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Article

CHEMICAL WEED CONTROL IN PADDY FIELDS INOCULATED WITH *Azospirillum lipoferum*

Controle Químico em Arrozais Inoculados com ***Azospirillum lipoferum***

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ABSTRACT - Herbicides, as a major part of weed control strategy in paddy fields, have different impact on growth and activity of soil-beneficial bacteria such as Azospirillum species. A field experiment was conducted at Sefid Rood Livestock and Agricultural Company, northern Iran, to investigate the possibility of chemical weed control in paddy fields inoculated with *Azospirillum lipoferum*. The experiment was designed in a factorial arrangement based on a randomized complete block with three replicates. The factors were Azospirillum application (inoculation with or without *Azospirillum lipoferum*) and weed management regime (butachlor application with supplementary hand-weeding, bensulfuron methyl application with supplementary hand-weeding, combination of butachlor and bensulfuron methyl application with supplementary hand-weeding, hand-weeding at 15, 30, and 45 days after transplanting, and no weeding [not weeded during the rice-growing period). The results showed that plants inoculated with *A. lipoferum* produced 19% higher grain yield compared to plants that were not inoculated. The highest grain yields were recorded for plots treated with butachlor with supplementary hand-weeding (4,512 kg ha^{-1}) and for those treated with a combination of butachlor and bensulfuron methyl with supplementary hand-weeding (4500.5 kg ha^{-1}). The lowest yield (3494.3 kg ha^{-1}) was recorded for weedy plots. No significant interaction was detected between *A. lipoferum* application and weed management regime for grain yield, indicating that the herbicides had no adverse effect on the efficiency of *A. lipoferum* in promoting growth and grain yield of rice. There was no significant difference in the dry weights of weed between inoculated and non-inoculated plots. The dry weights of weed in hand-weeded and herbicide-treated plots were significantly lower than that of the weedy plot. In conclusion, the result of this experiment confirms the possibility of chemical weed control in paddy fields inoculated with *A. lipoferum*.

Keywords: beneficial bacteria, bensulfuron methyl, butachlor, paddy fields, weed interference.

RESUMO - Os herbicidas, como uma parte importante da estratégia de controle de plantas daninhas em arrozais, têm diferentes impactos no crescimento e atividade de bactérias benéficas para o solo, como as espécies de Azospirillum. Um experimento de campo foi realizado no Sefid Rood Livestock and Agricultural Company, norte do Irã, para investigar a possibilidade de capina química em campos de arroz inoculados com ***Azospirillum lipoferum****. O experimento foi desenvolvido em esquema fatorial, com base em blocos casualizados com três repetições. Os fatores foram a aplicação de Azospirillum (inoculação com ou sem* ***Azospirillum lipoferum****) e o regime de manejo de plantas daninhas (butaclor – aplicação com capina manual suplementar; bensulfuron-methyl – aplicação com capina manual suplementar; combinação de butaclor e bensulfuron-methyl –*

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aplicação com capina manual suplementar, capina manual aos 15, 30 e 45 dias após o transplante e sem capina [não eliminados durante o período de cultivo de arroz). Os resultados mostraram que plantas inoculadas com ***A. lipoferum*** *apresentaram rendimento de grãos 19% superior em comparação com aquelas que não foram inoculadas. As maiores produções de grãos foram registradas para parcelas tratadas com butaclor com capina manual suplementar (4.512 kg ha^{-1}) e para aquelas tratadas com uma combinação de butaclor e bensulfuron-methyl com capina manual suplementar (4.500,5 kg ha^{-1}). O menor rendimento (3.494,3 kg ha^{-1}) foi registrado nos lotes que não foram capinados. Não houve interação significativa entre a aplicação de A. lipoferum e o regime de manejo de plantas daninhas para produção de grãos, indicando que os herbicidas não tiveram nenhum efeito adverso sobre a eficiência de* ***A. lipoferum*** *na promoção do crescimento e produtividade de grãos de arroz. Não houve diferença significativa nos pesos secos de plantas daninhas entre parcelas inoculadas e não inoculadas. O peso seco de plantas daninhas em parcelas capinadas manualmente e tratadas com herbicidas foi significativamente menor do que o do lote que não foi capinado. Em conclusão, o resultado desta experiência confirma a possibilidade de capina química em campos de arroz inoculados com* ***A. lipoferum****.*

Palavras-chave: bactérias benéficas, bensulfuron-methyl, butaclor, arrozais, interferência das plantas daninhas.

INTRODUCTION

The high prices of nitrogen fertilizers and their low use efficiency in rice fields (Choudhury and Khanif, 2004) have encouraged agronomists and producers to evaluate the use of alternative nutrient sources that are both sustainable and environment-friendly. Plant growth-promoting rhizobacteria (PGPR) colonize the rhizosphere of monocots (Fibach-Paldi et al., 2012; Kazi et al., 2016) and dicots (Jalilian et al., 2012) and stimulate plant growth by means of nitrogen fixation (Olivares et al., 2013), production of phytohormones (Lugtenberg, 2015) and siderophores (Bakker et al., 2013), and phosphorous solubilization (Barea et al., 2015). Among PGPRs, *Azospirillum* species are aerobic heterotrophs bacteria that fix N non-symbiotically in the rhizosphere of plants from the Poaceae family such as rice (Fibach-Paldi et al., 2012) and wheat (Kazi et al., 2016). These bacteria also produce plant-growth hormones (auxins, gibberellins, and cytokinin), siderophores, as well as antifungal and antibacterial compounds. They also enhance phosphate solubilization (Maksimov et al., 2011). Previous research studies have shown that *Azospirillum lipoferum*, *Azospirillum brasilense*, and *Azospirillum amazonense* are highly potent nitrogen-fixing organisms in the rhizosphere of rice plants (Sahoo et al., 2014). Rice grain yield was significantly increased by 20.4% (Garcia de Salamone, 2012) and 22% (Balandreau, 2002) in field experiments through *Azospirillum* inoculation. The positive effect of *Azospirillum brasilense* inoculation on rice growth and grain yield under field conditions was also reported by Pedraza et al., 2009, Garcia de Salamone et al. (2010), Mukhopadhyay et al. (2013). In contrast, Ruiz-Sanchez et al. (2011) reported that there were no significant differences in biomass production in *Azospirillum*-inoculated and non-inoculated rice plants, both under well-watered and drought stress conditions.

Weeds are one of the major constraints in rice production. Loss in rice yield resulting from weed competition has been estimated at 30-40% in Bangladesh, 36-56% in the Philippines, 40-100% in South Korea, and up to 90% in Iran (Mamun et al., 2013). Despite environmental concerns and increasing herbicide-resistance in weeds, herbicides are extensively used in paddy fields as a major part of weed control strategy. Nowadays, paddy fields in Iran are treated with a combination of butachlor (N-(butoxymethyl)-2-chloro-N-(2.6-diethylphenyl) acetamide) for barnyardgrass control and bensulfuronmethyl (2-(4.6-dimethoxypyrimidin-2-carbamoylsulfamoyl)-o-toluic acid methyl ester) for broadleaf and sedge control (Yaghoubi et al., 2010). In addition, almost all fields are hand-weeded once or twice that depending on rice genotype and weed flora. Both of the aforementioned herbicides are applied to paddy soils as pre-emergent herbicides and may have different impact on growth and activity of soil-beneficial bacteria, depending on dose, bacteria species, and soil properties. The adverse impacts of pesticides on soil microbial diversity and activities have been described by many researchers (Littlefield-Wyer et al., 2008). Moreover, Mahia et al. (2008) reported that total C mineralization and C biomass were reduced by 28% and 12%, respectively, when soil was treated with atrazine. In contrast, some research works have shown that herbicides may stimulate the activity of some bacteria. Min et al. (2001) reported that butachlor application stimulated the growth of anaerobic fermentative and sulphate-reducing bacteria while inhibiting the growth of acetogenic bacteria in paddy soil. They also reported that nitrogen fixation and nitrification increased at the initial stage of butachlor application, but decreased afterwards in a paddy soil. In a tropical rice soil, Sahoo et al. (2016) found that pretilachlor application at the recommended dose (600 g a.i. ha^{-1}) did not record any significant changes in the amount of bacteria, actinomycetes, fungi, nitrogen fixers, and microbial biomass carbon compared to the untreated control plot. El-Ghamry et al. (2001) reported that microbial biomass –C and –N decreased in Bensulfuron methyl-treated soils.

A survey of the literature uncovers extensive reports on the effect of pesticides on the activity and population of soil microorganisms, while little information is available on the effect of butachlor and Bensulfuron methyl – applied separately or in combination with each other on the growth and grain yield of rice plants inoculated with *Azospirillum lipoferum*. Therefore, the main objectives of this study were to evaluate the possibility of herbicide application in rice fields treated with *Azospirillum lipoferum* and the effect of herbicide application on yield and growth of inoculated and non-inoculated rice plants. However, the following objectives were also evaluated: 1) effect of weed management regime on rice growth and grain yield, and 2) effect of *Azospirillum lipoferum* inoculation on rice growth and grain yield.

MATERIALS AND METHODS

Site characteristics

The field experiment was conducted in 2014 at Sefid Rood Livestock and Agricultural Company (37°12' N, 49°38' E, 20 masl), Rasht, north of Iran. The physico-chemical conditions of the soil were: 2.2% organic matter content, 48% clay, 38% silt, 14% sand, 7.1 pH, total N 0.308%, available phosphorous 13.2 mg kg^{-1}, available potassium 146.0 mg kg^{-1}, and EC 1.44 dS m^{-1}. Figures 1 and 2 respectively show weekly air temperature (maximum, minimum, and average) and precipitation during the rice-growing period, measured at the weather station of Rasht located 10 km far from the experiment site.

Experimental design and treatments

The experiment was designed in a factorial arrangement based on a randomized complete block design with three replicates. The factors were *Azospirillum* application (inoculation with or without *Azospirillum lipoferum*) and weed management regime (butachlor [EC 60%] application with supplementary hand-weeding at 15 days after transplanting, bensulfuron methyl [DF 60%] application with supplementary hand-weeding at 15 days after transplanting, combination of butachlor and bensulfuron methyl application with supplementary hand-weeding at 15 days after transplanting, hand-weeding at 15, 30, and 45 days after transplanting, and no weeding [not weeded during rice growing period and no herbicide application]). Butachlor and bensulfuron-methyl were applied separately or in combination with each other seven days after transplanting on flooded plots using a hand-held bottle designed for soil-applied herbicides in paddy rice fields at the rate of 1800 and 75 g a.i. ha^{-1}, respectively.

Azospirillum lipoferum bacteria were obtained from the biology laboratory of Soil and Water Research Institute, Karaj, Iran. Before inoculation, Gum Arabic (10%) was applied to the seeds of the Hashemi cultivar. The seeds were then inoculated with *A. lipoferum* in the proportion of 10 g of peat (10^8 cells/g peat) kg^{-1} seed. The inoculated seeds were dried in the shade and then spread individually on the rice nursery on April 14, 2014. The three-leaf stage seedlings were transplanted to experiment plots (3 m x 4 m) at a hill spacing of 20 cm x 25 cm, with three seedlings per hill, on 12 May 2014. Non-inoculated controls were also included in the experiment.

Crop management

Nitrogen fertilizer (50 kg ha^{-1} as urea), phosphorus (70 kg ha^{-1} as Triple superphosphate), and potassium (30 kg ha^{-1} as K_2SO_4) were manually broadcast and incorporated before

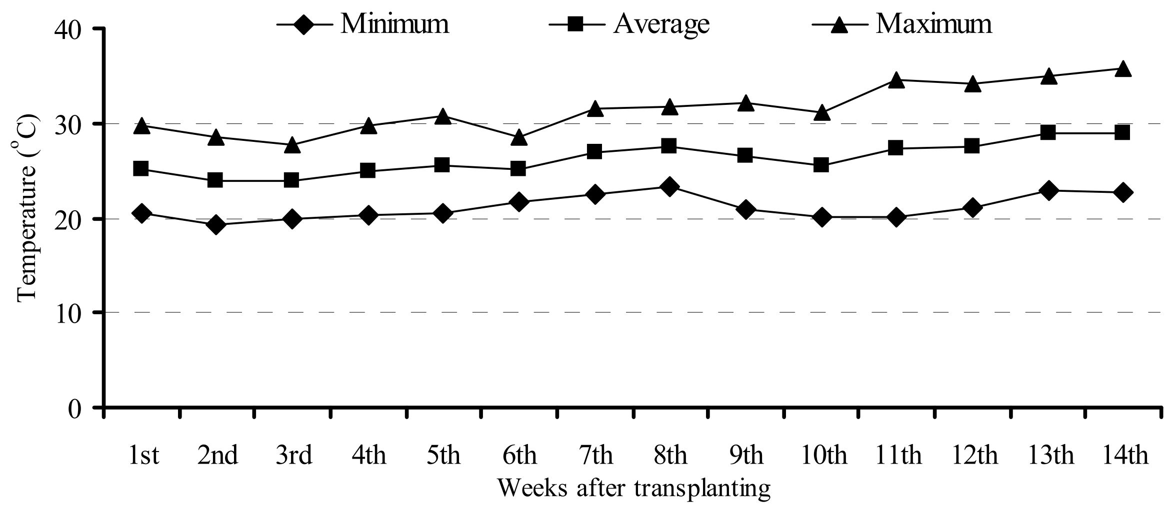

Figure 1 - Weekly temperatures (maximum, minimum and average) during rice growing period.

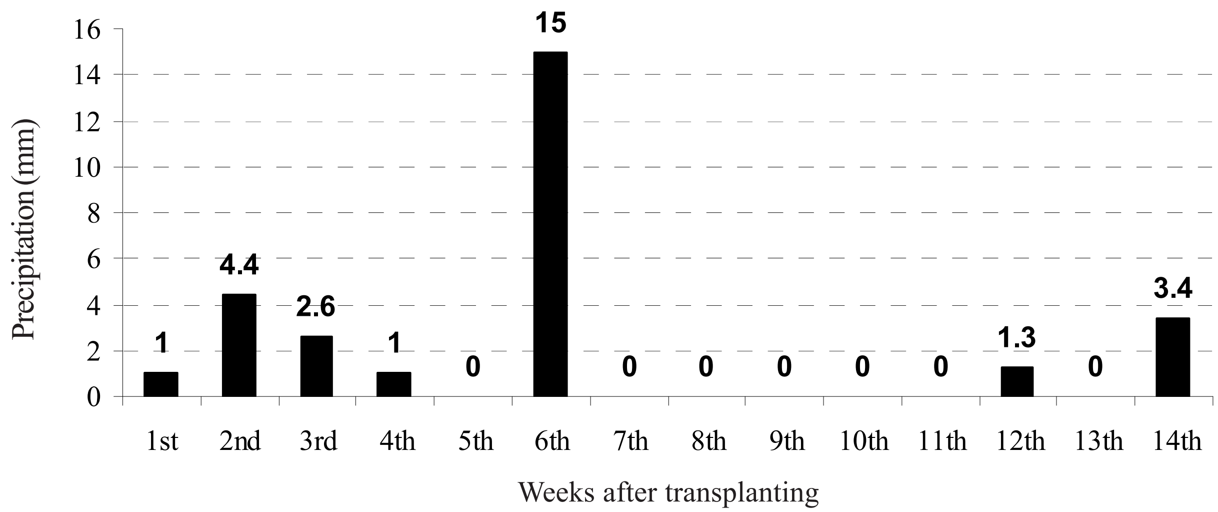

Figure 2 - Weekly precipitation during rice growing period.

transplanting. The remaining N (50 kg ha^{-1} as urea) was also applied as topdressing at panicle initiation. No pesticides were used in the experiment since the crop was not affected by pests or diseases. Consistent with the paddy field practices in northern Iran, a permanent flood of 5-7 cm depth was maintained after crop establishment until 20 days before harvesting time.

Crop measurements and calculations

At the flowering stage, five plants were randomly selected from each plot and the flag leaf area was measured with a leaf area meter (LI-3000A Leaf Area Meter (Li-Cor, USA)). Rice grain yield (14% moisture content) and yield components (panicle number m^{-2}, grain number per panicle, 1000 grain weight) were determined at the harvesting stage (Gomez, 1972). Panicle fertility was calculated as follows:

$$\text{Panicle fertility} = (\text{Number of filled grains/Total number of grains per panicle}) \times 100 \qquad \text{(eq. 1)}$$

Rice biomass was determined from a 1 m^2 area in each plot. Plants were randomly selected, clipped at the ground level, threshed, dried at 70 °C for three days, and weighed. Rice biomass was expressed as the dry weight of above-ground plant per hectare. Harvest index (HI) was calculated as the ratio between the dry mass of the grains and biomass.

Rice leaves and grains were separately ground to pass through a 1 mm sieve, after which the concentration of N in leaves and grains was determined using the micro-kjeldahl method described by Pregl (1945). Grain N uptake was calculated by the following equation:

$$\text{Grain N uptake} = \text{Grain dry weight} \times \text{grain N concentration} \quad \text{(eq. 2)}$$

Weed measurement

Weeds were collected by clipping at the soil surface from four random quadrates of 25 cm x 25 cm area in each plot at the harvesting stage. Weed species were identified and weed biomass was determined for samples dried at 70 ^{o}C for three days.

Statistical analyses

All data were subjected to analysis of variance (ANOVA) and means were compared using Fisher's protected LSD test at 5% level of significance. All statistical analyses were conducted using SAS (SAS, 2004).

RESULTS AND DISCUSSION

Composition of weed communities

Barnyardgrass (*Echinochloa crus galli*), water grass (*E. oryzoides*), arrowhead (*Sagittaria trifolia*), water-plantain (*Alisma plantago-aquatica*), pondweed (*Potamogeton nodosus*), saltmarsh bulrush (*Bolboschoenus maritimus* syn. *Scirpus maritimus*), and smallflower (*Cyperus difformis*) were the most dominant weed species in the experiment field.

Weed biomass

ANOVA showed that the main effect of *A. lipoferum* on weed biomass was not significant at 0.05 probability level (Table 2). This indicates that *A. lipoferum* has no significant negative or positive effect on weed growth or biomass accumulation. PGPR such as *A. lipoferum* may have positive effects on the weed biomass if they colonize in the rhizosphere and improve weed growth and biomass, while negative effects can be found if inoculation with *A. lipoferum* increases vigour in young rice plants and, hence, improves weed suppression. Contrary to this result, Massenssini et al. (2014) reported that weeds, compared to crops, have a greater ability to establish positive interactions with the soil microbial community, which allows them to acquire greater competitive ability in agro-ecosystems. Weed biomass was significantly affected by the weed management regime (Table 1). The highest weed biomass (299.0 g m^{-2}) was recorded in weedy plots. Weed biomass was reduced by 90%, 93%, 86%, and 92% in hand-weeded plots, plots treated with butachlor, plots treated with bensulfuron methyl, and plots treated with a combination of two management, respectively, compared to the weedy plots. The result also shows that there were no significant differences in weed biomass between hand-weeded plots and herbicide-treated plots. These results indicate that weeds in rice fields can be effectively controlled through herbicide application with supplementary hand-weeding.

Yield components and paddy yield

All yield components, panicle fertility, and paddy yield were significantly affected by inoculation with *A. lipoferum* and weed management regime (Table 1). Number of panicles per m^2, number of grains per panicle, and 1000 grain weight were increased by 19%, 20%, and 5%, respectively, after inoculation with *A. lipoferum* (Table 2). Panicle fertility was significantly lower in inoculated plants than in non-inoculated ones (Table 2). This result indicates that number of unfilled grains per panicle increased more than the total number of grains per panicle when plants were inoculated with *A. lipoferum*. Contrary to this result, Garcia de Salamone (2012) reported that

Table 1 - Mean squares for the effect *of Azospirillum lipoferum* inoculation and weed management regime on measured traits in rice

Source of variance	df	Weed dry weight	Paddy yield	Number of panicles per m^2	Number of filled grains per panicle	Panicle fertility	Thousand grain weight	Biomass	Harvest index	Flag leaf area	Leaf N concentration	Grain N concentration	Grain N uptake
Repeat	2	160 ^{ns}	181814**	546*	15 ^{ns}	6 ^{ns}	0.4 ^{ns}	227602 ^{ns}	15 ^{ns}	4805 ^{ns}	0.002 ^{ns}	0.005 ^{ns}	20 ^{ns}
Azospirillum (A)	1	2 ^{ns}	4141110**	24060**	4224**	363**	12.0 **	3839909*	143**	829670**	0.084 **	0.152**	1926**
Weed management regime (W)	4	90077**	1030161**	2885**	266**	3 ^{ns}	0.7**	2927848*	44 ^{ns}	60880**	0.056**	0.102**	646**
W × A	4	32 ^{ns}	41503 ^{ns}	181 ^{ns}	2 ^{ns}	19 ^{ns}	0.1 ^{ns}	1544558 ^{ns}	24 ^{ns}	3511 ^{ns}	0.001 ^{ns}	0.002 ^{ns}	14 ^{ns}
Erorr	18	351	27248	94	17	2	0.2	841941	17	2983	0.001	0.02	36
VC (%)	-	23.4	3.9	2.9	3.1	1.5	1.4	9.9	9.1	4.5	7.4	7.8	9.8

^{ns}, * and ** represent non-significant, significant at 5 and 1% probability level, respectively.

Table 2 - Mean comparison for the effect of *Azospirillum lipoferum* inoculation on measured traits in rice

Azospirillum lipoferum application	Weed dry weight ($g\ m^{-2}$)	Paddy yield ($kg\ ha^{-1}$)	Number of panicles per m^2	Number of grains per panicle	Panicle fertility (%)	Thousand grain weight (g)	Biomass ($kg\ ha^{-1}$)	Harvest index (%)	Flagleaf area ($cm^2\ hill^{-1}$)	Leaf N concentration (%)	Grain N concentration (%)	Grain N uptake ($kg\ ha^{-1}$)
Inoculation	80.1 a	4559.0 a	358.2 a	143.2 a	91.4 b	28.3 a	9582.6 a	47.7 a	1370.3 a	1.34 a	1.73 a	69.1 a
Un-inoculation	79.6 a	3815.9 b	301.5 b	119.5 b	98.4 a	27.0 b	8867.1 b	43.3 b	1037.7 b	1.23 b	1.59 b	53.0 b
LSD (0.05)	14.3	126.6	7.4	3.1	1.1	0.3	703.9	3.2	41.8	0.07	0.09	4.6

Means within a column followed by the same letter are not significantly different at the 5% level according to Fischer's Protected LSD test.

the percentage of chaffy grains was reduced in rice cultivars inoculated with *Azospirillum brasilense* and *Pseudomonas fluorescens*. Plants inoculated with *A. lipoferum* had significantly greater paddy yield (19%) compared to non-inoculated ones (Table 2). This value is similar to the values of 20.4% and 22% which were reported by Garcia de Salamone (2012) and Balandreau (2002), respectively. This increase in yield is mainly attributed to an improvement in root development resulting from an increase in water and mineral uptake (Richardson et al., 2009) and, to a lesser extent, from biological N_2 fixation (Steenhoudt and Vanderleyden, 2000). In addition to improving N status in inoculated plants, plant-growth promotion activity by *Azospirillum* spp. may also be related to synthesis of gibberellin (Cassan and Diaz-Zorita, 2016). Moreover, *Azospirillum* spp. are IAA-producing PGPR that are well-known for their ability to induce enhancement of plant growth and yield (Cassan and Diaz-Zorita, 2016). At the same time, the enhancement of seed germination and seedling vigour due to *Azospirillum* inoculation can lead to improvement of crop stands and increasing grain yield (Cassan et al., 2009). A similar result was reported by Panahi et al. (2015), who claimed that rice grain yield, number of panicles, and number of grains per panicle were significantly increased by 23%, 17%, and 12%, respectively, with bio-fertilizer application. However, they reported that 1000 grain weight was not significantly influenced by bio-fertilizer application.

The highest number of panicles was recorded both for the plots that had been treated with butachlor with supplementary hand-weeding (350.4 panicle m^{-2}) and plots managed with a combination of butachlor and bensulfuron methyl with supplementary hand-weeding (341.4 panicle m^{-2}). The lowest number of panicles (293.0 panicle m^{-2}) was recorded for weedy plots (Table 3). The highest number of grains per panicle was found in plots treated with butachlor with supplementary hand-weeding, which was statistically similar to the number of grains per panicle in plots treated with a combination of butachlor and bensulfuron methyl with supplementary hand-weeding and hand-weeded plots, followed by plots treated with bensulfuron methyl, and finally by weed plots (Table 3). Panicle fertility was significantly increased for plots treated with butachlor with supplementary hand-weeding and plots treated with a combination of butachlor and bensulfuron methyl with supplementary hand-weeding. The lowest panicle fertility was recorded for weedy plots (Table 3). Plants treated with butachlor by itself or in combination with bensulfuron methyl with supplementary hand-weeding produced heavier grains compared to plants under other regimes (Table 3). The reduction of yield components in weedy plots is due to the competition between rice plants and weeds for nutrients and light. Similarly,

Table 3 - Mean comparison for the effect of weed management regime on measured traits in rice

Weed management regime	Weed dry weight (g m^{-2})	Paddy yield (kg ha^{-1})	Number of panicles per m^2	Number of grains per panicle	Panicle fertility (%)	Thousand grain weight (g)	Biomass (kg ha^{-1})	Harvest index (%)	Flagleaf area (cm^2 $hill^{-1}$)	Leaf N concentration (%)	Grain N concentration (%)	Grain N uptake (kg ha^{-1})
Hand weeding	30.3 b	4188.0 b	332.7 b	133.5 ab	95.3 ab	27.6 bc	9223.2 ab	45.8 ab	1237.0 b	1.28 a	1.66 a	60.6 b
Weedy plot	299.0 a	3494.3 c	293.0 c	120.6 c	96.0 a	27.3 c	8229.3 b	42.4 b	1044.0 c	1.13 b	1.45 b	44.1 c
Butachlor	21.6 b	4512.0 a	350.4 a	138.3 a	94.1 b	28.2 a	9088.2 ab	49.8 a	1321.6 a	1.35 a	1.74 a	68.8 a
Bensulfuron methyl	40.8 b	4242.5 b	331.8 b	130.1 b	94.9 ab	27.4 bc	9404.8 a	45.5 ab	1206.8 b	1.28 a	1.66 a	61.4 b
Butachlor + Bensulfuron methyl	22.6 b	4500.5 a	341.4 ab	134.3 ab	94.2 b	27.9 ab	10178.7 a	44.3 ab	1210.6 b	1.38 a	1.79 a	70.3 a
LSD (0.05)	22.7	200.2	7.9	5.0	2.9	0.5	1113.0	5.0	66.2	0.11	0.15	7.2

Means within a column followed by the same letter are not significantly different at the 5% level according to Fischer's Protected LSD test.

Aminpanah et al. (2014) reported that rice grain yield was reduced by 54% because of weed competition. Heafele et al. (2004) reported that rice grain yield was reduced by 46% in weedy plots.

Paddy yields were significantly higher for plots treated with butachlor and supplementary hand-weeding (4,512 kg ha^{-1}) or a combination of butachlor and bensulfuron methyl with supplementary hand-weeding (4500.5 kg ha^{-1}) compared to the hand-weeded plot (Table 3). This result indicates that these herbicide treatments can control weeds effectively in rice fields. Manual weeding can be performed only when weeds have reached a sufficient size to be easily pulled out by hand. By that time, losses in yield may have already occurred. Moreover, some weed species such as *Echinochloa* spp. are difficult to distinguish from rice at the early stage growth; they escape from hand-weeding and reduce rice yield (Chauhan, 2012). At the same time, weed control by hand-weeding two or three times induces severe stress on rice plant and results in a decrease in grain yield. The results also showed that there was no significant difference in paddy yield between the hand-weeded plots and plots treated with bensulfuron methyl (Table 3). Bensulfuron methyl mainly controls broad leaves and sedges and provides less control of barnyardgrass. Thus, yield loss in plots treated with bensulfuron methyl is mainly due to competition between rice and barnyardgrass for nutrients and light. The results also showed that grain yield was reduced by 17% in weedy plots compared to hand-weeded plots (Table 3). The reduction in grain yield in weedy plots is mainly due to competition between rice and weeds for nutrients – principally nitrogen – and light. Some weed species such as *Echinochloa* spp. and *Cyperus* spp. used nitrogen more efficiently than rice and reduce nitrogen availability for the crop. *Echinochloa* spp. are often taller than rice plants and thus lead to a high reduction in rice growth and grain yield as a result of shading. In contrast, since the soil was kept moist by flooding in transplanted rice, competition for water can be expected to be minimal.

No significant interaction was found between *A. lipoferum* application and weed management regime in terms of grain yield and yield components (Table 1). This indicates that the herbicides had no inhibitory or stimulatory effect on the efficiency of *Azospirillum* in promoting growth and grain yield of rice. In other words, this result indicates that the effect of *A. lipoferum* on grain yield and yield components were the same in the weedy treatment, herbicide-treated, and hand-weeded plots. Therefore, it is feasible to apply the herbicide for weed control in paddy fields inoculated with *A. lipoferum*.

Rice biomass and harvest index

The main effects of *A. lipoferum* and the weed management regime were significant on rice biomass (Table 1). Plants inoculated with *A. lipoferum* produced 8% greater biomass compared to non-inoculated plants (Table 2). An increase in the number of lateral roots and root hairs in inoculated plants enlarge the root surface available for nutrients. This results in a higher nutrient uptake by inoculated roots, which in turn could be the main factor that contributes to enhancing plant growth and biomass production (Steenhoudt and Vanderleyden, 2000; Fibach-Paldi et al., 2012). The positive effect of *A. lipoferum* on rice growth was consistent with what has been shown in previous works (Panahi et al., 2015). Plots treated with herbicide or hand-weeded plots produced significantly greater biomass compared to weedy plots (Table 3). This is consistent with the results of Zhao et al. (2006). ANOVA also showed that the interaction between *A. lipoferum* and weed management regime was not significant for rice biomass. These results also indicate that the positive effect of *A. lipoferum* on rice growth can occur even when herbicides are used for weed control in paddy rice fields. In other words, herbicides had no adverse effect on *A. lipoferum* activity.

HI was significantly influenced by *A. lipoferum* inoculation and weed management regime, although the interaction between them was not significant (Table 2). HI increased by 9% when seeds were inoculated with *A. lipoferum*. (Table 2). This value is lower than the value of 16% reported by Garcia de Salamone (2012). This discrepancy may be attributed to the difference in rice cultivars, *Azospirillum* species and strains, degree of N fertilization, and soil characteristics. The result also indicates that *A. lipoferum* improved the paddy yield (19%) more than biomass (8%). Similarly, Garcia de Salamone (2012) reported that inoculation with plant growth-promoting bacteria increased aerial biomass production and grain yield of the Supremo 13 rice cultivar by 4.7% and 20.2% respectively. Significant differences in HI were found among non-weeded, hand-weeded, and herbicide-treated plots (Table 3). HI was significantly lower in weedy plots than hand-weeded or herbicide-treated plots (Table 3), which implies that weed competition reduces grain yield more than biomass. Consistent with our result, Heafele et al. (2004) reported that HI of Sahel108 cultivar was significantly reduced by 22% under weedy conditions. In contrast, Zhao et al. (2006) claimed that HI was not significantly influenced by weed competition.

Flag leaf area

The flag leaf is the topmost leaf on the cereal culm and is well-positioned to intercept light. Thus, it plays a very important role in small-grain crops such as rice during the grain-filling period. At the same time, photo-assimilate translocation from flag leaf to panicle (grain) is enhanced by the proximity of the flag leaf to the grain. ANOVA shows that the flag leaf area was significantly ($p \leq 0.01$) influenced by *A. lipoferum* and weed management regime, while the interaction between them was not significant (Table 1). The flag leaf area in plants inoculated with *A. lipoferum* was significantly higher compared to non-inoculated plants (Table 2). This may be due to better N status in inoculated plants. Nitrogen plays an important role in leaf expansion rate and tillering; therefore, N supply increases the amount of photosynthetic active radiation (PAR) intercepted by the canopy. At the same time, N supply increases leaf N concentration, which favourably affects the rate of leaf photosynthesis (Muchow and Sinclair, 1994). The highest (1321.6 cm^2 $hill^{-1}$) and the lowest (1044.0 cm^2 $hill^{-1}$) flag leaf area were recorded in butachlor-treated plots and weedy plots, respectively (Table 3). Similarly, Iqbal and Wright (1997) reported that flag leaf area of wheat was significantly reduced under weedy conditions.

Leaf N concentration

Azospirillum lipoferum and weed management regime had a significant ($p \leq 0.01$) effect on leaf N concentration (Table 1). Plants inoculated with *Azospirillum lipoferum* showed significantly higher leaf N concentration compared to non-inoculated plants (Table 2). *Azospirillum* can positively influence the N-content of plants through biological nitrogen fixation (Steenhoudt and Vanderleyden, 2000). High leaf N content has been linked to delayed leaf senescence in plants (van Oosterom et al., 2010), which in turn increases the duration of photosynthesis. Plants grown in weedy plots had significantly lower leaf N concentration compared to plants in other weed management regimes (Table 3). Moreover, no significant difference was found in leaf N concentration between herbicide-treated plots and hand-weeded plots. Lower leaf N concentration in weedy plots suggests N deficiency as a result of severe competition between rice and weeds. The decline in photosynthesis with decreasing leaf N concentration has been well documented (Muchow and Sinclair, 1994). Leaf N content is closely related to photosynthetic rate, biomass production, and Rubisco content (Ookawa et al., 2004). Similarly, Sinclair and Horrie (1989) reported that leaf N content influences biomass accumulation by means of alterations in both CO_2 assimilation and radiation use efficiency. Hence, decreased N supply for rice plants in plots that are not weeded may reduce rice yield directly by affecting photosynthetic productivity and indirectly by resulting in increased competition between weed and rice (Iqbal and Wright, 1997).

Grain N concentration and grain N uptake

Grain N concentration and grain N uptake were significantly (p≤0.01) affected by *A. lipoferum* and weed management regime (Table 1). Grain N concentration and grain N uptake were increased by 8% and 30%, respectively, because of *A. lipoferum* inoculation (Table 2). Our results are in agreement with the findings of Garcia de Salamone et al. (2010). Richardson et al. (2009) concluded that the higher N uptake is mainly due to morphological changes in inoculated rice roots, especially the enhancement in number of lateral roots and root hairs, which extend the root surface available for nutrients. The higher amount of grain N uptake in inoculated plants is mainly due to increased grain yield and increased grain N concentration after *A. lipoferum* inoculation. Plants grown in weedy plots had significantly lower grain N concentration compared to plants grown in other weed management regimes (Table 3). This was mainly due to the fact that weeds, especially C4 species such as *Echinochloa* spp., show a significantly stronger response to nitrogen than rice; therefore, nitrogen availability for rice plants is reduced in the heavy presence of weeds. It has been reported that 40-50% of the grain N uptake comes from the leaf (Liefferinga et al., 2004). Thus, it could be expected that decreases in leaf N concentrations in the heavy presence of weeds (Table 3) would have important repercussions on grain N concentration. The highest grain N uptake was found in plants grown in plots treated with butachlor by itself with supplementary hand-weeding (70.3 kg ha^{-1}) or in plots treated with butachlor in combination with bensulfuron methyl with supplementary hand-weeding (68.8 kg ha^{-1}), while the lowest grain N uptake (44.1 kg ha^{-1}) was recorded for plants grown in weedy plots (Table 3). The higher grain N uptake is attributed to both increased grain yields and higher grain N concentration in plots treated with butachlor by itself or in combination with bensulfuron methyl.

In conclusion, the results of this experiment illustrate that *Azospirillum* application significantly increases rice grain yield in non-weeded, herbicide-treated, and hand-weeded plots. Therefore, farmers can apply herbicides (butachlor by itself or in combination with bensulfuron methyl) for weed control in rice fields inoculated with *A. lipoferum*.

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