uptake by vegetative parts

The data on the effect of inoculants and herbicides on nitrogen uptake by vegetative parts (Table 1) reveals that on 30th day, treatments A₁+A₂+B (36.75mg) and A₁+A₂ (31.27

mg) had maximum nitrogen uptake which was significantly more than the control (28.12 mg) while others were at par.

When Arelon was applied at a normal field rate and half of it, there was a reduction in all the treatments. At half of the field rate, the maximum activity was found in A₁ (20.67 mg) followed by B (20.25 mg) and soil alone (18.97 mg) and all were significantly lower than the control (28.12 mg). The nitrogen uptake varied from 20.25- 16.00 mg in treatments at normal field rate and was significantly less compared to the control (28.12 mg).

On 60th day the activity increased significantly compared to values of 30th day in all cultural treatments and it was also more over control except x+A₁+A₂ (22.30mg)

Table 2. Effect of inoculants and herbicide (Arelon) on nitrogen uptake(mg/plant) (by Vegetative parts) at different stages of growth

Treatments	Stages of 30 days	Growth 60 days
Control	28.12	29.95
<i>Azospirillum lipoferum</i> (A1)	28.63	41.25
<i>Azotobacter chroococcum</i> (A2)	28.12	39.88
<i>Bacillus polymyxa.</i> (B)	28.70	56.22
A1+A2	31.27	53.68
A1+B	29.17	31.71
A2+B	27.50	33.07
A1+A2+B	36.75	45.13
½ x Arelon (1/2a)	18.97	35.68
1/2a+A1	20.67	34.93
1/2a+A2	15.53	37.29
1/2a+B	20.25	40.11
1/2a+A1+A2	15.33	43.89
1/2a+A1+B	17.48	44.41
1/2a+A2+B	16.93	40.95
1/2a+A1+A2	18.00	56.23
x Arelon (a)	17.25	35.88
a+A1	17.25	23.85
a+ A2	20.25	36.73
a +B	16.00	35.64
a+A1+A2	18.00	22.30
a+A1 +B	20.25	34.08
a+A2 +B	16.42	30.11
a +A1 +A2 +B	19.12	30.17

Treatments (T) Stages (S) (TXS)

S.em + 0.94 0.26 1.34
 C.D. 5% 2.66 0.75 3.76

In absence of herbicide, maximum activity was found in B (56.22 mg) followed by A₁+A₂ (53.68 mg) and A₁+A₂+ B (45.13g). At half of the normal field rate. The maximum activity was found in A₁+ A₂ + B (56.23) followed by A₁+B (44.4mg) and A₁+A₂ (43.89 mg). At normal field rate, the activity was significantly more over control except A₁+A₂ which was less (22.30 mg) than control (29.95 mg).

The results on nitrogen uptake by ears (Table 2) reveal that without herbicide level the inoculation with different bacteria increased the nitrogen uptake in ears. The maximum nitrogen uptake was found in A₁+A₂+B (48.48 mg) followed by A₁ (38.27mg), A₁+A₂ (34.28mg) and A₂ (33.92mg) which were significantly more than the control (29.20mg). At half of the normal field rate, there was no adverse effect of herbicide on

nitrogen uptake in ears. The nitrogen uptake in ears by A₁(49.25 mg) was significantly more than the control (33.28 mg) while others were at par with the control. At normal field rate, all treatments were having more values (39.27, 39.60, 43.7, 39.4, 42.3 mg) than control (29.92 mg) except A₂

Table 3. Effect of inoculants and herbicide (Arelon) on nitrogen uptake by emerged ear at flowering stage (mg/ear)

Treatments	Doses of herbicide		
	0x	½ x	X
Control	29.20	33.28	29.92
<i>Azospirillum lipoferum</i> (A ₁)	38.27	49.25	39.27
<i>Azotobacter chroococcum</i> (A ₂)	33.92	34.63	25.80
<i>Bacillus polymyxa</i> (B)	33.25	44.65	39.60
A ₁ + A ₂	34.28	34.12	43.70
A ₁ + B	24.27	34.00	24.47
A ₂ + B	28.33	34.00	39.45
A ₁ + A ₂ + B	48.48	30.03	42.30

S.em ± (Treatment) 1.46
 C.D. 5% 4.15

(25.80 mg) and A₁+B (24.4 mg) which were lower than control.

An adverse effect of herbicide (Arelon) was seen on the wheat crop when it was applied alone or in presence of

different bacteria. The inhibitory effect on the nitrate reductase activity affects the ability of the plant to utilize the nitrate. Similar studies on insecticides such as kelthane caused inhibition of nitrate reductase in fungi from root nodules and fabacean (vici a faba). The nitrate reductase activity was lowered when Arelon was applied at half the field rate and field rate. After the application, there was an immediate adverse effect on NR activity and in some cases completely inhibited NR activity. But after some time, it started recovering and there was good NR activity on 60th day and 75th days. The application of different bacteria alone or in combination produced more nitrate/g dry leaves compared to the uninoculated control. The herbicide also adversely affected the uptake of nitrogen in vegetative parts. The adverse effect was more on 30th day but after 60th day it became less, and activity started recovering. It was also around 60th day of growth that was not affected adversely except in one or two cases. Evaluation of oxidative stress on soya bean and wheat crops in response to chlorpyrifos was seen by (Singh and Katiyar, 2017). Chlorpyrifos is a broad-spectrum organophosphate insecticide being used for more than a decade to control foliar insects that affect agricultural crops to reduce pod damage (Khan et. Al., 2009; Kumar et. al., 2010). It also produces hazardous effects on the environment when it is applied directly to plants or mixed with soil (Howard, 1991). Several researchers proved that pesticide use in different plants leads to the inhibition of nitrate reductase. In the present investigation, the pesticide showed an inhibitory effect on plants and compelled us to use nitrate reductase-friendly methodology in place of the above herbicide.

Conclusion

The effect of inoculation of different bacteria with or without herbicide proved beneficial. The herbicide effect was also found to be less in inoculated samples.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

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