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Herbicide Application History and Different Biostimulators Effects on Bacterial Potency for Atrazine Bioremediation

Ayman H. Mansee *Doaa M. Abd El-Gwad*

Abd El-Salam M Marei

Department of Pesticide Chemistry and Technology,
Faculty of Agriculture,
Alexandria University,
Alexandria, Egypt

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Herbicide Application History and Different Biostimulators Effects on Bacterial Potency for Atrazine Bioremediation

Ayman H. Mansee[a,*], Doaa M. Abd El-Gwad[a], Abd El-Salam M. Marei[a]

[a] *Department of Pesticide Chemistry and Technology, Faculty of Agriculture, Alexandria University, Alexandria, Egypt*

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ABSTRACT

Different bacterial isolates from two types of soils differentially in atrazine applications history were isolated. Two consortiums from each soil sample (Sandy loam and Clay loam) were isolated using non-selective and then selective media and tested for their atrazine degradation efficacy. Effects of different biostimulators on consortiums growth and atrazine degradation efficacy were also studied. The consortium AyDds isolated from the soil of a long history of atrazine applications by selective media was capable of degrading 82.97% of applied atrazine in 60 days. The growth of consortiums reached the highest value when all nitrogen sources were removed from the media. The rate of atrazine degradation was increased by cells grown in a media supplemented with ammonium instead of atrazine as a sole source for nitrogen.

1. Introduction

Atrazine (6-chloro-N-ethyl-N-[1-methylethyl]-1,3,5-triazine-2,4-diamine) is considered one of the most regularly used herbicides to control broad-leaf weeds for corn, sorghum, sugarcane and other crops. The common use of this herbicide has led to its contamination in different environmental media [1-3]. Atrazine concentrations above the allowable contaminant levels for drinking water of 0.1 and 3 µg l^{-1} in Europe and the United States, respectively, have been frequently detected [4]. In addition, atrazine is described as a probable endocrine disruptor and as a carcinogenic and teratogenic agent [5]. Therefore, rapid removal of atrazine is crucial for a safe environment. Many microorganisms were isolated and studied for their capabilities for atrazine mineralization including members of genera *Pseudomonas*, *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Rastonia* and *Norcardioides* [6-10]. Of all the studied bacteria, *Pseudomonas* sp. strain ADP might be the best-characterized atrazine mineralizing one [11-12]. In the natural environment, organic compound degradation is often carried out by a mixed microbial community. Evidences suggested that rates of growth and substrate utilization are frequently higher in enriched mixed cultures than those rates in pure cultures isolated from the mixture [13]. For atrazine degradation, bacteria consortia appeared to be more common and more efficient than individual species [14-15]. Accordingly, current investigation studied using bacterial consortiums for degradation atrazine. In addition, bioremediation can be improved by enhancing microbial activity which could be stimulated through addition of organic matter [16].

Atrazine degrading bacteria normally use atrazine either as a nitrogen or carbon source thus; influences of nitrogen and carbon compounds availability on their efficiency for atrazine biodegradation has been targeted by several studies. Dehghani *et al.* [17] assessed the effects of carbon and nitrogen sources on atrazine biodegradation by mixed bacterial consortium. Sodium citrate and sucrose had the highest influences on atrazine biodegradation rates (87.22%) among different carbon sources. Atrazine biodegradation rate decreased more quickly by the addition of urea (26.76%) compared to ammonium nitrate. Effects of carbons, nitrogen, and Tween-80 supplements on the degradation efficiency of *Ps. putida* HK-6 in media containing simazine as target substrate were studied by Cho *et al.* [18]. The most effective simazine degradation was shown in the presence of molasses as supplemental carbon source. Addition of nitrogen sources produced a delayed effect for the target substrate. Tween-80 enhanced the degradation of target substrate. Kannika *et al.* [19], studied the atrazine degradation ability of atrazine-degrading bacterium (strain KU001) at various nitrogen sources. Atrazine degradation was not inhibited in cells grown on ammonium, nitrate, or urea as compared with cells cultivated on growth limiting nitrogen sources. During the atrazine degradation process, the supplementation of nitrate completely inhibited atrazine degradation activity in strain KU001, whereas ammonium and urea had no effect. The degradation ability of *Rhodococcus* sp. BCH2 at various temperatures (20–60 °C), pH (range 3–11), carbon (glucose, fructose, sucrose, starch, lactose, and maltose), and nitrogen (ammonium molybdate, sodium nitrate, potassium nitrate, and urea) for triumph optimum atrazine degradation were studied by Kolekar *et al.* [20]. This results indicate that atrazine degradation at higher concentrations (100 ppm) was pH and temperature dependent. However, glucose and potassium nitrate

* Corresponding author: Department of Pesticide Chemistry and Technology, Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

Tel.: +20 100 020 1988.

E-mail address: amansee@alex-agr.edu.eg (A.H. Mansee).

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were optimum carbon and nitrogen source, respectively. The current investigation aims to isolate bacterial isolates from soil with a different history of atrazine application, assessing their capabilities for degrading atrazine; and finally studying effects of different nutrients as bacterial biostimulation on atrazine bioremediation process.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Herbicide: -Atrazine (6- chloro - N^2 – ethyl - N^4 – isopropyl -1, 3, 5 - triazine-2, 4-diamine), standard (≥98%) and formulated (Atrazex 80% W.P) were obtained from Kafer El-Zayat Pesticides and Chemical Company, Kafer El-Zayat, Egypt.

Thiamine-HCl, biotin, folic acid, nicotinamide, and pyridoxine-HCl were obtained from Sigma–Aldrich Chemical Co., USA. Methanol, sulfuric acid, Ethylenediaminetetraacetic acid, potassium dihydrogen phosphate and potassium monohydrogen phosphate were obtained from El-Gomhouria Co. Alexandria, Egypt.

2.1.2. Medias and buffers

1. Atrazine medium (selective) comprising of (g/L) of K_2HPO_4 (1.6), KH_2PO_4 (0.4), $MgSO_4.7H_2O$ (0.2), NaCl (0.1), $CaCl_2$ (0.02), sucrose (1), sodium citrate (1), atrazine stock solution (2.5 ml), salt stock solution (20 ml.), and vitamin stock solution (20 ml).
2. The salt stock solution was prepared by dissolving (g/L) of EDTA (2.5), $ZnSO_4$ (11.1), $FeSO_4$ (5.0), $MnSO_4.H_2O$ (1.54), $CuSO_4.5H_2O$ (0.4), Co $(NO_3)_2$ $6H_2O$ (0.25), $Na_2B_4O_7.10H_2O$ (0.18), and 5.0 milliliters of concentrated H_2SO_4 to delay precipitation of salts. Also, vitamins stock solution was prepared by adding (mg/L) of thiamine-HCl (5), biotin (2), folic acid (2), nicotinamide (10), and pyridoxine-HCl (10). The atrazine stock solution was prepared in methanol (20 mg/ml), and the pH was adjusted to 7.3.
3. Non selective media is comprising of (g/L) of K_2HPO_4 (1.6), KH_2PO_4 (0.4), $MgSO_4.7H_2O$ (0.2), NaCl (0.1), $FeCl_3$ (0.02), glucose (1), sodium citrate (1), $(NH_4)_2SO_4$ (0.5), yeast extract (2.5), and tryptone (5).
4. Luria-Bertani media (L.B) is comprising of (g/L) of tryptone (10), yeast extract (5) and NaCl (10).
5. The U buffer containing: 10 mM sodium phosphate pH 7, and 0.1 mM $MgSO_4$ in one liter of deionized water.
6. Buffer: prepared by dissolving (g/L) of $Na_2HPO_4.12H_2O$ (0.2), and KH_2PO_4 (0.1).

2.1.3. Soil

Two types of soils are used in the current investigation. The first soil analysis (Sandy loam texture, organic matter 1.78%, pH 7.59), had two years history of different applications with herbicides including atrazine. The second soil analysis (Clay loam texture, organic matter 1.94%, pH 7.81), peering a long history of atrazine applications (more than 10 years). Soil samples are collected from the top layer 0-30 cm, air dried, crushed and sieved to pass through a 2 mm sieve.

2.2. Methods

2.2.1. Bacteria isolation

Isolation was carried out according to Mandelbaum *et al.* [14]. Five grams of soil sample were taken and inoculated into 20 ml of either selective or nonselective media. Cultures are incubating at 30°C for one week without shaking in dark conditions. After one week, 0.5 ml from incubated cultures was transferred to 20 ml of freshly prepared media for one week under the same conditions. Then, one ml from inoculated media is transferred onto nutrient agar media (NA) plates, and then incubates at 30ºC for 5 days. Isolation of bacterial colonies was carried out depends on the base of differentiation according to the morphological shape and growth pattern [21].

2.2.2. Influence of incubation time on isolates efficiency

Isolates efficiency were assayed with two different methods: UV and turbidity. In the first method the concentration of remaining atrazine in supernatant after incubation with bacterial isolate was determined at 221 nm, while in the second one, bacterial growth was measured at 600 nm after incubation with atrazine.

- Using UV Spectroscopy for measuring Atrazine degradation

The method of García-Gonzalez *et al.* [22] for assaying atrazine degradation spectrophotometrically was used as a tool for measuring bacterial isolates efficiencies. A 100 µl of consortium of the isolated bacteria incubated at 30°C overnight with shaking in 3 ml of atrazine media fortified with ammonium chloride as the sole source of nitrogen. After incubation period cells are harvested by centrifugation (15min/5000 rpm) and pellets were washed three times with phosphate-buffered saline solution pH 7.0 and resuspended in atrazine treated media. Cultures were subsequently shaken overnight at 30°C, cells were harvested by centrifugation (15 min/5000 rpm) and pellets are washed three times with U buffer and resuspended in the same buffer until the OD_{600} reaching 0.25. Three replicates for each consortium as well as control were prepared by taking 3 ml of the bacterial or control solution were incubated with 50 ppm atrazine for (2, 7, 14, 21, 28, and 60) days. At each time intervals, bacterial isolates efficiencies were assessment by centrifugation for 3 min. at 5000 rpm and supernatant was subject for atrazine determination spectrophotometrically by measuring the absorbance at 221 nm.

- Using turbidity or measuring the growth

The method of García-Gonzalez *et al.* [22] for growing isolates was carried out with minor modification in the current investigation as follow. One colony for bacterial isolates or 100 µl of consortium was added separately in 3 ml of atrazine medium modified by adding 50 ppm ammonium chloride instead of atrazine in the media. This mixture was incubated at 30°C overnight with shaking. For assaying the isolates efficiency, 100 µl of growing solution were added to 5 ml of atrazine medium (50 ppm atrazine) and incubated at 30°C for 2, 7, 14, 21, 28, and 60 days. At each interval the solution turbidity was determine by measuring the absorbance at 600 nm as indictor for growth of bacteria.

2.2.3. Biostimulation effects of different nutrients

Growing of soil bacterial isolates in different nutrients before incubated with targeted atrazine was used in the current investigation as tools for assaying consortium biostimulation influences on atrazine bioremediation process. A 100 µl of consortium of the isolated bacteria was incubated with 3 ml of L.B medium, at 30°C overnight with shaking. Then, 200µl of inoculation were transferred to 10 ml of M.S medium with one of the treatments presented in the Table1 and incubated for 48 hours at 30°C. Cells then were harvested by centrifugation (15min/5000 rpm) and pellets were washed three times with phosphate-buffer, pH 7.0 and resuspended in the buffer until the OD_{600} reaching 0.1. Ten µl of growing solution were added to

one ml of atrazine medium (50 ppm atrazine) and incubated at 30°C for 2,5,7,&9 days. At each interval, the solution turbidity was determined by measuring the absorbance at 600 nm as indictor for bacterial isolates growing.

Table 1 Contents of biostimulators treatments incubated with consortiums.

Treatments	A	B	C	D	E	F	G
NH_4Cl	-	-	+	+	-	+	+
Glucose	+	+	+	+	-	-	+
Atrazine(ppm)	-	50	-	50	50	50	100

2.2.4. Confirmation the biostimulation influence on isolate efficiency

A 100 µl of AyDds consortium isolated bacteria was incubated in 3 ml of L.B medium, at 30°C overnight with shaking. Two hundreds µl of inoculation are transferred to 10 ml of M.S medium with different treatments, as presented in Table 1, and incubated at 30°C, for 48 hours. Cells were harvested by centrifugation (15min/5000 rpm) and pellets were washed three times with phosphate-buffer, pH 7.0 and resuspended in the buffer until the OD_{600} reaching 0.1 (the lowest record). Ten µl of growing (incubated) solution were added to one ml of atrazine medium (50 ppm atrazine) and incubated at 30°C for 2,4,7, and 14 days. At each intervals the growth of bacteria was determine by measuring the absorbance at 600 nm. For atrazine determination, treated samples were centrifuged (5000 rpm) immediately for 3 min. Then atrazine concentration in the supernatant was determined at 221 nm as mentioned previously.

2.2.5. Statistical analysis

All data were statistically analyzed using SPSS version 19 statistical software. Statistical differences between treatment means ($P > 0.05$) were determined by Least Significant Differences (LSD) test.

3. Results and Discussion

3.1. Bacteria isolation

Different bacterial isolates from two types of soils samples were isolated using selective and nonselective media as mentioned earlier. Differentiation between isolates was conducted based on morphological and growth feature according to Bergey's manual of systemic bacteriology. Isolates from first soil using nonselective media were presented as AyDd (from 1 up to 6), while isolates from the same soil using selective media were presented as AyDds (from 1 up to 5). Whereas those from second soil using nonselective media were symbolled as AyDt from 1 to 7, and AyDts1, AyDts2, AyDts3, AyDts4, and AyDts5 when using selective media for isolation.

3.2. Efficiency of Bacterial Isolates on Atrazine Degradation

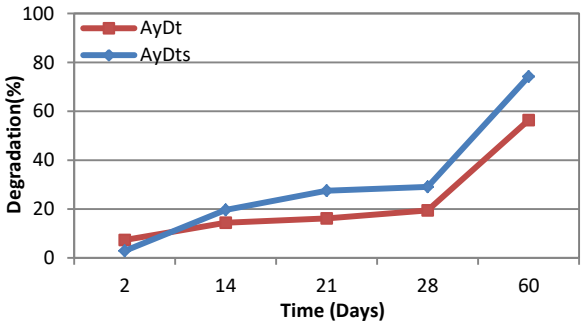
3.2.1. Measuring atrazine degradation by UV spectrophotometric

Bacterial isolates were incubated with 50 ppm of atrazine for 2, 14, 21, 28 and 60 days to assess their potentially for atrazine biodegradation. Table 2 and Fig. 1 illustrate the degradation percentages as a monitor of bacterial isolates efficiencies of first soil (AyDt and AyDts) as well as those of second soil (AyDd and AyDds).

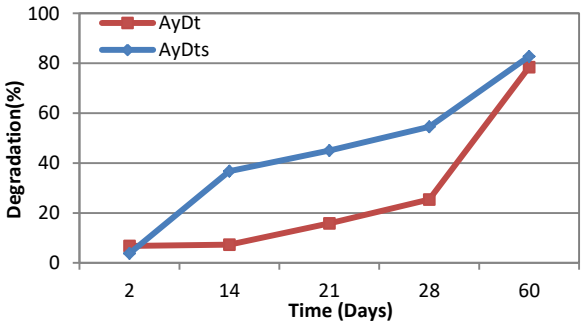
The atrazine degradation percent achieved when using consortium AyDt were 7.20, 14.11, 15.89, 19.35 and 56.67% at 2, 14, 21, 28 and 60 days, respectively. Consortium AyDt showed the maximum atrazine degradation percent at 60 days with corresponding values 56.67 %.

Table 2 Efficiency of different bacterial isolates for degrading atrazine

Degradation (%)	Time (day)					
Isolates	2	14	21	28	60	Mean
AyDt	7.20	14.11	15.89	19.35	56.67	22.64^{d}
AyDts	2.69	19.77	27.33	28.68	73.64	30.42^{b}
AyDd	6.66	7.4125	16.04	25.46	78.62	26.84^{c}
AyDds	3.74	36.519	44.99	54.22	82.97	44.49^{a}
Mean	5.0725^{e}	19.45^{d}	26.06^{c}	31.93^{b}	72.98^{a}	
LSD interaction				5.52		



(a) 1st soil efficiency as selective vs. non-selective.



(b) 2nd soil efficiency as selective vs. non-selective.

Fig. 1 Efficiency of different bacterial isolates for degrading atrazine.

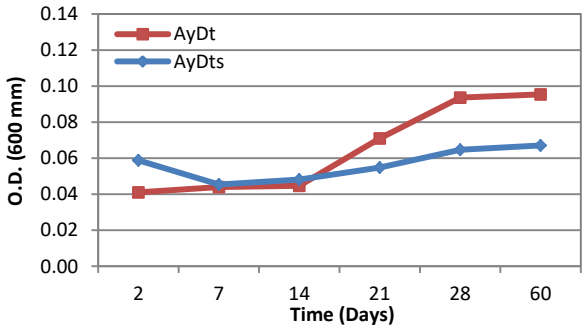
For the consortium AyDts, atrazine degradation percentages were 2.69, 19.77, 27.33, 28.68 and 73.64% at 2, 14, 21, 28 and 60 days, respectively. The atrazine degradation percentages were 6.66, 7.41, 16.04, 25.46 and 78.62% at 2, 14, 21, 28 and 60 days, respectively when consortium AyDd was used. The highest degradation percent was recorded at 60 days. The atrazine degradation percent when consortium AyDds tested were 3.74, 36.519, 44.99, 54.22 and 82.97% at 2, 14, 21, 28 and 60 days, respectively. This result showed significant differences between consortiums. The highest degradation percent was obtained from consortium AyDds (when recorded 44.49% as mean for all tested intervals). In addition, the obtained results clarify significant differences between degradation percent at each time intervals.

Bacterial isolates from the soil of long atrazine applications history were more potency for degrading atrazine than those of isolates of the other soil. In addition, the consortiums isolated by selective media showed higher atrazine degradation efficiency than those of nonselective consortiums. This finding was supported by Singh *et al.* [23] who isolated an *Acinetobacter* species from a soil heavily contaminated with atrazine and found are capable for degrading up to 250 ppm. Also, Vaishampayan *et al.* [24) were online with current results when they isolate different species of bacteria from different

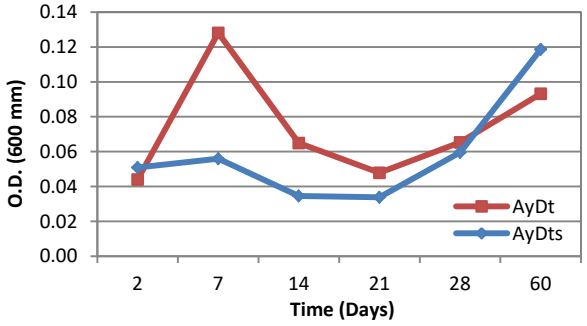
agriculture soil that had history of atrazine applications. They reported that their isolates had the capabilities for metabolizing atrazine. Moreover, Umar *et al.* [25] used a batch enrichment technique to isolate atrazine-degrading *Rhodococcus* sp strain from an agricultural land with history of atrazine application in Bauchi state, Northeastern Nigeria. The strain was identified on the basis of physiological, biochemical and 16S rRNA gene sequencing. Growth studies and HPLC analysis showed that the strain has potential of atrazine degradation. Finally, *Arthrobacter sp*. strain AK-YN10 was an s-triazine pesticide degrading bacterium isolated from a sugarcane field in Central India with history of repeated atrazine use by Sagarkar *et al.* [26]. AK-YN10 was shown to degrade 99 % of atrazine in 30 h from media supplemented with 1000 mg/l^{-1} of the herbicide.

3.2.2. Measuring isolates growth using turbidity

Figure 2 demonstrates the growth of bacterial isolates after incubated with 50 ppm of atrazine for (2, 7, 14, 21, 28 and 60) days using the corresponding turbidity values. The growth of bacterial isolates was increased by increasing incubation time. The turbidity value of bacterial isolates after 60 days incubation was ranged between 0.068 and 0.117 for consortium AyDts and AyDds, respectively.



(a) Growth 1st soil isolates (selective vs. non-selective media).



(b) Growth 2nd soil isolates (selective vs. non-selective media).

Fig. 2 Growth of Different Bacterial Isolates of the two tested soil.

The growth of consortiums AyDt, AyDts, AyDd and AyDds was measured by measuring optical density at 600 nm. All consortiums showed similar growth patterns with an increase in optical density from 0.05 to 0.093 within 60 days. The low growing values of consortiums could be a result of using ammonium chloride as a sole of nitrogen source before incubated with atrazine. Although, Sing *et al.* [23] found that no inhibition in growth of bacterial isolate was observed even at 250 ppm. Siripattanakul *et al*. [27] found that the growth of stable mixed cultures (MC1, MC2 and MC3 and J14a) increase in optical density of 0.05 to between 0.5 and 0.7 within 72 h in bacterial medium containing atrazine of 100 µg l^{-1}.

3.3. Biostmulation effects of different nutrients

3.3.1. Consortiums isolated by non-selective media

Figures 3 and 4 exemplify the effect of biostimulators (A, B, C, D, E, F and G) on growth of non-selective consortiums AyDd and AyDdt after incubated with 50 ppm of atrazine for (2 ,5, 7 and 9) days. The highest growth values were obtained from treatments A and C; however, treatments E and G lowered the growth of tested consortiums.

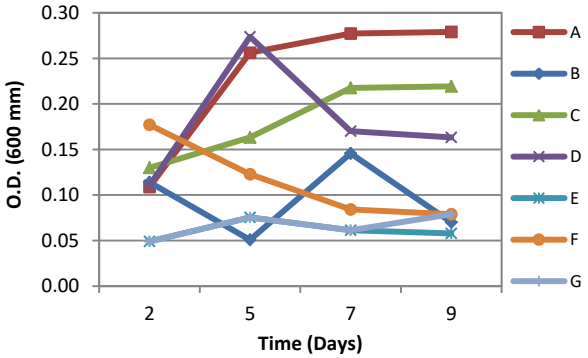


Fig. 3 Effects of Different Biostimulators Treatments on the Growth of AyDd Consortium.

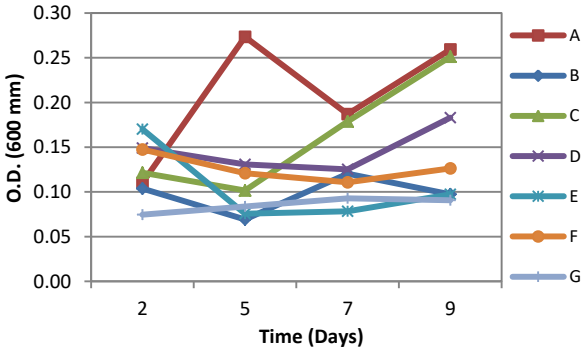


Fig. 4 Effects of Different Biostimulators Treatments on the Growth of AyDt Consortium.

3.3.2. Consortiums isolated by non-selective media

Figure 5 shows the effects of treatments (A, B, C, D, E, F and G) on growth of consortium AyDds following incubated with 50 ppm of atrazine for 2, 5, 7 and 9 days. The results show the same trend of non-selective consortiums. The highest growth values were recorded in treatment A however, treatments E and G lowered the growth of tested consortiums.

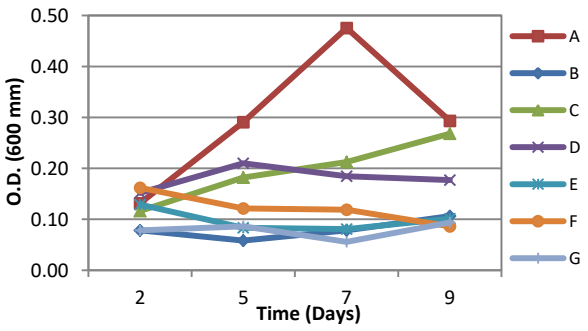


Fig. 5 Effects of Different Biostimulators Treatments on the Growth of AyDds Consortium.

Figure 6 represents the effect of treatments (A, B, C, D, E, F, and G) on growth of consortium AyDts after incubation with 50 ppm of atrazine for (2, 5, 7 and 9) days. The highest growth was obtained from treatments C and D, while treatment G was lowering the growth of tested consortiums. Current results revealed that the absence of all nitrogen sources (treatment A) is much better than the absence of one source of nitrogen (treatment B) in all consortiums except consortium AyDts. In addition, the growth of consortiums was decreased because of treatments E and F compared to C and D and this could be due to the absence of glucose in both E & F. The growth of consortiums in treatment G recorded the lowest value due to the concentration of the atrazine (100 ppm).

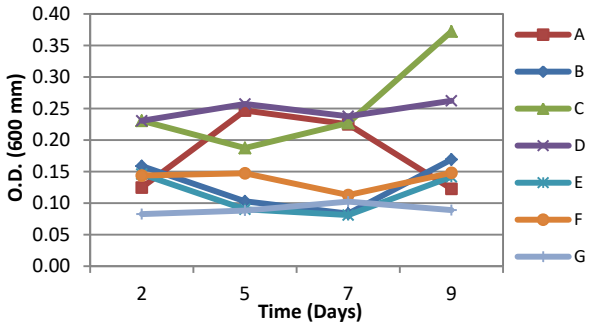


Fig. 6 Effects of Different Biostimulators Treatments on the Growth of AyDts Consortium.

Other researcher found that their isolates were much effective for degrading atrazine when it was the only source for carbon and nitrogen. Strong *et al*. [10] who clarify that *Arthrobacter aurescens* strain TC1 was grown in liquid minimal R medium containing 3,000 mg of atrazine per liter as the sole C and N source. After 7 days of growth at 30°C, the culture reached an A600 of 2.2. In addition, Cai *et al*. [28] found that the growth rate of strain AD1 was nearly twice as fast as *Pseudomonas* sp. strain ADP at 30°C when atrazine was used as the sole nitrogen source and sodium citrate as carbon source. The O.D.600 of strain AD1 reached 0.86 after 48-h incubation at 30°C, while under the same conditions the O.D.600 of strain ADP was 0.48. Qingyan *et al*. [29] found that the OD600 of strain AD26 reached 2.6 after 48 h incubation at 30°C when atrazine (500 mg/l) was used as the sole nitrogen source. Under the same conditions, the OD600 of strain ADP was only 1.33. Wang and Xie [30] studied that addition of nitrate, ammonia or urea on the growth of the strain DAT1. The addition of nitrate caused the highest growth of the strain DAT1. However, when atrazine was the sole nitrogen source for the strain DAT1, the growth was very slow. Moreover, the addition of cyanuric acid resulted in no higher growth of the strain DAT1, compared with the control treatment (atrazine as the sole nitrogen source), implying that cyanuric acid could not be used as nitrogen source for the strain DAT1.

3.4. Confirmation of biostimulation effluence on isolate efficiency

3.4.1. Using Turbidity

Figure 7 exemplifies effects of treatments (A, B, C, D, E, F and G) on growth of consortium AyDds after incubated with 50ppm of atrazine for (2, 4 7 and 14) days. Such figure conducted parallel, to confirm determination of atrazine degradation results, Fig. 8. The highest growth was obtained from treatment A, while the lowest growth value was record when treatment G was used. The current results showed that the absence of all nitrogen sources (treatment A) was the best biostimulator for the bacterial growth bacteria. The growth of consortiums recorded the lowest value when tested in treatment G due to the concentration of the atrazine (100 ppm).

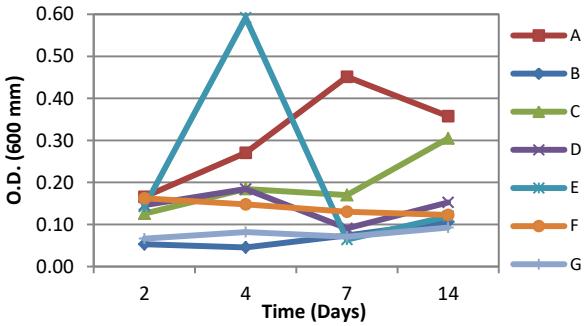


Fig. 7 Confirmation Effects of Different Biostimulators Treatments on the Growth of AyDds Consortium.

3.4.2. Using UV Spectroscopy

Table 3 and Fig. 8 exemplify the effect of treatments (A, B, C, D, E, F and G) on the efficiency of consortium AyDds on degrading 50 ppm of atrazine for 2,4,7 and 14 days.

Table 3 The atrazine degradation percent.

Treatments	Time (days)				
	2 days	4 days	7 days	14 days	Mean
A	5.18	24.19	31.98	32.05	23.35^{c}
B	0.00	11.00	26.71	31.68	17.33^{d}
C	21.54	26.91	30.49	31.17	27.53^{ab}
D	9.62	10.25	36.35	51.17	26.85^{abc}
E	14.68	16.24	26.67	35.47	23.27^{c}
F	13.12	14.58	44.14	47.72	29.89^{a}
G	11.46	14.56	31.17	40.39	24.40^{bc}
Mean	10.8^{d}	16.82^{c}	32.50^{b}	38.52^{a}	
LSD interaction				5.592	

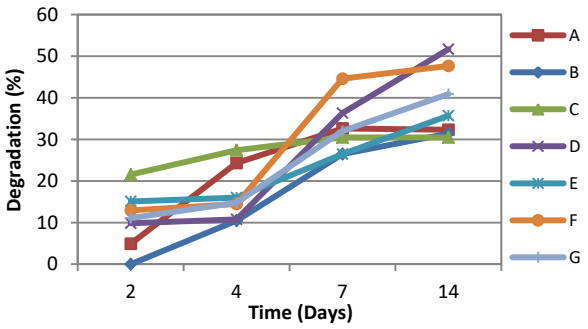


Fig. 8 Effects of Biostimulators Treatments on the Efficiency of AyDds Consortium for Degrading Atrazine.

The atrazine degradation percent achieved when using A treatment were 5.18, 24.19, 31.98 and 32.05% at 2, 4, 7 and 14 days, respectively. For treatment B the atrazine degradation percent were 0.00, 11.00, 26.71 and 31.68% at 2, 4, 7 and 14 days, respectively. The atrazine degradation percent were 21.54, 26.91, 30.49 and 31.17% at 2, 4, 7 and 14 days, respectively when treatment C is used. The atrazine degradation percent when treatment D tested were 9.62, 10.25, 36.35 and 51.17% at 2, 4, 7 and 14 days, respectively. The atrazine degradation percent achieved when using treatment E were 14.68, 16.24, 26.67 and 35.47% at 2, 4, 7 and 14 days, respectively. For treatment F the atrazine degradation percent were 13.12, 14.58, 44.14 and 47.72% at 2, 4, 7 and 14 days, respectively. The atrazine degradation percent were 11.46, 14.56, 31.17% and 40.39 at 2, 5, 7 and 14 days, respectively when treatment G was used.

The current results showed non-significant differences among treatments C, D, and F and there were no significant differences between treatments A and G. The highest degradation percent was recorded from treatment F (29.89). Moreover, results clarifying significant differences between degradation percentages at each interval time. The atrazine degradation rate was increased in cells grown when ammonium used as a sole source for nitrogen (treatments C, D and F) compared to cells cultivated on limiting nitrogen sources. The results in Table 2 were clarifying the role of biostimulators in enhancing the efficiency of consortium AyDds. The effect of treatment D (atrazine + glucose + ammonium chloride) after 2 and 14 days were 2.6 and 1.4 folds respectively. While when the treatment F (atrazine + ammonium chloride) used the efficiency were increased to 3.5 and 1.3 folds for the same intervals times respectively.

The obtained results were compatible with those reported by Wang and Xie [30] who found that the degradation rate of atrazine was increased by addition of nitrate, ammonia or urea. However, a marked degradation still could be observed when atrazine was the sole nitrogen source for the strain DAT1. In addition, the obtained results were opposite to those reported by García-Gonzalez *et al*. [22] who found that cells grown on ammonium as the sole nitrogen source failed to reduce the atrazine concentration in the supernatant significantly. The presence of atrazine in addition to ammonium in the growth medium did not stimulate atrazine degradation.

4. Conclusion

Isolated bacteria were differentiated according to the soil history of the atrazine application. In addition, influences of history and different biostimulator on isolates capabilities for degradation atrazine were observed. The consortiums isolated by selective media were high efficient atrazine degradation than those of nonselective consortiums. The highest atrazine degradation percentage was obtained from consortium AyDds (82.97%) at 60 days, while consortium AyDt showed the lowest percentage (56.67%) at the same time.

The growth of isolates was inhibited in cells grown on treatment G, as compared with cells cultivated on growth limiting nitrogen sources (treatment A). Finally, isolation of microorganisms from herbicides long history applied soil and using smart biostimulation, could be a good opportunity for degrading atrazine herbicide.

List of Abbreviations

NA	nutrient agar
L.B	Luria broth
M.S	Mineral salt
Treatment A	Glucose
Treatment B	atrazine + glucose
Treatment C	glucose + ammonium chloride
Treatment D	atrazine + glucose + ammonium chloride
Treatment E	Atrazine
Treatment F	atrazine + ammonium chloride
Treatment G	100 ppm atrazine + glucose + ammonium chloride
AyDd	Bacterial isolates from the soil of long atrazine applications history by nonselective media
AyDds	Bacterial isolates from the soil of long atrazine applications history by selective media
AyDt	Bacterial isolates from the soil of a minor atrazine applications history by nonselective media
AyDts	Bacterial isolates from the soil of a minor atrazine applications history by selective media

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