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Effect of *Azotobacter* cultures in relation to nitrogen application on growth, yield and alkaloidal composition of opium poppy *(Papaver somniferum)*

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ABSTRACT

A study conducted during 1987–91 to study the effect of *Azotobacter* cultures 'M 4', 'W 5' on 'Jawahar Aphim 16' and 'Trishna' ('IC 42') varieties of opium poppy (*Papaver somniferum* L.) grown at Delhi. It increased the yield parameters and these were at par with 40 kg N/ha. The *Azotobacter* cultures were compatible to use in combination with N fertilizer. Of the 2 *Azotobacter* strains W 5 was superior and its use through dripping gave better response than seed treatments. Further the use of bio-fertilizer saved expenditure on 40 kg N/ha in opium poppy.

In India, opium poppy (*Papaver somniferum* L.) is grown as a cold-weather, irrigated annual crop on fertile sandy-loam to medium heavy clay soils in 12 districts of Madhya Pradesh, Uttar Pradesh and Rajasthan. It demands a high level of nitrogen fertilization, applied through farmyard manure (10 tonnes/ha) and inorganic fertilizer (90 kg N/ha) in split doses. Nitrogen deficiency at early stage adversely affects growth, plant height and branching, whereas at later stage it reduces capsule size, volume of latex exudate and seed yield. This also decreases major opium alkaloids in the latex so collected (Jain, 1990, Jain *et al.*, 1990; Gaur and Rathore, 1991). In recent years, the price of inorganic fertilizers has gone up considerably which in turn has increased the cost of opium production. Use of bio-fertilizers (*Azotobacter* spp.) to supplement the N requirement in opium poppy gave encouraging results (Pareek *et al.*, 1992). besides fixing N, *Azotobacter* sp. provide growth-promoting substances and antifungal antibiotics which in turn facilitate a uniform germination, improve seedling vigour, resulting in maintaining a healthy plant stand leading to higher yields (Shende *et al.*, 1977; Chakrabarti and Yadav, 1991; Pareek *et al.*, 1992). In this context, an elaborate study was carried out on the use of M4 and W5 *Azotobacter* cultures applied both as seed treatment and through dripping on 2 popular high-yielding varieties of opium poppy along with the graded levels of N.

MATERIALS AND METHODS

Two separate trials were laid out at the National Bureau of Plant Genetic Resources Experimental Farm, Issapur, New Delhi, from 1987–88 to 1990–91 crop seasons. The soil of the experimental field was alluvial sandy loam in texture, having pH 7.8 and electrical conductivity 0.3 mmhos/cm

with 0.26% organic carbon. The soil was medium fertile and possessed 78, 25 and 350 kg/ha of total N, available P and total P respectively.

The first trial included 18 treatment combinations comprising 2 varieties ('Trishna' and 'Jawahar Aphim 16'), 3 levels of N (0, 40 and 80 kg/ha) and 3 levels of seed inoculations with *Azotobacter* cultures (no seed inoculation, seed inoculation each separately with M4 and W5 *Azotobacter* strains). These treatments were tested in randomized block design with 3 replications. In second trial (1989–91) treatments were slightly modified but retaining both the cultures and levels of nitrogen given in the first trial and thus making a total of 13 treatments combinations including an additional absolute control. The modification incorporated was in the methods of application of cultures, viz. seed treatment and dripping which mean a measured quantity of *Azotobacter* culture suspension in water was placed in soil near root-zone at 65 days age, followed by irrigation. These 13 treatments were tested in randomized block design with 4 replications.

As per practice seeds were directly sown in field during first week of November each year at a row spacing of 30 cm. The crop stand, after germination was maintained at 10 cm within rows through thinning at 45 days age. The gross plot size was 12.0 m^2 and net pot of 9 m^2 was harvested for each treatment. The crop was raised by following the standard cultural practices (Gupta, 1984). The physiologically mature capsules were lanced at 20 days after fall of petals. In all, 4 lancings were made on each capsule on alternate days to collect the latex. Data on growth, yield-contributing characters and yield were recorded. Major opium alkaloids were estimated in the crude latex by the method developed earlier at the Bureau by Srivastava and Maheshwari (1985).

RESULTS AND DISCUSSION

Effect of Azotobacter *cultures*

Analysis of data revealed that use of W5 *Azotobacter* gave 402.1, 331.75 and 102.69% higher yield of husk, seed and latex respectively over the absolute control (Tables 1, 2, 3). The corresponding increase owing to M4 culture was 378.4, 288.7 and 90.1%. The seed innoculation with *Azotobacter* improved the morphine (%) in latex and thus increased total morphine yield. The mean increase in morphine yield owing to M4 and W5 cultures was 8.93 and 12.27 kg/ha respectively over the absolute control. Both these treatments with cultures were at par in latex yield with 40 kg N/ha applied through inogranic fertilizer (Tables 1, 2, 3). The bacterial fertilization provided a favourable environment for growth and development of plant by fixing atmospheric N and releasing growth substances. Thus the treated plants showed better vigour and growth. This was reflected in values recorded for growth parameters like plant height, leaf area, number and size of capsule which ultimately contributed to higher yield.

Amongst the 2 methods of bio-fertilizer application, the dripping method significantly increased the seed yield and morphine content, whereas it has shown an edge in all other yield attributes (Tables 1, 2, 3); for other characters both the methods of culture application were at par. By and large, these results showed that bio-fertilizer was at par with 40 kg N/ha and thus saves expenditure on use inorganic fertilizer, costing Rs 300/ha at the present price level.

Effect of varieties

'Trishna' and 'JA 16' ('Jawahar Aphim 16') were compared for their growth and

Table 1. Effect of *Azotobacter* strains and nitrogen on yield attributes of opium poppy

Treatment	Days to flower	Plant height (cm)	Capsules/ plant	Capsule diameter (cm)	Capsule wall thickness (mm)	Leaves/ plant	6th leaf	
							Length (cm)	Width (cm)
N (kg/ha)								
0	104	109.2	3.07	4.09	1.66	20.1	20.4	13.3
40	106	113.7	3.67	4.26	1.61	21.7	21.9	16.1
80	110	116.2	4.15	4.78	1.65	24.8	22.8	17.6
CD (P = 0.05)	1.8	2.8	0.44	0.21	NS	1.9	2.3	2.1
Azotobacter *strain*								
M 4	108	109.5	3.53	4.05	1.60	22.1	21.5	14.5
W 5	110	115.3	3.70	4.74	1.61	21.9	22.1	16.8
CD (P = 0.05)	0.8	1.9	0.17	0.11	NS	NS	NS	NS
Inoculation methods								
Seed innoculation	108	110.8	3.72	4.05	1.59	21.4	20.7	15.7
Dripping	111	114.0	3.60	4.75	1.65	22.6	22.9	15.7
CD (P = 0.05)	0.8	1.9	NS	0.11	NS	NS	NS	NS
Control vs rest								
Absolute control	102	68.9	2.20	1.95	1.27	10.0	14.6	10.7
Rest	108	112.7	3.66	4.21	1.65	22.6	22.8	15.8
CD (P = 0.05)	3.6	5.6	0.82	0.42	0.28	3.8	5.0	3.8

yield in relation to *Azotobacter* and inorganic N fertilization. 'Trishna' gave 9.8, 43.8 and 40.5% higher seed, latex and morphine yield than 'Jawahar Aphim 16' (Tables 1, 2, 3). It has maintained its superiority in high morphine content vis-a-vis 'JA 16' under *Azotobacter* treatments also.

Effect of N

Application of N in general delayed the flowering, increased the plant height, capsules/plant, size of capsule and leaf area (Tables 1, 2, 3). This has in turn contributed towards higher latex and seed yields. In particular, 80 kg N/ha gave 37.5, 28.3, and 57.3% higher mean husk, seed and latex yields respectively over no N. It also showed marked effect in morphine yield; the mean values of morphine content and morphine yield were raised by 80 kg N/ha to 28.1 and 87% respectively over no N. It indicates that *Azotobacter* use partly meets the high N demand of this crop and its use is compatible with inorganic fertilizers.

The effect of N doses and *Azotobacter* strains were studied at Udaipur, Mandsaur and Faizabad also, which confirm the results obtained at Delhi.

Dry-matter production

The dry-matter production during growth period of opium poppy was studied to assess the individual treatments effect. The rate of dry-matter production was very low up to first 45 days and it increased gradually up to 75 days age. However, sharp increase in dry matter was noted during 105–155 days. The maximum dry-matter production was noted at 155 days age. The 80 kg N/ha gave the maximum dry matter, followed by 40 kg N/

Table 2. Effect of nirogen and *Azotobacter* strain on opium poppy

Treatment	Husk yield (kg/ha)		Seed yield (kg/ha)		Latex at 70°C (kg/ha)		Morphine (%)		Morphine yield (kg/ha)	
	1987–88	1988–89	1987–88	1988–89	1987–88	1988–89	1987–88	1988–89	1987–88	1988–89
Variety										
'Trishna'	1,523	1,307	1,033	1,046	57.49	46.76	10.64	16.11	6.12	7.53
'Jawahar Aphim 16'	1,473	946	924	969	34.91	37.59	9.29	12.98	3.24	4.87
CD (P = 0.05)	67	35	27	26	11.05	3.44	0.80	2.80	1.72	1.30
N (kg/ha)										
Control (without N)	1,257	840	865	930	32.02	29.91	8.97	12.50	2.87	4.00
40	1,503	1,087	977	1,031	45.64	44.62	10.21	15.65	4.65	6.98
80	1,683	1,247	1,067	1,162	60.47	52.00	10.81	16.33	6.56	8.49
CD (P = 0.05)	84	44	89	32	13.62	4.50	1.10	1.10	2.22	1.62
Azotobacter *strain*										
Control (without strain)	1,397	1,034	918	956	37.72	35.46	9.99	13.55	3.77	4.80
Strain M 4	1,403	1,079	949	1,018	45.36	44.68	9.30	14.91	4.22	6.67
Strain W 5	1,643	1,166	1,048	1,049	55.59	46.39	10.21	16.02	5.67	7.43
CD (P = 0.05)	84	44	89	32	13.62	4.50	NS	NS	2.20	1.62

Table 3. Response of opium poppy to *Azotobacter* strains and their methods of application in relation to nitrogen doses

Treatment	Husk yield (kg/ha)		Seed yield (kg/ha)		Latex at 70°C (kg/ha)		Morphine (%)		Morphine yield (kg/ha)	
	1989–90	1990–91	1989–90	1990–91	1989–90	1990–91	1989–90	1990–91	1989–90	1990–91
N (kg/ha)										
Control (without N)	1,008	971	707	736	43.75	44.73	13.87	12.64	6.08	6.91
40	1,194	1,090	872	803	50.53	48.62	15.77	15.90	7.97	7.34
80	1,430	1,249	983	929	56.65	60.32	16.45	17.95	9.32	10.82
CD (P = 0.05)	111	65	100	69	3.32	0.97	0.84	0.76	1.70	2.30
Azotobacter strain										
'M 4'	1,109	1,068	792	763	49.28	50.72	14.31	14.26	7.05	7.23
'W 5'	1,179	1,106	905	822	51.34	55.05	16.41	16.73	8.42	9.20
CD (P = 0.05)	NS	43	67	46	NS	0.64	0.56	0.53	NS	1.53
Inoculation methods										
Seed inoculation	1,069	1,046	807	803	50.79	51.68	15.30	14.96	7.77	7.73
Dripping	1,115	1,060	990	865	50.62	54.09	15.40	16.03	7.79	8.67
CD (P = 0.05)	74	NS	67	46	NS	0.64	0.56	0.53	NS	1.53
Control vs rest										
Absolute control	215	240	196	204	24.50	28.09	9.89	10.42	2.42	2.93
Rest	1,184	1,140	848	793	50.7	52.68	15.35	15.49	7.78	8.91
CD (P = 0.05)	100	102	122	138	1.74	1.93	2.62	2.02	3.40	4.60

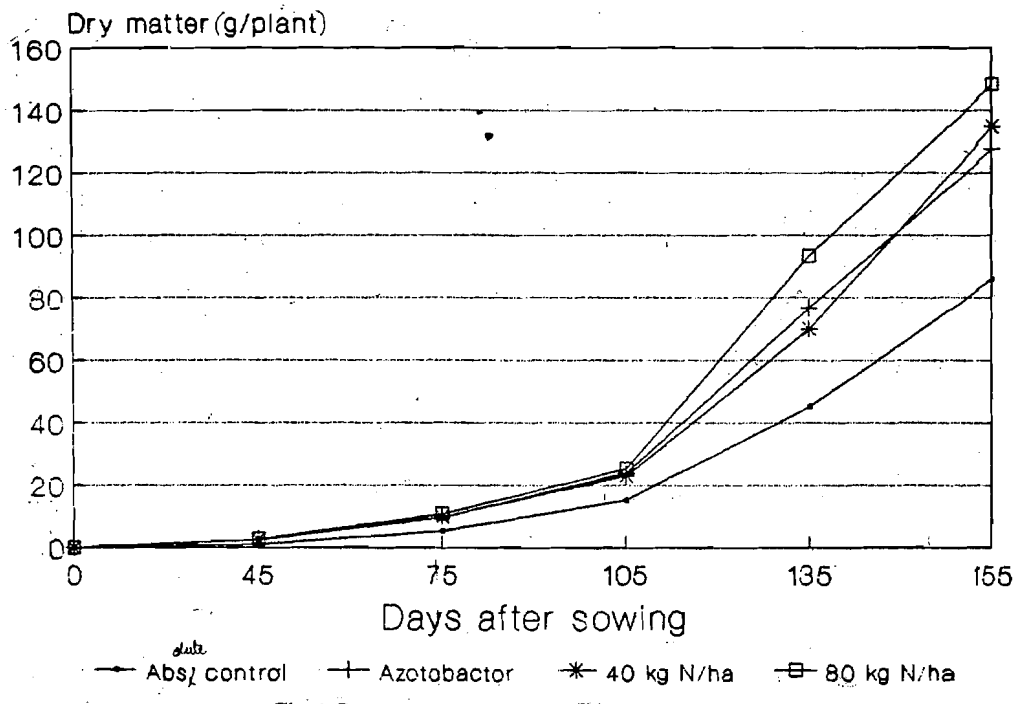

Fig. 1. Dry matter production in opium poppy

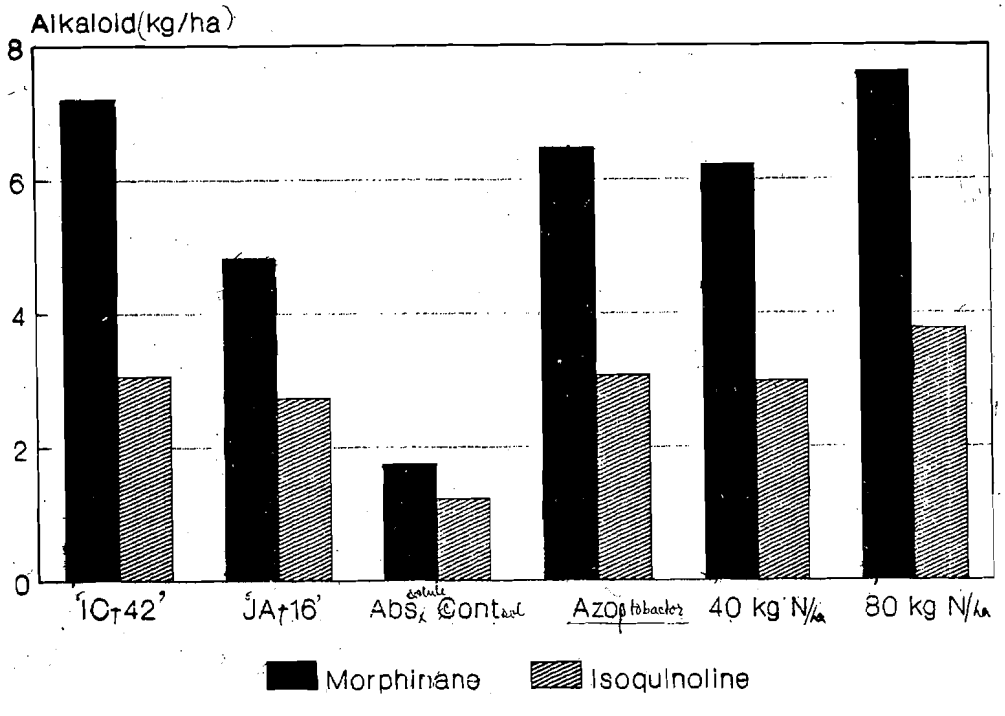

Fig. 2. Alkaloidal production in opium poppy

Table 4. Effect of nitrogen and *Azotobacter* on latex and seed yield (kg/ha) and morphine content under multilocation (mean data of 1989–90 and 1990–91)

Treatment	Latex yield at 70°C (kg/ha)				Seed yield (kg/ha)				Morphine (%)			
	Delhi	Udaipur	Mandsaur	Faizabad	Delhi	Udaipur	Mandsaur	Faizabad	Delhi	Udaipur	Mandsaur	Faizabad
Absolute control	26.29	20.98	13.47	17.00	200	239	291	278	10.15	10.40	11.40	11.40
40 kg N/ha	49.57	26.34	27.26	28.78	837	501	596	582	15.83	10.44	12.33	14.46
80 kg N/ha	58.48	29.42	26.72	34.00	956	610	592	654	17.20	10.59	11.63	14.17
M 4	50.00	26.44	25.72	28.69	778	524	564	638	14.28	10.47	11.48	13.51
W 5	53.19	26.18	26.86	29.58	863	533	566	662	16.57	10.53	12.43	13.68
CD (P = 0.05)	0.79	1.21	1.37	1.36	54	67	74	39	0.89	NS	NS	0.97

ha which compared well with W 5 *Azotobacter* culture treatment (Fig. 1).

Alkaloidal composition

The opium poppy latex has 5 major alkaloids, viz. morphine, codeine thebaine grouped as **morphinane** alkaloids whereas narcotine and **papaverine** are termed as isoquinoline **alkaloids**. The morphinane alkaloids are **more important**. 'Trishna' possessed higher **morphinane** alkaloids and maintained its original superiority in higher morphine content over 'JA 16' under treatment of *Azotobacter* cultures (Fig.2). Among the 2 *Azotobacter* cultures, W 5 gave maximum value of both the alkaloids. However, 80 kg N/ha proved the best treatment for obtaining the highest morphinane yield.

Thus N requirement of opium poppy crop can be reduced by 40 kg/ha through use of bacterial fertilization. This improved growth and yield parameters and were at par with 40 kg N/ha applied through chemical fertilizers. The use of these cultures makes no differences on quality of opium latex.

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