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EFFECT OF PESTICIDAL TREATMENT AND TYPE OF SOIL ON WHEAT AND COTTON SEEDLING LEAVES CONTENTS OF CHLOROPHYLL AND CAROTENOIDS

Rehab E.M.E. Salem*, M.A. El-Tantawy, A.A. Aioub and K.A. Gouhar

Plant Protection Dept., Faculty of Agric., Zagazig Univ., Egypt

ABSTRACT

The intrinsic phytotoxicity of six pesticides, namely: butralin, clodinafop – propargyl, bromoxynil, imidacloprid, malathion and carbosulfan against the three photosynthetic pigments chlorophyll a, chlorophyll b and carotenoids of cotton and wheat seedling leaves, cultivated in two types of soil was investigated. It was found that the content of imidacloprid, butralin, and carbosulfan – treated cotton seedling leaves of chlorophyll a, chlorophyll b and carotenoids were reduced in clay loam soil cultivation, i.e., the used pesticides acted as inhibitor for the biosynthesis of the three pigments. With the sandy clay loam cultivation, imidacloprid and butralin acted as inducers whereas the third compound carbosulfan exhibited inhibitory action. These criteria of toxicity was more obvious as time lapsed between pesticidal treatment and pigment assessment, i.e., the holding period.

Exposure of wheat plants, cultivated in both types of soil, to the two herbicides clodinafop – propargyl and bromoxynil and the insecticide malathion resulted in increase in the amounts of chlorophyll a, chlorophyll b and carotenoids during the whole experimental periods (4 days) with the exception of clodinafop – propargyl – treated plants which suffered from reduction in the level of the three pigments one day after treatment.

Keywords: Pesticides, types of soil, wheat, cotton, chlorophyll, carotenoids.

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* **Corresponding author:** Rehab E.M.E. Salem , **Tel.:** +201501496987
E-mail address: Rehab_Edarous@yahoo.com

INTRODUCTION

Using pesticides in agriculture is actually important to improve the quantity and quality of crops (Dierksmeier *et al.*, 1996). The phytotoxicity of pesticides does vary from pesticide to another. In the practice of pest control , the invisible phytotoxicity of pesticides is over looked, because evaluation far the phytotoxicity or subtle impact of pesticides on plant physiology is not required for their registrations, therefore, unless there is visible phytotoxicity of pesticides are commonly over used, especially for those pesticides with low mammalian toxicity or selective pesticides. In fact these pesticides not only often have inverse effects on the physiology and biochemistry of crop plants but also affect the plant tolerance to pest infestation (Wu *et al.*, 2003).

El-Aswad *et al.* (2008) studied the side effect of certain pesticides on the plant anatomical characteristics and the influence on the plant biochemical systems.

Therefore, present work focuses on the toxic effect of six pesticides (herbicides and insecticides) on the photosynthetic pigments chlorophyll (a and b) and carotenoids in leaves of two important crops (cotton and wheat) cultivated on two common types of soil in Egypt.

MATERIALS AND METHODS

Tested Pesticides

Six pesticides were used butralin, clodinafop-propargyl and bromoxynil (as herbicides); carbosulfan, imidacloprid and malathion as insecticides.

Tested Plants

Cotton (*Gossypium barbadence* L.) cv. Giza 86 and wheat (*Triticum aestivum* L.), cv. Sakha 69 were used.

Tested Soil

Two different soil types were chosen namely, clay loam soil and sandy clay loam soil were collected from Fakous and Salhaia regions, Sharkia governorate, respectively. Mechanical properties of the two tested types of soil are given in Table 1.

Experimental Design

Pots (40 cm diameter and 30 cm height) were filled with the tested soil after which cotton seeds or wheat grains were sown at April 2, 2007 and December 29, 2007, respectively. Three replicates for each treatment and untreated control were used. Pesticides used, recommended rates, the method and date of application are shown in Table 2.

Table 1. Mechanical properties of the soils under investigation (%)

Type of soil	Sand	Silt	Clay
Sandy clay loam	55.89	16.80	27.31
Clay loam	25.3	16.3	58.4

Table 2. Methods, rates and dates of pesticide applications on wheat and cotton plants

Plants	Rates of application	Methods of application	Dates of application
1.Wheat plant :			
Bromoxynil (Brominal W 24%EC)	1000 ml / fed.	Foliar	January 22,2008
Clodinafop – propargyl (Topik 15% WP)	150 ml / fed.	Foliar	January 28,2008
Malathion (Malathion 57 % EC)	150 ml / 100 L water	Foliar	January 12,2008
2. Cotton plant :			
Carbosulfan (Marshal 25%WP)	150 g / 100 L water	Foliar	April 14,2007
Imidacloprid (Confidor 20% SL)	50 ml / 100 L water	Foliar	April 10,2007
Butralin (Amex 48%EC)	2.5 L / fed.	Soil treatment	At sowing

Samples of leaves were taken before each application as control and after 24 and 96 hours of treatment and transferred immediately to the laboratory to determine chlorophyll (a), chlorophyll (b) and carotenoids .Leave samples of treated plants with butralin (Amex 48% EC) were taken after 10 days from sowing. The photosynthetic pigments were extracted from fresh leaves using 85% acetone according to Fideel (1962). The optical densities were measured spectrophotometrically using spectronic 20D colorimeter at 662, 644, and 440 nm to determine chlorophyll (a), chlorophyll (b) and carotenoids, respectively. The pigment concentrations were calculated using Wettstain's formula (Wettstain, 1957).

Chlorophyll (a) (mg / L) = $(9.784 \times E662) - (0.99 \times E644)$.

Chlorophyll (b) (mg / L) = $(21.426 \times E644) - (4.64 \times E662)$.

Carotenoids (mg/L) = $(4.695 \times E440) - [0.268 \times (\text{Chlorophyll } a+b)]$.

RESULTS AND DISCUSSION

The effect of six pesticides viz. the three herbicides butralin, clodinafop - propargyl and bromoxynil as well as the three insecticides imidacloprid, malathion and carbosulfan on wheat and cotton leaves contents of chlorophyll a, chlorophyll b and carotenoids is presented in Tables 3 and 4.

Data in Table 3 show that the three pesticides imidacloprid, butralin and carbosulfan displayed two different criteria of phytotoxicity in pesticide – treated cotton leaves. In clay loam soil, cotton leaves contents of chlorophyll a, chlorophyll b and carotenoids were, however, lower in pesticide-treated leaves than with the corresponding control ones. In other words, pesticides acted as inhibitors for the biosynthesis of the three photosynthetic pigments. It is obvious that there is wide range in activity in this respect, on ground of the used pesticide, the type of pigment and the time lapsed between pesticidal treatment and chemoassay. Reduction percentages in chlorophyll a, b and carotenoids ranged between ca. 0.0 to 0.9 , 18 to 19 imidacloprid-, butralin-, and carbosulfan – treated leaves one day after pesticidal treatment. These figures refer to the superiority of butralin in this respect. The picture differed after four days. Imidacloprid revealed

Table 3. Effect of pesticidal treatments and soil type on the content of cotton seedling leaves of chlorophyll a , chlorophyll b and carotenoids

Pesticides	Amounts (in mg /kg) of chlorophyll a, chlorophyll b, and carotenoids at the indicated period from pesticidal treatment (in days)											
	One day						Four days					
	Clay loam soil											
	Chlorophyll a	Control	Chlorophyll b	Control	Carotenoids	Control	Chlorophyll a	Control	Chlorophyll b	Control	Carotenoids	Control
Imidacloprid	3.91	3.92	11.55	11.66	3.62	3.42	3.05	3.92	9.41	11.66	2.17	3.42
Carbosulfan	3.47	3.66	10.66	11.63	2.99	3.27	4.02	3.66	12.34	11.63	3.44	3.27
	Ten days						Fourteen days					
Butralin	3.43	4.18	10.22	12.33	2.96	3.66	3.82	4.18	13.78	12.33	3.18	3.66
	Sandy clay loam											
Imidacloprid	3.04	2.87	9.25	8.73	2.66	2.48	5.21	2.87	17.65	8.73	4.89	2.48
Carbosulfan	2.89	4.51	9.14	17.36	2.37	4.55	4.17	4.51	13.09	17.36	5.82	4.55
	Ten days						Fourteen days					
Butralin	3.93	1.87	11.90	7.59	3.47	2.16	4.51	1.87	17.36	7.59	4.55	2.16

L.S.D0.05 between treatments (chlorophyll a) = 0.532

L.S.D0.05 between treatments (chlorophyll b) = 0.052

L.S.D0.05 between treatments (carotenoids) = 0.138

Table 4. Effect of pesticidal treatments and soil type on the content of wheat seedling leaves of chlorophyll a, chlorophyll b and carotenoids

Pesticides	Amounts (in mg /kg) of chlorophyll a, chlorophyll b, and carotenoids at the indicated period pesticidal treatment (in days)											
	One day						Four days					
	Clay loam soil											
	Chlorophyll a	Control	Chlorophyll b	Control	Carotenoids	Control	Chlorophyll a	Control	Chlorophyll b	Control	Carotenoids	Control
Clodinafop - propargyl	2.69	3.28	2.29	2.73	1.81	2.10	3.62	3.28	3.91	2.73	1.48	2.10
Malathion	3.16	2.79	3.23	2.34	2.64	1.15	2.92	2.79	2.34	2.34	1.61	1.15
Bromoxynil	2.40	1.62	2.44	1.95	1.02	0.40	3.38	1.62	1.95	1.95	092	0.40
	Sandy clay loam											
Clodinafop - propargyl	2.30	2.73	2.19	4.59	0.95	1.72	3.76	2.73	4.59	4.59	2.54	1.72
Malathion	2.52	2.01	2.21	1.98	1.56	0.57	2.17	2.01	1.98	1.98	1.27	0.57
Bromoxynil	2.84	1.28	2.88	2.20	1.11	0.39	3.26	1.28	2.20	2.20	0.79	0.39

L.S.D0.05 between treatments (chlorophyll a) = 0.434
L.S.D0.05 between treatments (chlorophyll b) = 0.563
L.S.D0.05 between treatments (carotenoids) = 0.468

the highest deleterious effect; reduction percentages ranged between 19.3 to 36.5. Data also show that the three pesticides behaved different with the three pigments, especially after 4 days from assessment. It is obvious that the level of chlorophyll a and b in butralin – and carbosulfan – treated leaves were higher than with the control ones. This phenomenon was absent with the third pigment carotenoids.

Strange enough to note that the picture differed greatly with sandy clay loam cultivation. Imidacloprid and butralin acted as inducers; the amount of chlorophyll a, chlorophyll b and carotenoids in pesticide – treated leaves surpassed those of the control treatments especially as the time was lapsed between pesticidal treatment and chemoassay. Increase percentages in the level of chlorophyll a, b and carotenoids were 5.7 , 5.9 and 7.2 in imidacloprid treatment. Magnitude increase took place after 4 days; the corresponding figure percentages were 81.2 , 102 and 97.1 . With butralin treatment revealed much higher inductive effect; increase percentages were 109.4, 56.9 and 60.2 for the corresponding preceding mentioned pigments after one day compared with ca. 14 , 129 and 110 four days later. Curiously enough to note that the third compound carbosulfan, however, displayed inhibitory action against the three pigments. Reduction percentages ranged between ca. 36 to 48 ; 8 to 28 after one and four days post pesticidal treatment, respectively. The differences between the behavior of the three tested pesticides toward the biosynthesis of the three pigments in the two types of soil may be due to the capacity of the pesticide to tolerate the biotransformation processes, i.e. , the persistence of the active compound which may be the parent compound or metabolites . With this way the opportunity factor may play an important role in this respect. Also, binding the used pesticide and its active metabolites with soil components may affect the available free active compound to be uptaked, penetrate, translocate via plant sap.

Data in Table 4 show that, in clay loam – cultivated wheat plants , the herbicide clodinafop – propargyl inhibited the biosynthesis of the three pigments one day post treatment ; reduction percentages were ca. 18, 16 and 14 in chlorophyll a , chlorophyll b and carotenoids contents, respectively. After four days the content of chlorophyll a and b were increased and surpassed the control treatments, whereas reduction in carotenoids was continued and amounted to ca. 29%. On the other hand, the other herbicide bromoxynil as well as the insecticide malathion acted as inducer and resulted in increase in the level of all the three pigments during the whole experimental periods. Increase percentages ranged between ca. 5 to 130, and 25 to 156 in malathion and bromoxynil – treated wheat leaves, respectively. Data show that the inductive effect of the herbicide bromoxynil was higher than with the other herbicide clodinafop – propargyl as well as the insecticide malathion.

The three compounds, however, behaved similar in sandy clay loam cultivated wheat plants. Clodinafop – propargyl – treated wheat plants suffered from reduction in the three pigments one day post treatment. The other two compounds malathion and bromoxynil displayed inductive effect during the whole experimental period simultaneously with clodinafop – propargyl during the second period of assessment. The amounts of chlorophyll a, chlorophyll b and carotenoids surpassed those of the control treatments; increase percentages in the preceding corresponding pigments ranged between ca. 8 to 170 and 31 to 180 in bromoxynil and malathion – treated plants during the whole experimental period. Data show, in general, that the inductive effect of the herbicide bromoxynil was higher than with clodinafop – propargyl as well as the insecticide malathion.

As previously mentioned the intrinsic phytotoxicity of the tested pesticides correlated with the used pesticide, soil type, pigments, plant species and the holding period. The obtained results cope with those found by other investigators. The high, poor and medium effect, respectively, of the used herbicides, insecticides and fungicides on chlorophyll (a and b) and carotenoids contents was also found by Walker (1975), Turgut and Fomin (2002) and Petit *et al.* (2008). Some insecticides, e.g., methyl parathion reduces photosynthesis. This was due to the degradative metabolite p–nitrophenol which has a similar structure as dinitrophenol, a compound with herbicidal properties (Youngman *et al.,* 1990). Also, Su *et al.* (1997) showed that accumulation of insecticides in the above ground tissue can result in phytotoxicity. Thus it is difficult to make generalization about the impact of a pesticide on crop plants.

Regarding the relative susceptibility of the pigments chlorophylls (a and b) and carotenoids , the chlorophyll content of leaves was reduced as well as a/b ratio in favor of chlorophyll b (Bolhar – Nordenkampf , 1975 and Dobrzanski and Bakowski, 1976) or greater loss of chlorophyll a than b after few days (Gokhale and Bansal , 2006). Also , the opposite effects, i.e., the increase or decrease of pigments content in the pesticide – treated cotton and wheat leaves was also observed by Gur *et al.* (1979), Whitehead (2003), Hector *et al.* (2004), and Gokhale and Bansal (2006).

The obtained results show that the soil type played an important role in pigments content of the pesticide–treated plants. This is in full agreement with Dobrazanski and Barowaski (1976) who found that soil type affect the phytotoxicity of pesticides; more phytotoxicity in sand than in a mixture of green house soil with sand . In this respect, Hector *et al.* (2004) indicated that the mechanisms of the phytotoxicity are not direct phytoactivity of the compound, but to indirect effect via non – target organisms, and in particular via, bacteria, fungi, and invertebrates in soil. Regarding the relative susceptibility of the used plant species, Qiu *et al.* (2004) showed that this phenomenon is due to their contents of hormones which result in a holistic impact of plant including photosynthesis in leaves, or metabolism of the plants (Barta, 1996).

The herbicide atrazine inhibited photosynthesis a few hours after application and resulted in a loss of total chlorophyll a , greater loss of chlorophyll a than b after a few days (Bolhar – Nordenkampf, 1975). With the holding period, Gur *et al.* (1979) found that the herbicide dalapon reduced the chlorophyll content of leaves in June and increased it in July.

In conclusion, the tested six pesticides revealed opposite intrinsic phytotoxicity against the biosynthesis of the three pigments viz., chlorophyll a and b as well as carotenoids, i.e., acted as inhibitors and inducers. These two criteria of toxicity correlated with the used pesticide, the holding period, the plant species and the soil type.

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تأثير المعاملة بمبيدات الآفات ونوع التربة على محتويات أوراق بادرات القمح والقطن من الكلوروفيل والكاروتينات

رحاب عيداروس محمد السيد سالم– مصطفى عبد الحفيظ الطنطاوي
علــى أحمــد علــى أيــوب – كمــال عبــد الــرازق جــوهــر
قسم وقاية النبات – كلية الزراعة – جامعة الزقازيق – مصر

قُيمت السمية النباتية الداخلية لــثلاث مــن مبيــدات الحشــائش هــى **butralin** ، **bromoxynil** ، **clodinafop - propargyl** وثلاث مــن المبيــدات الحشــرية هــى **malathion** ، **imidacloprid** ، **carbosulfan** على محتــوى أوراق نبــات القمــح والقطن المنزرعة بالتربة الطينية السلتية و الطينية الســلتية الرمليــة مــن الكلوروفيــل أ والكلوروفيل ب والكاروتينات. وجد أن محتوى أوراق نبــات القطــن المعاملــة بمبيــدات **carbosulfan** ، **butralin** ، **imidacloprid** مــن كلوروفيــل أ ، ب والكاروتينــات تناقصت فى النباتات المنزرعة بالتربة الطينية السلتية حيث قامت المبيدات المذكورة بــدور تثبيطي لعمليات التخليق الحيــوي للــثلاث صــبغات ســالفة الــذكر. كــان فعــل مبيــدي **butralin** ، **imidacloprid** فى النباتات المنزرعة بالتربــة الطينيــة الرمليــة حثــي "**inductive**" (تحفيزي) بينما أظهر المركب الثالث **carbosulfan** فعل تثبيطي. تزايد هذا التأثير بمرور الوقت من بدء المعاملة بالمبيدات وإجراء التقدير الكيماوي للصبغات.

فى حالة أوراق نبات القمح المنزرعة فى كلا النوعين من التربة المذكورة، وجــد أن الأوراق المعرضــة لمبيــدي الحشــائش **bromoxynil** ، **clodinafop - propargyl** والمبيد الحشري **malathion** تحتوى على كميات من الصبغات الثلاث أعلي من نظيرهــا فى النباتات الغير معاملة خلال فترة التقدير بالكامل فيما عدا النباتات المعاملة بالمبيد الأول بعد يوم من بدء المعاملة حيث عانت النباتات من نقص واضح فى الثلاث صبغات.