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Interaction between Terbutryn and Soil Microorganisms: Population Changes and Biosynthesis of Indole-3-Acetic Acid

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ABSTRACT. Terbutryn at concentrations of 10, 30 and 100 μg/g soil showed a significant stimulatory effect on the population of total bacteria, azotobacters, actinomycetes and fungi over those of the nontreated soil samples. The increases in the microbial populations were in proportion to the level of terbutryn, and persisted for at least 2 months. In liquid media, these levels caused substantial increase in the biosynthesis of indole-3-acetic acid by streptomycetes, fungi and azotobacters. The sensitivity of these organisms to the applied herbicide is a specific feature depending on the strain variation and the dose of the herbicide. The maximal biosynthesis of indole-3-acetic acid was 1.6, 2.8 and 8.1 fold, respectively, over that in the herbicide-free media.

Terbutryn (N-*tert*-butyl-N^1-ethyl-6-methylthio-1, 3, 5-triazine-2, 4-diyldiamine) is a selective herbicide for pre-emergence control of most annual and biennial monocotyledonous and dicotyledonous weeds.

Some reports proved that, under soil conditions, there is no or little effect on soil microflora (Cole and Baston 1974, and Paeschke and Heitfuss 1978), whereas other workers (Krezel and Kosinkiewiez 1972, Naguib *et al.* 1981, and Mohammed 1984) reported that herbicides may be either stimulatory or inhibitory to specific groups of microorganisms, or interfere with the beneficial and pathological microorganisms.

The present work was carried out in order to study the changes which take place in microbial populations in soil upon applying terbutryn. The effect of the herbicide on the potentiality of certain microorganisms to produce indole-3-acetic acid (IAA) was also investigated.

Materials and Methods

Application of the Herbicide to the Soil

A clay Egyptian soil collected from a cultivated field in which no pesticides were applied for about 5 years was used. The physical characteristics of the soil sample were as follows: water holding capacity (WHC), 13.33%; organic matter content, 6.96%; electrical conductivity, 0.25 ml mohs; and pH value, 8.47. Soil was sieved (2.0 mm meshes) and stored in closed plastic bags at 15% moisture.

Wettable powder (Igran-80 WP) was suspended in water and thoroughly mixed with soil to attain terbutryn concentrations of 10,30, and 100 μg/g air dried soil. The soil-terbutryn mixtures were incubated at 28-30°C and the moisture content was maintained by adding sterile distilled water periodically.

Enumeration of Soil Microbes

The numbers of propagules of soil bacteria, azotobacters, actinomycetes and fungi in clay soil were estimated by drawing samples at the following time intervals, 7, 14, 21, 28, 35, 42, 49, and 56 days using the dilution plate method. A modified soil extract agar was used for the enumeration of total bacteria (Farley and Lockwood 1968). Nitrogen-free agar (El-Essawy *et al.* 1984) was used for the azotobacter count. Starch nitrate agar and Czapek-Dox agar to which was added 1/15000 rose bengal were used for the determinations of the number of actinomycetes and fungi, respectively.

Determination of IAA

Herbicide suspension was added to the aforementioned sterilized liquid media at 5, 10, 30, and 100 μg/ml. The media (48 ml in 250 ml Ehrlenmeyer flasks) were inoculated with 2 ml aliquots of 48 h vegetative growth, and incubated at 28°C in a dark orbital shaker (160 rpm) for 7 days. Triplicate flasks were used for each treatment. The fermented cultures were centrifugated at 4000 rpm for 40 min.

Extraction, Chromatography and Assay of IAA

The post-culture liquids were acidified to pH 2.0-3.0 with IN HCl and extracted twice with 100 ml of peroxide-free ethyl ether. The ether extracts were evaporated at 40°C to dryness, the residue was dissolved in 2 ml methanol, and 0.2 ml aliquots were applied to Whatman No. 1 filter paper. The ascending method was used with the solvent system: isopropanol, ammonia, water (10:1:1) V/V/V). The developed spots were sprayed by a reagent system composed of 3% H_2SO_4 in methanol and 0.5% ferric chloride. The air-dried chromatogram was heated at 60-80°C for 3-5 min. The spots of IAA acquired violet red colorations (Rf value of 0.3-0.4). The portions of the chromatogram corresponding in position to the Rf value of the standard IAA were used for bioassay as described by Youssef *et al.*

(1970). The amount of IAA produced by the experimental organisms was estimated fluorimetrically according to Knegt and Bruinsma (1973).

Results

Effect of Terbutryn on Soil Microflora

Population of total bacteria, azotobacters, actinomycetes and fungi were counted over a period lasting fifty six days in clay soil supplemented with 10, 30 or 100 μg terbutryn per gram of soil. Sustained significant ($P < 0.005$) increases in the populations of these microorganisms were observed after 7-21 days of incubation. The increases continued throughout the experimental period (56 days). As a result of this experiment, the data (Table 1) showed that the herbicide at 10, 30 and 100 μg terbutryn/g soil reduced the bacterial number in the first seven days. At the fifty sixth day of incubation, the number increased 2.5 fold of the control treatment in soil treated with 30 or 100 μg terbutryn/g.

Table 1. Counts of bacteria as influenced by various doses of terbutryn

Periods of treatment (days)	Counts/g dry soil ($\times 10^4$)			
	Doses (μg/g soil)			
	0	10	30	100
0	48.7 ± 4.0	48.7 ± 4.0	48.7 ± 4.0	48.7 ± 4.0
7	46.3 ± 1.8	33.8 ± 3.4	34.7 ± 3.7	37.2 ± 1.2
14	52.3 ± 3.8	51.3 ± 3.8	56.7 ± 1.1	94.0 ± 3.5
21	53.2 ± 1.3	53.3 ± 1.5	65.4 ± 4.6	95.0 ± 9.1
28	58.6 ± 2.1	53.4 ± 7.3	75.0 ± 2.4	95.8 ± 9.5
35	68.0 ± 5.8	66.7 ± 4.7	78.0 ± 5.1	95.9 ± 9.3
42	55.6 ± 2.3	52.1 ± 1.5	78.0 ± 5.7	95.8 ± 9.0
49	43.5 ± 9.5	41.7 ± 4.9	81.3 ± 4.7	95.7 ± 9.2
			***	***
56	40.0 ± 5.8	38.3 ± 3.2	100.0 ± 10.0	105.0 ± 8.0

Each value is the mean of 20 determinations ± standard error.
The results were statistically analysed using "f" test.
***: Highly significant different at $P < 0.005$.

The population of azotobacters is presented in Table 2. The data suggest that the population was inhibited by the herbicide in the first 14 days. Beginning at twenty one days and continuing through fifty six days, the population of azotobacters in soil treated with 10, 30 and 100 μg terbutryn/g had increased 3.5, 4 and 5 times, respectively by day fifty six over those in the non-treated soil.

Table 2. Counts of azotobacters as influenced by various doses of terbutryn

Periods of treatment (days)	Counts/g dry soil ($\times 10^4$)			
	Doses (μg/g soil)			
	0	10	30	100
0	43.0 ± 3.2	43.0 ± 3.2	43.0 ± 3.2	43.0 ± 3.2
7	36.3 ± 1.9	33.8 ± 3.4	34.7 ± 3.8	27.2 ± 1.2
14	28.8 ± 0.3	22.1 ± 0.7	29.9 ± 0.7	28.3 ± 2.5
21	28.9 ± 2.0	43.7 ± 1.3	53.2 ± 3.1	24.7 ± 0.2
28	17.8 ± 3.6	29.6 ± 2.6	39.2 ± 1.4	43.1 ± 1.4
35	16.7 ± 0.7	27.0 ± 1.0	38.7 ± 0.5	44.2 ± 1.1
42	15.0 ± 1.8	34.3 ± 1.3	44.7 ± 1.1	45.3 ± 0.8
49	16.3 ± 1.8	33.3 ± 1.8	45.0 ± 1.8	59.3 ± 5.2
		***	***	***
58	16.0 ± 1.3	53.7 ± 4.6	60.7 ± 2.0	75.2 ± 1.4

Each value is the mean of 20 determinations ± standard error.
The results were statistically analysed using "f" test.
***: Highly significant different at $P < 0.005$.

The data (Table 3) show that when the soil was supplemented with 10 μg terbutryn/g soil, an earlier stimulation of actinomycetes resulted by seven days, followed by a return to the population level of non treated soil in the first fourteen days. After twenty one days continuing to thirty five days a depressing effect was observed, then a second stimulation occurred at forty two days and lasted to fifty six days, which recorded the maximum number of actinomycetes, being 1.5 times higher those in the untreated control, Supplementation of the soil with 30 and 100 μg terbutryn/g soil enhanced the population of actinomycetes during almost the entire incubation period. The numbers had increased 2.1 and 3 times, respectively, by the end of the experimental period (56 days) over those in the non-treated soil.

The population of fungi in the soil treated with terbutryn is presented in Table 4. The data suggest that the doses 10, 30 and 100 μg terbutryn/g were without effect on the fungal population during the first twenty one days. Soil treated with 10 μg terbutryn activaed the fungal number starting from twenty eight days and lasted forty nine days followed by a return to the population level of non-treated soil. However, the fungal numbers were enhanced in soil treated with 30 and 100 μg terbutryn/g which recorded the maximum numbers 1.5 and 2.1 times, higher respectively, than those in the untreated control.

Effect of Terbutryn on the Biosynthesis of IAA

Terbutryn stimulated the biosynthesis of IAA by the experimental organisms

Table 3. Counts of actinomycetes as influenced by various doses of terbutryn

Periods of treatment (days)	Counts/g dry soil ($\times 10^4$)			
	Doses (μg/g soil)			
	0	10	30	100
0	15.3 ± 1.0	15.3 ± 1.0	15.3 ± 1.0	15.3 ± 1.0
7	13.1 ± 0.4	15.7 ± 0.5	16.9 ± 1.4	18.1 ± 1.9
14	5.0 ± 0.4	6.0 ± 0.9	15.2 ± 0.9	14.8 ± 0.7
21	19.4 ± 1.4	12.2 ± 0.2	23.2 ± 0.05	22.4 ± 0.4
28	18.5 ± 2.9	11.3 ± 0.3	24.3 ± 0.3	27.7 ± 1.2
35	16.7 ± 3.7	14.9 ± 0.9	24.9 ± 0.2	26.9 ± 0.4
42	16.4 ± 0.3	16.9 ± 1.1	25.7 ± 0.6	28.9 ± 0.2
49	16.0 ± 1.7	17.3 ± 1.2	31.7 ± 1.7	48.0 ± 3.1
		***	***	***
56	18.0 ± 1.6	36.7 ± 3.3	37.7 ± 2.9	50.5 ± 3.9

Each value is the mean of 20 determinations ± standard error.
The results were statistically analysed using "f" test.
***: Highly significant different at $P < 0.005$.

(Table 5). The data show that the supplementation of terbutryn to the culture media of *Streptomyces corchorusii, S. mutabilis* and *S. atroolivaceus* enhanced the biosynthesis of IAA, with minor differences in regard to the species and herbicide concentration. The biosynthesis of IAA was more prominent at 100 μg/ml dose, being 1.6, 1.5, 1.4 times greater, respectively, than those of the control.

On the contrary, the doses 30 and 100 μg/ml remarkably attenuated the biosynthesis of the auxin by *Trichoderma viride*. Similarly, the latter dose also showed a pronounced depressing effect on *Fusarium moniliforme*. However, the production of the auxin by *Trichoderma viride* was stimulated at 5 and 10 μg terbutryn/ml to 1.9 and 2.6 times, respectively, greater than the untreated control. The herbicide remarkably enhanced the biosynthesis of IAA by *Fusarium moniliforme* with differences in response with 5, 10 and 30 μg/ml to 1.1, 2.4 and 1.2 times over the respective control.

Supplementation of terbutryn to the culture medium of *Azotobacter chroococcum* 1, induced high gain of IAA. The maximum level was 2.3 times over the control with administration of 100 μg/ml of the herbicide. The biosynthesis of IAA by *A. chroococcum* 3 and 21 was also induced by the herbicide at 5 and 10 μg/ml doses. The maximum gains of IAA were recorded to be 2.6 and 8.1 times higher, respectively, than those of the control. On the contrary, the biosynthesis of the auxin by *A. chroococcum* was attenuated at dose levels 30 and 100 μg/ml.

Table 4. Counts of fungi as influenced by various doses of terbutryn

Periods of treatment (days)	Counts/g dry soil ($\times 10^4$)			
	Doses (μg/g soil)			
	0	10	30	100
0	9.6 ± 0.7	9.6 ± 0.7	9.6 ± 0.7	9.6 ± 0.7
7	5.2 ± 0.3	3.6 ± 0.3	4.6 ± 0.3	3.8 ± 0.7
14	3.3 ± 0.2	3.7 ± 0.3	2.7 ± 0.2	2.5 ± 0.0
21	3.0 ± 0.1	2.8 ± 0.3	2.7 ± 0.2	3.8 ± 0.2
28	3.5 ± 0.3	4.3 ± 0.3	5.1 ± 0.2	6.2 ± 0.2
35	3.0 ± 0.2	4.5 ± 0.1	5.2 ± 0.3	12.5 ± 0.0
42	3.4 ± 0.2	4.6 ± 0.1	5.5 ± 0.2	13.7 ± 1.8
49	5.2 ± 0.2	6.3 ± 0.8	10.7 ± 0.1 ***	14.7 ± 0.9 ***
56	7.3 ± 1.0	7.5 ± 0.5	10.7 ± 1.0	14.7 ± 0.3

Each value is the mean of 20 determinations ± standard error.
The results were statistically analysed using "f" test.
***: Highly significant different at $P < 0.005$.

Table 5. Effect of various doses of terbutryn on the biosynthesis of indole-3-acetic acid (IAA)

Organism*	IAA production (μg/100 ml culture medium)				
	Doses (μg/ml culture medium)				
	0	5	10	30	100
Streptomyces corchorusii	11.5±0.8	11.6±0.9	14.9±0.7	15.7±1.0	18.2±1.6
S. mutabilis	13.2±0.9	15.1±1.0	16.4±1.1	17.2±0.9	19.9±1.6
S. atroolivaceus	10.9±1.0	12.4±1.0	13.9±0.9	14.1±1.0	16.0±1.1
Trichoderma viride	6.6±0.5	12.6±1.0	17.4±1.1	4.9±0.3	1.6±0.08
Fusarium moniliforme	7.6±0.4	8.7±0.3	18.1±1.2	9.5±0.6	5.6±0.04
Azotobacter chroococcum 2	9.1±1.0	10.3±1.0	16.4±1.8	19.4±1.6	21.4±2.0
A. chroococcum 3	8.2±0.8	10.4±1.0	16.3±1.1	18.5±1.8	21.5±2.0
A. chroococcum 21	2.5±0.02	13.5±1.0	19.6±1.6	11.1±1.8	10.0±2.8

Each value is the mean of 3 determinations ± standard error.
* organisms isolated from soil treated with terbutryn.

Discussion

Population of total bacteria, azotobacters, actinomycetes and fungi initially decreased followed by a return to normal or even an increase in population number confirming the results of earlier workers (Spiridonov and Yakovlev 1968, and Percich and Lockwood 1978). Moreover, although the concentrations of terbutryn, used in the present study, were in excess of field rates (10 μg/g is about 2.5 times the usual rate), the population of total bacteria, azotobacters, actinomycetes and fungi increased and were sustained for at least 2 months.

In this connection, it may be mentioned that Bakalivanov (1976) and Percich and Lockwood (1978) reported stimulation of microorganisms during the first 2-3 months of soil treated with simazine or atrazine in accordance with our findings.

Triazines affect soil microflora to a certain extent; simazine had no influence on metabolically active bacteria and fungi (Paeschke and Heitefuss 1978). On the other hand, Gruzdyev *et al.* (1981) showed that terbutryn stimulated the growth of bacteria, azotobacters and actinomycetes which is in accordance with the finding of Mohammad (1984).

These observations may lead to the conclusion that the stimulatory effect of terbutryn on soil microflora may be due to direct stimulation by terbutryn itself, degradation products of terbutryn or a "growth factor" effect enabling a more efficient use of refractory organic substrates in soil.

In culture media, terbutryn stimulated the biosynthesis of IAA with minor differences in regard to species and strains. The stimulatory effect of the herbicide may be due to its effect in increasing amino acids or to the chemical structure of terbutryn itself as an organic compound containing nitrogen and sulphur.

In this connection, it may be mentioned that Fletcher and Kirkwood (1982) reported that terbutryn at a concentration of 10-50 μg/ml increased the amino acid content of the treated plant. However, most of organisms required tryptophan for auxin production (Brown and Norman 1970, Strzelezyk and Polokska-Burdziej 1984, and El-Shanshoury 1985). On the other hand Zhivka (1967) reported that nitrogen and sulphur- containing organic compounds possessed favorable influences on the growth and secretion of growth regulating substance. El-Essawy *et al.* (1984) also noticed a beneficial effect of combined nitrogen containing organic compounds on IAA production by *A. chroococcum*.

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التفاعل بين مبيد الحشائش تيربيوترين وكائنات التربة الدقيقة: التغير في تعدادها وإنتاج أندول ـ ٣ ـ حمض الخليك

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يهدف هذا البحث إلى تبيان تأثير التركيزات المختلفة من أحد طرز المبيدات العشبية وهو التيربيوترين على التعداد الكلي لكل من البكتريا والاكتينوميستات والفطريات وكذلك إنتاج أندول ـ ٣ ـ حمض الخليك بواسطة مجموعة من الكائنات الدقيقة المعزولة من التربة المعاملة بالمبيد نفسه.

وقد دلت نتائج الدراسة على أن معاملة التربة بثلاثة تركيزات مختبرة، لها تأثير معنوي في زيادة التعداد الكلي للبكتريا والازوتوباكتر والاكتينومستات وكذلك الفطريات، مع ملاحظة أن هذا التأثير يزداد بزيادة الجرعة. كما دلت النتائج أنه عند إضافة المبيد بتركيزات مختلفة للمنبت الغذائي السائل لكل من الاستربتومسيتات والفطريات وكذلك بكتريا الازوتو باكتر كروكوكم، ازداد إنتاج أندول ـ ٣ ـ حمض الخليك ولكن بكميات متفاوتة باختلاف السلالة وجرعة المبيد المضافة للمنبت. وجد أيضا أن المبيد أكثر تأثيراً على إنتاجية أندول ـ ٣ ـ حمض الخليك في المنابت الغذائية للازوتو باكتر كروكوكم يليها الفطريات ثم الاستربتومسيتات عنه في المنابت الغذائية الخالية منه.