EFFECTS OF ATRAZINE AND BUTACHLOR ON GROWTH OF MUNG BEAN (*Vigna radiata*), WATER SPINACH (*Ipomoea aquatica*) AND SOIL BACTERIA

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Abstract

Atrazine and butachlor are herbicides extensively applied to control weeds. In this study, the effects of these compounds on the growth of mung bean, water spinach and soil bacteria were determined. The results showed that all offsets of mung bean and water spinach were killed after amendment with atrazine. However, the effects of butachlor on both plants in the dry season were unapparent, while this compound used at 2.0× (4.0 L/ha) severely reduced the growth of the plants in the rainy season. For example, the length of beanstalks, and weight of beanstalks and roots of mung bean grown in the rainy season at 2.0× butachlor were lower by 36.2% and 44.3% compared to the control, respectively. Moreover, the application of butachlor decreased the yield of mung bean. In addition, both atrazine and butachlor inhibited the growth of soil bacteria even after two months. This study showed that these herbicides are highly toxic to mung bean, water spinach, and soil bacteria.

Keywords: *Atrazine, butachlor, mung bean, soil bacteria, water spinach.*

ẢNH HƯỞNG CỦA ATRAZINE VÀ BUTACHLOR ĐẾN SỰ SINH TRƯỞNG CỦA ĐẬU XANH (*Vigna radiata*), CÂY RAU MUỐNG (*Ipomoea aquatica*) VÀ VI KHUẨN ĐẤT

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Tóm tắt

Atrazine và butachlor là những hoạt chất của chất diệt cỏ được sử dụng rộng rãi để kiểm soát cỏ dại. Trong nghiên cứu này, ảnh hưởng của atrazine và butachlor đối với sự sinh trưởng của đậu xanh, rau muống và số lượng vi khuẩn đất đã được khảo sát. Kết quả cho thấy rằng tất cả đậu xanh và rau muống đều bị chết sau khi phun xịt atrazine. Tuy nhiên, tác động của butachlor đối với cả hai loài cây trồng trong mùa khô là không rõ ràng, trong khi hợp chất này làm giảm sự sinh trưởng của cây trồng trong mùa mưa khi sử dụng ở nồng độ 2,0× (4.0 L/ha). Ví dụ, chiều dài của cây đậu, và trọng lượng của thân đậu và rễ của đậu xanh trồng trong mùa mưa ở nồng độ 2,0× butachlor giảm lần lượt là 36,2% và 44,3% so với đối chứng. Hơn nữa, việc sử dụng butachlor làm giảm năng suất của đậu xanh. Ngoài ra, cả atrazine và butachlor đều ức chế sự phát triển của vi khuẩn đất sau khi sử dụng. Nghiên cứu này chỉ ra rằng cả atrazine và butachlor có độc tính cao đối với đậu xanh, rau muống và vi khuẩn trong đất.

Từ khóa: *Atrazine, butachlor, đậu xanh, vi khuẩn đất, rau muống.*

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1. Introduction

Herbicides have been widely applied to control weeds, contributing significantly to increase crop yields. The application of herbicides reduces labor and production costs. Herbicides are the key to sustainable crop production throughout the world and will remain the mainstay for weed control in the foreseeable future. However, the intensive use of herbicides resulted in serious environmental pollution. Herbicides persist on the top soil, and can be leached down in groundwater (Ayansina et al., 2003). The accumulation of the toxic chemicals is harmful to microorganisms, plants, animals, and humans. Soil microorganisms play an important role in pesticide detoxification as well as pesticide degradation (Ayansina & Oso, 2006). However, herbicides possibly have harmful effects on the soil micro flora by reducing the flora's activities. The changes in community of soil microorganisms caused by pesticides are an important indicator to evaluate pesticides' effect on soil ecology.

Butachlor (N-butoxymethyl-2-chloro-2',6'-diethylacetanilide) is a pre-emergence herbicide widely used in rice fields and other crops viz. corn, soybean (Dwivedi et al., 2012). The herbicide can be applied at doses of 1.0-5.0 L/ha (Aminpanah et al., 2013). It is one of the most commonly used to control a wide range of annual grass and broad leaf weeds (Wang et al., 2013). Butachlor has been shown to affect microbial populations and enzyme activities (Chen et al., 1981; Min et al., 2001).

Atrazine (6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine) is another selective pre-emergence herbicide to control broadleaf and grassy weeds. Atrazine has become one of the world's most popular herbicides (Yan et al., 2015). It is usually used at doses of 1.0-2.0 kg/ha. The compound kills sensitive plants by blocking the electron flow in photosystem II (PSII), leading to the inhibition of CO_2 assimilation and the generation of large amounts of reactive oxygen species (Hess, 2000). Atrazine is considered to be high persistent in the environment, leading to the contamination of food, soils, and water resources (Croll, 2011; Graymore et al., 2001).

InVietnam, mung bean (Vigna radiata) and water spinach (Ipomoea aquatica) are widely cultivated. Herbicides are used to control weeds for these crops. Water spinach is considered to be an aquatic plant, but it is also cultivated in upland fields. In the Mekong delta, these plants are usually cultivated in rotation with rice and other crops. Therefore, herbicides used in former crops may affect the later ones. However, no study on the effects of these herbicides on the growth and development of mung bean and water spinach has been carried out. Therefore, this study determined the effects of atrazine and butachlor on the growth of mung bean, and water spinach and soil bacteria.

2. Materials and methods

2.1. Soil collection and fertilizing

Soil samples were collected from a mango garden, at a depth of 10 - 50 cm, in Cao Lanh city, Dong Thap Province. Soil samples were ground and removed large debris to obtain soil size smaller than 1.0 cm. Chemical and physical properties of soil were analyzed using the APHA method (APHA, 1998). Soil samples used for doing experiments in the dry season and the rainy season were collected from the same site as shown in Table 1. The soil was then transferred into thermocol boxes up to the depth of 15 cm. The dimension of each thermocol box was 41×60×15 cm (width×length×depth). Four holes were separately punched through the bottom of each box so that redundant water could be removed. NPK fertilizer (Binh Bien Company, Ninh Binh) containing 20% total N, 10% P_2O_5 and 5% K_2O was used at 200 kg/ha as its label package instructed. The fertilizer was diluted in tap water and sprayed on soil using a hand-held sprayer with a volume of 2.0 L, and mixed thoroughly.

Table 1. Physicochemical properties of the dried soil

	Units	Soil
Silt	%	28.6 ± 5.5
Sand	%	50.2 ± 4.8
Clay	%	21.1 ± 4.4
Total organic carbon	%	3.0 ± 0.4
Total N	%	0.15 ± 0.05
P_2O_5	ppm	25.5 ± 4.4
K_2O	ppm	5.5 ± 0.5
pH		6.6 ± 0.1

2.2. Seed sowing and herbicide amendment

The seeds of mung bean and water spinach purchased from Vinaseed Company (Dong Thap) were pregerminated for 24 hours at room temperature (~30°C) by placing them in petri dishes on wet paper towels and incubating in darkness. Thereafter, twelve seeds were sown in each box at equidistant positions to give a density of 0.02 m^2 per seed.

To avoid the effects of adjuvants added in herbicides, butachlor and atrazine (purity > 98%) bought from Sigma-Aldrich were used in all experiments. The compounds were diluted in absolute ethanol at 0.1 mM and used as stock solutions. The stocks were added into water and sprayed on soil at 2.0 L of butachlor or 2.0 kg of atrazine per ha (1.0×). The experiments were also carried out at doses of 0.5× (1.0 kg atrazine or 1.0 L butachlor/ha), and 2.0× (4.0 kg atrazine or 4.0 L butachlor/ha). Soil without any herbicide served as a control. The herbicides were sprayed after sowing four days, before germinating any weeds. All experiments were conducted four replicates.

Before seed sowing, tap water was sprayed to obtain soil moisture content of 30% of field capacity. The cultivated plants were also watered regularly from seed sowing to harvesting.

2.3. Weather conditions

The experiments were conducted in two independent stages. The first stage was started at the beginning of February to the end of March, 2020. The second stage was from mid-August to mid-October, 2020. The weather conditions were described in Table 2. The first stage was carried out in the dry season with long sunshine duration and low rainfall, while the second one was conducted in the rainy season with short sunshine duration and high rainfall.

Table 2. Weather conditions during experiment. All data were showed on average (provided by Center of Meteorological and Hydrological Administration, Dong Thap)

Weather conditions	First stage	Second stage
Temperature (oC)	27.7	27.5
Air humidify (%)	78.5	84.4
Sunshine hour (hours/day)	9.0	5.3
Rainfall (mm/day)	0.057	10.13

2.4. Plant harvesting

After two months, all mung bean and water spinach were harvested. Water was added until the soil became fully saturated, so that all plants were easily uprooted. The plants were cleaned with tap water, air-dried and measured their fresh weight and length. Mung bean and water spinach were then dried using a Memmert oven (Germany) at 75°C for two days before reweighing.

2.5. Enumeration of soil bacteria

Bacteria enumeration was conducted immediately after herbicide amendment, 20 days, 40 days and 60 days. Soil samples were collected by a sterilized spoon at a depth of 2.0 - 3.0 cm. The soil was transferred to a lab within several hours. Populations of bacteria and fungi were enumerated and expressed as number of CFUs/g soil. Soil samples were serial diluted and placed on agar plates containing mineral salt medium supplemented with glucose (1.0 g/L) and ammonium sulfate (1.0 g/L).

2.6. Statistical analysis

All obtained data from at least three experiment replicates are shown as the mean ± standard deviation. Significant differences among means were statistically analyzed using one-way Duncan's test ($p < 0.05$) in SPSS program version 22.0.

3. Results and discussion

3.1. Effects of butachlor and atrazine on soil bacteria

Soil samples were collected at the same place, so bacteria numbers were the same at the beginning. As described at Figure 1, 2, and 3, the numbers of bacteria increased in all soil samples probably because soil was enriched with NPK. Moreover, suitable conditions such as soil moisture content might have favored the growth of microorganisms. However, both atrazine and butachlor negatively affected bacteria growing in soil, in both dry and rainy seasons in most treatments. The increase of bacteria numbers in soil without any herbicide was significantly higher than in soil treated with atrazine and butachlor in the dry season in most treatments.

The numbers of bacteria in soil cultivated with water spinach were not statistically different at 0.0×, 0.5×, 1.0× and 2.0× after 20 days of treatment with burachlor (Figure 1). However, bacteria numbers in untreated soil in the dry season were significantly higher than those in treated soil on the day of 40 and 60. On the other hand, the differences of bacteria population among treatments were unapparent in the rainy season (Figure 1B).

For bacteria population in soil cultivated with mung bean, butachlor also inhibited the growth of bacteria at all concentrations (Figure 2). The difference of weather conditions in both dry and rainy seasons did not significantly change the density of soil bacteria. The higher butachlor concentrations resulted in smaller bacteria numbers in soil after 60 days, suggesting the toxicity and long-term effect of the herbicide. Only exception was found on 40 days after the treatment in the dry season, when the density of bacteria in untreated soil decreased. In addition, legume was not found on root bean cultivated in soil treated with butachlor in the dry season and the numbers of root legume were significantly smaller when the herbicide was amended at 2.0×. This result showed that butachlor seriously inhibited Rhizobium sp. which symbiotically established inside the root nodules.

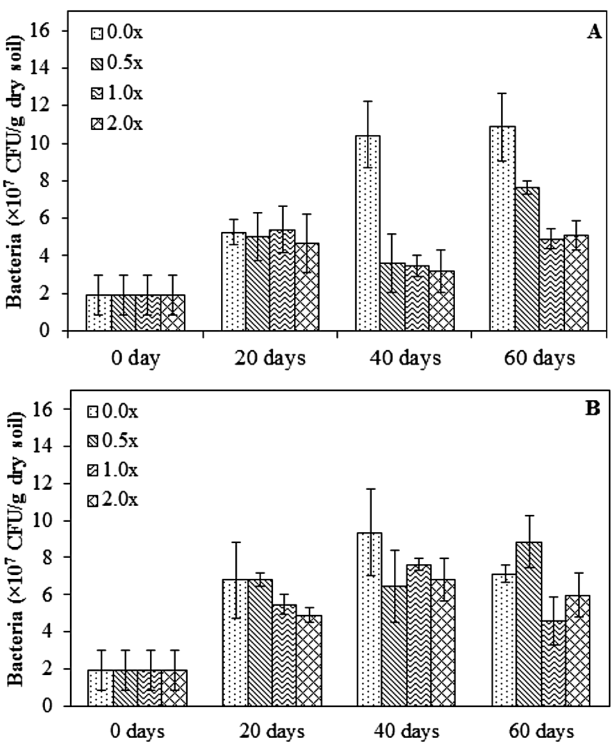

Figure 1. Effects of butachlor on bacterial populations in soil cultivated with water spinach in (A) the dry season and (B) rainy season

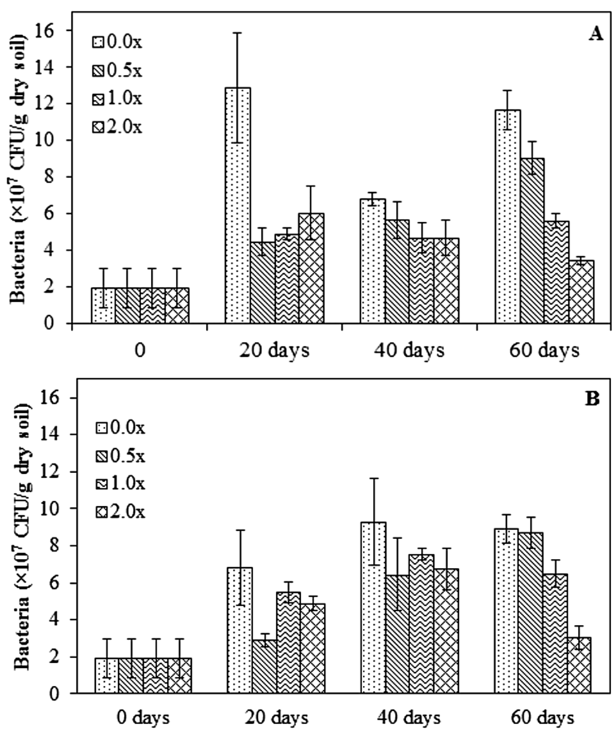

Figure 2. Effects of butachlor on bacterial populations in soil cultivated with mung bean in (A) the dry season and (B) rainy season

In previous reports, the effects of butachlor on soil bacteria were varied, depending on soil types. Both atrazine and butachlor caused significant decrease of viable bacterial community in soil (Emurotu & Anyanwu, 2016). Min et al. (2001) presented that butachlor stimulated the growth of anaerobic bacteria. In another study, population of methanogens was slightly stimulated by adding butachlor in paddy soil (Zhou et al., 2012).

After amendment with atrazine, all mung bean and water spinach were dead within several days, so the plants did not affect the changes of bacteria. This soil can be used as the baseline to determine the role of plants on soil bacteria. Obtained data in this study showed that both cultivated water spinach and mung bean did not apparently affect density of soil bacteria.

The effect of atrazine on bacteria was only determined in the dry season. The density of bacteria population significantly increased over time in soil without atrazine. The bacteria numbers in control outnumbered bacteria in treated soil. However, no significant difference of numbers of bacteria density was found in soil at all atrazine concentrations (Figure 3).

Previous reports also showed that atrazine negatively affected the growth of soil bacteria (Ayansina & Oso, 2006; Sebiomo el al., 2011; Ha et al., 2017). The numbers of bacteria was decreased after treated with an herbicide containing atrazine as the active component, but bacteria density recovered after 30 days (Ha et al., 2017). The long-term effects caused by herbicides emerged in this study probably because native bacteria had not adapted these toxic substrates. Moreover, the long effects of atrazine on bacteria were probably due to the persistence of atrazine in soil. Previous reports presented that half-life values of atrazine in soil stayed on from several weeks to more than one year (Arthur et al., 2000; Ma et al., 2011), which inhibited soil microorganisms for a long time.

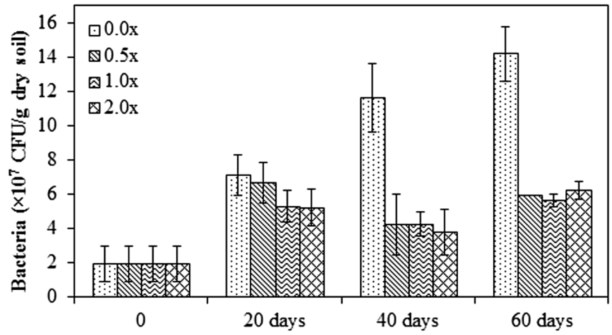

Figure 3. Effects of atrazine on bacterial populations in soil in the dry season

3.2. Effects of butachlor and atrazine on the characteristics of mung bean

After supplementation with atrazine at any concentration, all mung bean and water spinach died. This result showed that these plants were so sensitive to the herbicide even at a low concentration. Atrazine is resistant to degradation in water and soil, which may cause phytotoxicity to subsequent crops (Gao et al., 2018). Even though atrazine is not used to control weeds in cultivating mung bean at the time, the herbicide used in former crops can affect this plant. Moreover, atrazine in soil may be dissolved in the soil water and moved into water sources where mung bean is cultivated.

The application of butachlor negatively affected the growth of mung bean and water spinach, especially at the dose of 2.0×. Figure 4 showed that both plants grown at 2.0× butachlor were undersized compared to the control. For mung bean cultured in the dry season, the effects of butachlor on length and weight of roots and beanstalks were unapparent, while harvested biomass of chickpea was significantly reduced at 1.0× and 2.0× (Table 3). When mung bean was cultured in the rainy season at 2.0×, herbicide caused reduction of all characteristics (Table 4). The length of beanstalks and weight of beanstalks and roots of the plant grown in the rainy season at 2.0× butachlor were lower than those of the control by 36.2% and 44.3% on average, respectively. Moreover, harvested chickpea was reduced by 57.1% at 2.0× butachlor in the rainy season and by 31.9% in the dry season at 2.0× butachlor.

Weather conditions in the rainy season favored the growth of mung bean. The length and weight of roots and beanstalks of mung bean cultured in the rainy season were higher than those grown in the dry season at concentrations smaller than 2.0×. We assumed that higher numbers of root nodules might have fixed higher amount of nitrogen, resulting in the higher plant length and weight. However, its weight of legume and chickpea were lower when mung bean was grown in rainy season.

Herbicides containing butachlor are usually used for rice field, but sometimes they are used for other crops. Butachlor may remain in soil for several years (Carlson et al., 2006). The rotation of mung bean and other crops is encouraged because the plant can enrich the soil with life-giving nitrogen. However, this study showed that yield capacity of the plant reduced by the herbicide application. Thus, the use of butachlor should be considered in the crop of mung bean.

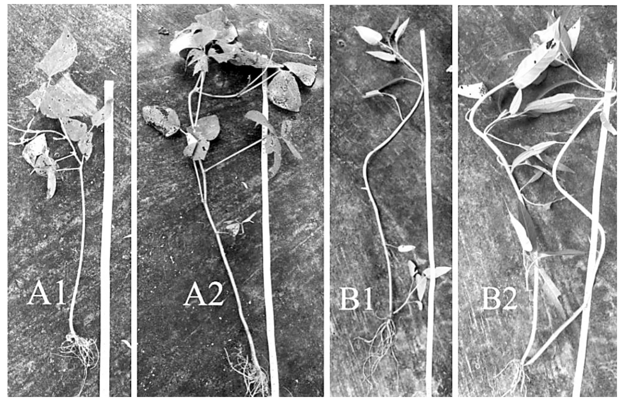

Figure 4. (A) Mung bean *(Vigna radiata)* and (B) water spinach *(Ipomoea aquatica)* grown on (1) soil treated with butachlor (2.0×) and (2) soil without butachlor cultured in the rainy season

Table 3. Effects of butachlor on some characteristics of mung bean in the dry season

Butachlor	Length of plant (cm)		Fresh weight of beanstalk and root (g/plant)		Dried weight of beanstalk and root (g/plant)	Dried chickpea (on average, g/plant)	Root nodule/ plant
	Beanstalk	Root	Beanstalk	Root			
0.0×	37.57±5.21^{a}	14.18±3.37^{a}	9.74±1.65^{ab}	2.66±0.84^{b}	5.48±1.67^{a}	2.70	3.33±1.30
0.5×	31.38±3.94^{b}	12.69±2.49^{a}	12.12±4.20b^{c}	2.93±0.87b	5.31±1.53^{a}	2.81	0
1.0×	30.79±5.16^{b}	13.88±2.40a	15.75±5.86^{c}	2.65±0.55^{b}	8.84±2.71^{b}	1.73	0
2.0×	36.69±3.00^{a}	13.41±2.06^{a}	7.23±1.56^{a}	1.74±0.66^{a}	5.08±2.29^{a}	1.84	0

Notes: Different superscript letters indicate statistically significant differences ($p < 0.05$) among treatments within a column. Data are means of the results from at least three individual experiments, and mean values and standard deviations are shown.

Table 4. Effects of butachlor on some characteristics of mung bean in rainy season

Butachlor	Length of plant (cm)		Fresh weight of beanstalk and root (g/plant)		Dried weight of beanstalk and root (g/plant)	Dried chickpea (g/plant)	Root nodule/ plant
	Beanstalk	Root	Beanstalk	Root			
0.0×	69.38±8.81^{c}	29.70±5.70^{c}	12.22±2.05^{b}	3.58±0.94^{b}	8.33±1.92^{bc}	1.33	15.58±3.03
0.5×	57.99±8.30^{b}	18.91±4.19^{ab}	14.31±4.36^{b}	3.10±1.19^{b}	6.99±0.96^{b}	0.95	14.33±2.84
1.0×	68.10±10.97^{c}	22.74±4.79^{b}	21.57±3.94^{c}	3.71±0.60^{b}	9.04±1.77^{c}	1.15	15.33±2.64
2.0×	44.29±5.60^{a}	14.73±1.48^{a}	7.00±2.29^{a}	1.38±0.53^{a}	4.64±0.88^{a}	0.57	6.33±2.50

Notes: Different superscript letters indicate statistically significant differences ($p < 0.05$) among treatments within a column. Data are means of the results from at least three individual experiments, and mean values and standard deviations are shown.

Table 5. Effects of butachlor on some characteristics of water spinach in the dry season

Butachlor	Length of plant (cm)		Fresh weight of stem shoots and root (g/plant)		Dried weight of stem shoots and root (g/plant)
	Stem shoots	Root	Stem shoots	Root	
0.0×	93.78±10.81[d]	30.79±3.04[d]	44.37±6.75[c]	9.38±1.95[c]	9.15±1.92[c]
0.5×	73.66±8.59[c]	16.07±1.90[c]	31.68±6.65[b]	4.71±1.23[b]	7.54±2.04[b]
1.0×	56.45±9.64[b]	11.25±1.89[b]	18.85±2.84[a]	3.49±0.85[a]	5.43±1.24[b]
2.0×	38.49±5.19[a]	7.62±1.46[a]	15.49±3.02[a]	2.52±0.71[a]	3.57±0.94[a]

Notes: Different superscript letters indicate statistically significant differences ($p < 0.05$) among treatments within a column. Data are means of the results from at least three individual experiments, and mean values and standard deviations are shown.

Table 6. Effects of butachlor on some characteristics of water spinach in rainy season

Butachlor	Length of plant (cm)		Fresh weight of stem shoots and root (g/plant)		Dried weight of stem shoots and root (g/plant)
	Stem shoots	Root	Stem shoots	Root	
0.0×	79.32±10.13[b]	26.57±2.13[d]	41.57±8.66[c]	8.08±3.20[b]	8.86±2.55[b]
0.5×	51.45±6.79[a]	23.00±2.62[c]	36.59±2.39[b]	7.58±1.06[b]	7.43±0.63[b]
1.0×	52.51±9.15[a]	14.98±3.12[b]	21.74±5.68[a]	3.59±0.86[a]	5.96±0.95[a]
2.0×	47.27±12.51[a]	10.15±2.09[a]	27.81±6.56[a]	4.55±1.49[a]	6.53±0.53[ab]

Notes: Different superscript letters indicate statistically significant differences ($p < 0.05$) among treatments within a column. Data are means of the results from at least three individual experiments, and mean values and standard deviations are shown.

The supplementation with butachlor in soil significantly reduced length and weight of water spinach in both seasons (Table 5 and 6), with more severely in the dry season (Table 5). Some values of size and weight of water spinach were not significantly at 1.0× and 2.0× butachlor. The lengths of stem shoots and roots of water spinach grown at 2.0× butachlor in the dry season were reduced by 59.0% and 75.3% on average compared to those of the control, respectively, while these data obtained in the rainy season were reduced by 40.4% and 61.8%. Total dried biomasses of stem shoots and roots of water spinach grown at 2.0× butachlor were reduced by 61.0% and 26.3% when the plants were cultured in the dry and rainy season, respectively.

4. Conclusion

Both atrazine and butachlor inhibited the growth of soil bacteria even after two months in both dry and rainy seasons. The application of atrazine resulting in all mung bean and water spinach killed within several days. Butachlor added into soil at 4.0 L/ha caused the reduction of body length and biomass of water spinach by 36.2% and 44.3% in the rainy season. The application of butachlor at 4.0 L/ha decreased yield capacity of mung bean by 57.1% in the rainy season and by 31.9% in the dry season. This study indicated that atrazine and butachlor were toxic to soil bacteria and caused damages to mung bean and water spinach.

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References

Aminpanah, H., Sorooshzadeh, A., Zand, E., & Moumeni, A. (2013). Using more competitive cultivar against barnyardgrass (Echinochloa crusgalli) to reduce herbicide application rate in lowland rice fields. *Thai Journal of Agricultural Science, 46*(4), 201-207.

APHA. (1998). *American public health association*, 20th ed. American Public Health Assoc, Washington, DC.

Arthur, E. L., Perkovich, B. S., Anderson, T. A., & Coats, J. R. (2000). Degradation of an atrazine and metolachlor herbicide mixture in pesticide-contaminated soils from two agrochemical dealerships in Iowa. *Water, Air, and Soil Pollution*, *119,* 75-90.

Ayansina, A., & Oso, B. A. (2006). Effect of two commonly used herbicides on soil microflora at two different concentrations. *African Journal of Biotechnology*, *5*(2), 129-132.

Ayansina, A. D. V., Ogunshe, A. A. O., & Fagade, O. E. (2003). Environment impact assessment and microbiologist: An overview. *Proceedings of 11th annual national conference of environment and behaviour association of Nigeria (EBAN)*, 26-27.

Chen, Y. L., & Chen, J. S. (1979). Degradation and dissipation of herbicide butachlor in paddy field. *Journal of Pestcide Science*, *4*, 431-438.

Croll, B. T. (1991). Pesticides in surface waters and groundwaters. *Journal of the Institution of Water and Environmental Management*, *5*(4), 389-395.

Dwivedi, S., Singh, B. R., Al-Khedhairy, A. A., Alarifi, S., & Musarrat, J. (2010). Isolation and characterization of butachlor-catabolizing bacterial strain *Stenotrophomonas acidaminiphila JS-1 from soil and assessment of its biodegradation potential. Letters in Applied Microbiology, 51*(1), 54-60.

Emurotu, M. O., & Anyanwu, C. U. (2016). Effect of atrazine and butachlor on soil microflora in agricultural farm in Anyigba, Nigeria. *European Journal of Experimental Biology*, *6*(2), 16-20.

Gao, J., Song, P., Wang, G., Wang, J., Zhu, L., & Wang, J. (2018). Responses of atrazine degradation and native bacterial community in soil to Arthrobacter sp. strain HB–5. *Ecotoxicology and Environmental Safety*, *159*, 317-323.

Graymore, M., Stagnitti, F., & Allinson, G. (2001). Impacts of atrazine in aquatic ecosystems. *Environment International*, *26*(7-8), 483-495.

Ha, D D., Nguyen, T. K. K., & Bui, M. T. (2018). Effects of hercicides on bacteria num and chemical components of soil. *Journal of Science and Technology - The University of Da Nang*, *9*(118), 89-93.

Hess, D. F. (2000). Light-dependent herbicides: an overview. Weed Research, 48(2), 160-170.

Ma, T., Zhu, L., Wang, J., Wang, J., Xie, H., Su, J., Zhang, Q., & Shao, B. (2011). Enhancement of atrazine degradation by crude and immobilized enzymes in two agricultural soils. *Environmental Earth Sciences*, *64*, 861-867.

Min, H., Ye, Y.-f., Chen, Z.-y., Wu, W.-x., & Yufeng, D. (2001). Effects of butachlor on microbial populations and enzyme activities in paddy soil. *Journal of Environmental Science and Health*, Part B, *36*(5), 581-595.

Sebiomo, A., Ogundero, V.W., & Bankole, S.A. (2011). Effect of four herbicides on microbial population, soil organic matter and dehydrogenase activity. *African Journal of Biotechnology*, *10*(5), 770-778.

Wang, S., Li, H., & Lin, C. (2013). Physiological, biochemical and growth responses of Italian ryegrass to butachlor exposure. *Pesticide Biochemistry and Physiology*, *106*(1-2), 21-27.

Zhou, X., Shi, X., Zhang, L., & Zhou, Y. (2012). Effects of pesticide-contamination on population and activity of bacteria in purple paddy soil. *Energy Procedia*, *16*, 284-289.