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Growth Stimulatory Effects of *Azospirillum* Strains on *Triticum aestivum* and *Vigna radiata*

Amina Munir, Iram Munir, Shazia Afrasyab and Shahida Hasnain
Botany Department, Punjab University, Quiad e Azam Campus, Lahore 54590, Pakistan

Abstract: Five bacterial strains of *Azospirillum* (As-2, As-4, As-5, As-6 and As-8) were used both as monocultures and mixed cultures (twenty-six combinations for each plant) for the inoculation of ***Triticum aestivum*** (non leguminous) and ***Vigna radiata*** (leguminous) seeds. Inoculated as well as non inoculated seeds were germinated and grown under controlled temperature and light conditions for 10 days. Growth parameters revealed that in both plants, mono culture inoculations manifested the better growth as compared to non inoculated treatments. Mixed cultures inoculation 3j (three strain combination) and 4a (four strain combination) in ***T. aestivum*** and 4a (four strain combination) and 3i (three strain combination) in ***V. radiata*** exhibited synergistic responses as compared to control treatment. Stimulatory effects were more pronounced in non leguminous seedlings relative to leguminous one.

Key words: *Azospirillum*, *Triticum aestivum*, *Vigna radiata*, growth stimulatory effects, plant microbe interaction

Introduction

Plant microbe interaction has become a major area of research as far as improved crop quality is concerned. Nitrates availability, better root development, ensuring enhanced uptake of nutrients and improvement in physiological processes of plant, are some beneficial aspects attributed to the plant by microbes. Bacteria are major inhabitants of rhizosphere, rhizoplane and histoplane and play a vital role in pronounced plant growth (Riggs ***et al.***, 2001). Bacteria can increase the availability of nutrients to the plants (Alami ***et al.***, 2000), colonize the rhizosphere (Lubeck ***et al.***, 2000) and regenerate the quality of soil (Alami ***et al.***, 2000). Regarding these qualities of bacteria, significant work has been done on plant growth promoting bacteria (Bashan, 1998; Pan ***et al.***, 2000; Bashan and de-Bashan, 2002; Bashan ***et al.***, 2002). ***Azospirillum*** spp., are plant associated diazotrophs of alpha class of proteobacteria (Martin-Didonet ***et al.***, 2000). They are capable of performing the nitrogen fixation endophytically (Kennedy ***et al.***, 1997; Swensen and Mullin, 1997). The ability to successfully manipulate endophytic bacteria in agricultural production system will depend upon ability to select, incorporate and maintain beneficial microbial population in the field (Sturz and Nowak, 2000). Feasibility of use of mixture of bacterial strains has been reported by Bashan ***et al.*** (2000). Hence the present work deals with inoculation studies of mono and mixed cultures of ***Azospirillum*** strains on the early growth of ***Triticum aestivum*** (non leguminous) and ***Vigna radiata*** (leguminous).

Materials and Methods

Five bacterial strains of ***Azospirillum*** i.e., As-2, As-4, As-5, As-6 and As-8 were used for the present work. Bacterial isolates As-2, As-4, As-5, As-6 and As-8 were obtained from roots of different weeds. Mono and mixed cultures (all possible combinations; Table 1) of these 5 strains were used for inoculating ***T. aestivum*** and ***V. radiata*** which were subsequently used for germination experiments. Five mono and 26 mixed cultures were used for each plant.

Seeds were sterilized and soaked in bacterial suspension (mono and mixed bacterial suspension were prepared by following the method of Afrasayab and Hasnain, 2000) for 15-20 min while for control treatment seeds were soaked in sterilized distilled water for same duration. Experimental layout was as described previously (Afrasyab and Hasnain, 2000). The growth parameters of 10 days old seedlings were taken. In addition to growth measurements protein contents (Lowery ***et al.***, 1951), auxin contents (Mahadevan, 1984), acid phosphatases (Iqbal and Rafique, 1987) and peroxidases (David and Murray, 1965) of the inoculated and non-inoculated seedlings were also determined.

Results

The percentage germination in non-inoculated treatment was 77.5% for ***Triticum aestivum*** (Table 1). Whereas, the monoculture inoculated treatments provoked germination maximally as compared to non inoculated treatment. In this respect 100% germination was manifested by 1e (As-8) inoculation. With all mixed cultures enhancement in this parameter was recorded as compared to non inoculated treatment. 1a (As-2) and 1d (As-6) provoked the germination (3.03-21.21% As-2, 9.90-21.1% As-6) in combination with any other bacterial strain as compared to when inoculated alone. 1b (As-4) and 1c (As-5) enhanced percentage germination in majority of cases. Whereas 1e (As-8) affected this parameter adversely in all mixed cultures as compared to inoculated alone. Generally the %age germination in ***Vigna radiata*** decreased with the majority of mono and mixed cultures inoculations (Table 2). Enhancement in this parameter was recorded with only few inoculations i.e., 1b (As-4), 1c (As-5) and 1d (As-6) mono cultures and in mixed cultures 2b (As-2, As-5) and 2d (As-2, As-8).

Seedling length of ***Triticum aestivum*** was significantly stimulated in mono-culture inoculations (17.91-24.23%) as compared to non inoculated treatment. 1a (As-2) and 1c (As-5), separately or in combination whenever inoculated as mixed cultures, reduced the seedling length up to 25% over control. Maximum increase was recorded in 3j mixed culture (As-4, As-6 and As-8). Seedling lengths of ***Vigna radiata*** with monoculture inoculations were higher as compared to control treatment. Mixed cultures 2a (As-2, As-4), 2d (As-2, As-8), 3a (As-2, As-4, As-6), 3b (As-2, As-5, As-6) and 3d (As-4, As-5, As-6) exhibited increase, over control, in seedling lengths. Dry weight g^{-1} fresh weight in both of the plants i.e., ***T. aestivum*** and ***V. radiata*** decreased in almost all of monoculture treatments except 1d (As-6) and 1a (As-2), 1e (As-8). In ***T. aestivum*** treatments 1a (As-2), 1b (As-4) and 1c (As-5) inoculations generally caused increases in the dry weight g^{-1} fresh weight in combination with one another and any other strain. In the seedlings of ***V. radiata*** this parameter was mostly decreased both with mono and mixed culture inoculations but with a few bacterial combinations i.e., 2c (As-2, As-6) and 5a (As-2, As-4, As-5, As-6, As-8) increases were observed.

Table 1: Effect of *Azospirillum* (As) inoculation (mono and mixed cultures) on percentage germination, growth, activity of enzymes (peroxidase, acid phosphatase) protein contents and auxin contents of *Triticum aestivum* (mean of four replicates

Strain/ Combination	Sym-bol	%age germi-nation	Seedling length (cm)	Dry wt./ gram fresh wt. mg g^{-1}	Soluble protein content μg g^{-1}	Auxin content μg g^{-1}	Acid phosphatase unit g^{-1} fresh wt.	Peroxidase unit g^{-1} fresh wt.
Control	Ctl	77.50±1.77	22.61±1.10	113.18±19.00	935.00±24.80	1.77±0.06	394.00±2.80	141.77±5.30
As-2	1a	82.50±1.77	28.09±0.60	104.85±7.75	1096.00±45.40	2.43±0.04	415.00±2.54	161.82±1.11
As-4	1b	90.00±0.00	26.66±1.40	94.76±5.76	1296.00±56.73	2.29±0.06	416.00±0.70	171.55±3.11
As-5	1c	92.50±1.77	28.01±1.70	93.43±6.10	998.00±27.00	1.96±0.17	420.00±0.52	210.60±4.68
As-6	1d	82.50±1.77	27.14±1.10	114.68±6.98	1108.00±22.70	2.17±0.13	401.65±0.90	184.18±3.76
As-8	1e	100.00±0.00	27.12±0.50	97.61±0.59	962.00±15.60	2.08±0.124	408.30±3.31	116.23±2.17
As-2, As-4	2a	97.50±1.77	24.56±2.20	148.5±14.00	914.00±32.60	1.625±0.27	308.00±1.40	129.39±3.53
As-2, As-5	2b	92.50±1.77	23.18±1.60	125.50±11.80	728.00±45.40	2.32±0.48	589.00±6.00	164.33±0.79
As-2, As-6	2c	90.00±3.54	23.59±1.94	116.10±0.34	796.00±17.02	5.57±0.76	379.60±6.80	126.34±1.98
As-2, As-8	2d	92.50±1.77	24.80±1.49	117.15±7.00	626.00±27.00	3.23±0.55	422.50±1.77	242.60±2.00
As-4, As-5	2e	97.50±1.77	24.32±1.10	114.10±8.30	782.00±12.80	5.38±0.08	179.30±3.10	101.75±2.50
As-4, As-6	2f	90.00±3.54	24.89±1.90	108.01±1.95	900.00±42.50	2.69±0.70	422.50±1.70	103.06±7.50
As-4, As-8	2g	95.00±0.00	25.61±1.90	136.72±22.00	1226.00±12.28	1.90±0.28	68.80±0.90	211.90±5.17
As-5, As-6	2h	92.50±1.77	26.51±1.80	130.00±7.77	1148.00±17.02	1.81±0.03	239.22±6.54	129.22±6.30
As-5, As-8	2i	92.50±1.77	24.50±1.20	119.38±7.50	1064.00±17.02	4.78±0.04	805.30±2.70	30.46±5.50
As-6, As-8	2j	97.50±1.77	25.70±1.18	117.60±6.80	1168.00±36.90	2.81±0.80	517.30±1.68	320.10±3.69
As-2, As-4, As-5	3a	85.00±10.60	22.60±1.60	129.54±3.80	1254.00±49.60	1.85±0.40	639.37±3.90	120.19±2.12
As-2, As-5, As-6	3b	90.00±3.54	22.82±2.00	130.30±3.00	850.00±21.30	0.44±0.00	296.00±2.83	185.20±3.48
As-2, As-6, As-8	3c	90.00±0.00	21.07±2.00	107.60±0.30	1302.00±21.28	3.29±0.49	768.25±1.24	79.21±4.97
As-2, As-4, As-8	3d	92.50±1.77	23.23±2.00	144.90±0.62	1340.00±31.20	2.38±0.09	1186.50±4.20	139.51±7.43
As-2, As-5, As-8	3e	97.50±1.77	25.37±1.90	96.00±12.00	1388.00±14.18	3.86±0.35	812.90±0.02	24.54±2.64
As-4, As-5, As-6	3f	97.00±1.77	21.12±0.70	99.15±3.40	1383.00±40.40	4.54±0.40	1111.00±7.40	85.50±1.89
As-4, As-5, As-8	3g	90.00±3.54	24.30±0.45	114.7±4.0	1302.00±21.27	4.37±0.90	841.50±1.50	353.12±7.82
As-2, As-4, As-6	3h	82.50±8.36	23.87±1.80	110.82±11.0	1192.00±11.32	1.57±0.03	143.50±2.48	558.30±5.80
As-5, As-6, As-8	3i	90.00±7.09	23.78±1.00	108.15±7.30	1154.00±27.00	2.36±0.70	405.50±1.70	55.50±0.64
As-4, As-6, As-8	3j	97.50±1.77	28.81±0.61	113.79±0.28	1232.00±17.02	4.37±0.37	964.20±2.85	89.38±3.45
As-2, As-4, As-5,As-6	4a	100.00±0.00	26.06±1.70	113.8±2.10	1334.00±27.00	1.68±0.24	468.02±1.06	85.81±3.14
As-2, As-4, As-5, As-8	4b	85.00±7.09	24.11±2.00	107.52±1.07	458.00±18.44	1.70±0.08	480.00±14.18	94.58±1.00
As-2, As-4, As-6, As-8	4c	92.50±1.77	24.45±0.87	79.79±1.75	1034.00±21.28	3.18±0.09	853.60±1.10	43.06±1.57
As-2, As-5, As-6, As-8	4d	95.00±1.77	24.80±2.00	100.7±0.50	1090.00±41.30	1.48±0.02	351.20±1.61	76.09±2.02
As-4, As-5, As-6, As-8	4e	82.50±8.86	25.02±1.39	114.5±3.50	1404.00±5.65	4.44±0.57	476.20±5.00	84.97±3.34
As-2, As-4, As-5, As-6, As-8	5a	87.50±8.86	25.47±1.30	97.03±6.40	1254.00±29.80	1.51±0.30	380.30±3.31	75.85±3.88
L.S.D. at P=0.05		3.10	0.79	9.55	19.56	0.30	2.99	2.97

Table 2: Effect of *Azospirillum* (As) inoculation (mono and mixed cultures) on percentage germination, growth, activity of enzymes (peroxidase, acid phosphatase) growth, protein contents and auxin contents of *V. radiate* (mean of four replicates

Strain/ Combination	Sym-bol	%age germi-nation	Seedling length (cm)	Dry wt./ gram fresh wt. mg g^{-1}	Soluble protein content µg g^{-1}	Auxin content µg g^{-1}	Acid phosphatase unit g^{-1} fresh wt.	Peroxidase unit g^{-1} fresh wt.
Control	Ctl	91.65±3.6	18.96±0.8	52.9±6.5	251.0±4.9	0.55±0.08	144.9±3.6	9.8±0.2
As-2	1a	89.97±3.7	19.48±0.2	64.5±8.3	288.0±2.8	0.59±0.03	366.5±3.1	10.6±2.9
As-4	1b	94.9±2.7	20.1±0.4	49.1±3.2	486±2.4	0.65±0.2	267.2±6.1	10.7±0.5
As-5	1c	96.65±1.6	19.9±0.4	19.8±2.0	360.0±5.6	0.64±0.03	408.0±6.3	29.9±1.0
As-6	1d	94.97±2.7	20.0±0.3	48.0±5.5	603.0±6.3	0.74±0.01	508.0±4.9	12.4±0.3
As-8	1e	86.65±3.3	20.3±0.1	52.9±3.5	229.0±10.6	0.70±0.01	341.5±3.8	17.7±8.4
As-2, As-4	2a	83.3±3.7	21.0±0.07	28.2±3.1	317.5±6.0	0.66±0.03	512.2±8.6	13.3±1.2
As-2, As-5	2b	93.3±3.5	18.8±0.09	32.6±7.1	668.0±12.7	0.50±0.04	409.7±3.7	14.4±0.9
As-2, As-6	2c	91.65±3.6	18.6±0.3	99.3±3.2	762.0±1.4	0.36±0.03	835.4±13.5	7.8±0.2
As-2, As-8	2d	95.0±4.3	20.2±0.3	55.9±3.0	813.5±10.2	0.47±0.10	868.7±3.3	45.9±0.7
As-4, As-5	2e	88.3±4.9	19.5±0.1	38.4±4.2	766.0±4.2	0.53±0.01	310.1±2.8	8.9±0.1
As-4, As-6	2f	80.0±0.0	18.9±0.4	61.5±2.5	612.5±3.8	0.63±0.01	736.5±8.8	12.2±0.7
As-4, As-8	2g	84.9±1.4	19.6±0.5	50.8±0.7	512.0±1.4	0.83±0.02	346.0±15.5	6.8±0.2
As-5, As-6	2h	91.6±7.2	18.5±0.1	54.3±0.9	812.0±4.2	0.82±0.01	357.2±5.8	15.0±2.8
As-5, As-8	2i	84.9±5.4	19.5±0.2	52.5±2.0	711.0±5.6	0.65±0.03	287.7±4.7	5.9±0.3
As-6, As-8	2j	91.6±5.4	18.7±0.1	39.0±0.9	574.5±6.0	1.11±0.06	470.5±7.4	25.7±1.4
As-2, As-4, As-5	3a	91.6±2.7	20.1±0.08	52.3±4.2	765.5±7.4	0.53±0.16	740.5±10.2	7.7±0.1
As-2, As-5, As-6	3b	83.2±5.0	21.5±0.2	43.8±1.8	600.0±4.2	0.37±0.13	604.5±3.8	14.1±0.9
As-2, As-6, As-8	3c	83.3±5.0	19.9±0.3	52.8±9.7	590.5±4.5	2.36±0.11	633.7±7.6	3.7±1.3
As-2, As-4, As-8	3d	88.2±1.4	20.1±0.3	43.5±1.6	502.5±2.4	0.38±0.03	370.4±10.3	13.2±0.6
As-2, As-5, As-8	3e	86.6±2.3	18.8±0.2	39.7±2.5	576.5±2.4	0.38±0.02	412.4±4.3	16.6±0.04
As-4, As-5, As-6	3f	91.6±3.6	19.1±0.4	49.7±3.8	806.0±4.2	0.63±0.007	520.7±10.4	26.7±1.2
As-4, As-5, As-8	3g	88.3±4.9	19.8±0.1	52.2±6.1	619.5±1.06	0.57±0.07	325.0±5.2	16.7±0.05
As-2, As-4, As-6	3h	86.6±3.3	19.6±0.2	62.2±2.2	607.5±7.4	0.87±0.09	629.0±4.2	16.1±0.14
As-5, As-6, As-8	3i	86.6±5.7	19.0±0.1	50.6±4.6	721.5±1.7	0.42±0.04	691.2±5.1	27.9±0.16
As-4, As-6, As-8	3j	83.2±5.0	19.3±0.07	49.4±3.7	755.0±4.9	0.28±0.02	348.0±2.8	6.1±0.18
As-2, As-4, As-5, As-6	4a	91.6±2.7	19.2±0.3	55.0±4.2	455.0±6.3	0.41±0.02	482.2±3.7	4.9±0.4
As-2, As-4, As-5, As-8	4b	86.6±0.0	18.3±0.2	47.6±2.3	746.0±2.8	0.88±0.03	459.9±5.0	11.2±0.5
As-2, As-4, As-6, As-8	4c	71.6±4.9	18.4±0.2	43.2±4.4	761.0±6.3	0.56±0.01	520.0±4.5	12.8±1.6
As-2, As-5, As-6, As-8	4d	89.9±1.6	19.5±0.2	47.0±4.0	583.5±7.4	0.53±0.02	701.9±1.4	23.7±0.5
As-4, As-5, As-6, As-8	4e	86.6±2.3	17.6±0.3	46.0±4.1	361.5±1.7	0.60±0.01	428.2±4.4	7.7±0.4
As-2, As-4, As-5, As-6, As-8	5a	89.9±3.7	19.5±0.3	74.2±3.7	707.0±4.9	0.16±0.03	591.0±5.6	9.8±1.2
L.S.D. at P=0.05		12.8	0.89	5.82	38.92	0.13	21.36	4.97

With monoculture inoculation in both plants, enhancement in protein contents (except 1e i.e., As-8 inoculation in ***V. radiata***) auxin contents, acid phosphatases and peroxidases (except 1e i.e., As-8 inoculation ***T. aestivum***) were observed. Protein contents in ***V. radiata*** seedlings were increased with all mixed cultures and maximum increase was observed with mixed-culture 2d (As-2, As-8) while in ***T. aestivum*** 1a (As-2) inoculation generally enhanced protein synthesis in mixed cultures. Same was case with 1c (As-5) and 1e (As-8) as compared to monoculture inoculations. Auxin contents in ***T. aestium*** with mixed culture inoculations having As-8 generally enhanced the auxin level whereas As-2 (1a), As-4 (1b), As-5 (1c), As-6 (1d) caused decreases in the auxin contents in majority of mixed cultures. In ***V. radiata*** bacterial inoculation generally caused decrease in this parameter. Acid phosphatase increased with all mixed cultures in ***V. radiata*** as compared to control treatment. In ***T. aestivum*** 1a (As-2) inoculation caused reduction in acid phosphatase activity in majority of cases except 2d (As-2, As-8) inoculation which stimulated increase in this parameter. Whenever 1b (As-4) and 1d (As-6) were together in any mixed culture they caused enhancement in acid phosphatase activity but 1a (As-2) in mixed cultures had inhibitory effects on the activity of acid phosphatase, relative to their monocultures. Peroxidase activity was maximum in all monoculture inoculations as compared to control treatment in ***V. radiata***. In ***T. aestivum*** all monocultures except As-8 (1e) exhibited maximum peroxidase activity as compared to control treatment. This parameter in ***T. aestivum*** was negatively stimulated in majority of mixed cultures inoculations. Overall maximum increase in the activity of this enzyme was exhibited by 3 h (As-2, As-4, As-6), where it was four times as compared to non inoculated treatment. Peroxidases were positively stimulated in ***V. radiate*** with mixed cultures 2a, 2b, 2d, 2f, 2h, 2j, 3b, 3d, 3e, 3f, 3g, 3h, 3j, 4b, 4c and 4d.

Discussion

Five bacterial strains isolated from histoplane of ***Medicago*** (As-2), ***Rumas dentatus*** (As-4), ***Coronopus didymus*** (As-5) and ***Cinchorium intybus*** (As-6 and As-8) growing in S.S. Farm, Badian Road, Lahore were used as mono and mixed cultures for inoculating ***Triticum aestium*** and ***Vigna radiata***. All the isolates were gram negative, obligatory aerobic bacteria and were having characteristics of ***Azospirillum*** (Holt ***et al.***, 1994). They are known among other root associated bacteria (Martin-Didonet ***et al.***, 2000).

The percentage germination in the monoculture inoculated seeds of ***T. aestivum*** was increased as compared to control treatment, enhancement in germination with bacterial inoculation was also reported by Hasnain and co-worker (Hasnain ***et al.***, 1995; Siddique ***et al.***, 1997; Hasnain and Afrasayab, 2000). 1e (As-8) inoculation hampered the germination in mixed cultures as compared to when inoculated alone. As-2 and As-6 have synergistic effects in all mixed cultures. In case of ***V. radiata*** generally mixed cultures inoculations hindered the germination but most of the monocultures caused increase in this parameter. There was not any pronounced effect on number of leaves of seedlings of ***V. radiata*** with the inoculation of mono and mixed cultures as compared to control treatment.

In ***T. aestivum*** number of roots and root hair was maximum in all inoculated treatments as compared to non-inoculated treatment. According to reports the bacterial strains which stimulate the growth of plant colonize the roots, release some chemotaxis to root exudates due to which competition among micro organisms decreased and thereby increasing the rate of root colonization with microbes (Bashan and Holguin, 1994), ***Azospirillum*** strains are good colonizer of wheat (Bashan ***et al.***, 1987). Seedling lengths of both of the plants were maximum with monoculture inoculations and with majority of mixed cultures as compared to control treatments. ***Azospirillum lipoferum*** is considered as potential phytostimulator, since it significantly increases plant growth. Growth improvement depends upon plant bacterium associations (Ramamoorthy ***et al.***, 2000). Dry wt g^{-1} fresh weight was decreased in all monocultures treatments except As-6 in case of ***T. aestivum*** and As-4, As-5, As-6 and As-8 exhibited synergism in most of the mixed cultures. In case of ***Vigna radiata*** inoculation of mono and mixed cultures generally reduced this parameter but some increases were also recorded. In ***T. aestivum*** soluble protein contents were improved with bacterial inoculation as compared to control treatment. As-2 inoculation caused increased protein synthesis in most of the mixed cultures except 2c, 2d, 3b and 4b. The mixed culture inoculations of As-2, As-4, As-6 and As-8 exibited increase in this parameter except few combinations as compared to their mono culture inoculations. In ***T. aestivum*** treatments, auxin level was maximum in inoculated treatments (monocultures) as compared to non inoculated treatment. As-8 (1e) elevated the auxin level in all mixed cultures except 4d and 5a, whereas As-2 (1a), As-4 (1b), As-5 (1c) and As-6 (1d) decreased the auxin level in most of the mixed culture which might be due to their antagonistic effect over each other leading to reduction in auxin level. In case of ***V. radiata***, with monoculture bacterial inoculations increased auxin content of seedlings as compared to non-inoculated treatment and among them As-6 monoculture inoculation showed pronounced increase. Generally As-2, As-4 and As-6 showed antagonistic effects in mixed culture inoculations as compared to their mono culture inoculations. Whereas As-5 and As-8 stimulated auxin contents in their mixed culture inoculations as compared to when inoculated alone. Protein contents of ***V.radiata*** seedlings were increased with monoculture inoculation relative to non inoculated treatment except As-8, As-2 caused significant increase. In mixed culture inoculations As-2, As-4, As-6 and As-8 showed synergism over respective monocultures. Hence the bacterial strains interfere with the activity of each other and exhibit increase or decrease in soluble protein contents over non-inoculated control. Bacterial strains stimulate the acid phosphatase as reported by Saleh and Belisle (2000). In case of both enzymes analysis in ***T. aestivum*** and ***V. radiata*** shoot, generally monoculture inoculations increase the activity of the acid phosphatase. In case of ***T. aestivum*** some mixed cultures i.e., 3 f, 3 j and 3 d caused maximum increase in acid phosphates than non-inoculated and monoculture treatments. Acid phosphatase were positively stimulated in all of mixed cultures. Peroxidases catalyze the peroxidase dependent oxidation of a range of inorganic and organic compounds. They are primarily the intracellular enzymes with important roles in cellular processes (Everse ***et al.***, 1990) and bacterial inoculations may increase the activity of peroxidase (Fang and Kao, 2000). In case of ***T. aestivum*** the peroxidase activity was decreased with inoculation of As-8 (1e) in monoculture inoculations and in mixed cultures significant decrease was manifested by 3e. Activity of peroxidase was significantly increased in case of ***V. radiata*** with inoculations of As-8 except for 2 g, 2 l, 3 c and 4 e as compared to control treatment and in majority of mixed cultures this parameter was positively stimulated.

The ongoing discussion imparts that generally with monoculture inoculations of As-2, As-4, As-5, As-6, As-8 in ***T. aestivum*** and ***V. radiata***, enhancement in all parameters (i.e., growth and biochemical parameters) as compared to control treatment was recorded Whereas mixed culture inoculations 2 h, 3 j and 4 a in ***T. aestivum*** exhibited pronounced effects in majority of parameters. In ***V. radiata***, many inoculations exhibited better growth. Majority of monocultures inoculates have stimulatory effects and inoculations with specific mixed culture combinations exhibited synergistic responses in growth parameters. Two plants responded differently with different combinations. Hence it can be concluded that strain plant combination is important for growth improvement These results also reflect the impact of ***Azospirillum*** inoculation on leguminous plants. Nevertheless the increases in ***V. radiata*** was not that pronounced as in ***T. aestivum*** but the positive role of ***Azospirillum*** is clearly depicted here.

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